Bioenergetics in Photosystem I: Time-Resolved Step-Scan FTIR and Visible Spectroscopic Studies of the Secondary Electron Acceptor A1

Hiroki Makita
Georgia State University

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BIOENERGETICS IN PHOTOSYSTEM I: TIME-RESOLVED STEP-SCAN FTIR AND VISIBLE SPECTROSCOPIC STUDIES OF THE SECONDARY ELECTRON ACCEPTOR $A_1$

by

HIROKI MAKITA

Under the Direction of Gary Hastings, PhD

ABSTRACT

Time-resolved infrared and visible absorption difference spectroscopy was applied for the study of electron transfer (ET) reactions involving $A_1$, the secondary electron acceptor in photosystem I (PSI). In PSI, the secondary electron acceptor $A_1$ is a phylloquinone (PhQ) molecule. Flash-induced absorption changes at room and cryogenic temperatures in the infrared and visible spectral ranges were probed for PSI with a series of native and non-native quinones in the $A_1$ binding site. Obtained kinetic and spectral data were analyzed for the functional and structural properties of $A_1$ and PSI.

Using transient absorption spectroscopy in the visible spectral range, the rates and directionality of ET processes in PSI with modified $A_1$ were determined. A detailed kinetic
A simulation model was constructed and solved in the context of Marcus ET theory, and midpoint redox potentials of $A_1$ was predicted within a tight range. The transient absorption kinetics for ten different quinones and the kinetic simulation revealed that the wasteful charge recombination process in native PSI occurs in the inverted region. Although inverted-region ET had been widely suggested to be an important mechanism contributing to photosynthetic efficiency, the mechanism had never been demonstrated in any native photosynthetic system. The result presented here is the first demonstration of inverted-region ET in a native photosynthetic reaction center in physiological conditions. Through Marcus theory-based simulation, inverted-region ET is quantitatively shown to be an important mechanism underlying the high efficiency in PSI ET.

Time-resolved infrared difference spectroscopy was undertaken using step-scan FTIR technique with a microsecond temporal resolution. Highly-resolved double difference spectrum was constructed to identify infrared bands due to PhQ in the $A_1$ binding site. Assisted by the DFT-based vibrational frequency calculations, vibrational modes due to anionic PhQ$^-$ were identified. The calculations suggest that PhQ is asymmetrically H-bonded, and that this interaction is especially strong for PhQ$^-$, but not for PhQ. Additionally, discrepancies that previously existed between FTIR and EPR studies on PSI with plastoquinone-9 in the $A_1$ site were resolved. A method to incorporate a benzoquinone was established, and $(A_1^- - A_1)$ FTIR difference spectra for a series of benzoquinones were produced for the first time.

INDEX WORDS: Photosynthesis, Photosystem I, Electron transfer, Time-resolved spectroscopy, FTIR, $A_1$, Phylloquinone
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HIROKI MAKITA

Committee Chair: Gary Hastings

Committee: Vadym Apalkov

Gennady Cymbalyuk

Mukesh Dhamala

Brian Thoms

Electronic Version Approved:

Office of Graduate Studies
College of Arts and Sciences
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AS CAN BE SEEN IN THE DDS DIAGRAM, THE SHIFT IN PEAKS BETWEEN
TWO DS IS SMALL AND RESULTS IN A DIMINISHED SIGNAL SIZE IN A DDS. THEREFORE, HIGH SIGNAL-TO-NOISE RATIO IN DS ARE USUALLY REQUIRED FOR A CONSTRUCTION OF DDS.

FIGURE 2.1 (A) ARRANGEMENT OF THE TWO BRANCHES OF ET COFACTORS IN PSI. NUMBER SUBSCRIPT REFERS TO COFACTOR. LETTER SUBSCRIPT REFERS TO PROTEIN SUBUNIT (PSAA OR B) TO WHICH COFACTOR IS BOUND. THE A/B -BRANCH REFERS TO THE SET OF ET COFACTORs ON THE LEFT/RIGHT SIDE, RESPECTIVELY. FIG. 2.1A IS GENERATED USING THE 2.5 Å X-RAY CRYSTAL STRUCTURE OF TRIMERIC PSI PARTICLES FROM THE CYANOBACTERIUM THERMOSYNECHOCOCCUS ELONGATUS (PDB FILE ACCESSION NUMBER PDB ID: 1JB0) (10). (B) VIEW OF PHQ IN THE A1A BINDING SITE. POSSIBLE H-BONDING INTERACTIONS ARE SHOWN (DOTTED), AND PHQ NUMBERING SCHEME IS INDICATED. NITROGEN/OXYGEN/SULFUR ATOMS ARE BLUE/RED/YELLOW, RESPECTIVELY.

FIGURE 2.2 RT FLASH-INDUCED ABSORPTION CHANGES AT 800 (A, B) AND 703 (C, D) NM ON A 350 NS TIMESCALE FOR MENB− PSI WITH PQ9 (BLUE), 2MNQ (GREEN), AND PHQ (RED) INCORPORATED. DATA FOR BOTH DILUTE (B, D) AND CONCENTRATED (A, C) PSI SAMPLES ARE SHOWN. AT 800 NM, FOR PSI WITH PQ9 INCORPORATED (BLUE), THE DATA IS BEST FIT TO TWO EXPONENTIALS WITH LIFETIMES OF 33 AND 107 NS (A) OR 23 AND 105 NS (B). THE FITTED BI-EXPONENTIAL FUNCTIONS (BLACK) ARE ALSO SHOWN IN A AND B. AT 703 NM, THE DATA IS ADEQUATELY FIT TO A
SINGLE EXPONENTIAL WITH LIFETIME OF 96 NS (C) OR 103 NS (D). THESE FITTED SINGLE EXPONENTIAL FUNCTIONS ARE ALSO SHOWN IN C AND D (BLACK). DATA IS ALSO SHOWN FOR DILUTE WT PSI PARTICLES FROM S6803 (MAGENTA) (B, D). FIGURE 2.3 RT FLASH-INDUCED ABSORPTION CHANGES AT 703 NM ON 10–35 MS TIMESCALES FOR MENB− PSI SAMPLES WITH (A) PQ₀ AND (B) 2MNQ INCORPORATED INTO A₁ BINDING SITE. MEASUREMENTS ARE SHOWN FOR DILUTE SAMPLES BUT SIMILAR RESULTS ARE FOUND FOR CONCENTRATED SAMPLES (NOT SHOWN). THE DATA IN BOTH FIGURES WERE FITTED TO A SINGLE EXPONENTIAL FUNCTION (PLUS A CONSTANT). THE FITTED FUNCTIONS ARE SHOWN (RED) ALONG WITH THE CALCULATED TIME CONSTANTS. FIGURE 2.4 ROOM TEMPERATURE FLASH-INDUCED ABSORPTION CHANGES AT 487 NM FOR MENB− PSI SAMPLES WITH (A) PHQ, (C) PQ₀ AND (D) 2MNQ INCORPORATED INTO A₁ BINDING SITE. FOR COMPARISON, MEASUREMENTS FOR WT PSI PARTICLES FROM S6803 ARE ALSO SHOWN (B). MEASUREMENTS ARE FOR DILUTE SAMPLES ONLY AS THE ABSORBANCE AT 487 NM FOR CONCENTRATED SAMPLES PROHIBITS SPECTROSCOPIC MEASUREMENT. THE CALCULATED FITTED FUNCTIONS (RED) AND TIME CONSTANTS ARE ALSO SHOWN. INSET IN C AND D SHOWS ABSORPTION CHANGES OVER SHORTER 0.9 AND 1.8 MS TIME WINDOWS, RESPECTIVELY.
FIGURE 2.5 Low temperature (77 K) flash-induced absorption changes at 800 (A, C, E) and 703 nm (B, D, F) for concentrated PSI samples with PQ₉ (A, B), PHQ (C, D) and 2MNQ (E, F) incorporated. The data at both probe wavelengths were fitted simultaneously to a single exponential function (red) and a lifetime of 250/338/240 ms was calculated, respectively.

FIGURE 2.6 Low temperature (77 K) flash-induced absorption changes at several IR frequencies obtained using PSI with (A) PHQ and (B) 2MNQ incorporated into the A₁ binding site. Data were collected in 5 ms increments. The four kinetics in each caption were fitted simultaneously to a single exponential plus a constant. The fitted functions are shown (red) and are characterized by a time constant of 301 and 241 ms, respectively.

FIGURE 2.7 Simulated temporal evolution of the population of radical pair states for PSI with (A) PHQ, (B) 2MNQ and (C) PQ₉ incorporated. Evolution of the [P700⁺A₁⁻] (red), and [P700⁺A₁B⁻] (blue) populations are shown along with the sum of the two [P700⁺A₁⁻] (black). (D) Kinetic model used in simulations. ΔGₐ₁ₐ/FX₀/ΔGₐ₁B/FX₀ used are +15/-10, +85/+60 and +175/+150 MEV for PSI with PHQ, 2MNQ and PQ₉ incorporated, respectively. Using these values in Eq. 2.2 (with R = 9.0 Å, Λ = 0.7 EV, T = 298 K) the rate constants indicated in (D) are calculated (in units of ns⁻¹).
THE TEMPORAL PROFILES IN A, B AND C ARE A SUM OF SEVERAL EXPONENTIAL COMPONENTS. THE WEIGHTED AVERAGE OF THE TIME CONSTANTS (CALCULATED AS INDICATED IN (39) FOR [P700+A1−] ARE 142 NS, 1.8 MS AND 60 MS FOR PSI WITH PHQ, 2MNQ AND PQ9 INCORPORATED, RESPECTIVELY.

FIGURE 3.1 (A) ROOM TEMPERATURE (298 K) FLASH-INDUCED ABSORPTION CHANGES AT 703 NM FOR PSI SAMPLES WITH PHQ INCORPORATED INTO THE A1 BINDING SITE. SAMPLES WERE EXCITED USING 532 NM LASER PULSES WITH PUMP ENERGIES RANGING FROM 0.4 TO 1.7 MJ/PULSE. (B). PLOT OF SIGNAL AMPLITUDE AT 703 NM AS A FUNCTION OF PUMP PULSE ENERGY. THE DATA POINTS (SQUARES) REPRESENT THE AVERAGE VALUE IN THE 1-4 μS RANGE, WHILE THE ERROR BARS INDICATE THE MIN/MAX DATA POINTS IN THE 1-4 μS RANGE.

FIGURE 3.2 RT FLASH-INDUCED ABSORPTION CHANGES AT 800 (A), 703 (B) AND 487 NM (C) FOR PSI WITH CL2NQ INCORPORATED. DATA WAS COLLECTED OVER SEVERAL TIME WINDOWS IN A LINEAR FASHION (SEE INSETS) AND IS PLOTTED HERE ON A LOGARITHMIC TIMESCALE. FITTED CURVES ARE ALSO SHOWN (RED). THE PARAMETERS DERIVED FROM FITTING ARE SUMMARIZED IN TABLE 3.1. THE DATA AT 487 NM WAS COLLECTED USING LESS CONCENTRATED SAMPLES BUT WAS SCALED TO MATCH THE OPTICAL DENSITY OF SAMPLES USED TO COLLECT THE DATA AT 703 AND 800 NM. INSETS: ABSORPTION CHANGES ON A (LINEAR) 0–25 MS TIMESCALE.
FIGURE 3.3 $77\text{ K}$ FLASH-INDUCED ABSORPTION CHANGES AT 800 NM (A) AND 703 NM (B) FOR PSI WITH CL$_2$NQ INCORPORATED. THE DATA IS BEST FITTED TO TWO STRETCHED EXPONENTIALS WITH LIFETIMES OF 2.3 MS AND 78.2 MS. THE FITTED CURVES ARE SHOWN IN RED, AND THE FITTED PARAMETERS SUMMARIZED IN TABLE 3.1. Insets: Absorption changes on a 0–25 MS timescale.

FIGURE 3.4 $77\text{ K}$ FLASH-INDUCED ABSORPTION CHANGES OF SEVERAL INFRARED WAVELENGTHS (IN CM$^{-1}$) AT 298 (A) AND 77 K (B). THE KINETICS AT 298/77 K ARE FITTED SIMULTANEOUSLY TO A STRETCHED EXPONENTIAL FUNCTION WITH TIME CONSTANT OF 129.4 MS (B = 0.70)/75.6 MS (B = 0.70), RESPECTIVELY. DIFFERENT SAMPLES WERE USED FOR THE RT AND LT MEASUREMENTS.

FIGURE 3.5 (A) $77\text{ K}$ FLASH INDUCED ABSORPTION CHANGES AT 703 NM FOR PSI WITH CL$_2$NQ INCORPORATED. EACH TRACE IS THE AVERAGE OF 640 LASER FLASHES. (B) RELATIVE INITIAL AMPLITUDES OF FLASH-INDUCED ABSORPTION CHANGES AS A FUNCTION OF THE NUMBER OF LASER FLASHES FOR PSI WITH CL$_2$NQ (DOTS) AND PHQ (SQUARES) INCORPORATED, PROBED AT 703 NM. THE RELATIVE AMPLITUDE IS TAKEN AS THE RATIO OF THE INITIAL ABSORPTION CHANGES AT 77 K TO THAT AT 298 K. THE 298 K INITIAL AMPLITUDE IS INDEPENDENT OF THE NUMBER OF LASER FLASHES. THE AMPLITUDE OF THE FLASH-INDUCED ABSORPTION CHANGES AT LT BEFORE ANY LASER FLASHES IS SIMILAR TO THAT FOUND AT RT. IN (B) THE DATA FOR PSI WITH CL$_2$NQ
INCORPORATED IS FITTED TO EQ. 3.1, WITH BEST FIT PARAMETERS \( \Delta A_R = 0.578 \) AND \( \Phi = 0.9989 \). THE QUANTUM YIELD FOR THE FORMATION OF AN IRREVERSIBLE STATE IS THEREFORE 0.0011. ............................... 76

FIGURE 4.1298 K FLASH-INDUCED ABSORPTION CHANGES AT 487 NM FOR PSI WITH (A) AQ, (B) PHQ, (C) 2MNQ, (D) 2CLNQ, (E) 2BRNQ, AND (F) CL\(_2\)NQ INCORPORATED INTO THE \( A_1 \) BINDING SITE. THE DATA ASSOCIATED WITH FORWARD ET (A–C) ARE FITTED TO A SUM OF EXPONENTIAL FUNCTIONS PLUS A CONSTANT. THE DATA ASSOCIATED WITH CHARGE RECOMBINATION (D–F) ARE FITTED TO A SUM OF STRETCHED EXPONENTIAL FUNCTIONS PLUS A CONSTANT. THE FITTED FUNCTIONS ARE ALSO SHOWN (RED). THE INITIAL SIGNAL AMPLITUDES WERE SCALED. THE TIMESCALES ARE SELECTED TO HIGHLIGHT THE MOST PROMINENT DECAY PHASES. THE TIME CONSTANTS OBTAINED FROM FITTING THE DATA ARE LISTED ON TABLE 4.1. THE TIME CONSTANTS OF MINOR PHASE ASSOCIATED WITH P700\(^+\)A\(_1\)\(^-\) CHARGE RECOMBINATION (SEE CH. 3) ARE NOT INCLUDED................................................................................................. 86

FIGURE 4.2298 K FLASH-INDUCED ABSORPTION CHANGES AT 703 NM FOR PSI WITH (A) 2CLNQ, (B) 2BRNQ, (C) CL\(_2\)NQ, AND (D) BR\(_2\)NQ INCORPORATED INTO THE \( A_1 \) BINDING SITE. THE DATA ARE FITTED TO A STRETCHED EXPONENTIAL FUNCTION PLUS A CONSTANT. THE FITTED FUNCTIONS ARE ALSO SHOWN (RED). THE INITIAL SIGNAL AMPLITUDES HAVE BEEN SCALED. THE OBTAINED TIME CONSTANT ARE LISTED ON TABLE 4.1. 87
FIGURE 4.3 77 K FLASH-INDUCED ABSORPTION CHANGES AT 703 NM FOR PSI WITH (A) AQ, (B) PHQ, (C) 2MNQ, (D) PQₐ, (E) 2CLNQ, (F) 2BRNQ, (G) CL₂NQ AND (H) BR₂NQ INCORPORATED. FITTED FUNCTIONS ARE SHOWN IN RED AND THE CALCULATED TIME CONSTANTS ARE LISTED IN TABLE 4.1. .......... 88

FIGURE 4.4 298 K TIME-RESOLVED ABSORPTION CHANGES AT SEVERAL INFRARED WAVELENGTHS (WAVENUMBERS) FOR PSI WITH (A) 2CLNQ, (B) 2BRNQ, (C) CL₂NQ, AND (D) BR₂NQ INCORPORATED. IN EACH CAPTION THE SHOWN WAVELENGTHS WERE FITTED SIMULTANEOUSLY TO A STRETCHED EXPONENTIAL FUNCTION AND A CONSTANT (RED). THE CALCULATED TIME CONSTANTS ARE LISTED IN TABLE 4.1................. 89

FIGURE 5.1 ARRANGEMENT OF THE ET COFACTORS IN PSI FROM THERMOSYNECHOCOCCUS ELONGATUS (PDB FILE 1JB0, (10)). EDGE-TO-EDGE DISTANCES (IN Å) BETWEEN COFACTORS IS REPRESENTED BY THE DOTTED VERTICAL LINES. THE ESTABLISHED REDOX POTENTIALS FOR P700/P700⁺ (36), Fₓ/Fₓ⁻ (7), FA/FA⁻ AND FB/FB⁻ (39) ARE SHOWN. THE KINETIC MODEL USED TO SIMULATE THE LIGHT-INDUCED POPULATION EVOLUTION OF RADICAL STATES IN PSI AT BOTH 298 AND 77 K IS OUTLINED. THE TERMINAL IRON-SULFUR CLUSTERS (FA AND FB) WERE INCLUDED FOR SIMULATIONS AT 298 K BUT NOT AT 77 K. ET FROM A₀⁻ TO A₁ IS ASSUMED TO BE MUCH FASTER THAN ET FROM A₁⁻ TO THE OTHER COFACTORS, AND THUS ONLY THE POPULATION DYNAMICS OF THE COFACTORS FOLLOWING P700⁺A₁⁻ FORMATION ARE CONSIDERED. THE INITIAL POPULATION OF A₁A⁻/A₁B⁻ WAS ASSUMED TO BE 50/50 AT 298
K AND 95/5 AT 77 K. INSET: SIMPLIFIED KINETIC MODEL USED PREVIOUSLY TO MODEL ONLY THE KINETICS OF FORWARD ET FROM A1⁻ TO Fₓ AT 298 K.

FIGURE 5.2 OUTLINE OF THE SYSTEMATIC PROCEDURES APPLIED IN SIMULATIONS. THE SIMULATION STARTS WITH A KINETIC MODELING OF PSI WITH PHQ INCORPORATED INTO THE A₁ BINDING SITE (LEFT COLUMN) AND CONTINUES TO THE KINETIC MODELING OF PSI WITH NON-NATIVE QUINONES (RIGHT COLUMN).

FIGURE 5.3 A HEAT MAP OF CALCULATED AGREEMENT BETWEEN THEORETICAL AND EXPERIMENTAL WEIGHTED AVERAGE TIME CONSTANTS. AGREEMENT IS CALCULATED AS [1–(T–E)/E], WHERE T/E IS THE THEORETICAL/EXPERIMENTAL WEIGHTED AVERAGE TIME CONSTANT, RESPECTIVELY. THE SHADED AREA INDICATES THE REGION WHERE EM(A₁B) IS MORE POSITIVE THAN EM(A₁A).

FIGURE 5.4 SIMULATED POPULATION EVOLUTION OF RADICAL STATES AT 298 K FOR PSI WITH (A) AQ, (B) PHQ, (C) 2MNQ, (D) PQ₀, (E) 2CLNQ, (F) 2BRNQ, (G) CL₂NQ, AND (H) BR₂NQ INCORPORATED. TIME EVOLUTION OF THE POPULATION OF A₁⁻ (BLUE), A₁A⁻ (MAGENTA), A₁B⁻ (PURPLE), Fₓ⁻ (GREEN), Fₐ⁻ (DARK YELLOW), Fₜ⁻ (CYAN), AND RECOVERY OF P700 (RED). THE WEIGHTED AVERAGE TIME CONSTANT OF THE SIMULATED POPULATION EVOLUTIONS ARE COMPARED TO THE EXPERIMENTAL WEIGHTED AVERAGE TIME CONSTANTS IN TABLE 5.2. QUINONE STRUCTURES ARE ALSO SHOWN (CARBON, OXYGEN, CHLORINE, AND
BROMINE ATOMS ARE BLACK, RED, GREEN, AND PURPLE, RESPECTIVELY).

FIGURE 5.5 POPULATION EVOLUTION OF THE RADICAL STATES IN PSI WITH EIGHT DIFFERENT QUINONES INCORPORATED (SAME ORDER AS IN FIG. 5.4) AT 77 K. THE TEMPORAL PROFILES FOR A_1^- (BLUE), F_X^- (GREEN) AND P700 (RED) ARE SHOWN. AT 77 K, FOR PSI WITH ALL OF THE DIFFERENT QUINONES INCORPORATED, THE POPULATION EVOLUTION OF A_1B^- IS ESSENTIALLY COMPLETE WITHIN 10^-5 S (NOT SHOWN). THEREFORE, THE POPULATION EVOLUTION CURVE SHOWN FOR A_1^- IS ESSENTIALLY THAT OF A_1A^-.

FIGURE 5.6 PLOT COMPARING THE E_M FOR THE EIGHT QUINONES REPORTED IN TABLE 5.2 [A_1A IN BLACK, A_1B IN RED] WITH E_1/2 IN APROTIC SOLVENT. THE EQUATIONS RESULTING FROM FITTING THE DATA TO A LINEAR FUNCTION IS ALSO SHOWN. THE LINEAR RELATIONSHIPS OBTAINED PREVIOUSLY FOR SOLVENT-EXTRACTED PSI ARE ALSO SHOWN FOR COMPARISON. THE EQUATIONS GOVERNING THE LATTER ARE E_M-
\[ A_1(V) = 0.720E_{1/2}(V) - 0.408 \text{ (BLUE) AND } E_{M-A_1}(V) = 0.690E_{1/2}(V) - 0.433 \text{ (GREEN)} \] (40).

**FIGURE 6.1** ARRANGEMENT OF THE ET COFACTORS (PIGMENTS) IN PSI WITH THE TWO POSSIBLE ROUTES OF ET INDICATED. FIGURE WAS DERIVED FROM THE 2.5 Å CRYSTAL STRUCTURE OF PSI FROM THERMOSYNECHOCOCCUS ELONGATUS (10). COFACTOR HYDROCARBON TAILS HAVE BEEN TRUNCATED. A SIMILAR FIGURE IS OBTAINED USING THE 2.8 Å CRYSTAL STRUCTURE OF PSI FROM S6803 (137). EDGE-TO-EDGE DISTANCES (IN Å) BETWEEN COFACTORS (DOTTED) AS WELL AS THE COFACTOR REDOX POTENTIALS FOR P700 (36), A\textsubscript{1A} AND A\textsubscript{1B} (48), F\textsubscript{X} (138), F\textsubscript{A} AND F\textsubscript{B} (14, 39) ARE SHOWN. ARROWS INDICATE THE KINETIC MODEL THAT WAS USED PREVIOUSLY TO ANALYZE THE LIGHT-INDUCED DYNAMICS OF RADICAL PAIR STATES IN PSI (48). P700\textsuperscript{+}F\textsubscript{A/B}\textsuperscript{−} AND P700\textsuperscript{+}F\textsubscript{X}\textsuperscript{−} RADICAL PAIR RECOMBINATION PROCEEDS VIA REPOPULATION OF A\textsubscript{1−} (30), AS INDICATED.

**FIGURE 6.2 (A)** 77 K FLASH INDUCED ABSORPTION CHANGES AT 703 NM (INVERTED) FOR PSI WITH TEN DIFFERENT QUINONES INCORPORATED. FITTED FUNCTIONS ARE ALSO SHOWN (SOLID LINES). (B) PLOT OF THE OBSERVED P700\textsuperscript{+}A\textsubscript{1−} RECOMBINATION RATE AT 77 K VERSUS THE REACTION FREE ENERGY CALCULATED USING THE QUINONE IN VITRO MIDPOINT POTENTIALS \([-\Delta G^0 = -E(E_{1/2} - 450)]\). DATA IS FIT TO A PARABOLIC FUNCTION (DOTTED) AND IS NUMBERED ACCORDING TO TABLE 6.1.
FIGURE 6.3(A) MARCUS PLOT OF P700\(^+\)A\(_{1A}^-\) INTRINSIC (\(T_{\text{INT}}\), ■) AND OBSERVED (\(T_{\text{OBS}}\), ×) RECOMBINATION RATES AT 77 K VERSUS THE REACTION FREE ENERGY \([-\Delta G^0 = -E(E_M - 450)\]) FOR TEN DIFFERENT QUINONES INCORPORATED INTO PSI. NUMBERING IS ACCORDING TO TABLE 6.1. THE PARABOLIC FITTED FUNCTION IS BASED ON ET PARAMETERS DERIVED PREVIOUSLY (48), ALONG WITH THE INTRINSIC TIME CONSTANTS LISTED IN TABLE 6.1. THE HORIZONTAL ERROR BARS (ITEMS 5 AND 10) ARE ESTIMATES OF THE IN SITU POTENTIAL BASED ON A POSSIBLE ±0.2 Å ERROR IN THE EDGE-TO-EDGE DISTANCE BETWEEN P700 AND A\(_1\). (B) MARCUS PLOT DETAILING P700\(^+\)A\(_{1A}^-\) INTRINSIC RECOMBINATION RATES AT 298 K VERSUS THE REACTION FREE ENERGY FOR TEN DIFFERENT QUINONES INCORPORATED. THE PARABOLIC FITTED FUNCTION IS BASED ON ET PARAMETERS DERIVED PREVIOUSLY (48) ALONG WITH THE INTRINSIC TIME CONSTANTS LISTED IN TABLE 6.2. A REORGANIZATION ENERGY OF 580 MEV AND A MEAN VIBRATIONAL MODE WITH ENERGY OF 56 MEV WAS USED.

FIGURE 6.4 MODEL USED IN KINETIC SIMULATIONS FOR PSI IN SITU. THE CIRCLED REGION HIGHLIGHTS THE EXTENSION FROM PREVIOUSLY CONSIDERED KINETIC MODELS (48).

FIGURE 6.5 POPULATION DYNAMICS OF (A\(_{1A}^-\)+A\(_{1B}^-\)) (CYAN), F\(_X^-\) (RED), F\(_A^-\) (GREEN), F\(_B^-\) (ORANGE) AND P700 (BLACK) SIMULATED USING THE FIRST AND SECOND-ORDER TIME CONSTANTS AND AMPLITUDES ASSOCIATED WITH ET FROM F\(_B^-\) TO FD. DOTTED LINES ARE FOR PSI WHERE P700\(^+\)A\(_{1A}^-\)
AND P700⁺A₁B⁻ RADICAL PAIR RECOMBINATION OCCUR IN THE INVERTED REGION. SOLID LINES ARE FOR PSI WHERE RADICAL PAIR RECOMBINATION IS OPTIMIZED BY MODIFYING THE REORGANIZATION ENERGY TO MATCH THE FREE ENERGY. IN THE INVERTED REGION THE EXTENT OF RECOVERY OF P700 REMAINS LOW (BLACK, DOTTED) WHILE IT IS CONSIDERABLY INCREASED FOR THE SITUATION WHERE THE RATE IS OPTIMIZED (−ΔG⁰ = Λ) (BLACK, SOLID). ............................................................. 137

FIGURE 6.6 KINETIC MODEL THAT INCLUDES BOTH A MOBILE DONOR (PC) AND ACCEPTOR (FD) SPECIES. MOBILE DONOR/ACCEPTOR IS RED/BLUE, RESPECTIVELY. THIS MODEL EXTENDS ON THE MODEL OUTLINED IN FIG. S4 BY INCLUDING A PC COFACTOR THAT CAN RE-REDUCE P700⁺. NUMBERS IN BRACKETS INDICATE THE INITIAL POPULATIONS OF THE VARIOUS STATES IN THE SIMULATIONS. ............................................................. 139

FIGURE 6.7 EXAMPLE INDICATING HOW SOLAR ENERGY CONVERSION EFFICIENCY IS CALCULATED IN THE PC-PSI-FD KINETIC MODEL SHOWN IN FIG. 6.6. POPULATION DYNAMICS FOR (A₁A⁻⁺A₁B⁻) (BLUE), Fₓ⁻ (RED), Fₐ⁻ (PURPLE), Fₜ⁻ (BROWN), P700 (YELLOW) AND FD (BLUE). POPULATION OF A₁⁻, Fₓ⁻, Fₐ⁻, Fₜ⁻ AND FD⁻ REMAINING WHEN P700 HAS FULLY Recovered ARE INDICATED BY DOTTED LINES. THESE RADICALS ACCOUNT FOR ~99% OF THE TOTAL POPULATION. DOTTED LINES ARE FOR PSI WHERE P700⁺A₁⁻ RADICAL PAIR RECOMBINATION OCCURS IN THE INVERTED REGION. SOLID LINES ARE FOR PSI WHERE P700⁺A₁⁻ RADICAL PAIR
RECOMBINATION IS OPTIMIZED BY MODIFYING THE REORGANIZATION ENERGY TO MATCH THE REACTION FREE ENERGY.

FIGURE 6.8 EXTENDED KINETIC MODEL THAT INCLUDES PC⁺ RE-REDUCTION. IN THIS PQH₂-PC-PSI-FD MODEL, AN INITIAL POPULATION (INDICATED IN GRAY) IS ASSIGNED TO A₁ (50 EACH TO A₁A AND A₁B) AND PQH₂ (100). THE RELATIVE POSITION OF THE COFACTORS (ON A VERTICAL SCALE) IS ARBITRARY, AND DOES NOT REFLECT DIFFERENCES IN COFACTOR REDOX POTENTIALS.

FIGURE 7.1 (A) ARRANGEMENT OF THE ET COFACTORS IN PSI, GENERATED USING THE 2.5 Å X-RAY CRYSTAL STRUCTURE OF PSI FROM T. ELONGATUS (PDB 1JB0) (10). (B) VIEW OF PHQ IN THE A₁A BINDING SITE. POSSIBLE H-BONDING INTERACTIONS ARE SHOWN (DOTTED). (C) MOLECULAR MODEL FOR NEUTRAL PHQ IN THE A₁ BINDING SITE. NITROGEN/OXYGEN/SULFUR/CARBON ATOMS ARE COLORED BLUE/RED/YELLOW/GREY, RESPECTIVELY. HIGH QM LAYER SHOWN IN BALL AND STICK REPRESENTATION. LOW QM LAYER SHOWN IN STICK REPRESENTATION. MM LAYER SHOWN AS THIN STICKS. (D) MODEL DEMONSTRATING BOND LENGTHS AND ANGLES CALCULATED FOR NEUTRAL AND REDUCED PHQ. BLACK/RED/BLUE: NEUTRAL/REDUCED/X-RAY.

FIGURE 7.2 FTIR ABSORPTION SPECTRA FOR (A) PHQ AND (B) 2MNQ IN TETRAHYDROFURAN (THF). THE SPECTRA WERE SCALED SO THAT THE INTENSITIES OF THE BANDS AT 1662 AND 1666 CM⁻¹ ARE SIMILAR.
FIGURE 7.3 KINETICS OF ABSORPTION CHANGES AT SEVERAL INFRARED WAVELENGTHS OBTAINED AT 77 K FOLLOWING 532 NM LASER FLASH EXCITATION OF PSI PARTICLES WITH PHQ (LEFT) AND 2MNQ (RIGHT) INCORPORATED. TIME RESOLUTION WAS 6 µS. SPECTRAL RESOLUTION WAS 4 CM\(^{-1}\).

FIGURE 7.4 TIME-RESOLVED \([P700^+A_{1}^- - P700A_{1}]\) FTIR DS IN THE 1800-1390 CM\(^{-1}\) REGION, OBTAINED FOLLOWING LASER FLASH EXCITATION OF MENB\(^{-}\) PSI PARTICLES WITH PHQ (A) AND 2MNQ (C) INCORPORATED. SPECTRA AT T = 0, 12, 54, 102, 150, 204, 252, 300, 248, 402, 450, 498, 552, 600, 648, 702, AND 750 µS AFTER THE LASER FLASH ARE SHOWN. EXCEPT FOR THE SPECTRUM SHOWN AT T = 0 µS (GREEN), THE SPECTRA SHOWN ARE THE AVERAGE OF THREE SPECTRA CENTERED AT THE INDICATED TIME POINT. FROM GLOBAL ANALYSIS, THREE DAS ARE OBTAINED FOR PSI WITH PHQ (B) AND 2MNQ (D) INCORPORATED. A ~15 µS PHASE (RED) IS ASSOCIATED WITH A HEATING ARTIFACT RELATED TO LASER FLASH EXCITATION. A ~360/240 µS PHASE FOR PSI WITH PHQ/2MNQ IS DUE TO P700\(^{+}\)A\(_{1}\)^{−} CHARGE RECOMBINATION. A DAS ASSOCIATED WITH THE NON-DECAYING COMPONENT IS SHOWN IN BLACK.

FIGURE 7.5 \([P700^+A_{1}^- - P700A_{1}]\) TR FTIR DAS FOR MENB\(^{-}\) PSI PARTICLES WITH PHQ (A) AND 2MNQ (B) INCORPORATED INTO THE A\(_{1}\) BINDING SITE. THE SPECTRA SHOWN ARE THE AVERAGE OF 3 SEPARATE EXPERIMENTS. THE STANDARD ERROR SPECTRA ARE ALSO SHOWN (DOTTED). CORRESPONDING \([P700^+ - P700]\) FTIR DS ARE SHOWN IN FIG. 7.4. (C)
(2MNQ – PHQ) FTIR DDS OBTAINED BY SUBTRACTING SPECTRUM A FROM B. PROPAGATED STANDARD ERROR IS OVERLAI
D TO THE DDS (SHADED)................................................................. 161


FIGURE 7.8: CALCULATED [A\textsuperscript{−} – A\textsubscript{1}] DS FOR PHQ (A) AND 2MNQ (B) IN THE PROTEIN BINDING SITE. THE CALCULATED FREQUENCIES ARE SCALED BY 0.968/0.977 FOR THE NEUTRAL/ANION STATE, RESPECTIVELY. (C) CALCULATED [2MNQ – PHQ] DDS, OBTAINED BY SUBTRACTING SPECTRUM A FROM B.

FIGURE 8.1: ARRANGEMENT OF ET COFACTORS IN PSI. STRUCTURE WAS DERIVED FROM THE 2.5 Å X-RAY CRYSTAL STRUCTURE OF PSI FROM THERMOSYNECHOCOCCUS ELONGATUS (PDB ID 1JB0) (10). F\textsubscript{x}, F\textsubscript{A}, AND F\textsubscript{B} ARE IRON-SULFUR CLUSTERS, A\textsubscript{0} IS CHLOROPHYLL-A, AND A\textsubscript{1} IS PHQ. PIGMENT HYDROCARBON CHAINS ARE REMOVED FOR CLARITY.

FIGURE 8.2: DAS OF THE MAJOR DECAY PHASE FOUND USING GLOBAL ANALYSIS OF TR FTIR DS OBTAINED USING (A) PRE-FLASHED AND (B) NON-FLASHED INTACT PSI (SOLID) AND PSI–F\textsubscript{x} SAMPLES (DOTTED) AT 77 K. TRANSIENT ABSORPTION CHANGES AT SEVERAL WAVE NUMBERS ARE SHOWN FOR PRE-FLASHED (C) AND NON-FLASHED (D) INTACT PSI SAMPLES. SIMILAR ABSORPTION CHANGES ARE FOUND FOR PSI–F\textsubscript{x} SAMPLES.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>µs</td>
<td>microsecond</td>
</tr>
<tr>
<td>¹³C-PSI</td>
<td>¹³C-labeled PSI</td>
</tr>
<tr>
<td>¹⁸O-PhQ</td>
<td>¹⁸O-labeled PhQ</td>
</tr>
<tr>
<td>2BrNQ</td>
<td>2-bromo-1,4-naphthoquinone</td>
</tr>
<tr>
<td>2ClNQ</td>
<td>2-chloro-1,4-naphthoquinone</td>
</tr>
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<td>2OHNQ</td>
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<tr>
<td>AceQ</td>
<td>acequinocyl</td>
</tr>
<tr>
<td>AQ</td>
<td>9,10-anthraquinone</td>
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<tr>
<td>AQS</td>
<td>sodium anthraquinone-2-sulfonate</td>
</tr>
<tr>
<td>Asc</td>
<td>sodium ascorbate</td>
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<tr>
<td>BQ</td>
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</tr>
<tr>
<td>Br₂NQ</td>
<td>2,3-dibromo-1,4-naphthoquinone</td>
</tr>
<tr>
<td>C. reinhardtii</td>
<td><em>Chlamydomonas reinhardtii</em></td>
</tr>
<tr>
<td>C=O</td>
<td>carbonyl</td>
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<tr>
<td>CaF₂</td>
<td>calcium fluoride</td>
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<td>Chl-a</td>
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<tr>
<td>cyt</td>
<td>cytochrome</td>
</tr>
<tr>
<td>DAS</td>
<td>decay-associated spectrum, spectra</td>
</tr>
<tr>
<td>DDS</td>
<td>double difference spectrum, spectra</td>
</tr>
<tr>
<td>DFT</td>
<td>density functional theory</td>
</tr>
<tr>
<td>DMBQ</td>
<td>2,6-dimethyl-1,4-benzoquinone</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
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<tr>
<td>DMNQ</td>
<td>2,3-dimethyl-1,4-naphthoquinone</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DS</td>
<td>difference spectrum, spectra, spectroscopy</td>
</tr>
<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
</tr>
<tr>
<td>ET</td>
<td>electron transfer</td>
</tr>
<tr>
<td>Fd</td>
<td>ferredoxin</td>
</tr>
<tr>
<td>FNR</td>
<td>ferredoxin-NADP⁺ oxidoreductase</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>FWHM</td>
<td>full width at half maximum</td>
</tr>
<tr>
<td>H-bond</td>
<td>hydrogen bond</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>Lhc</td>
<td>light harvesting complex</td>
</tr>
<tr>
<td>LT</td>
<td>low temperature (77 K)</td>
</tr>
<tr>
<td>MeONQ</td>
<td>2-methoxy-1,4-naphthoquinone</td>
</tr>
<tr>
<td>MIR</td>
<td>mid-infrared</td>
</tr>
<tr>
<td>MM</td>
<td>molecular mechanical</td>
</tr>
<tr>
<td>ms</td>
<td>millisecond</td>
</tr>
<tr>
<td>NHE</td>
<td>normal hydrogen electrode</td>
</tr>
</tbody>
</table>
NIR  near-infrared
NQ   1,4-naphthoquinone
ns   nanosecond
ONIOM our own n-layered integrated molecular orbital and molecular mechanics
OPD  optical pathlength difference
pbRC purple bacterial reaction center
Pc   plastocyanin
PED  potential energy distribution
PhQ  phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone)
PMS  phenazine methosulfate
PQ, PQ0, PQ-9 plastoquinone, plastoquinone-9
PQH2 plastoquinol
ps   picosecond
PSI  photosystem I
PSI-FX PSI devoid of terminal acceptors, $F_{A/B}$
PSII photosystem II
QM  quantum mechanical
Rb. sphaeroides Rhodobacter sphaeroides
RC   reaction center
RT   room temperature (~298 K)
S6803 Synechocystis sp. PCC6803
S7002 Synechococcus sp. PCC7002
SHE  standard hydrogen electrode
T. elongatus Thermosynechococcus elongatus
THF  tetrahydrofuran
TR   time-resolved
TRRS time-resolved rapid-scan
TRSS time-resolved step-scan
UQ   ubiquinone
WT   wild type
ZPD  zero path difference
1 INTRODUCTION

1.1 Photosystems

With mankind’s ever-increasing demand for fossil fuels, and dwindling supplies, a global energy crisis is looming. One possible solution to this crisis is to harness and convert only a very tiny fraction of the solar energy that impinges on earth (1). Harnessing solar energy is a difficult task, but it is a problem that has been solved by photosynthetic organisms. In photosynthesis, solar energy is harvested and used for a synthesis of chemical products. These organic materials serve as the source for most of food and energy consumed by humanity (2). The primary photochemistry of photosynthesis, harvesting and converting solar energy, is managed by a protein complex called photosystem (3). A variety of photosystems exist that are broadly grouped as a type I or II photosystem. In plants, algae, and bacteria which carry out oxygenic photosynthesis, solar energy is captured and converted independently but cooperatively by two systems called photosystem I and II (PSI and PSII) (4). One common but remarkable feature of photosystems is the ability to capture and convert light energy at high efficiency. In PSI, the photochemical quantum yield is exceptionally high and is reported to be approaching unity (4, 5). Consequently, PSI has been widely studied by many groups of different background for the development of a bio-mimicking or bio-hybrid solar energy conversion device that is efficient and cost-effective (6). However, despite such expectations, many of the molecular and thermodynamic properties of PSI, including the mechanism of efficient energy conversion reactions, still remain unresolved (7). Understanding such properties of a natural solar energy converter is of great importance for the development of efficient artificial solar cells. For this reason, this dissertation focuses on elucidation of the molecular mechanisms underlying the highly efficient solar energy conversion processes in PSI.
In oxygen-evolving photosynthetic organisms, two photosystems are found embedded in the membrane of thylakoid which, in plants and algae, is organized in chloroplasts (5). Along with cytochrome \( b_{6}f \) complex and ATP synthase, which are also embedded in the thylakoid membrane, PSI and PSII participate in the light reaction of photosynthesis. An organization of the protein complexes that perform the light reaction of photosynthesis are depicted in Fig. 1.1.

**Figure 1.1** An organization of the four protein complexes in the thylakoid membrane that perform the light reaction of photosynthesis. The four complexes are photosystem II (PSII), cytochrome \( b_{6}f \) complex (Cyt \( b_{6}f \)), photosystem I (PSI), and ATP synthase. The schematic also shows the diffusible electron donors and acceptors, plastocyanin (PC), ferredoxin (Fd), and ferredoxin-NADP\(^{+}\) oxidoreductase (FNR).

PSII uses light energy to produce a highly oxidizing species that leads to the splitting of water molecules into oxygen and protons (8). PSI, on the other hand, uses sunlight to produce reducing species that ultimately leads to the assimilation of carbon dioxide into organic molecules through the Calvin-Benson-Bassham cycle (9). In both photosystems, solar energy conversion is achieved by a series of electron transfer (ET) reactions through protein-bound pigments that transport an electron across the thylakoid membrane. A result of this ET process is a charge separation across the membrane with a net negative charge on the stromal side and net positive on the lumenal side. The light-induced transfer of electrons across a biological membrane via a series of protein-bound
cofactors is the fundamental mechanism of solar energy conversion in all photosystems. In PSI, the quantum yield of this process is nearly 100% (4).

### 1.2 Photosystem I

Structurally, cyanobacterial PSI isolated from *Thermosynechococcus elongatus* (*T. elongatus*) is composed of 12 protein subunits, 96 chlorophylls (Chl), 22 carotenoids, 3 iron-sulfur clusters, 2 phylloquinones (PhQ), and 4 lipids (10, 11). The number of subunits and the molecules vary slightly between different cyanobacterial species. In cyanobacterial PSI isolated from *Synechocystis* sp. PCC6803 (*S. elongatus*), 11 protein subunits, 24 carotenoids, 3 iron-sulfur clusters, 2 PhQ, and 17 lipids are found (12). In higher plants and algae, a functional unit called light-harvesting complex (Lhc) forms a supercomplex with PSI (13). Lhc serves as an antenna to collect incident light and transfer energy to PSI. In cyanobacteria, Lhc is lacking and chlorophyll and carotenoid molecules in PSI serves as antenna pigments. The energy collected by antenna pigments are transferred to an inner complex called a reaction center (RC). A RC is the location of light-induced ET, and organizes within a set of pigments that function as electron donor and acceptors. These ET cofactors are bound in three protein subunits: heterodimeric PsaA and PsaB units at the stromal side, and a PsaC unit at the lumenal side (11). At the base of PsaA and PsaB subunits is a heterodimeric pair of Chl-*a* and Chl-*a*’ termed P700. Chl-*a*’ is the 13\(^2\) epimer of Chl-*a*. The Chl-*a* and Chl-*a*’ are bound to PsaB and PsaA, and are referred as P\(_B\) and P\(_A\), respectively. Extending from P700, the ET cofactors bound to PsaA and PsaB are organized to form two near-symmetric ET chains, which are termed A-branch and B-branch. In each of the two ET branches, a Chl-*a* molecule serves as the primary electron acceptor A\(_0\) and a PhQ molecule as the secondary electron acceptor A\(_1\). The acceptors in a specific branch are referred to by their branch letter in subscript (for example, A\(_1\) in A-branch is referred as A\(_{1A}\)). Beyond A\(_1\), the two ET chains
converges at Fx, a [4Fe-4S] iron-sulfur cluster positioned between PsaA, PsaB, and PsaC. From Fx, the ET chain extends to terminal electron acceptors in PsaC, called Fa and Fb, both of which are [4Fe-4S] iron-sulfur clusters. The X-ray crystal structures of cyanobacterial PSI isolated from thermophilic T. elongatus (resolved at 2.5 Å) (10), and of PSI-Lhc supercomplex isolated from a pea plant (resolved at 2.6 Å) (13) were reported in 2001 and 2017, respectively. At the time of this dissertation (2018), determination of the first highly-resolved trimeric structure of PSI isolated from mesophilic cyanobacterium S6803 was announced (12). In this dissertation, although much of the experimental work was undertaken on PSI isolated from S6803, the theoretical work was based on the T. elongatus structure. The numbering scheme for the molecules in PSI is based on the T. elongatus structure as well. Inspection of the X-ray crystal structure of the PSI RC reveals the local environment of the cofactor binding site and potential interactions between protein and cofactor. For the secondary acceptor A1, the indole ring of tryptophan (TrpA697 for the A1A binding site, T. elongatus numbering) seems to be π-stacked with the ring plane of PhQ, the cofactor in the binding site. Additionally, and more notably, one of the carbonyl group of PhQ, C4=O, appears to be subjected to hydrogen (H) bonding by the backbone NH group of leucine residue (LeuA722 for the A1A binding site, T. elongatus numbering), while the other carbonyl group (C1=O) is likely not H-bonded. The exact role, or the effect to the function of ET, of the quinone-protein interactions remains unresolved. The structure of PSI, the arrangement of the ET cofactors within the protein complex, molecular details of PhQ, and the protein environment of the A1A binding site highlighting the possible quinone-protein interactions are organized in Fig. 1.2.
Figure 1.2  The structure of PSI and the cofactors involved in the light-induced ET, constructed from the 2.5 Å X-ray crystal structure of PSI isolated from *T. elongatus* (pdb 1JB0).  (A) An organization of trimeric PSI viewed from the stromal side to the lumenal side.  Dotted circle encloses a single PSI unit.  (B) The stromal view of a single unit of PSI.  The cofactors involved in ET and the surrounding antenna pigments are highlighted.  (C) A side view of a single unit of PSI.  The highlighted molecules are the cofactors involved in ET and the antenna pigments.  (D) Arrangement of the ET cofactors in the PSI RC.  Hydrocarbon tails of cofactors are truncated for clarity.  Possible ET pathways are indicated by blue arrows.  (E) Enlarged view of the A1A binding site.  Amino acid residues near PhQ are included.  The numbering scheme for carbon atoms in PhQ is indicated in red.  Possible H-bonding interaction is shown in dotted blue line.
Incident light energy is collected by an antenna complex or pigments and transferred to cofactors in RC to initiate the ET reactions. A traditional model for the primary photochemical events involves an energy transfer to P700 (which would form the excited state of P700, P700*) followed by an ET from P700* to A_0 (which would form a radical pair P700^+A_0^-) (14). Recent studies have revealed the initial charge separation to be a more complex process, however. In one proposed model, the initial charge separation is not between P700* and A_0, but with P700* and the accessory Chl-a that is located between P700 and A_0, often referred as A_1 (unlabeled Chl-a in Fig. 1.2D) (15). An ET process from A_1^- to A_0 follows to form the P700^+A_0^- radical pair. In another model, the energy transfer occurs to A_1, forming A_1^* (16). The charge separation occurs between A_1^* and A_0, creating A_1^+A_0^- state. In the following step, P700 reduces A_1^+ and results with P700^+A_0^- . In the first model, A_1 is the primary electron acceptor and A_0 is the secondary acceptor, and in the second scenario, the primary electron donor is A_1. In this dissertation, for simplicity, P700 is referred as the primary electron donor and A_0 the primary electron acceptor following the traditional convention.

Multiple models predict the mechanism of the initial photochemical reaction in the PSI RC, but the earliest radical pair that is explicitly observed experimentally is the P700^+A_0^- state (17). The radical pair is stabilized by an ET from A_0^- to A_1 occurs on a ps time scale, which forms the P700^+A_1^- state (17-19). As described earlier, the ET cofactors in the PSI RC are arranged to form two nearly symmetrical ET chains, each containing A_1, A_0, and A_1. Experimental evidences confirm that both branches are utilized, and the ET in PSI is bidirectional (20). That is, the ET processes in the PSI RC could proceed through the A-branch to form P700^+A_0A^- and P700^+A_1A^- radical pairs, or through B-branch by an ET process from A_0B^- to A_1B^- . Irrespective of the charge separation mechanism or the ET chain being utilized, within ~50 ps after light excitation, an ET
to $A_1$ is complete and a radical pair state $P700^+A_{1A}^-$ or $P700^+A_{1B}^-$ is formed (17-19). Reported estimates of the relative utilization of the two branches show variations, but in most cases the use of A-branch is favored over the B-branch (21-25). Beyond $A_1$, the two branches are converged by an iron-sulfur cofactor $F_X$. The ET process between $A_1$ and $F_X$ is one of the characteristic reaction in the PSI RC due to its biphasic nature. The rate of forward ET from $A_{1}^-$ to $F_X$ through the A-branch is approximately an order of magnitude slower than the same reaction through the B-branch, despite the similarities in the cofactor organization: lifetime of the ET process from $A_{1B}^-$ to $F_X$ through is reported to be in the range of $\sim 10-25$ ns, while the rate for $A_{1A}^-$-to-$F_X$ ET is in the range of $\sim 250-340$ ns (20, 26-30). Following the bidirectional and biphasic ET from $A_1$ to $F_X$, the charge separation is further stabilized by ET from $F_X$ to terminal electron acceptors $F_A$ and $F_B$ in $\sim 500$ ns (31). Due to the reduction-oxidation (redox) potentials of the terminal acceptors, which are discussed more in detail below, the population of $F_A^-$ and $F_B^-$ are in equilibrium and the radical pair state is noted as $P700^+F_{AB}^-$ to reflect this equilibration.

In its natural environment in the thylakoid membrane, upon completion of light-induced ET across the membrane, an electron transferred to the terminal acceptors $F_{AB}$ are collected by a diffusible cofactor ferredoxin (Fd) (32), and an electron is replenished to P700 by a plastocyanin (Pc) or cytochrome (cyt) (33), which are also diffusible cofactors. Reflecting the overall redox reaction that PSI performs through its light-induced ET process, PSI is often described as plastocyanin-ferredoxin-oxidoreductase (9). For the study of the PSI RC, the RC is often isolated from the neighboring protein complexes and thylakoid membrane. In the isolated PSI RC, after an electron transferred to the terminal acceptor, the radical pair $P700^+F_{AB}^-$ undergoes through a charge recombination reaction with a lifetime of $\sim 80-100$ ms (34). $F_{AB}^-$, as a result, is oxidized and $P700^+$ is re-reduced. At cryogenic temperature, ET in the PSI RC becomes heterogeneous.
The forward ET is restricted, and approximately 40% of PSI undergoes P700⁺A₁⁻ charge recombination reaction. About 10% of PSI recombines from the P700⁺Fₓ⁻ state. The remaining 50% of the fraction forms the radical pair state P700⁺Fₐₐ⁻ that is virtually irreversible once formed. The P700⁺A₁ recombination proceeds with a reaction lifetime of ~350 µs, and the P700⁺Fₓ with ~1 ms. Several hypotheses are proposed for the origin of this heterogeneity, but the exact mechanism remains unresolved.

The energetics of charge separation in PSI predicts that the reactions are mostly thermodynamically downhill, with an exception to the excitation of P700 (35). The redox potential of P700, which is species-dependent, is approximately ~+450 mV for S6803 (36), and is shifted to -1300 mV upon P700* (estimated by subtracting 700 nm photon energy from P700). This highly negative potential makes P700* one of the most reducing species in the nature. The redox potentials of Fₐ and Fₐ are in the range of ~-520 mV and ~-550 mV, respectively, and the estimate for A₀ is in the -1100 mV range (see Ref. (37) for a survey of reported values). A range of values has been reported for the redox potential of Fₓ, but a consensus value is in the range of -680 mV (7). Direct redox titration of A₁ has not been possible, but a variety of indirect method has been applied for the estimation of the A₁ redox potential in the past. The indirect approaches, however, have resulted in a wide range of values ranging from -810 mV to -530 mV (38-43). The lowest potential reported would suggest the ET from A₁⁻ to Fₓ is a highly exergonic, or a thermodynamically downhill reaction, while the highest reported value would indicate that the same process is highly endergonic, or thermodynamically uphill. The energetics scheme of ET in PSI that lists the known ET pathway and rates along with the cofactor redox potentials are summarized in Fig. 1.3.
Figure 1.3  Energetics of PSI ET at 298 K (left) and 77 K (right). The forward ET from A₁ to Fₓ is highlighted in orange, the charge recombination through A₁A at 298 K is highlighted in red, and the P700⁺A₁A⁻ charge recombination pathway at 77 K is indicated in blue. The dotted arrows for a 77 K diagram refers to inhibited or blocked ET pathways.

One approach to investigate the functional and structural properties of the PSI RC is to induce mutations. A variety of mutagenesis has been applied for the study of PSI in the past and has successfully introduced modifications to the function and structure of specific cofactors, ET chains, antenna pigments, and protein subunits. One modified PSI that is utilized often in this work is produced by a mutation on menB gene in S6803 (44). The products of a series of men genes are responsible for the biosynthesis of PhQ, the cofactor in the A₁ binding site. Targeted inactivation of these genes interferes with the synthesis of PhQ, and as a result the men mutant strains produce PSI that lacks PhQ in the A₁ binding site. The A₁ binding site in these men mutant strains are instead occupied by a plastoquinone-9 (PQ), an ET cofactor present in PSII RC (44). The subsequent studies have found that these PQ recruited into the A₁ binding site are functional as an ET cofactor in the PSI RC, but are only loosely bound as they can be replaced in vivo or in
*vitro* by PhQ and other non-native quinones upon incubation with excess amount of quinone of interest (30, 45-48). Methods to remove the native PhQ and introduce non-native cofactors into the A₁ binding site have existed prior to the identification of the *men* genes but relied on harsh chemical treatments that modified antenna pigments as well. The *men* mutant strain has provided a non-invasive and minimally disruptive method to modify the A₁ binding site in PSI, both *in vivo* and *in vitro*. In this work, PSI isolated from the *menB* deletion mutant strain of *S6803* (hereafter referred as *menB⁻* PSI for simplicity) is used to modify the A₁ cofactor. The ET kinetics of *menB⁻* PSI with PQ serving as A₁ is modified from the wild-type (WT) PSI with PhQ in the A₁ binding site. Modified kinetics and comparison to the kinetics of WT PSI have been described previously and was the focus of my thesis in 2012 (49).

While the focus of this dissertation is mainly on the PSI RC, the other RCs are also commonly studied. PSII RC and purple bacterial RC (pbRC) are the two most commonly studied kinds, both of which are type II RCs. The X-ray crystal structures of the two RCs are resolved at much higher 1.9 Å (50-52). Features such as the ET cofactors arranged in two pseudo-symmetrical branches, and a quinone molecule serving as one of the electron acceptors resemble the PSI RC. In PSII RC, a PQ molecule serves as electron acceptors QA and QB, and a ubiquinone-10 (UQ-10) occupies the QA and QB binding sites in pbRC from *Rhodobacter sphaeroides* (*Rb. sphaeroides*). The special pairs are termed P680 and P870 for PSII RC and pbRC, respectively. Unlike the PSI RC, however, in these type II RCs only one ET chain is utilized, and a quinone in the QB binding site serves as a terminal acceptor. ET reactions proceed from the primary donor to QA, and charge separation is completed by an inter-quinone ET from QA to QB. Upon successive ET from QA accompanied by two proton transfer reactions, QB is doubly reduced and protonated to form a quinol, which is then released from the binding site.
As introduced above, quinone molecules are involved as ET cofactors in PSI, PSII, and pbRC. Variants of these RCs are all found to utilize quinones as ET cofactors as well: pbRC from Blastochloris viridis (Bl. viridis, formerly Rhodopseudomonas viridis) contain menaquinone-9 in the QA binding site and UQ-9 in QB (52), PSI from green algae and plants contain 5’-monohydroxyphylloquinone (53), and a type I RC from green sulfur bacteria contains menaquinone-7 (54). Menaquinone is present in heliobacterial RC, the simplest known RC, yet a lack of quinone in the recently reported X-ray crystal structure reveals that the quinone is not tightly bound and is not an intermediate ET cofactor in this type I RC (55). Quinones are ubiquitous in biology beyond photosynthetic RCs and play important roles in a wide range of bioenergetic systems. The molecules are commonly categorized by the number of aromatic rings into three classes: benzoquinone (BQ), naphthoquinone (NQ), and anthraquinone (AQ). The two carbonyl groups may be placed in ortho, meta, or para positions, but in this dissertation the quinones considered are analogues of 1,4-BQ, 1,4-NQ, and 9,10-AQ, which belong to the para series and referred as p-quinones. Different substituent groups on a quinone modifies the redox potential differently, and a wide range of redox potential can be covered by slight modifications on the side chain. Generally, ranges of redox potentials are ordered, from the highest (more positive) to the lowest (more negative), as 1,4-BQs, 1,4-NQs, and 9,10-AQs (56). A quinone may function as both electron and proton acceptors, and in total nine redox states exist for a quinone (57). A quinone in the A1 binding site normally functions as a single electron acceptor and donor, so the two redox states of a quinone discussed in this dissertation are an unprotonated neutral state (Q) and an unprotonated but singly reduced state (Q−). Additionally, a doubly-reduced and doubly-protonated quinone (a quinol, QH2) is mentioned. Here, the singly reduced state is referred to as a semiquinone or an anion state. Structures of quinones of interest are shown in Fig. 1.4.
Figure 1.4  Chemical structures of (A) PhQ, (B) PQ, (C) 2-methyl-1,4-naphthoquinone, and (D) AQ. The numbers in the aromatic or quinonic rings indicate the carbon numbering schemes. (A) and (C) are examples of 1,4-NQ, (B) is of 1,4-BQ, and (D) is of 9,10-AQ. (C) is an example of modified NQ that is incorporated into the A$_1$ binding site.

1.3 Electron Transfer Theory

The basic mechanism of highly efficient solar energy conversion in photosynthetic organisms is the serial ET through protein-bound cofactors. Understanding the energetics of ET, therefore, is essential for an understanding of the solar energy conversion. The energetics of ET is commonly discussed in terms of a rate constant, half-life, or a time constant/lifetime, and a driving force of reaction which is tied to redox potentials of the donor/acceptor pair. These elements for ET between a weakly coupled (widely separated) pair is described by the non-adiabatic Marcus theory, originally developed by Rudolph Marcus starting in 1950s (58). The theory, which is often termed Marcus theory or simply ET theory, is based on a premise that the ET is carried out as a quantum tunneling process. As such, the non-adiabatic ET rate, $k_{ET}$, is described by Fermi’s golden rule (Eq. 1.1).

$$k_{ET} = \frac{2\pi}{\hbar} |V|^2 FC$$

Equation 1.1
$|V|^2$ represents the square of the electronic coupling between the donor and acceptor, and $FC$ refers to the Franck-Condon factor. The conditions which the ET theory is built upon are: 1) ET is a tunneling event, 2) structural rearrangement of donor, acceptor, and solvent occurs to optimize the environment for ET, 3) ET occurs with the maximum overlap of nuclear wavefunctions of the reactant and product (Franck-Condon principle), and 4) the nuclear motions can be separated from the electronic motions (Born-Oppenheimer approximation). Following the conditions 1) and 4), the electronic and nuclear terms are separated as $|V|^2$ and $FC$ in Eq. 1. Since the ET is defined as a tunneling event through a uniform energy barrier that exists between the donor and acceptor species, the electronic coupling element is sensitive to the thickness of the barrier. The coupling term therefore is dependent on the actual distance between the donor and acceptor. The distance considered in this case is the edge-to-edge distance ($R$). With an increasing distance, the coupling element falls off exponentially. Therefore, the coupling element can be rewritten to include the distance relationship as in Eq. 1.2.

$$|V|^2 = |V_0|^2 e^{-\beta R} \quad \text{Equation 1.2}$$

$|V_0|$ represents the electronic coupling element at zero distance, and $\beta$ is the medium-dependent coefficient that alters the sensitivity to the distance. An interpretation of the Franck-Condon factor is derived from a set of parabolic curves known as the Marcus diagram, which is outlined in Fig. 1.5.
Figure 1.5  Reactant and product potential surfaces described as potential wells of a single simple harmonic oscillator. $q$ and $q'$ correspond to the nuclear coordinate of the minima for the reactant and product, respectively. $E_a$ is the activation energy, and $\Delta G^0$ is the standard free energy difference. $\lambda$ is the reorganization energy. The relation between these three parameters are given in the text.

In this diagram, Marcus expresses the nuclei potential energy levels as a simple harmonic oscillator. The parabolic curve of the oscillator is plotted against the nuclear reaction coordinate. The potential curves for the reactant and the product are shown as similar curves with displaced (both in reaction coordinate and in energy level) minima. Due to the Franck-Condon principle (condition 3) in the four conditions listed above), ET is expected to take place at the cross-over point of the two curves. A difference in energy level between the reactant’s minimum to the cross-over point is the activation energy of the reaction, $E_a$. While the $E_a$ term and its relation to the reaction rate have been described by the transition state theory and the Eyring equation for the standard chemical reaction, a direct computation or measurement of the activation energy for an ET reaction is challenging. Instead, the activation energy for ET can be represented by the other
energy terms, such as the standard free energy difference and the reorganization energy. The standard free energy difference, $\Delta G^0$, is the difference in the energy minima of the reactant and the product. The standard free energy difference is related to redox potentials of the donor/acceptor as $-\Delta G^0 = e[E_{ox} - E_{red}]$, where $E_{ox}$ and $E_{red}$ are the redox potentials of oxidized and reduced species, respectively. $-\Delta G^0$ is also referred to as the driving force of the reaction. The reorganization energy, $\lambda$, is associated with the energy required to modify the nuclear coordinate of the donor, acceptor, and the immediate environment such as the solvent, without ET occurring. This energy term follows from condition 2). In the Marcus diagram, the reorganization energy corresponds to a difference in energy level of the reactant at the reaction coordinate $q'$ and $q$. Using these two energy terms, the activation energy can be expressed as Eq. 1.3

$$E_a = \frac{(\Delta G^0 + \lambda)^2}{4\lambda}$$  \hspace{1cm} \text{Equation 1.3}

The Franck-Condon factor for the classical Marcus expression is:

$$FC = \frac{1}{\sqrt{4\pi\lambda k_B T}} e^{-\frac{(\Delta G^0 + \lambda)^2}{4\lambda k_B T}}$$  \hspace{1cm} \text{Equation 1.4}

Combining the electronic and nuclear term in Eq. 1.2 and 1.4 into the original Eq. 1, the classical Marcus equation takes the form of Eq. 1.5, which is commonly known as the Marcus equation.

$$k_{ET} = \frac{2\pi |V_0|^2 e^{-\beta R}}{\hbar \sqrt{4\pi\lambda k_B T}} e^{-\frac{(\Delta G^0 + \lambda)^2}{4\lambda k_B T}}$$  \hspace{1cm} \text{Equation 1.5}

$k_B$ is the Boltzmann constant and $T$ is the temperature. Since the development of this classical expression, the Marcus equation has been modified to include quantum mechanical corrections and allow for a low temperature dependence (59). With quantum mechanical correction for low temperature ET, the $FC$ term in the Marcus equation is given by the Hopfield
expression (60), which replaces the term \(2\lambda k_B T\) with \(\sigma^2 = \lambda \hbar \omega \coth(\hbar \omega / 2k_B T)\). The semi-classical expression of the Marcus equation then takes the form given by Eq. 1.6.

\[
k_{\text{ET}} = \frac{2\pi}{h} \frac{|v_0|^2 e^{-\beta R}}{\sqrt{2\pi \lambda \hbar \omega \coth(\hbar \omega / 2k_B T)}} e^{\frac{-(\Delta \sigma^0 + \lambda)^2}{2\lambda \hbar \omega \coth(\hbar \omega / 2k_B T)}}
\]

Equation 1.6

\(\omega\) is the characteristic vibrational mode coupled to ET (60). For applications to biological ET, a simplified empirical expression has been developed through an extensive survey of ET processes in photosynthetic RCs and semi-synthetic systems (61, 62). The derivation of the three-parameter expression, commonly known as Moser-Dutton’s ruler, is as follows:

1) The semi-classical expression in Eq. 1.6 is put in a logarithmic form.

\[
\ln k_{\text{ET}} = \ln \left(\frac{2\pi |v_0|^2}{h \sqrt{2\pi \lambda \hbar \omega}}\right) - \beta R - \frac{(\Delta \sigma^0 + \lambda)^2}{2\sigma^2}
\]

\[
2.303 \log k_{\text{ET}} = 2.303 \left[\ln \left(\frac{2\pi |v_0|^2}{h \sqrt{2\pi \lambda \hbar \omega}}\right) - \beta R - \frac{(\Delta \sigma^0 + \lambda)^2}{2\sigma^2}\right]
\]

2) Common vibrational frequency of 56 meV is used for \(\hbar \omega\), and 298 K for \(T\).

\[
\log k_{\text{ET}} = \log \left(\frac{2\pi |v_0|^2}{h \sqrt{2\pi \lambda \hbar \omega}}\right) - \frac{\beta R}{2.303} - \frac{(\Delta \sigma^0 + \lambda)^2}{2.303 \cdot 2.0762627 \lambda}
\]

\[
\log k_{\text{ET}} = \log \left(\frac{2\pi |v_0|^2}{h \sqrt{2\pi \lambda \hbar \omega}}\right) - \frac{\beta R}{2.303} - 3.1 \frac{(\Delta \sigma^0 + \lambda)^2}{\lambda}
\]

3) A mean value of \(\beta = 1.4 \, \text{Å}^{-1}\) is suggested by studies on wide range of biological systems for intraprotein ET.

\[
\log k_{\text{ET}} = \log \left(\frac{2\pi |v_0|^2}{h \sqrt{2\pi \lambda \hbar \omega}}\right) - 0.6R - 3.1 \frac{(\Delta \sigma^0 + \lambda)^2}{\lambda}
\]

4) For systems at \(-\Delta G^0 = \lambda\), in which the third term drops out, the extrapolated \(\log k_{\text{ET}}\) at van der Waals contact \((R = 3.6 \, \text{Å})\) is 13. This value is used to calculate a constant that replaces the first term with the coupling element.
\[
\log k_{ET} = 15 - 0.6R - 3.1 \frac{(\Delta G^0 + \lambda)^2}{\lambda} \quad \text{Equation 1.7}
\]

In this simplified empirical relationship (Eq. 1.7), the ET rate is expressed in three parameters. In this expression, the units are eV for \(\Delta G^0\) and \(\lambda\), Å for \(R\), and s\(^{-1}\) for \(k_{ET}\). Alternatively, the distance sensitivity \(\beta\) can be expressed in terms of a protein packing density, \(\rho\), as \(\beta = 0.9\rho + 2.8(1 - \rho)\). The four-parameter version built by using this expression takes the form of Eq. 1.8

\[
\log k_{ET} = 13 - (1.2 - 0.8\rho)(R - 3.6) - 3.1 \frac{(\Delta G^0 + \lambda)^2}{\lambda} \quad \text{Equation 1.8}
\]

With judicious choices of \(R\), \(\lambda\), and \(\rho\), the simplified empirical relationships (Eq. 1.7 and Eq. 1.8) allow for expression of the ET rate, \(k_{ET}\), as a function of the standard free energy gap, \(\Delta G^0\). As such, in the studies of PSI energetics, an inverse of the experimentally observed rate of population dynamics have often been taken as \(k_{ET}^{-1}\) and applied to these empirical expressions to deduce \(\Delta G^0\) (27, 63). In more recent studies, however, it was found that \(\Delta G^0\) and the midpoint potentials calculated by this approach are unreasonable (30, 39). The assumption that associates the experimentally observed rate of population evolution to \(k_{ET}^{-1}\) is only appropriate when the ET reaction is largely exothermic and unidirectional, a situation that may not apply to ET in PSI. The appropriate rate constant to use in Eq. 1.7 and Eq. 1.8 could only be ascertained within the context of an appropriate kinetic model as described below.

### 1.4 Inverted Region

For all forms of the equations introduced above, the Marcus equation predicts that an ET rate has a Gaussian dependence on the standard free energy difference. In a fully quantum mechanically corrected version, the predicted dependence is an inverted asymmetric parabolic profile (64, 65). In a plot of the reaction rate constant against the driving force of a reaction known
as the Marcus curve, initially an increase in the reaction rate is observed with an increase in the driving force. This is expected as the reaction becomes less thermodynamically uphill and/or more thermodynamically downhill with an increase in the driving force. The relationship is observed in a region where $-\Delta G^0 < \lambda$, and is called a normal region. At the point where the driving force matches the reorganization energy ($-\Delta G^0 = \lambda$), the reaction becomes activation-less (i.e., $E_a = 0$) and the reaction rate reaches an optimal rate. Beyond this optimal rate, however, the rate of ET decreases upon further increase in the driving force into a range where $-\Delta G^0 > \lambda$ (66). This rather counterintuitive range of driving forces in which a larger free energy gap results in slower ET is often referred as the Marcus inverted region, or simply as the inverted region. The Marcus curve and its three regions are summarized in Fig. 1.6.

**Figure 1.6** The Marcus curve and its three regions. A region where $-\Delta G^0 < \lambda$, an increase in the driving range induces an increase in the reaction rate, and is called a normal region. At the point where $-\Delta G^0 = \lambda$, an optimal (or maximal) reaction rate is observed. In a region where $-\Delta G^0 > \lambda$, an increase in the driving force results in a decrease in the reaction rate. This region is referred to as the inverted region.
The inverted region effect, which predicts a lower reaction rate for more exothermic reaction, is one of the most important predictions made by Marcus ET theory. Given its potential implications for the biological systems which are discussed in detail below, the effect was subject to experimental verification. The experimental demonstration of the inverted region effect, however, was not achieved until the 1980s, nearly 30 years after the initial work by Marcus (67).

To observe the Marcus inverted region, a system with a small reorganization energy and/or a large driving force is required. Even when these conditions are met, a demonstration of the inverted-parabolic feature of the Marcus curve in a biomolecular system was complicated by contributions from the diffusion rate that concealed the fast ET rate (68). One approach to circumvent this obstacle is to construct a rigid spacer molecule between donor and acceptor molecules. Using a rigid hydrocarbon spacer and a common electron donor on a variety of electron acceptors, Miller et al. reported the first experimental evidence for the Marcus inverted region in 1984 (67).

Since its prediction, the inverted region effect has been proposed to have crucial roles in biological ET systems, especially photosynthetic RCs (69, 70). Particularly, the inverted region effect has long been suggested to be one of the major factors that governs the high efficiency of photosynthetic ET (69, 71). To facilitate ET at high quantum efficiency, forward reactions must outcompete wasteful back reactions. That is, forward ET processes with small driving forces must proceed rapidly while highly exothermic charge recombination reactions proceed slowly. It is thought that the photosynthetic RCs effectively achieve this contradictory condition by placing forward ET in the normal region and recombination reactions in the inverted region. The inverted region effect has been observed experimentally in the number of artificial or semi-artificial systems (70, 72-76). In native biological systems, however, only a limited number of cases have been identified. For photosynthetic RCs, the $\text{P}^+\text{Q}_\lambda^{-}$ charge recombination reaction of purple bacterial
RCs with non-native quinones in $Q_A$ (65, 77), the forward $A_0^- \rightarrow A_1$ ET in the modified PSI RCs also with non-native quinones in $A_1$, and more recently (78), the long-range $Y'_Z^{ox}Q_A^-$ charge recombination in PSII RCs at cryogenic temperature (79) have been identified as ET process in the inverted region. Although the inverted region ET is proposed as the integral reaction in biological ET, experimental verification of such kinetics in unmodified systems under physiological conditions (e.g. room temperature) has been elusive and a contribution of the inverted region effect to the overall efficiency of photosynthetic ET has remained unverified.

1.5 Spectroscopic Studies of Photosynthetic Reaction Centers

Diverse types of spectroscopic techniques have been applied for the studies of kinetic, structural, and energetic properties of photosynthetic RCs. Different absorptivity of the ET cofactors, a wide range of the kinetic rates, and a photoactivation as a reaction trigger are some of the features that allow applications of a variety of spectroscopy methods. Types of spectroscopy applied for the study of the PSI RC in this dissertation can be grouped largely into two categories: transient absorption spectroscopy in the visible to near-infrared (NIR) spectral region, and Fourier transform infrared (FTIR) spectroscopy in the mid-infrared (MIR) spectral region. Both methods are applied as a form of difference spectroscopy (DS) to monitor the cofactor or radical pair of interest effectively.

Much of the details on transient visible absorption spectroscopy was given in my previous thesis (49). Briefly, in what is also known as pump-probe spectroscopy, a probing light source (“probe”) is sent through a sample to monitor a change in absorption at select wavelength over time, and an actinic light source (“pump”) is directed to generate a transient species. Transient absorption spectroscopy for the study of photosynthetic RCs has been applied on broad timescales, ranging from femtoseconds (fs) to seconds (s). In this work, transient absorption spectroscopy on
nanosecond (ns) to tens of millisecond (ms) timescales at 487, 703, and 800 nm are used. Bleaching of ground state P700 and absorption of P700\(^+\) are monitored at 703 and 800 nm, respectively (36). At 487 nm, kinetics of A\(_1^-\) can be indirectly monitored as an electrochromic shift induced on nearby chlorophyll and carotenoid molecules (22).

1.6 Fourier Transform Infrared Spectroscopy

The second type of spectroscopy utilized in this study, FTIR spectroscopy, is categorized under a genre of infrared (IR) spectroscopy. IR absorption spectroscopy is aimed to probe molecular vibration by conducting an absorption spectroscopy in the IR spectral region. Absorption in the IR region is due to transitions between the vibrational energy levels. IR absorption occurs when the energy of impinging IR radiation matches the difference in two vibrational energy levels and a transition through these two levels induces only molecular vibrations that result in a change in net dipole moment. A various type of vibration is induced on a molecular bond upon IR absorption. For non-linear molecules with \(N\) atoms, molecules have \(3N-6\) degrees of freedom for vibrational motions. These vibrational motions are called normal modes. The vibrational motion of molecular bonds is described as stretching mode if a change in the bond length is experienced, and bending mode if a bond angle is modified. Stretching modes can be symmetrical or asymmetrical, and bending movement includes scissoring, rocking, wagging, and twisting. In each normal mode, all atoms of a molecule vibrate at the same frequency. A set of normal modes and associated frequencies are unique to each molecule and susceptible to environmental effect. The vibrational frequency \((\tilde{\nu})\) is dependent on a force constant \((k)\) and masses of vibrating atoms as \(\tilde{\nu} \propto \sqrt{k/\mu}\), where \(\mu\) is the reduced mass. For a diatomic molecule with atomic masses of \(m_1\) and \(m_2\), the reduced mass is given as \(\mu = m_1m_2/(m_1 + m_2)\). The dependence on mass indicates an isotope labeling of an atom, which modifies the atomic mass,
will induce a shift in the frequency. The force constant dependence suggests that a bond order and bond length will impact the vibrational frequency. For example, the vibrational frequencies associated with C-O, C=O, and C≡O all appear in a different range. Additionally, even with a same bond order, isolated C-H stretching mode and C-H stretching mode in H-C=O group exhibit different vibrational frequencies due to modification in the force constant. Lastly, the force constant is also modified by the ionization state of a molecule. That is, anion and cation formation will induce a shift in vibrational frequency respect to its neutral state. These sensitivities make IR spectroscopy a great tool to understand molecular details such as chemical structure, bond/angle/orientation parameters, and intramolecular interactions.

Several methods exist for collection of an IR spectrum, but FTIR spectroscopy arguably is the most widely applied approach. At the core of FTIR spectroscopy is the Michelson interferometer. The two-beam interferometer divides a source light into two paths with different pathlengths. The divided beams are reflected off mirrors, and then recombined. The recombining beams experience interference due to the pathlength difference. In a conventional FTIR spectrometer, one of the mirror is fixed while the other mirror is made to move, or scan, continuously at constant velocity. The scanning mirror creates a difference in optical pathlength (Optical Pathlength Difference), and the recombining beams produces an interference pattern. When the pathlengths are equidistant (a condition known as zero path difference (ZPD)), two beams are in phase and constructive interference occurs for light at all wavelength. For polychromatic light, at non-ZPD, some wavelengths interfere constructively while some interfere destructively. The resulting interference pattern of the recombined beams is known as an interferogram, and an interferogram intensity against a pathlength difference is the data recorded by a detector. Since an interferogram contains information of all the wavelengths in it, separation
of light is not required: each time a measurement is made, data on all the wavelengths are collected simultaneously. This multiplexity is one of the major advantages of FTIR spectrometry over dispersive spectrometry (Fellgett’s advantage). Additionally, the slits used in a dispersive spectrometer for a selection of wavelength is not required in an FTIR spectrometer, so more light can be delivered to the detector. This advantage is known as the throughput or Jacquinot’s advantage. Instead of slit widths, an OPD determines a spectral resolution for an FTIR spectrometer. Although an interferogram contains information of all the spectral region, a relationship between intensity and OPD is of little use. The intensity of an interferogram recorded by a detector against an OPD is given as Eq. 1.9.

\[
S(\delta) = \int_{-\infty}^{+\infty} B(\tilde{\nu}) \cos 2\pi \tilde{\nu} \delta \ d\tilde{\nu}
\]

Equation 1.9

Equation 1.9 is in a form of the Fourier transform pair, where the other half of the pair is described as Eq. 1.10.

\[
B(\tilde{\nu}) = \int_{-\infty}^{+\infty} S(\delta) \cos 2\pi \tilde{\nu} \delta \ d\delta
\]

Equation 1.10

The Fourier-transformed interferogram in Eq. 1.10 gives an intensity as a function of frequency, \(\tilde{\nu}\). The processed spectrum of intensity vs. frequency is known as a single-beam spectrum. The name FTIR originates from the Fourier transformation of the interferogram into single-beam spectrum. Calculation of an absorption spectrum from the single-beam spectrum is similar to other absorption spectroscopy and follows the Beer-Lambert law. This concept was described previously in my thesis in 2012 (49).

1.7 Difference Spectrum and Double Difference Spectrum

While an ability to measure all infrared-active vibrational modes of a molecule simultaneously is an advantage of FTIR spectroscopy, measurement of a specific molecule, cofactor, or bonding interaction in a biological system becomes troublesome due to this exact
advantage. For a biological system, when measured, vibrational modes of an entire system including its environment (such as solvent) contribute to the absorption profile. Oftentimes, contribution from the system is many orders of magnitude greater than an absorption by a target molecule. In a biological system, the major contributions are due to water in the environment and the amino acid. The repetitive unit of the peptide backbone gives rise to large characteristic absorption bands, two of which are known as amide I and amide II. To study the vibrational properties of the target cofactor, the absorption by the cofactor of interest must be selectively extracted. One method for such an extraction is an application of difference spectroscopy. In difference spectroscopy, an absorption is measured for two different states of the molecule/bond/interaction of interest. Commonly measured states include cation/anion, changes in oxidation state, and formation/dissociation of a bond. By subtracting one spectrum from the other, a difference spectrum (DS) is produced. In DS, all the bands due to part of the system that did not change between two states cancel out and only the bands that have experienced a shift in frequency appear. The method can, therefore, effectively reveal the absorption bands due cofactor of interest by removing contributions from the environment.

Often, a major difficulty in the IR difference spectroscopy is in its analysis where IR band composition is determined. Even after a removal of the unwanted protein and water absorption, FTIR DS carries high information content and assignment of bands to specific molecular structure is not straightforward. To facilitate this analysis, one method that is effective is a construction of double difference spectrum (DDS). To generate DDS, DS are calculated for systems with highly specific change introduced to the cofactor of interest. The specific changes introduced to the cofactor include isotope labeling, site-directed mutagenesis, and a replacement of a cofactor with an analogous molecule. The changes are aimed to shift frequencies of specific vibrational modes.
By subtracting one DS from the other, therefore, all the unaffected modes disappear and only the modes shifted by the introduced change will be visible. To summarize this section, a set of diagrams, starting from a schematic of an FTIR spectrometer and leading to a generation of DDS, is compiled in Fig. 1.7.

**Figure 1.7** A set of diagrams describing a process of collecting infrared absorption spectrum from an FTIR spectrometer and creating a difference and double difference spectra (DS and DDS, respectively). (Top) A schematic of core components of an FTIR spectrometer. An IR detector records an interferogram, as an IR intensity against optical pathlength difference (OPD). An interferogram is converted to a single-beam spectrum by applying a Fourier transformation. By recording this single-beam spectra for both the background and for the sample (shown as gray and green spectra, respectively, in the diagram for single-beam spectrum), an absorption spectrum can be calculated. (Bottom) A schematic describing a process of a DS and DDS constructions. In a DS, peaks associated with state I appear as negative signals, and state II as positive signals. Similarly, in a DDS, peaks associated with species in DS I appear as negative signals, and DS II as positive signals. Oftentimes, as can be seen in the DDS diagram, the shift in peaks between two DS is small and results in a diminished signal size in a DDS. Therefore, high signal-to-noise ratio in DS are usually required for a construction of DDS.
1.8 Time-Resolved Step-Scan FTIR Difference Spectroscopy

To create DS, two different states need to be generated in a sample being measured. A measurement of two states is facilitated if a second state is triggered by an actinic light. In such a system, a measurement of DS can be controlled simply by on/off of the actinic light source. As described in previous sections, reactions in photosynthetic RCs are light-activated. Therefore, light-induced FTIR difference spectroscopy has been widely applied as a method of vibrational spectroscopy for the study of photosynthetic RCs. In many studies, the light-induced FTIR difference spectroscopy for photosynthetic RCs is applied as a photoaccumulation experiment. Absorption spectra are measured before, during, and after an exposure to actinic light, with a duration of the exposure ranging on a second to minute timescale. Continuous exposure to the actinic light accumulates a radical pair state of the interest, and allows for its spectrum to be measured and averaged on a sub-minute to minute timescale. Spectra before and after the light-activation is used to cancel the undesired absorption bands and correct for the environmental effect. Many of the FTIR DS for the primary donors are collected by this method.

The photoaccumulation method, however, fails when the cofactors are short-lived or when the states cannot be accumulated. In such cases, the FTIR difference spectroscopy must be applied in a time-resolved fashion. To distinguish from the time-resolved technique, measurements that do not monitor the temporal evolution of the spectra, such as the photoaccumulation measurement mentioned above, are referred as “static” or “steady-state” measurements. One approach to the time-resolved measurement of FTIR DS is to take an advantage of the fast scanning ability of the moving mirror in the modern FTIR spectrometer, and a symmetry of an interferogram. The method is referred as time-resolved rapid-scan (TRRS) FTIR difference spectroscopy. The scanning mirror in research-grade FTIR spectrometers can reach the modulation frequency of over
300 kHz or over 9.49 cm/s in terms of mirror velocity (80). An interferogram can be collected when a scanning mirror moves from a position A to B. An identical interferogram can be measured, however, when a mirror returns from a position B to A. Therefore, by collecting interferograms on both forward scan and backward scan, amount of information collected in a given time course can be doubled. Such a scanning method is called a bi-directional acquisition or forward-backward acquisition. An interferogram is symmetric across a center-burst at ZPD. The information stored in either side of a center-burst, often referred as wings, are identical. Each interferogram can, therefore, be “split” into two identical copies. A scanning mode which places a center-burst in the middle is called a double-sided scan, as opposed to a single-sided scan which places a center-burst at the edge. Given a situation where a detector acquisition rate is not a limiting factor, with a rapidly scanning mirror operated in double-sided, forward-backward acquisition mode and splitting interferogram in post-acquisition process, a single-beam spectrum can be collected on a ms timescale.

Many of the radical states in the PSI RC, however, decay on sub-ms timescale and cannot be photoaccumulated. Whereas in type II RCs in which FTIR DS of Q_A and Q_B can be constructed from a static or TRRS measurement, the radical state of A_1 cannot be photoaccumulated and decays on a ns timescale at room temperature and on a µs timescale at cryogenic temperature. Clearly, neither a photoaccumulation method nor a rapid scan technique can be applied for a measurement of A_1; a measurement technique with much faster temporal resolution is required. By operating an interferometer to scan in discrete “steps” instead of in continuous motion as in rapid-scan mode, it is possible to achieve this required time resolution. In a technique known as time-resolved step-scan (TRSS) FTIR difference spectroscopy, transient absorption kinetics is measured at a distinct mirror position, while the scanning mirror is held still. A method of kinetic measurement is similar
to that of the transient absorption spectroscopy in the visible to NIR region described above. The movable mirror’s position is then “stepped” slightly in OPD to the next position, and the kinetic measurement is repeated. By completing all the mirror positions in this manner, time-resolved interferograms can be re-constructed. In contrast to the steady-state or rapid scan techniques where a data acquisition follows the spectral axis and a temporal axis is a product of post-acquisition data analysis, a data acquisition in the step-scan measurement is on the temporal axis and the spectral profile is computed by the data analysis.

Several differences between static/rapid-scan methods and a step-scan technique are noteworthy. In a step-scan method, the scanning mirror speed is no longer relevant to the temporal resolution as the mirror is moved in discrete steps. The time resolution is determined by the width of actinic light source, the detector response time, and the analog-to-digital converter. To conduct a step-scan measurement appropriately in a reasonable time frame, certain conditions must be satisfied by the measured sample and instrumental setup. For each step-scan measurement, a number of mirror steps required to reconstruct an interferogram is often in the range of 500 to 1000, and at least one kinetic measurement must be made at each step. To improve a signal-to-noise ratio, multiple measurements are coadded at each step. The reaction, therefore, must be precisely reproducible. Any variations in the sample state or the measured reaction will introduce spectral and/or kinetic artifacts. Because each step-scan experiment requires the reaction to be repeated many (tens to hundreds of thousands) times, the time course of one experiment is much longer than a typical static or rapid-scan experiment. The duration of the step-scan experiment is determined by the reaction timescale, number of repetition required, and a number of steps required to complete an interferogram. At each step, before a new actinic pulse can be sent the reaction kinetics generated by the previous actinic flash must have decayed/relaxed completely. The
timescale of the reaction kinetics, therefore, sets the maximum repetition rate at which the actinic pulse can be triggered. To improve signal-to-noise ratio, often 10 to 20 coadditions are made per step, and the entire experiment is repeated \( \sim 20 – 60 \) times and averaged. For a measurement with a repetition rate of 10 Hz, with 20 coadditions per step and 60 experiments averaged, an entire set of experiments would take approximately \( (120 \times \text{number of steps}) \) seconds. With over 1000 stepping positions, the experimental duration exceeds well over 30 hours. One approach to reduce the measurement time is to minimize the number of stepping points. The number of stepping points are tied to the spectral region; reducing the number of points reduces the spectral region that can be measured accurately (the Nyquist criterion). In the FTIR spectroscopy, if the light intensity outside the measurable spectral region falls on a detector, the intensity is incorrectly associated with the frequency and creates an artifact known as “folding”. Therefore, to reduce the stepping points properly, and in turn reduce the measurement time, use of optical filters is necessary.

For the studies in this dissertation, the TRSS FTIR difference spectroscopy was applied at 77 K with a 4 cm\(^{-1}\) spectral resolution and 946 stepping points, which corresponds to the spectral range of 2106.39 to 1065.54 cm\(^{-1}\). The repetition rate is 10 Hz. Two 2000 cm\(^{-1}\) – 1000 cm\(^{-1}\) bandpass filters ensured the light beyond the measured spectral range is completely blocked, along with 4 sets of CaF\(_2\) windows that block light below \( \sim 1200 \) cm\(^{-1}\). The temporal resolution was 6 \( \mu \)s, and 590 time slices are measured per kinetic trace (36 points before the actinic flash). 20 coadditions are made per stepping point, and the experiment is usually repeated 40-60 times. With these instrumentation parameters, the overall time required to complete the set of experiments is \( \sim 20 – 30 \) hours. The standard error is obtained as measure of the experimental variability by repeating the entire process.
1.9 Aim of this Research

The focus in this dissertation is the functional and structural properties of the PSI RC and, more specifically, of the secondary electron acceptor $A_1$. With the emergence of the high-resolution X-ray crystal structure and experimental evidence for bidirectional ET in the early 2000s (10, 20), the studies on PSI RC have experienced great advancement. The determination of the relative positions of the ET cofactors and the surrounding protein environment at a sub-Å level has enabled the high-level computational modeling of the molecular interactions, and a number of calculations have been undertaken to describe the spectroscopic features from the theoretical perspectives. The ET kinetic rates and pathways, and the temperature dependence are now well-characterized. However, much of the energetic aspects of ultra-efficient ET processes, and the molecular details of the pigment-protein interactions that promote biologically common pigments to function as unique ET cofactors, are far from being fully understood.

The cofactor of the focus in this work, $A_1$, is a quinone molecule. Quinones are ubiquitous in biology and play a variety of roles in a wide range of bioenergetic system. In PSI, PhQ in the $A_1$ binding site serves as an important cofactor in both the forward and backward ET reactions. Given its roles in the biphasic forward ET and in the wasteful recombination reaction, the functions of $A_1$ is hypothesized to be a key factor in the underlying mechanism of highly efficient ET in PSI. Therefore, one focus of this research is to understand the energetics of the secondary electron acceptor $A_1$. Specifically, the goal is to estimate the range of $A_1$ redox potentials, which reported values still vary significantly between different studies, and determine the impact of $A_1$’s energetics to the overall properties of ET in PSI. The study is carried out as a kinetic measurement of the ET reactions involving $A_1$ and a development of a theoretical kinetic simulation model. For the kinetic measurement, the quinone in the $A_1$ binding site is strategically modified and its effect
on the ET kinetics is studied. Instrumentation for the kinetic measurement and establishment of sample preparation methods are direct continuation of the work reported in the thesis in 2012. By developing a computational model, the experimentally observed ET kinetics are explained from the perspectives of ET theory.

The second goal is to elucidate the molecular interaction between PhQ and the A$_1$ binding site through FTIR difference spectroscopy. FTIR is a molecular specific probe and is useful for investigating the molecular-level features of the pigments involved in ET. Because ET processes through A$_1$ occur very rapidly in PSI, the properties of A$_1$ is best probed using time-resolved techniques. The research includes the instrumentation, sample preparation methods, and data analysis methods. The aim in this part of the study is to construct highly-resolved DDS for the A$_1$ cofactors, and to identify and assign the vibrational modes due to PhQ in the A$_1$ binding site. Specifically, the effect of asymmetrical H-bonding to the quinone is investigated.

The work presented in this dissertation is intended to explore the aspects of both the energetics and molecular details of the secondary acceptor A$_1$. The ultimate goal of the research that the current work is leading to is to bridge the wide gap that currently exists between the two aspects.

1.10 Dissertation Overview

This chapter introduces the background information on the photosynthetic RCs. The basic function and molecular composition of the PSI RC are outlined. Brief introductions to the ET theory and FTIR spectroscopy are summarized here as well.

The work presented in the 2012 thesis was composed of the instrumentation of transient absorption spectroscopy in the visible and NIR spectral region and applications to the measurement of transient kinetics in the PSI RC (49). Both the WT and menB PSI were measured on ns to ms
timescales, and differences in the ET kinetics between the WT and modified PSI were characterized. Additionally, re-introduction of native PhQ into \( \text{menB}^- \) PSI was undertaken. PhQ was successfully incorporated into the A\(_1\) binding site by replacing PQ, resulting in the identical ET kinetics as the WT PSI. This dissertation starts as a continuation of the previous thesis.

In Ch. 2, in addition to the previously studied PhQ, a non-native quinone is incorporated into the A\(_1\) binding site of \( \text{menB}^- \) PSI. 2-methyl-1,4-napthoquinone (2MNQ) is introduced to \( \text{menB}^- \) PSI, and the transient absorption kinetics were measured. A method for preparing a thin-film sample that allows the transient absorption measurement at both room and cryogenic temperature without cryoprotectant is established in this chapter. This type of sample enables both the visible and IR measurements at 298 and 77 K to be made under identical conditions. Using both the standard liquid sample in a spectroscopic cuvette and the thin-film sample, transient absorption kinetics at the visible, NIR, and MIR spectral regions are obtained at room and cryogenic temperatures. The biphasic forward A\(_1^-\) \( \rightarrow \) F\(_X\) ET rates, the P700\(^+\)F\(\text{A/B}\) charge recombination rate, and P700\(^+\)A\(_1^-\) charge recombination rate at 77 K for PSI with PhQ, 2MNQ, and PQ are determined. Inspection of the ET rate of three quinones suggests that the experimentally observed rate cannot be applied directly to the Marcus equation to solve for the reaction driving force. The observed rates are results of quasi-equilibration of forward and backward rates. Estimation of the cofactor potentials can only be achieved through an appropriate kinetic modeling. The three cofactors (A\(_1\)A, A\(_1\)B, and F\(_X\)) model is constructed as an initial attempt at simulating the ET kinetics for the estimation of A\(_1\) midpoint potentials.

Ch. 3 details the study on the directionality of ET in PSI at 298 and 77 K. In this study, a high potential naphthoquinone (2,3-dichloro-1,4-napthoquinone, Cl\(_2\)NQ) is incorporated into the A\(_1\) binding site of \( \text{menB}^- \) PSI. Incorporation of a high potential quinone effectively inhibits the
forward ET at 298 K, and blocks the same process at 77 K. At both temperatures, the observed recombination phase is the P700⁺A₁⁻ charge recombination reaction. Using this property, the fractional usage of the A- and B-branches at two temperatures are investigated. Additionally, the quantum yield for the formation of the irreversible state at 77 K is compared for PSI with PhQ and Cl₂NQ incorporated.

In Ch. 4, in continuation of the work in Ch. 2 and 3, the transient absorption kinetics at 487 nm and 703 nm at 298 K, at 703 nm at 77 K, and at select IR wavenumbers at 77 K are collected for PSI with eight different quinones in the A₁ binding site. The series of quinones incorporated into PSI ranges over 500 mV of in vitro midpoint potentials. Collection of the reaction time constants for a wide range of midpoint potential is crucial for the development of kinetic simulation models, which is undertaken in Ch. 5.

Ch. 5 details the development of kinetic simulation model using the time constants obtained from ns to ms time-resolved absorption spectroscopy. A detailed kinetic model is constructed and solved within the context of Marcus ET theory, and it is found that the data for all of eight quinones can be well described only if the in situ midpoint potentials of the quinones fell in a tightly defined range. For PSI with PhQ incorporated into the A₁ binding site all of the time-resolved optical data is best modeled when the in situ midpoint potential of PhQ on the A/B-branches is -635/-690 mV, respectively. With the midpoint potential of Fₓ set at -680 mV, this indicates that forward ET from A₁⁻ to Fₓ is slightly endergonic/exergonic on the A/B branch, respectively. The same kinetic model is used for the eight different quinones incorporated, and in situ redox potentials for all of the incorporated quinones on both branches are determined. A linear correlation is found between the in situ and in vitro midpoint potentials of the quinones on both branches.
With the Marcus theory-based kinetic simulation model and the midpoint potentials of cofactors estimated through the model, the mechanism behind the high quantum yield of light-induced ET in PSI is investigated in Ch. 6. The key phenomenon to be investigated is the effect of inverted region ET in the charge recombination processes. It has been long suggested that the unproductive charge recombination in photosynthetic RCs are suppressed due to the back reaction occurring in the Marcus inverted region. However, inverted region ET has never been demonstrated in any native photosynthetic system. In this chapter, it is demonstrated that the unproductive charge recombination in native PSI RC does occur in the inverted region, at both room and cryogenic temperatures. Computational modeling of light-induced ET processes in PSI demonstrate a marked decrease in photosynthetic quantum efficiency, from 98% to below 72%, if the unproductive charge recombination process does not occur in the inverted region. Inverted region ET is therefore demonstrated to be an important mechanism contributing to efficient solar energy conversion in PSI.

Chapters 7 through 9 address the structural aspects of A1. In Ch. 7, TRSS FTIR difference spectroscopy is applied for the study of PSI with PhQ and 2MNQ incorporated into the A1 binding site. Time-resolved FTIR DS are fitted globally to multi-exponential functions, and decay-associated spectra (DAS) are constructed based on the reaction lifetimes. By applying a global analysis, the [P700\(^{+}\)A\(_1^-\) – P700A\(_1\)] DS that is free of previously inseparable spectral artifacts is produced at high spectral resolution. The global analysis procedure requires very high signal-to-noise ratio in kinetics, and such ratio is achieved by removing oscillatory noise that previously existed in the TRSS FTIR data from our group. The [P700\(^{+}\)A\(_1^-\) – P700A\(_1\)] DAS for PSI with PhQ and 2MNQ incorporated are used to construct [PhQ – 2MNQ] DDS. In the anion spectral region, two clear difference features are observed, and are assigned to the bands due to the quinonic
carbonyl groups that have split due to intense H-bonding to one group but not the other. The spectral interpretation is aided by the multi-layered quantum mechanical/molecular mechanical (QM/MM) calculation of vibrational frequency. Much of the calculations undertaken in this study is the work of Leyla Rohani and Nan Zhou in our group.

Chapters 8 and 9 focus on the properties of BQ in the A_1 binding site of PSI. Previously, for PSI with PQ in the A_1 binding site, discrepancies existed between EPR and FTIR spectroscopic data: from the EPR measurement at cryogenic temperature, the formation P700^+A_1^- radical state is observed, while from the FTIR measurement at 77 K, the P700 triplet state (^3P700) is observed. In Ch. 8, TRSS FTIR study on PSI with PQ is revisited to resolve this disagreement between two studies, and in Ch. 9 the (A_1^- – A_1) FTIR DS of PSI with three BQ analogues, including PQ, are analyzed. The band assignment is supported by the DFT-based vibrational frequency calculation. For the calculation of vibrational frequency, three computational models were developed for consideration of asymmetric H-bonding.


2 TIME-RESOLVED VISIBLE AND INFRARED DIFFERENCE SPECTROSCOPY FOR THE STUDY OF PSI WITH NON-NATIVE QUINONES INCORPORATED INTO THE A₁ BINDING SITE

2.1 Introduction

In photosynthetic oxygen evolving organisms, solar energy is captured and converted independently, but cooperatively, in two separate photosystems called photosystem I (PSI) and photosystem II. Photosystem II uses light to catalyze the generation of products that eventually lead to the oxidation of water and the subsequent liberation of molecular oxygen as a by-product. PSI on the other hand uses light to catalyze the formation of reducing products that eventually leads to the reduction of carbon dioxide, and its eventual incorporation into glucose.

In each of the two photosystems light energy conversion is realized via the transfer of electrons via a series of protein bound acceptors across a biological membrane. The nature of the acceptors in terms of their electronic and structural organization within the protein environment has been a subject of interest for decades.

The arrangement of the electron transfer (ET) cofactors in the PSI RC is outlined in Fig. 2.1A. PSI contains two almost identical chains of ET cofactors bound to the protein subunits PsaA or PsaB. The cofactors bound to the PsaA or PsaB proteins are labeled with a subscript A or B, respectively. P700, the primary electron donor in PSI, is a heterodimeric Chl-a/Chl-a' species, where Chl-a' is a 13² epimer of Chl-a (10). The primary electron acceptor, A₀, is a monomeric Chl-a molecule (81), and A₁ is a phylloquinone (PhQ) molecule (82). PhQ is a 2-methyl-3-phytyl-1,4-naphthaquinone, the structure of which is outlined in Fig. 2.1B. F₅, F₆ and F₇ are (4Fe–4S) iron sulfur clusters (83, 84).
Figure 2.1 (A) Arrangement of the two branches of ET cofactors in PSI. Number subscript refers to cofactor. Letter subscript refers to protein subunit (PsaA or B) to which cofactor is bound. The A/B -branch refers to the set of ET cofactors on the left/right side, respectively. Fig. 2.1A is generated using the 2.5 Å x-ray crystal structure of trimeric PSI particles from the cyanobacterium *Thermosynechococcus elongatus* (PDB file accession number PDB ID: 1JB0) (10). (B) View of PhQ in the A1A binding site. Possible H-bonding interactions are shown (dotted), and PhQ numbering scheme is indicated. Nitrogen/oxygen/sulfur atoms are blue/red/yellow, respectively.

In PSI, following light excitation of P700, an electron is transferred to A1 (via A0) in < 50 ps (17, 19). To further stabilize the charge separated state, the electron is then transferred from A1− to Fx. A1− to Fx ET is characterized by two time constants of 10–25 ns and 260–340 ns at room temperature (RT) (20, 26-29). From Fx− an electron is then transferred to FA and FB, also on a nanosecond timescale (31).

As mentioned, in cyanobacterial PSI at RT, forward ET from A1− to Fx is characterized by two phases with time constants of 10–25 ns and 260–340 ns (20, 26-29). These “fast” and “slow” kinetic phases have been associated with ET down the B and A branches, respectively (20, 26-29). As the temperature is lowered, in a portion of the RCs, forward ET diminishes and is replaced by a P700*A1− direct recombination reaction, which is characterized by a time constant of ~ 245 μs
at 77 K (26). This time constant is found using PSI particles from *Thermosynechococcus elongatus*. In PSI from *S6803* the corresponding time constant is \(~ 285–340 \mu s\) (see below and (27)). In cyanobacterial PSI particles at 77 K, the P700\(^+\)A\(_1^−\) state recombines in \(~ 45\%\) of the particles, the P700\(^+\)F\(_X^−\) state recombines in greater than 5 ms in \(~ 20\%\) of the PSI particles, and in \(~ 35\%\) of the PSI particles ET is irreversible (26). The degree to which each branch is active in ET is becoming clearer (85, 86), with the 260–340 ns/285–340 \(\mu s\) components at RT/77 K, respectively, being associated with ET along the A branch (7, 85, 86). The 10–25 ns phase and the irreversible fraction at RT and 77 K, respectively, have been associated with ET along the B branch (7, 39). The different rates of ET from A\(_1^−\) to F\(_X\) at RT, as well as the different functionality at 77 K is primarily ascribed to different midpoint potentials of PhQ in the A\(_1^+\) binding site on the A and B branches.

The reported range of the midpoint potential for PhQ occupying the A\(_1^+\) binding site is \(~ -670 to \sim -850 \text{ mV}\) (see (7) for a review), making it one of the most reducing quinones in biology. The estimated difference in the midpoint potentials between A\(_1A^+\) and A\(_1B^+\) ranges from 25 to 173 mV, with ET from A\(_1A^−\) to F\(_X\) being “thermodynamically uphill” while A\(_1B^−\) to F\(_X\) is “thermodynamically downhill” (39, 41) (see below).

The unprecedented redox potential of PhQ in the A\(_1^+\) binding site is in part a result of interactions of PhQ with the surrounding protein environment. Fig. 2.1B shows a view of PhQ in the A\(_1A^+\) binding site and several of the surrounding amino acids. The B-side is similar. Fig. 2.1B indicates that the C\(_1=O\) group of PhQ is not H-bonded whereas the C\(_4=O\) is H-bonded to the backbone NH group of LeuA722 (*T. elongatus* numbering).

Recently it has been demonstrated that different quinones can be incorporated into the A\(_1^+\) binding site in *menB* null mutant PSI particles simply by incubating the particles in a large molar
excess of the quinone of interest (46, 47, 87, 88). This quinone incubation method relies on mutant cells (from the cyanobacterium *Synechocystis* sp. 6803) in which genes that code for enzymes involved in PhQ biosynthesis have been disrupted (44, 89). For example, in mutants where the *menB* gene has been deactivated PhQ biosynthesis is inhibited and plastoquinone-9 (PQ₉) is recruited into the A₁ site instead (44, 63, 89). PSI particles from these mutant cells will be referred to as *menB*⁻ PSI particles. In *menB*⁻ PSI particles foreign quinones can displace PQ₉ in the A₁ binding site, simply by incubating particles in the presence of the quinone of interest (46, 47, 87, 88).

In this chapter, work undertaken using regular *menB*⁻ PSI particles, with PQ₉ in the A₁ binding site, and *menB*⁻ PSI particles with PhQ or menadione [2-methyl-1,4-naphthquinone (2MNQ)] incorporated into the A₁ binding site is described. This chapter focuses on measurement of the kinetics of forward and reverse ET in PSI samples with the three different quinones incorporated, at both RT and 77 K. The kinetics of ET are sensitive to the redox potential of the quinone in the A₁ binding site, and here Marcus theory is used in combination with a quasi-equilibration model to estimate the redox potentials of the quinones in the A₁ binding site.

### 2.2 Materials and Methods

Trimeric PSI particles from *menB* null mutant cells from *Synechocystis* sp. PCC 6803 (*S6803*) were isolated and stored as described previously (44). To incorporate quinones into the A₁ binding site, PSI particles are incubated in the presence of a ~ 1000× molar excess of the quinone of interest (quinone/RC ratio). Quinones were dissolved in ethanol and added in such a way that the ethanol concentration never exceeds 2% of the total volume. PSI particles were incubated in the presence of quinone at 4 °C in the dark for ~ 24 h, with stirring. Alternatively, PSI particles could be incubated at RT with stirring for 4 h.
2.2.1 Time-resolved absorption spectroscopy in the visible spectral range

Nanosecond (ns) to millisecond (ms) transient absorption spectroscopy in the visible spectral region was undertaken using an Edinburgh Instruments LP920 flash photolysis spectrometer. 532 nm, 5 ns pulses from a Continuum Minilite Nd:YAG laser were used for excitation. A flash repetition rate of 10 Hz was typically used, although measurements at 1 or 0.2 Hz were found to yield similar results (not shown). A xenon arc lamp was used as probe light source in either CW mode (for measurements over tens of milliseconds) or pulsed mode (for temporal changes on a timescale less than 1 ms). In pulsed mode the xenon arc lamp produces flashes of ~ 2 ms duration with a relatively flat intensity profile over most of this range. A 1 cm water cell was placed in front of the probe lamp to reduce heating effects.

Flash-induced absorption changes were measured at 800, 703 and 487 nm. After passing through the sample, probing light was directed through a monochromator (Bentham Instruments TMc 300) to select the probe wavelength. Probe light was detected using a Hamamatsu R928 photomultiplier tube. To minimize actinic effects of the probing light narrow-band (10 nm FWHM) interference filters were placed in front of the sample. Optical filters were also placed after the sample, in front of the entrance slit to the monochromator, in order to attenuate 532 nm laser scattered photons.

2.2.2 Time-resolved step-scan FTIR

Time-resolved step-scan FTIR experiments with 6 μs time resolution, at 77 K, were undertaken as described previously (90-92). Data were collected in the 1950–1100 cm⁻¹ region at 4 cm⁻¹ spectral resolution. For time-resolved step-scan FTIR measurements PSI samples are concentrated to a thick paste and then squeezed between two calcium fluoride windows. The
windows were pressed until the sample has an amide II absorbance (at ~ 1550 cm⁻¹) of ~ 0.8 at RT. The path-length in the sample cells is estimated to be < 5 μm. Samples were mounted in a cryostat (APD cryogenics or Cryo Industries of America) and the temperature was lowered to 77–81 K (hereafter referred to as 77 K).

PSI samples used in FTIR measurements formed a clear glass at both RT and 77 K. Both visible and infrared measurements were undertaken using the same sample, in the same sample cell, without cryoprotectant. Samples prepared for FTIR spectroscopy have very high absorption in the visible region, with the OD at the peak of the Q₇ absorption band (at ~ 679 nm) being greater than 5.0. The sample OD at 703 and 800 nm is still well below 1.0, so probing at these wavelengths is still feasible. So for samples used in FTIR measurements, time-resolved visible measurements probing at 703 and 800 nm, at both RT and 77 K were undertaken. For samples prepared for FTIR spectroscopy measurements probing at 487 nm are not possible. Measurements on “dilute” samples are still possible, however. The term “dilute samples” refers to samples prepared under ‘more-standard’ conditions, in a 10 × 5 mm cuvette, with concentration adjusted to achieve an optical density of ~1.6 at the peak of the Q₇ band at ~679 nm.

2.3 Results

Below menB⁻ PSI particles that have been incubated in the presence of either PhQ or 2MNQ are considered. It will be shown that PhQ or 2MNQ is incorporated into the A₁ binding site in these PSI particles. These menB⁻ PSI particles are simply referred as PSI with PhQ or 2MNQ incorporated. Regular menB⁻ PSI particles are referred as PSI with PQ₈ incorporated.

PSI samples with “low” Q₇ peak absorption (1.6 or less) will be called dilute samples, to distinguish them from the samples used in FTIR measurements, which will be called “concentrated” samples. Concentrated samples can be probed at 703 and 800 nm, but not at
487 nm. Concentrated samples can also be studied spectroscopically at 77 K without cryoprotectant, while dilute samples cannot.

2.3.1 Nanosecond time-resolved visible absorption spectroscopy

Fig. 2.2 shows RT flash-induced absorption changes at 703 and 800 nm for PSI with PQ₉, 2MNQ and PhQ incorporated, on a 350 ns timescale. Data are shown for concentrated (left) and dilute (right) samples. Also shown are data obtained using WT cyanobacterial PSI particles from S6803 (with PhQ naturally present in the A₁ binding site). The “spike” observed in the first ~ 10 ns following laser excitation is an artifact due to imperfect subtraction of changes related to scattering and possibly fluorescence from the sample (Data associated with scattering from the sample was collected using the laser to excite samples but without probing light. This data was subtracted from the experimental data but, clearly, such a subtraction is imperfect). This “spike” should have a temporal width close to the instrument response, and is an artifact that in no way impacts any of the conclusions in this chapter.

For the data in Fig. 2.2, several points are noteworthy: 1) The ns kinetics are similar for both concentrated and dilute samples. 2) The flash-induced absorption changes are identical for WT PSI from S6803 and for menB⁻ PSI with PhQ or 2MNQ incorporated. 3) The ns decay phases observed for PSI with PQ₉ incorporated are not observed for the other PSI samples.
Figure 2.2  RT flash-induced absorption changes at 800 (A, B) and 703 (C, D) nm on a 350 ns timescale for menB− PSI with PQ9 (blue), 2MNQ (green), and PhQ (red) incorporated. Data for both dilute (B, D) and concentrated (A, C) PSI samples are shown. At 800 nm, for PSI with PQ9 incorporated (blue), the data is best fit to two exponentials with lifetimes of 33 and 107 ns (A) or 23 and 105 ns (B). The fitted bi-exponential functions (black) are also shown in A and B. At 703 nm, the data is adequately fit to a single exponential with lifetime of 96 ns (C) or 103 ns (D). These fitted single exponential functions are also shown in C and D (black). Data is also shown for dilute WT PSI particles from S6803 (magenta) (B, D).

Probe wavelengths at 703 and 800 nm are used primarily to monitor absorption changes associated with P700 and P700+, respectively (93). Since the P700 ground state recovers in tens of milliseconds, via P700*F_{AB}− charge recombination (34, 63, 94), no kinetic evolution of the absorption changes associated with P700 and P700+ is expected in a 350 ns time range. This is essentially the case for WT PSI, and menB− PSI with PhQ or 2MNQ incorporated, where the kinetic traces are essentially flat over the 350 ns timescale. For PSI with PQ9 incorporated, however, the absorption changes evolve considerably over the 350 ns timescale.

For PSI with PQ9 incorporated the data at 703 nm is adequately described by a single exponential function with time constant of 96–103 ns. If the data at 800 nm is fitted to a single
exponential function, a lifetime of 57–70 ns is calculated. From an evaluation of the residuals associated with this fit, it is clear that a single exponential function does not adequately describe the data. If the data at 800 nm is fitted to a bi-exponential function, lifetimes of 33 and 107 ns (Fig. 2.2A) or 23 and 105 ns (Fig. 2.2B) are calculated.

For PSI with PQ\textsubscript{9} incorporated the initial \((t = 0)\), positive, flash-induced absorption change at 800 nm is greater than it is for PSI with PhQ incorporated. As a result of the ns kinetic phases, however, the positive absorption change at \(t = 350\) ns, is smaller for PSI with PQ\textsubscript{9} incorporated compared to PSI with PhQ incorporated. On the other hand, for PSI with PQ\textsubscript{9} incorporated, the initial \((t = 0)\), negative, flash-induced absorption change at 703 nm is \textit{smaller} than it is for PSI with PhQ incorporated, and as a result of the ns kinetic phases, this difference becomes larger.

The \(\sim 23–107\) ns decay phases in PSI with PQ\textsubscript{9} incorporated have not been observed before, and their origin is uncertain. In addition to these nanosecond decay phases, microsecond decay phases are also observed in PQ\textsubscript{9}-incorporated PSI (data not shown). Since decay phases are observed at both 800 and 703 nm, wavelengths that are normally associated with P700\textsuperscript{+} and P700, respectively, it is tentatively suggest that these nanosecond decay phases could be due to \(^3\text{P700}\) formation, and the microsecond phase due to the decay of \(^3\text{P700}\). When ET from \(A_0^-\) to \(A_1\) is blocked, the formation and decay of \(^3\text{P700}\) state are known to occur on ns and \(\mu\)s timescales, respectively \cite{32}. While the features of the kinetics obtained at 800 nm for PSI with PQ\textsubscript{9} incorporated (Fig. 2.2A, B; blue) are comparable to the absorption change measured at 820 nm on PSI RC devoid of \(A_1\), \(F_X\), and \(F_{A/B}\) \cite{95}, it is not clear if the extinction coefficients of P700 and \(^3\text{P700}\) at 703 nm could give rise to the observed absorption changes at 703 nm.

Time-resolved visible spectroscopy on \textit{menD1} \textsuperscript{−} PSI from \textit{Chlamydomonas reinhardtii} also exhibit \(\sim 30\) ns and tens of microseconds decay phases when ET beyond \(A_0^-\) is blocked (because
plastoquinol is present in the A₁ binding site) (96, 97). These observed ns and μs decay phases were probed at both 320 nm and 430 nm, and were assigned to P700⁺A₀⁻ radical pair recombination leading to P700⁺ formation and then its subsequent decay (96, 97).

A more detailed study of the ns and μs decay phases observed in PSI particles with PQ₉ incorporated will be presented elsewhere. However, since the replacement of PQ₉ with other quinones is correlated to the disappearance of these ns and μs decay phases (Fig. 2.2), the differences in the kinetics between PSI with PQ₉ incorporated, and the other PSI samples, are often used to assess the extent of quinone incorporation into the A₁ binding site.

In Fig. 2.2 the data for the dilute PSI samples are scaled to a sample absorbance at ~ 680 nm (Q₉ peak) of 1.6. Fig. 2.2 indicates that the concentrated PSI samples lead to absorption changes that are nearly six times greater than that found for dilute PSI samples. The kinetics are the same for both concentrated and dilute samples, however. In addition the ratio of the amplitudes of the absorption changes for both concentrated and the dilute samples are the same. That is:

\[
\frac{\Delta A_{800}^{t=0}(PhQ)}{\Delta A_{800}^{t=0}(PQ_9)} \approx 0.7, \quad \frac{\Delta A_{350ns}^{t=0}(PhQ)}{\Delta A_{350ns}^{t=0}(PQ_9)} \approx 1.6, \quad \frac{\Delta A_{703}^{t=0}(PhQ)}{\Delta A_{703}^{t=0}(PQ_9)} \approx 0.7, \quad \text{and} \quad \frac{\Delta A_{350ns}^{t=0}(PhQ)}{\Delta A_{350ns}^{t=0}(PQ_9)} \approx 1.8,
\]

where an expression of the form \( \Delta A_{800}^{t=0}(PhQ) \) represents the initial \((t = 0)\) flash-induced absorption change at 800 nm for PSI with PhQ incorporated.

From the data in Fig. 2.2 at RT (as well as the data discussed below), since the same kinetics with the same amplitude ratios for both dilute and concentrated samples are observed, it is concluded that there are no concentration dependent artifacts to consider, and that conclusions drawn from work on concentrated samples are equally applicable to dilute samples.

2.3.2 Room temperature P700⁺Fₐ₋ₐ radical pair recombination

P700⁺Fₐ₋ₐ charge recombination is most easily probed at ~ 703 nm on a millisecond (ms) timescale (93). At RT, for WT PSI (or menB⁻ PSI with PhQ incorporated), radical pair
recombination is characterized by a time constant of ~ 80 ms (see (94) and references therein). Fig. 2.3 shows RT flash-induced absorption changes on millisecond timescales for dilute PSI samples with (A) PQ₉ and (B) 2MNQ incorporated. The data in each are fitted to a single exponential function (plus a constant). The fitted decays are also shown in Fig. 2.3A and B, and are characterized by time constants of 3.2 and 14.4 ms, respectively. A decay constant of 3.2 ms is the same as that found previously for menB⁻ PSI with PQ₉ incorporated (63, 98). For PSI with 2MNQ incorporated, a P700⁺Fₐ/ₕ⁻ lifetime of ~ 14 ms has not been reported (although see (99)). The fact that millisecond decay phases are observed indicates that the A₁ binding site is occupied. The fact that the time constants observed are very different to that found for WT PSI, or for menB⁻ PSI with PhQ incorporated, indicates that PQ₉ or 2MNQ is present in the A₁ binding site.

![Figure 2.3](image)

**Figure 2.3** RT flash-induced absorption changes at 703 nm on 10–35 ms timescales for menB⁻ PSI samples with (A) PQ₉ and (B) 2MNQ incorporated into A₁ binding site. Measurements are shown for dilute samples but similar results are found for concentrated samples (not shown). The data in both figures were fitted to a single exponential function (plus a constant). The fitted functions are shown (red) along with the calculated time constants.

For PSI with PQ₉/2MNQ incorporated ~23/12% of the absorption change at 703 nm (Fig. 2.3) does not decay on the timescale considered. This is reminiscent of that found in WT PSI
particles, where it is found that ~10% of the initial absorption change does not decay with a characteristic ~80 ms time constant. These longer lived components are presumably associated with ET from the terminal iron sulfur clusters to an external acceptor, such as oxygen (94).

2.3.3 $P700^+ A_1^- \rightarrow P700^+ F_x^-$ forward electron transfer at room temperature

Forward ET from $A_1^-$ to $F_x$ in PSI is most easily monitored at ~ 487 nm (22, 27). Fig. 2.4 shows flash-induced absorption changes at 487 nm for (dilute) $menB^-$ PSI with (A) PhQ, (B) PQ$_9$ and (D) 2MNQ incorporated. Fig. 2.4B shows absorption changes at 487 nm for (dilute) WT PSI samples. Different timescales were covered for each sample so that the dominant decay phase can be easily visualized.

**Figure 2.4** Room temperature flash-induced absorption changes at 487 nm for $menB^-$ PSI samples with (A) PhQ, (C) PQ$_9$ and (D) 2MNQ incorporated into $A_1$ binding site. For comparison, measurements for WT PSI particles from S6803 are also shown (B). Measurements are for dilute samples only as the absorbance at 487 nm for concentrated samples prohibits spectroscopic measurement. The calculated fitted functions (red) and time constants are also shown. Inset in C and D shows absorption changes over shorter 0.9 and 1.8 μs time windows, respectively.
For PSI with PhQ incorporated, a decay phase with time constant of ~ 20 ns is observed (Fig. 2.4A, B). The amplitude of this decay phase cannot be reliably estimated given the available time resolution. There is little doubt, however, that it is at least partially associated with B branch ET, which is known to occur on such a time scale (27).

Excluding the data in the first ~ 30 ns following the laser flash, and by fitting the data in Fig. 2.4A and B to a single exponential function, a time constant of 275–309 ns is calculated. For PSI from S6803 at RT, a time constant of 340 ns has been found previously (27), in relatively good agreement with the data presented here. The data in Fig. 2.4A and B have been scaled, so the signal at 487 nm is slightly larger for menB⁻ PSI with PhQ incorporated, compared to WT.

For regular menB⁻ PSI with PQ₉ incorporated the kinetics are clearly bi-exponential (Fig. 2.4C). Fitting the data in Fig. 2.4C to a bi-exponential function, time constants of 13.9 and 210.8 μs are calculated. Previously, for menB⁻ PSI with PQ₉ incorporated, two phases of forward ET from A₁⁻ to Fₓ have been found with lifetimes of 11.4–18.1 and 306–377 μs (63), in good agreement with observations made here. These fast and slow phases have tentatively been assigned to A₁B⁻ → Fₓ and A₁A⁻ → Fₓ ET, respectively (7).

The RT flash-induced absorption changes at 487 nm for dilute menB⁻ PSI with 2MNQ incorporated, on an 18 μs timescale, are shown in Fig. 2.4D. In addition to a microsecond decay phase, Fig. 2.4D indicates that faster, nanosecond phases also contribute to the absorption change. To better resolve these components measurements were made on a 1.8 μs timescale (inset). By fitting the data to a bi-exponential function, time constants of 420 ns and 3.1 μs are found. The rates of forward ET from A₁⁻ to Fₓ with 2MNQ occupying the A₁ binding sites have not been reported previously. However, following on from the suggested origin of the fast and slow phases
for PSI with PQ₉ incorporated, it is tentatively suggest that the fast and slow phases observed at 487 nm for PSI with 2MNQ incorporated are due to A₁B⁻ to Fₓ and A₁A⁻ to Fₓ ET, respectively.

2.3.4 \textbf{P700}⁺\textit{A₁}⁻ charge recombination at 77 K

Flash-induced absorption changes at 703 and 800 nm at 77 K were measured using only concentrated PSI samples. Fig. 2.5 shows flash-induced absorption changes at 800 and 703 nm at 77 K for concentrated PSI with PQ₉ (A, B), PhQ (C, D) and 2MNQ (E, F) incorporated, on a 1 ms timescale. For PSI with PQ₉/PhQ/2MNQ incorporated into the A₁ binding site, the data at 703 and 800 nm was fitted simultaneously to a single exponential function, and a 250/338/240 μs time constant was calculated, respectively.

\textbf{Figure 2.5} Low temperature (77 K) flash-induced absorption changes at 800 (A, C, E) and 703 nm (B, D, F) for concentrated PSI samples with PQ₉ (A, B), PhQ (C, D) and 2MNQ (E, F) incorporated. The data at both probe wavelengths were fitted simultaneously to a single exponential function (\textit{red}) and a lifetime of 250/338/240 μs was calculated, respectively.
From previous TR step-scan FTIR DS studies on menB− PSI samples at 77 K (with PQ9 incorporated), it has been shown that 3P700 is formed in a fraction of the PSI particles, presumably because in this fraction PQ9 is not functional, or perhaps not even present (100). At 77 K, the decay of 3P700 and radical pair recombination are characterized by similar time constants. Thus it is not possible to distinguish between the two processes at 77 K on the basis of time constants. For this reason the data obtained at 77 K for PSI with PQ9 incorporated will not be considered, and only data obtained at 77 K for PSI with PhQ or 2MNQ incorporated will be considered.

2.3.5 Time-resolved step-scan FTIR difference spectroscopy at 77 K

Fig. 2.6 shows absorption changes at four IR frequencies, obtained using concentrated PSI particles with PhQ or 2MNQ incorporated at 77 K. The four kinetic traces were fitted simultaneously to a single decaying exponential component plus a constant (non-decaying) component. The fitted functions are shown, and are characterized by a time constant of 301/241 μs for PSI with PhQ/2MNQ incorporated, respectively. The absorption changes at 1754 cm−1 contain contributions that are associated with the decay of P700+ and A1−, while the changes at 1748 cm−1 contain contributions that are associated with the recovery of P700 and A1 (90). The absorption changes at 1495 and 1415 cm−1 in Fig. 2.6A are mostly due to the decay of PhQ− in the A1 binding site (90). The absorption changes at 1504 and 1427 cm−1 in Fig. 2.6B are mostly due to the decay of 2MNQ− in the A1 binding site (101).
Figure 2.6 Low temperature (77 K) flash-induced absorption changes at several IR frequencies obtained using PSI with (A) PhQ and (B) 2MNQ incorporated into the A\textsubscript{1} binding site. Data were collected in 5 \( \mu \)s increments. The four kinetics in each caption were fitted simultaneously to a single exponential plus a constant. The fitted functions are shown (red) and are characterized by a time constant of 301 and 241 \( \mu \)s, respectively.

For PSI with PQ\textsubscript{9} incorporated, absorption changes at several IR frequencies have been presented previously, and a time constant of 208 \( \mu \)s was found [39]. However, this 208 \( \mu \)s decay phase, which accounts for > 80\% of the signal amplitude at 1594 cm\(^{-1}\), is due to the decay of \(^3\)P700 and not P700\(^+\)A\textsubscript{1}\(^-\) (100).

Fig. 2.6 demonstrates that radical pair recombination at 77 K is faster for PSI with 2MNQ incorporated than for PSI with PhQ incorporated (241 vs. 301 \( \mu \)s). Such a result also follows from Fig. 2.5, where radical pair recombination at 77 K is characterized by a time constant of 240/338 \( \mu \)s for PSI with 2MNQ/PhQ incorporated, respectively. Thus the low temperature (77 K) TR visible and IR data are in good agreement. This comparison is a strong indication of the validity and applicability of the TR step-scan FTIR DS approach for the studies of quinones in the A\textsubscript{1} binding site in PSI at 77 K.

The time constants and associated decay amplitudes discussed in this chapter for the various PSI particles, at 298 and 77 K, are summarized in Table 2.1.
Table 2.1  Summary of time constants calculated for PSI particles with different quinones incorporated. At 77 K only concentrated samples were studied. The ratio of the initial amplitudes at 77 K and RT (column 5) indicates the percentage of PSI particles in which reversible ET processes are occurring at 77 K. The ratio of the monoexponential decay amplitude at 77 K to the initial amplitude at RT is listed also (column 6). This latter ratio gives a measure of the percentage of PSI particles in which P700\(^+\)A\(_1\)\(^-\) charge recombination occurs at 77 K. At 298 K, for PSI with PQ\(_9\) incorporated, the signal amplitudes after the ~30–100 ns decay phases are used.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temp. (K)</th>
<th>(\lambda_{pr}) (nm)</th>
<th>(\Delta A_{77K}/\Delta A_{RT}) lifetime/Amplitude (%)</th>
<th>(\Delta A_{77K}^{decay}/\Delta A_{RT}) (%)</th>
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</thead>
<tbody>
<tr>
<td>PQ(_9)</td>
<td>298</td>
<td>703</td>
<td>3.2 ms/79.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>298</td>
<td>487</td>
<td>13.9 (\mu s)/65.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>703</td>
<td>250 (\mu s)/73.9</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>800</td>
<td>250 (\mu s)/69.1</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>IR</td>
<td>208 (\mu s) (100)</td>
<td></td>
</tr>
<tr>
<td>PhQ</td>
<td>298</td>
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<td>~15 ns (27)</td>
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<tr>
<td></td>
<td>77</td>
<td>703</td>
<td>338 (\mu s)/79.1</td>
<td>51.8</td>
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<td></td>
<td>77</td>
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<td>49.3</td>
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<tr>
<td></td>
<td>77</td>
<td>IR</td>
<td>301 (\mu s)</td>
<td></td>
</tr>
<tr>
<td>2MNQ</td>
<td>298</td>
<td>703</td>
<td>14.4 ms/98.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>298</td>
<td>487</td>
<td>420 ns/20.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>703</td>
<td>240 (\mu s)/90.6</td>
<td>60.8</td>
</tr>
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<td></td>
<td>77</td>
<td>800</td>
<td>240 (\mu s)/86.1</td>
<td>66.3</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>IR</td>
<td>241 (\mu s)</td>
<td></td>
</tr>
</tbody>
</table>

2.4 Discussion

2.4.1 Indicator of quinone incorporation into the A\(_1\) binding site in menB\(^-\) PSI

All of the data presented here indicate the successful incorporation of foreign quinones into the A\(_1\) binding site of menB\(^-\) PSI. The RT forward ET (A\(_1\)\(^-\) to F\(_X\)) and charge recombination (P700\(^+\)F\(_{A/B}\)\(^-\)) rates probed at 487 nm and 703 nm respectively, for PSI with PQ\(_9\) and PhQ incorporated, agree well with previously reported values. In PSI with 2MNQ incorporated, the forward ET (Fig. 2.4) and charge recombination rates (Fig. 2.3) are highly distinguishable from those for PSI with PQ\(_9\) or PhQ incorporated. The amplitudes of the decay phases suggest a very high level of 2MNQ incorporation into the A\(_1\) binding site.
The ns kinetics probed at 800 or 703 nm (Fig. 2.2) also serve as a useful indicator of successful quinone incorporation, since PSI with PQ\textsubscript{0} in the A\textsubscript{1} binding site exhibits ~30–100 ns decay phases that are not observed using PSI with PhQ or 2MNQ incorporated. Since the ns decay phases disappear when PhQ or 2MNQ is incorporated into the A\textsubscript{1} binding site, these ns phases probably relate to a lack of functionality of the A\textsubscript{1} binding site in a portion of the PSI particles, either because the A\textsubscript{1} binding sites are unoccupied, or because the A\textsubscript{1} binding sites are occupied by non-functional species, such as plastoquinol (PQH\textsubscript{2}). In either case, ET to A\textsubscript{1} is blocked and P700\textsuperscript{+}A\textsubscript{0}\textsuperscript{−} could recombine to yield \textsuperscript{3}P700. Previous TR FTIR DS studies do indicate that \textsuperscript{3}P700 is formed in a fraction of PSI samples with PQ\textsubscript{0} incorporated at 77 K, but it is unclear if it is this fraction of PSI particles that give rise to the ns decay phases at RT. As mentioned above, it is unclear if a difference in the extinction coefficients between P700\textsuperscript{+} and \textsuperscript{3}P700 could explain the absorption changes at 800 and 703 nm. Therefore, the origin of the ~30–100 ns decay phases observed at RT for PSI with PQ\textsubscript{0} incorporated remains an open question that will be addressed in future publications. The main point here is that the loss of ~30–100 ns decay phases for \textit{menB}\textsuperscript{−} PSI incubated in the presence of other quinones is an indicator that the new quinone has been incorporated into the A\textsubscript{1} binding site.

2.4.2 Redox potential of quinones in the A\textsubscript{1} binding site

Observed rates of forward ET can be used to estimate the redox potential of the quinone in the A\textsubscript{1} binding site, and Eq. 2.1 outlines an empirical approximation relating the rate of intra-protein ET (\(k\)) to the edge-to-edge distance between the electron carriers (\(R\)), the standard reaction free energy (\(\Delta G^0\)), and the reorganization energy (\(\lambda\)) (61). In Eq. 2.1, \(k\) is measured in s\textsuperscript{−1}; \(R\) in Å; \(\Delta G^0\) and \(\lambda\) in eV.

\[
\log k = 13 - (1.2 - 0.8\rho) \cdot (R - 3.6) - \gamma (\Delta G^0 + \lambda)^2 / \lambda \quad \text{Equation 2.1}
\]
The parameter $\rho$ gives a measure of the protein packing density, a value that is correlated to the tunneling barrier of the medium. A mean value of 0.76 is obtained in a survey of packing densities in a series of ET systems (102), and this number is used here. In the classical treatment of Marcus, $\gamma$ is given by $F/(4 \times 2.303 k_B T)$ (103, 104). In the quantum mechanical treatment recommended by Moser and Dutton, $\gamma$ is modified by the Hopfield approximation that replaces $k_B T$ with $\hbar \omega / 2 \coth(\hbar \omega / 2 k_B T)$ (60, 62, 103). With $\hbar \omega = 0.056 \text{ eV}$ and $T = 298 \text{ K}$, $\gamma$ is calculated to be 3.1. Substituting the values for $\rho$ and $\gamma$ into Eq. 2.1 results in Eq. 2.2. Eq. 2.2 indicates that a change in ET rate may reflect a change in $R$, $\Delta G^0$ and/or $\lambda$.

$$\log k = 15 - 0.6 R - 3.1 (\Delta G^0 + \lambda)^2 / \lambda$$

Equation 2.2

Previous EPR studies have shown that the distances from P700 to PQ$_9$, 2MNQ, or PhQ in the different PSI particles are nearly identical (89, 105). The reorganization energy associated with ET from A$_1^-$ to F$_X$ is also commonly assumed to be unaffected by the type of quinone in the A$_1$ binding site. Therefore, a change in the rate of ET from A$_1^-$ to F$_X$ is generally considered to be associated with a change in the Gibbs standard reaction free energy.

The average edge-to-edge distance (established using the 2.5 Å PSI crystal structure) between the quinone in the A$_1$ binding site and F$_X$ is $\sim 9.0$ Å (37, 39). However, distances in the 6.8–11.3 Å range have also been used (35, 37, 41, 63). This wide range of edge-to-edge distances will be considered in calculations presented below. Commonly used reorganization energies typically fall in the 0.7–1.0 eV range (35, 41, 63), and values in this range will also be considered in calculations presented below.

Earlier estimates of the midpoint potential of PhQ in the A$_1$ binding site were undertaken assuming unidirectional ET, and a single midpoint potential is assigned to PhQ in both the A$_{1A}$ and A$_{1B}$ binding sites. As experimental support for bi-directional ET emerged, differences in A$_{1A}$
and A\textsubscript{1B} midpoint potentials between 25–173 meV have been proposed to account for differences in A and B branch ET rates (39, 41). Furthermore, simulations suggested that the A\textsubscript{1A} \rightarrow F\textsubscript{X} ET process is slightly endergonic, while the A\textsubscript{1B} \rightarrow F\textsubscript{X} process is slightly exergonic (37, 39).

Forward ET from PhQ\textsuperscript{-} to F\textsubscript{X} on the A branch is characterized by a time constant of 309 ns (Fig. 2.4A and Table 2.2). Using this time constant in Eq. 2.2, along with \( R = 9.0 \) Å and \( \lambda = 0.7 \) eV, \( \Delta G^0 \) is calculated to be +135 meV. ET from A\textsubscript{1B} to F\textsubscript{X} is reported to be in the 10–25 ns range (20, 27-29). Using these time constants, along with \( R = 9.0 \) Å and \( \lambda = 0.7 \) eV in Eq. 2.2 yields \( \Delta G^0 \) values in the −98 to −28 meV range. Thus the midpoint potential of A\textsubscript{1A} is calculated to be 163–233 mV more positive than A\textsubscript{1B}. The lower end of this difference in free energy between A\textsubscript{1A} and A\textsubscript{1B} is in agreement with the range computed by Ishikita and Knapp (155–166 mV), and with that calculated by Ptushenko et al. (173 mV) (38, 41).

**Table 2.2** Calculated Gibbs standard reaction free energy (in meV) for A\textsubscript{1} \rightarrow F\textsubscript{X} ET in PSI, with PhQ, 2MNQ, and PQ\textsubscript{9} in the A\textsubscript{1} binding site.

<table>
<thead>
<tr>
<th>R (Å)</th>
<th>( \lambda ) (eV)</th>
<th>( \tau = 15 ) ns PhQ</th>
<th>( \tau = 309 ) ns PhQ</th>
<th>( \tau = 420 ) ns 2MNQ</th>
<th>( \tau = 3.1 ) μs 2MNQ</th>
<th>( \tau = 13.9 ) μs PQ\textsubscript{9}</th>
<th>( \tau = 211 ) μs PQ\textsubscript{9}</th>
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<td>6.8</td>
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<td>298</td>
<td>313</td>
<td>405</td>
<td>470</td>
<td>579</td>
</tr>
<tr>
<td>0.8</td>
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<td>283</td>
<td>382</td>
<td>451</td>
<td>579</td>
<td>567</td>
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<tr>
<td>1.0</td>
<td>–0.6</td>
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<td>211</td>
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<td>–79</td>
<td>–55</td>
<td>82</td>
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<td>313</td>
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</tbody>
</table>

For PSI with PQ\textsubscript{9} incorporated, previously reported values of the Gibbs standard reaction free energy for the A\textsubscript{1} \rightarrow F\textsubscript{X} ET process are +12, +35, and +95 meV (63, 98). However, with the time constant of 13.9 μs measured here (Fig. 2.4, Table 2.2), along with \( R = 9.0 \) Å and
$\lambda = 0.7 \text{ eV}$, $\Delta G^0$ is calculated to be $+335 \text{ meV}$ using Eq. 2.2. If the observed 211 $\mu$s time constant is taken as the rate of forward ET in the second branch, then $\Delta G^0$ is calculated to be $+456 \text{ meV}$, and there is a 121 meV difference in $\Delta G^0$ between the quinones on each branch.

Similarly, for PSI with 2MNQ incorporated, with forward ET being characterized by time constants of 420 ns and 3.1 $\mu$s, $\Delta G^0$ is calculated to be $+154 \text{ meV}$ and $+261 \text{ meV}$, respectively. The calculated difference in $\Delta G^0$ between 2MNQ on the A and B branches is therefore 107 meV.

For all three quinones incorporated, a difference in midpoint potentials of $A_{1A}$ and $A_{1B}$ is calculated to be in the 100–200 mV range.

As indicated above, a wide range of parameters have been used in various applications of Marcus ET theory to PSI. Therefore, in addition to the parameters calculated above, values for $\Delta G^0$ using other commonly reported parameters are listed in Table 2.2.

For PSI with PhQ incorporated, $\Delta G_{A1A/FX}^0 = 135 \text{ mV}$ ($R = 9.0 \text{ Å}$ and $\lambda = 0.7 \text{ eV}$) is calculated using the experimentally observed rate as input in Eq. (2). However, using this approach for PSI with 2MNQ and PQ$_9$ incorporated places the calculated $\Delta G_{A1A/FX}^0$ value at $+261$ and $+457 \text{ mV}$ (Table 2.2), both of which are unreasonably large ($> 10k_B T$).

If the experimentally observed ET rates are used directly in Eq. 2.2 then this implies that the ET processes is virtually irreversible, requiring the forward ET rate to be much larger than the reverse ET rate. Another approach is to explicitly consider forward and reverse ET processes, in a so-called “quasi-equilibrium” model, that was initially proposed by Brettel et al., and later extended by Santabarbara et al. to include bi-directional ET (14, 26, 29, 39, 106). In this quasi-equilibrium model, experimentally observed rates do not represent the intrinsic rate of ET that should be used in Eq. 2.2. Using this quasi-equilibrium modeling approach, Santabarbara et al. have suggested $\Delta G_{A1A/FX}^0$ and $\Delta G_{A1B/FX}^0$ values of $+15$ and $-10 \text{ meV}$, respectively, for PSI with
PhQ incorporated (39). The very different values (compared to that listed in Table 2.2) are a reflection of the fact that the observed decay rates (Fig. 2.4) and intrinsic rates are quite different.

Fig. 2.7D shows the quasi-equilibrium model used to simulate the experimental time-resolved data for PSI with PhQ, 2MNQ and PQ$_9$ incorporated. Simulation involves numerical solution of a series of first order, linear differential equations associated with the various radical pair state populations, with the various rate constants used as input (described in detail in (39)). Forward ET rates (shown in Fig. 2.7D) were computed using Eq. 2.2 with $R = 9.0$ Å and $\lambda = 0.7$ eV and appropriate values for $\Delta G^0$. Reverse ET rates were calculated assuming a Boltzmann distribution ($T = 298$ K). In the presented model (Fig. 2.7D) the initial radical pair state populations ([P700$^+$$A_{1A}^-$] and [P700$^+$$A_{1B}^-$]) were distributed evenly (50%/50%), although uneven distributions were found to yield similar results (not shown). In addition, the $F_X^- \rightarrow F_A$ ET rate was assumed to be unaffected by the type of quinone in the $A_1$ binding site.
Figure 2.7  Simulated temporal evolution of the population of radical pair states for PSI with (A) PhQ, (B) 2MNQ and (C) PQ₉ incorporated. Evolution of the [P700⁺A₁⁻] (red), and [P700⁺A₁⁺] (blue) populations are shown along with the sum of the two [P700⁺A₁⁻] (black). (D) Kinetic model used in simulations. ΔG⁺A₁A/FX₀/ΔG⁺A₁B/FX₀ used are +15/−10, +85/+60 and +175/+150 meV for PSI with PhQ, 2MNQ and PQ₉ incorporated, respectively. Using these values in Eq. 2.2 (with R = 9.0 Å, λ = 0.7 eV, T = 298 K) the rate constants indicated in (D) are calculated (in units of ns⁻¹). The temporal profiles in A, B and C are a sum of several exponential components. The weighted average of the time constants (calculated as indicated in (39) for [P700⁺A₁⁻] are 142 ns, 1.8 µs and 60 µs for PSI with PhQ, 2MNQ and PQ₉ incorporated, respectively.

For PSI with 2MNQ incorporated, ΔG⁺A₁A/FX₀/ΔG⁺A₁B/FX₀ was taken as +85/+60 meV, respectively (outlined in Fig. 2.7D). Substituting these values into Eq. 2.2 gave the rate constants in Fig. 2.7D, which were then used to calculate the temporal evolution of the P700⁺A₁⁺ population,
which is shown in Fig. 2.7B. This solution is by no means unique, and represents merely a possible starting point for more refined analysis. The P700+A1− population evolution shown in Fig. 2.7B can be described by three time constants of 1.8 ns, 64.4 ns, and 1.92 μs, with associated decay amplitudes of 0.02, 0.01, and 0.97. By weighting the time constants by the appropriate amplitude and summing the results, an averaged time constant can be calculated (39). For PSI with 2MNQ incorporated the averaged time constant is calculated to be 1.86 μs (Fig. 2.7B), which agrees well with the experimental weighted average time constant (average of 420 ns and 3.1 μs components).

For PSI with PQ9 incorporated, ΔGA1A/FX0/ΔGA1B/FX0 were taken as +175/+ 150 meV (Fig. 2.7D), and an average time constant of 60 μs is calculated. Taking the observed time constants of 14 μs and 211 μs (with amplitudes of ~0.7 and ~0.3), an average time constant of 73 μs is calculated, in good agreement with the simulation.

For PSI with PhQ incorporated, ΔGA1A/FX0/ΔGA1B/FX0 were taken as +15/− 10 meV (Fig. 2.7D), and an average time constant is 142 ns is calculated, which is similar to that obtained previously using slightly different parameters and initial conditions (39).

In summary, the averaged time constant obtained using the quasi-equilibrium model for PSI with PhQ, 2MNQ or PQ9 incorporated simulates well the overall trend in the lifetimes observed experimentally. Some specific details, such as the amplitude ratios associated with the fast and slow kinetic components observed experimentally is not well modeled. The modeling outlined here represents a starting point for more detailed modeling that will include further experimental work on PSI with several more quinones incorporated into the A1 binding site.

To obtain the quinone midpoint potential, Em, from the calculated ΔG0, the midpoint potential of FX is required. A range of FX midpoint potentials have been reported (−650 to −730 mV), however (7). The calculated midpoint potential for PhQ in A1A binding site, using the
range of reported midpoint potentials for \( F_x \), and \( \Delta G^0 = +15 \text{ mV} \) \((R = 9.0 \text{ Å} \text{ and } \lambda = 0.7 \text{ eV})\) is −635 to −715 mV. Also by taking the values used in the quasi-equilibrium model, the ranges for the midpoint potential of 2MNQ and PQ\(_9\) are 70 and 160 mV more oxidizing, respectively.

The midpoint potentials of PhQ, PQ\(_9\) and 2MNQ in dimethylformamide (DMF) are −465 mV, −369 mV and −414 mV \( \text{vs. NHE} \), respectively \((40, 47)\). These values place 2MNQ and PQ\(_9\) 51 and 96 mV more positive than PhQ, respectively.

According to an empirical formula derived by Iwaki et al., \((40)\) the relationship between the midpoint potential in DMF \( (E_{1/2}) \) and that in the A\(_1\) binding site of PSI \( (E_m) \) is:

\[
E_m + 700 \text{ mV} = 0.69 \left( E_{1/2} + 387 \text{ mV} \right)
\]

Equation 2.3

Applying Eq. 2.3, the \( E_m \) of PQ\(_9\), 2MNQ, and PhQ in the A\(_1\) binding site is −682 mV, −718 mV, and −754 mV, respectively. This suggests that 2MNQ and PQ\(_9\) are 36 and 72 mV more positive than PhQ \textit{in situ}, respectively.

The differences in midpoint potentials calculated by directly using the experimental ET rates in Eq. 2.2 \( \text{[for } A_{1A}^- : +125 \text{ mV (2MNQ–PhQ); } +324 \text{ mV (PQ}_9\text{–PhQ)}] \) disagree considerably with the values associated with the quinone in DMF \( (+51 \text{ mV, } +96 \text{ mV}) \), or after correction using Eq. 2.3 \( (+36 \text{ mV, } +72 \text{ mV}) \). However, the differences in midpoint potentials calculated using the quasi-equilibrium model \( (+70 \text{ mV, } +160 \text{ mV}) \) are in good agreement with that estimated using Eq. 2.3.

These (positive) changes in midpoint potential on going from PhQ to PQ\(_9\) or 2MNQ will result in the lowering the equilibrium constant between A\(_1\) and F\(_X\). Consequently, forward ET slows while recombination (between P700\(^+\) and F\(_{AB}^-\)) accelerates \((98)\). Such an effect is clearly observed in the data presented here, with recombination time constants going from \( \sim 80 \text{ ms (PhQ)} \) to \( \sim 14 \text{ ms (2MNQ)} \) and \( \sim 3 \text{ ms (PQ}_9\)\).
2.4.3 **Heterogeneity in ET processes in PSI at 77 K**

Forward ET from A\textsubscript{1} to F\textsubscript{X} is the predominant process in cyanobacterial PSI particles at RT. As the temperature is lowered ET in PSI becomes heterogeneous, with different fractions undergoing P700\textsuperscript{+}A\textsubscript{1}\textsuperscript{−} and P700\textsuperscript{+}F\textsubscript{X}\textsuperscript{−} charge recombination, while in a third fraction a virtually irreversible P700\textsuperscript{+}F\textsubscript{A/B}\textsuperscript{−} state is formed (26, 107). Unless F\textsubscript{X} is pre-reduced, the P700\textsuperscript{+}A\textsubscript{1}\textsuperscript{−} recombination reaction at low temperature occurs only along the A branch (23). The reasons underlying the changes in photochemistry as the temperature is lowered are not entirely clear. It has been proposed that the different fractions are due to the formation of frozen-in conformational substates that differ in free energy (14, 23). In support of this hypothesis, heterogeneous ET is observed to be correlated to the glass transition temperature of the medium (~180 K in 65% glycerol), at which the viscosity reaches 10\textsuperscript{13} poise (26).

The data presented here can be used to establish to what extent each of the above three processes (P700\textsuperscript{+}A\textsubscript{1}\textsuperscript{−}, P700\textsuperscript{+}F\textsubscript{X}\textsuperscript{−}, and P700\textsuperscript{+}F\textsubscript{A/B}\textsuperscript{−}) contributes to PSI with the different quinones incorporated. Since the same samples are used for measurement at 298 and 77 K, the amplitude of the flash-induced signals at the two temperatures can be directly compared. The initial (t = 0) absorption changes give a measure of PSI particles in which reversible ET reactions occur. At RT this fraction is assumed to be 100%. By comparing the initial amplitude of the flash-induced absorption changes at 298 and 77 K the fraction of PSI particles that undergo irreversible ET at 77 K can be estimated, assuming temperature independent extinction coefficients.

For PSI with PhQ incorporated at RT, the initial amplitude of the flash-induced absorption change at 800/703 nm is ~6.5/−28 mOD, respectively (Fig. 2.2B and D). At 77 K the initial amplitude is ~3.2/−14.5 mOD, respectively (Fig. 2.5C and D). Thus 49–52% of the PSI particles participate in reversible ET at 77 K (Table 2.1), or 48–51% undergo irreversible ET at 77 K. From
the absorption changes at 77 K (Fig. 2.5C and D), about 20% of the PSI particles decay with a time constant considerably longer than the 338 μs (Table 2.1). This long-lived component is due to \( P700^+F_x^- \) recombination. So, \( P700^+A_1^- \) radical pair recombination occurs in about 37–39% of PSI particles at 77 K, while \( P700^+F_x^- \) occurs in about 10–15% of PSI particles at 77 K. In WT PSI particles from \( T. \ elongatus \) at 77 K, \( P700^+A_1^-/P700^+F_x^- \) charge recombination was shown to occur in ~45/20% of the PSI particles at 77 K, respectively, with irreversible ET occurring in ~35% of the PSI particles (26). These results are in good agreement with the data presented here for \( menB^- \) PSI from \( S6803 \) with PhQ reincorporated.

For PSI with 2MNQ incorporated, at 77 and 298 K, the initial amplitude of the flash-induced absorbance change at 800/703 nm is ~ 4/− 16.5 mOD (Fig. 2.5E, F) and 6/− 27 mOD (Fig. 2.2A, B), respectively. So ~ 61–66% of the RCs participate in radical pair recombination at 77 K (Table 2.1), or 34–39% undergo irreversible ET. For PSI with 2MNQ incorporated at 77 K, ~ 6% of the RCs decay with a time constant considerably longer than 240 μs. So \( P700^+F_x^- \) recombination occurs in ~6% of the PSI particles with 2MNQ incorporated at 77 K.

For PSI with \( PQ_9 \) incorporated at RT, after the ~ 100 ns phase is complete, the amplitude at 800/703 nm is 3.5/− 14 mOD (Fig. 2.2A, B). At 77 K, the initial amplitude decreased by 70–75% to 0.9/− 4.2 mOD at 800/703 nm, respectively (Fig. 2.5A and B). Of these diminished amplitudes, ~65–70% was accounted by the 250 μs decay phase. However, TR FTIR DS of PSI with \( PQ_9 \) in the \( A_1 \) binding site reveals this ~200 μs phase to be due to the decay of \( ^3P700 \). Consequently, the contributions of the three processes are undetermined for PSI with \( PQ_9 \) incorporated at 77 K.

In summary, for PSI with 2MNQ incorporated the proportion of RCs undergoing \( P700^+A_1^- \) recombination is considerably higher than for PSI with PhQ incorporated (~58% vs. ~39%,
Table 2.1), while the fraction of RCs undergoing $P700^+F_X^-$ recombination is smaller (~6% vs. ~15%). An explanation for this observation will be discussed below.

2.4.4 Quinone redox potential and low temperature ET heterogeneity

Extending the frozen conformational substate hypothesis to include bi-directional ET, it was initially suggested that a certain fraction of the RCs are frozen in a conformation that facilitates ET in either the A branch or B branch (7, 39, 108). In this model, the thermodynamically downhill ET from $A_{1B}^-$ to $F_X$ would form the irreversible state, while thermodynamically uphill ET from $A_{1A}^-$ to $F_X$ would result in $P700^+A_{1A}^-$ recombination reaction. This model does not provide an explanation for the fraction of $P700^+F_X^-$ state formed in repetitive flash experiments, however. In an attempt to address this issue, a distribution of cofactor midpoint potentials has been considered (14, 23). In purple bacterial reaction centers cofactor energy levels have been estimated to have a Gaussian width of $2\sigma \approx 100$ meV (109). In the case of PSI, where the free energy gaps between cofactors is similar to the width of possible distributions, such a spread in midpoint potentials could give rise to a set of substates that results in heterogeneous ET at low temperature (14, 23).

In this model, the substates are no longer required to use the A or B branch exclusively. However, EPR data does appear to indicate that the $P700^+A_{1A}^-$ state observed at low temperature is associated only with $A_{1A}$ (23, 47). Given this EPR data the 340/240 $\mu$s time constants (for PSI with PhQ/2MNQ incorporated at 77 K) are associated to $P700^+A_{1A}^-$ recombination.

$P700^+A_{1A}^-$ recombination is clearly faster for PSI with 2MNQ incorporated compared to PSI with PhQ incorporated. Such an observation is difficult to explain given that the free energy gap between $A_{1A}$ and $P700$ is calculated to be smaller for 2MNQ in the $A_1$ binding site compared to PhQ. An increase in rate when the free energy gap decreases can be explained, however, by considering the large free energy gap between $A_{1A}^-$ and $P700^+$ (~1.10 eV) (37). If a
reorganization energy of ~0.6 eV is assumed, and thus $\Delta G^0 > \lambda$, the recombination process would take place in the inverted region (66). This idea has been suggested previously for PSI ET reactions at 77 K (37). In the inverted region a decrease in free energy would result in an increased rate, as is observed here.

An increase in the P700$^+$A1$^-\cdot$ recombination rate as the free energy gap is lowered also follows from Eq. 2.1 appropriately adjusted so that it is applicable at 77 K ($R = 18.1$ Å, $\lambda = 0.6$ eV, $\rho = 0.88$, $\gamma = 3.9$). From the measured rates (240 and 340 ms) the free energy gap for PSI with PhQ incorporated is $\sim -1.20$ eV, which is $\sim 19$ meV more negative than that found for PSI with 2MNQ incorporated. That is, a faster rate is observed for a system in which the free energy gap is smaller. However, a $\sim 19$ meV difference in free energy between PhQ and 2MNQ incorporated into PSI is more than a factor of three smaller than the difference estimated using the quasi-equilibrium model along with the forward ET rates at RT (70 meV). Alternatively, if the free energy difference between PhQ and 2MNQ in PSI is the same at RT and 77 K ($\sim 70$ meV) then one would expect the time constant for $P^+A^-\cdot$ recombination to decrease from $\sim 340$ ms to $\sim 104$ ms for 2MNQ in the $A_1$ binding site.

Clearly, there are factors that lead to discrepancies when the Marcus ET theory is applied to PSI at RT and 77 K. For example, a $\sim 20$ meV difference in free energy between PhQ and 2MNQ in PSI could be explained by a small change ($\sim 0.2$ Å) in the edge-to-edge distance when 2MNQ is substituted for PhQ. In the above calculation it was assumed that the edge-to-edge distance between the pigments is unaltered on going from PhQ to 2MNQ. This assumption is based on EPR data, but a distance change of $\sim 0.2$ Å is below the accuracy achievable in the EPR measurement (47, 89). Thus such a small distance change cannot be ruled out based on a consideration of EPR data. Given such complications, it is difficult to establish simply on the basis
of the weak changes in ET rate whether light induced ET reactions in PSI at 77 K do indeed occur in the inverted region.

Perhaps the discrepancy between room and low temperature calculations could be explained on the basis of a change in the distribution of midpoint potentials of conformational substates and their effect on the rate of P700⁺A₁A⁻ recombination. In a case where a substate is frozen in an energetic configuration that places the free energy gap of A₁A⁻ and Fₓ to be positive but A₁B⁻ and Fₓ to be negative, an indirect accumulation of A₁A⁻ by inter-quinone ET via Fₓ may take place (23). In such a scenario, the observed kinetics (which monitors P700 and P700⁺) does not simply represent the oxidation of directly populated A₁A⁻, but includes contributions from the indirect accumulation of A₁A⁻ from A₁B⁻. These additional phases of ET would be expected to decrease the overall rate of the P700⁺A₁⁻ charge recombination reaction. Thus, the underestimation of difference in midpoint potentials of A₁A⁻ may be the result of this additional contribution included in the observed rates. Again, a number of mechanisms can explain the weak increase in ET rate (on going from PhQ to 2MNQ in the A₁ binding site), making it difficult to establish whether P700⁺A₁A⁻ recombination at 77 K occurs in the inverted region.

At 77 K PSI photochemistry is heterogeneous. The variation in the degree of heterogeneity for PSI with the different quinones incorporated was discussed above: For PSI with PhQ incorporated, irreversible charge separation occurs in 50% of the particles. P700⁺A₁⁻ recombines in ~40% of the particles, and P700⁺Fₓ⁻ recombines in ~10% of the particles (Table 2.1). In contrast, for PSI with 2MNQ incorporated, irreversible charge separation occurs in ~35% of the particles, P700⁺A₁⁻ occurs in about 60% of the particles, and P700⁺Fₓ⁻ recombines in ~5% of the particles (Table 2.1). Although detailed modeling has yet to be undertaken, the observed changes in heterogeneity can qualitatively be understood given the changes in the redox potentials that
occur in exchanging PhQ for 2MNQ. If substitution of PhQ by 2MNQ shifts the midpoint potential of $A_1^-$ by approximately +70 mV, then the reduction of terminal electron acceptors become less favorable, which could then potentially lead to a decrease in the amount of irreversible ET, as is observed here. Furthermore, a decrease in the number of reaction centers displaying $P700^+F_X^-$ recombination when 2MNQ is incorporated may also be explained as ET from $A_{1A}^-$ to $F_X$ becomes less favorable and $A_{1A}^-$ to $P700^+$ ET becomes more favorable. So the alterations in the degree to which the different ET processes contribute at 77 K for PSI with the different quinones incorporated can be qualitatively explained on the basis of the calculated redox potentials of the different quinones in the $A_1$ binding site.

### 2.5 Conclusions

1. 2MNQ and PhQ can displace PQ$_9$ and be incorporated almost quantitatively into the $A_1$ binding site in $menB^-$ PSI.

2. 30–100 ns decay phases are observed in native $menB^-$ PSI particles, but not in PSI with 2MNQ or PhQ reintroduced into the $A_1$ binding site.

3. At either room temperature or 77 K, light induced ET processes are governed by identical kinetics in dilute and highly concentrated PSI samples.

4. Highly concentrated samples form a clear and transparent glass at both room and low temperatures in ultrathin sample cells of ~5 μm thickness. This allows spectroscopy to be undertaken at 77 K using PSI samples in the absence of cryoprotectant.

5. Identical low temperature kinetics are observed in both the visible and infrared spectroscopy experiments.
6. The observed kinetics of $A_1^- \rightarrow F_X$ ET allow an estimate of the redox potential of PhQ, 2MNQ, and PQ in the $A_1$ binding site.

7. Observed alterations in the degree of heterogeneity of ET processes at 77 K for PSI with different quinones incorporated can be understood in terms of the differences in redox potential of the quinones incorporated.
3 DIRECTIONALITY OF ELECTRON TRANSFER IN CYANOBACTERIAL PHOTOSYSTEM I

3.1 Introduction

In photosynthetic oxygen-evolving organisms, photosystem I (PSI) utilizes light to drive electrons across a biological membrane from plastocyanin to ferredoxin (9). In PSI light induces the transfer of electrons from a donor chlorophyll containing species called P700, via a series of acceptors called A₀, A₁, Fₓ, Fₐ, and Fₜ (15, 82). In PSI the electron transfer (ET) cofactors are arranged to form two pseudo-symmetrical “branches”, termed the A- and B-branches (10). In recent years, it has become clear that both branches participate in ET (20, 22, 110). The fractional utilization of both branches still remains incompletely understood, however, especially for prokaryotic PSI where reported estimates show large variations (22-25). The branching ratio, as well as its temperature dependence, is of considerable interest as such information is required to model ET in PSI (23, 27). Here a series of experiments aimed at establishing this branching ratio, as well as its temperature dependence are described.

In PSI, the secondary electron acceptor, sometimes termed A₁, is a highly reducing phylloquinone (PhQ, 2-methyl-3-phytyl-1,4-naphthoquinone) species (111). In, so-called, menB⁻ mutant PSI particles, a gene that codes for a protein involved in PhQ biosynthesis has been deleted, and in these mutant PSI particles plastoquinone-9 (PQ₉, 2,3-dimethyl-5-prenyl-1,4-benzoquinone) is instead incorporated into the A₁ binding site (44). Recent studies have further shown that PQ₉ is weakly bound in the A₁ binding site, and can be displaced by different quinones both in vivo or in vitro (30, 45-47).

In this chapter the focus is on the study of menB⁻ PSI particles from the cyanobacterium Synechocystis sp. PCC 6803 (S6803) in which 2,3-dichloro-1,4-naphthoquinone (Cl₂NQ) has been
incorporated into the A₁ binding site. In aprotic solvent, Cl₂NQ has a very high midpoint potential of -49.5 mV, compared to -465 mV for PhQ (47). By incorporating a high potential quinone, forward ET from A₁⁻ to Fₓ is inhibited, resulting in P700⁺A₁⁻ radical pair recombination at both 298 and 77 K. This allows, for the first time, a direct study of this recombination reaction in identical samples at both 298 and 77 K.

In this chapter time-resolved visible and infrared (IR) difference spectroscopy is used to study P700⁺A₁⁻ radical pair recombination in isolated PSI particles at both 298 and 77 K. P700⁺A₁⁻ radical pair recombination is found to be biphasic at both temperatures, reflecting ET on both the A and B branches. Analysis of the kinetic phases allows an estimate of the degree of directionality (branching ratio) of ET in PSI at both temperatures. It is found that B-branch ET decreases considerably as the temperature is lowered.

### 3.2 Materials and Methods

Trimeric PSI particles were isolated from \textit{menB}⁻ mutant cells from \textit{S6803} and then stored as described previously (44). PhQ and Cl₂NQ, as well as all solvents and other chemicals, were obtained from Sigma-Aldrich (St. Louis, MO) and were used as received. Incorporation of PhQ into the A₁ binding site of \textit{menB}⁻ PSI was performed as described previously (30). To incorporate Cl₂NQ into the A₁ binding site, Cl₂NQ dissolved in dimethyl sulfoxide (DMSO) was added to a suspension of \textit{menB}⁻ PSI particles at ~500x molar excess (Cl₂NQ/RC ratio), while keeping the total volume of DMSO below 2%. The mixture was stirred in the dark at 277 K for 24 hours. Samples were diluted and then pelleted by ultracentrifugation (408,000g for 3 hours), and a cryoprotectant-free concentrated thin-film sample was prepared as described previously (30). Sodium ascorbate (20 mM) and phenazine methosulfate (10 µM) were added to the film to ensure rapid re-reduction of P700⁺ in repetitive flash experiments.
Visible and IR time-resolved experiments were undertaken in an identical fashion to that described previously (30). Briefly, for all time-resolved experiments, a 10 Hz, 532 nm saturating pump pulse of 5-7 ns duration from a Minilite or Surelite III Nd:YAG laser (Continuum, San Jose, Ca.) was used.

For concentrated PSI samples at room temperature (298 K) with PhQ incorporated into the A₁ binding site, absorption changes at 703 nm were monitored as a function of the pump pulse energy in order to verify that the pulses used were saturating. Absorption changes at several pump pulse energies are shown in Fig. 3.1A. As assessed using laser burn paper, the spot size of the excitation pulse at the sample is ~7 mm (1.54 mJ/cm² for a 1 mJ pulse). Fig. 3.1B shows the signal amplitude as a function of the pulse energy. Within the noise level of the experiment, the signal amplitude is the same at all pulse energies investigated, certainly for energies above 0.7 mJ per pulse. In the experiments discussed in this dissertation, pump pulse energies of 1.2 mJ is typically used, which is saturating according to the data in Fig. 3.1, but also sufficiently low to minimize contributions from triplet states of antenna pigments. Very similar data was obtained for PSI with Cl₂NQ incorporated.

Flash-induced absorption changes at 487, 703, and 800 nm were measured using an LP920 flash photolysis spectrometer (Edinburgh Instruments, Livingston, UK) as described previously (30). Time-resolved step-scan FTIR difference spectroscopy measurements in the 1950-1100 cm⁻¹ spectral region, with 4 cm⁻¹ spectral resolution and 6 μs temporal resolution, were undertaken using a Bruker Vertex80 or IFS66 FTIR spectrometer (Bruker Optics Inc. Billerica, MA) as described previously (30, 90, 100). For measurements at 77 K samples were mounted in a Model ND110H liquid nitrogen cryostat (Cryo Industries of America Inc., Manchester, NH).
Flash-induced absorption changes were fitted to a sum of stretched exponential functions and a constant, using the Levenberg-Marquardt algorithm, as implemented within Origin 7.5 software (OriginLab Corporation, Northampton, MA).

Figure 3.1  (A) Room temperature (298 K) flash-induced absorption changes at 703 nm for PSI samples with PhQ incorporated into the A1 binding site. Samples were excited using 532 nm laser pulses with pump energies ranging from 0.4 to 1.7 mJ/pulse. (B). Plot of signal amplitude at 703 nm as a function of pump pulse energy. The data points (squares) represent the average value in the 1-4 μs range, while the error bars indicate the min/max data points in the 1-4 μs range.

3.3 Results

3.3.1 Time-resolved visible absorption changes

Below it will be shown that the incubation procedure used here leads to a high level of incorporation of Cl2NQ into the A1 binding site in menB+ PSI particles. Hereafter such particles are simply referred as PSI with Cl2NQ incorporated.

Fig. 3.2 shows room-temperature (RT, 298 K) flash-induced absorption changes at 800, 703, and 487 nm for PSI with Cl2NQ incorporated. At 800 and 703 nm, flash-induced absorption changes are associated with P700+ and P700, respectively (93, 112, 113). Absorption changes near 487 nm are due to P700+/P700 and electrochromic shifts of carotenoid and/or chlorophyll
molecules near the reduced quinone species (22, 28, 114). It is well established that probe wavelengths between 475-490 nm can be used to follow light-induced reduction and re-oxidation of the quinone in the A1 binding site in PSI (22, 28, 114).

![Image of absorption changes](image)

**Figure 3.2**  RT flash-induced absorption changes at 800 (A), 703 (B) and 487 nm (C) for PSI with Cl2NQ incorporated. Data was collected over several time windows in a linear fashion (see insets) and is plotted here on a logarithmic timescale. Fitted curves are also shown (red). The parameters derived from fitting are summarized in Table 3.1. The data at 487 nm was collected using less concentrated samples but was scaled to match the optical density of samples used to collect the data at 703 and 800 nm. *Insets:* absorption changes on a (linear) 0–25 μs timescale.

The absorption changes at the three probe wavelengths shown in Fig. 3.2 were fitted simultaneously to a sum of two stretched exponential functions and a constant. The best fit yielded time constants of 3.8 and 139.5 μs. The fitted curves are also shown in Fig. 3.2, and the fitting
parameters are listed in Table 3.1. At all three probe wavelengths, the amplitude of the 139.5 μs component accounts for 62–73% of the total signal amplitude (Table 3.1).

**Table 3.1** Summary of fitting parameters obtained for visible and infrared transient absorption data at both 298 and 77 K. Relative amplitudes (ΔA) of two time constants (τ1 and τ2, with the stretch factor β1 and β2), and of non-decaying (n.d.) component, are listed.

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>ΔA1 (%)</th>
<th>ΔA2 (%)</th>
<th>ΔA3 (%)</th>
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<tr>
<td>800</td>
<td>19.7</td>
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<td>17.9</td>
<td>62.0</td>
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</tr>
<tr>
<td>487</td>
<td>14.8</td>
<td>72.7</td>
<td>12.5</td>
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<table>
<thead>
<tr>
<th>λ (cm⁻¹)</th>
<th>ΔA1 (%)</th>
<th>ΔA2 (%)</th>
<th>ΔA3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1754</td>
<td>–</td>
<td>88.2</td>
<td>11.8</td>
</tr>
<tr>
<td>1748</td>
<td>–</td>
<td>90.2</td>
<td>9.8</td>
</tr>
<tr>
<td>1717</td>
<td>–</td>
<td>83.8</td>
<td>16.2</td>
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<tr>
<td>1697</td>
<td>–</td>
<td>85.7</td>
<td>14.3</td>
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<tr>
<td>1509</td>
<td>–</td>
<td>94.4</td>
<td>5.6</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>τ1 = 2.3 μs / β1 = 1.0</th>
<th>τ2 = 78.2 μs / β2 = 0.72</th>
<th>n.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>4.9</td>
<td>89.4</td>
<td>5.7</td>
</tr>
<tr>
<td>703</td>
<td>4.4</td>
<td>91.4</td>
<td>4.2</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>λ (cm⁻¹)</th>
<th>ΔA1 (%)</th>
<th>ΔA2 (%)</th>
<th>ΔA3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1754</td>
<td>–</td>
<td>96.8</td>
<td>3.2</td>
</tr>
<tr>
<td>1748</td>
<td>–</td>
<td>94.9</td>
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<tr>
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<td>–</td>
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<td>1697</td>
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<td>4.2</td>
</tr>
<tr>
<td>1509</td>
<td>–</td>
<td>99.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Flash-induced absorption changes at 77 K for PSI with Cl2NQ incorporated are shown in Fig. 3.3 at (A) 800 and (B) 703 nm. By fitting the absorption changes at both wavelengths simultaneously to a sum of two stretched exponential functions, lifetimes of 2.3 and 78.2 μs were obtained. The fitted curves are also shown in Fig. 3.3, and the best fit parameters are summarized in Table 3.1. In PSI at 77 K under repetitive illumination, absorption changes associated with P700⁺A1⁻ radical pair recombination dominate (26).
Figure 3.3  77 K flash-induced absorption changes at 800 nm (A) and 703 nm (B) for PSI with Cl₂NQ incorporated. The data is best fitted to two stretched exponentials with lifetimes of 2.3 μs and 78.2 μs. The fitted curves are shown in red, and the fitted parameters summarized in Table 3.1. Insets: absorption changes on a 0–25 μs timescale.

3.3.2 Time-resolved IR absorption changes

Fig. 3.4 shows flash-induced absorption changes at selected IR wavelengths, at 298 and 77 K, for PSI with Cl₂NQ incorporated. Absorption changes at 1754 cm⁻¹ contain contributions that are associated with the decay of P700⁺ and Cl₂NQ⁻ (90, 115) while the changes at 1748 cm⁻¹ contain contributions that are associated with the recovery of P700 and Cl₂NQ (90, 115, 116). The absorption change at 1509 cm⁻¹ is most likely due to the formation and decay of Cl₂NQ⁻ (88, 115).

The IR kinetic traces were fitted simultaneously to a stretched exponential function plus a constant, and the best fit yielded time constants of 129 and 76 μs for the kinetics at 298 and 77 K, respectively. Although the temporal resolution of 6 μs in FTIR experiments is inadequate to resolve the faster phase observed in visible spectroscopy experiments, the time constant of the
slower phase is in excellent agreement with that found in the visible experiments, at both 298 and 77 K (Table 3.1).

Figure 3.4  Flash-induced absorption changes of several infrared wavelengths (in cm\(^{-1}\)) at 298 (A) and 77 K (B). The kinetics at 298/77 K are fitted simultaneously to a stretched exponential function with time constant of 129.4 μs (β = 0.70)/75.6 μs (β = 0.70), respectively. Different samples were used for the RT and LT measurements.

3.3.3  Irreversible Charge Separation in PSI at 77 K

At cryogenic temperatures, ET processes in PSI are heterogeneous, with fractions undergoing P700\(^+\)A\(_1\)\(^-\) and P700\(^+\)F\(_X\)\(^-\) radical pair recombination, and a third fraction undergoing irreversible P700\(^+\)F\(_{A/B}\)\(^-\) charge separation (26, 107). In repetitive flash experiments on identical samples, the amount of irreversible P700\(^+\)F\(_{A/B}\)\(^-\) state formed can be quantified by considering the initial signal amplitude at 298 and 77 K (30), assuming no irreversible charge separation at 298 K, and similar extinction coefficients at 298 and 77 K.

Figure 3.5A shows flash-induced absorption changes at 703 nm (and 77 K) for PSI with Cl\(_2\)NQ incorporated, monitored as a function of the number of laser flashes. The initial amplitude of the absorption change at 77 K relative to the initial amplitude at RT is plotted as a function of
the flash number in Fig. 3.5B (circles). For comparison corresponding data for PSI with PhQ incorporated (squares), collected using identical procedures is also shown.

Figure 3.5  (A) 77 K flash induced absorption changes at 703 nm for PSI with Cl\textsubscript{2}NQ incorporated. Each trace is the average of 640 laser flashes. (B) Relative initial amplitudes of flash-induced absorption changes as a function of the number of laser flashes for PSI with Cl\textsubscript{2}NQ (dots) and PhQ (squares) incorporated, probed at 703 nm. The relative amplitude is taken as the ratio of the initial absorption changes at 77 K to that at 298 K. The 298 K initial amplitude is independent of the number of laser flashes. The amplitude of the flash-induced absorption changes at LT before any laser flashes is similar to that found at RT. In (B) the data for PSI with Cl\textsubscript{2}NQ incorporated is fitted to Eq. 3.1, with best fit parameters $\Delta A_r = 0.578$ and $\Phi = 0.9989$. The quantum yield for the formation of an irreversible state is therefore 0.0011.

The data in Fig. 3.5B for PSI with Cl\textsubscript{2}NQ incorporated was fitted to Equation 3.1:

$$\Delta A_n = (\Delta A_1 - \Delta A_r)\Phi^{n-1} + \Delta A_r \quad \text{Equation 3.1}$$

Where $\Delta A_n$ is the amplitude induced by the $n$th flash, $\Delta A_r$ is the absorption change remaining after >8000 flashes, and $\Phi$ is the quantum yield for the formation of the reversible states. No significant decrease in the relative absorption change is observed beyond ~8000 laser flashes. Note that the data in Fig 3.3 is the average of more than 8000 laser flashes, and so the changes in Fig. 3.3 are representative of maximal accumulation of irreversible state. Importantly, when the sample was returned to RT, absorption changes identical to those in Fig 3.2 were
obtained. From fitting the data in Fig. 3.5B to Eq. 3.1, the quantum yield for the formation of irreversible state is found to be 0.0011.

For PSI with PhQ incorporated (Figure 3.5B, squares) the irreversible fraction is produced in less than 160 laser flashes, in good agreement with the previously reported 30 flashes for PSI particles from *Thermosynechococcus elongatus* (*T. elongatus*) (26). The quantum yield calculated previously for PSI from *T. elongatus* (with PhQ in the A₁ binding site) was ~0.55 (26), very much larger than the 0.0011 estimated here for PSI from *S6803* with Cl₂NQ incorporated. For PSI with PhQ/Cl₂NQ incorporated, the initial signal amplitude decreases by ~49/44% on going from 298 to 77 K (Figure 3.5B, Table 3.2), suggesting irreversible charge separation occurs in 49/44% of the PSI particles, respectively.

**Table 3.2** Fractions of PSI particles forming reversible P700⁺A₁⁻ and P700⁺Fₓ⁻ states, and the irreversible P700⁺Fₓ⁻ states, in PSI with PhQ, 2MNQ, or Cl₂NQ incorporated at 77 K measured in the identical experimental conditions. (* - Ref. (30))

<table>
<thead>
<tr>
<th></th>
<th>reversible</th>
<th>irreversible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P700⁺A₁⁻</td>
<td>P700⁺Fₓ⁻</td>
</tr>
<tr>
<td>PhQ</td>
<td>38 %</td>
<td>13 %</td>
</tr>
<tr>
<td>2MNQ⁻*</td>
<td>58 %</td>
<td>6 %</td>
</tr>
<tr>
<td>Cl₂NQ</td>
<td>53 %</td>
<td>3 %</td>
</tr>
</tbody>
</table>

**3.4 Discussion**

3.4.1 *Cl₂NQ incorporation into the A₁ binding site*

For PSI with PQ₉ incorporated, at RT, P700⁺Fₓ⁻ recombination is characterized by a time constant of ~3 ms (30, 45, 63, 98). In PSI with Cl₂NQ incorporated, this 3 ms phase is replaced by two μs phases that account for 85% of the amplitude of the absorption changes (Table 3.1). These μs decay phases are associated with P700⁺A₁⁻ radical pair recombination (see below) and so the data suggests at least and 85% level of incorporation of Cl₂NQ into the A₁ binding site in the PSI particles studied here.
For regular menB− PSI particles with PQ9 incorporated a rather intense decay phase with a lifetime of ~50 ns is observed at 800 nm (30). This 50 ns decay phase is most likely associated with P700+A0− radical pair recombination (117, 118) in PSI particles with no PQ9, or dysfunctional PQ9, in the A1 binding site. This 50 ns decay phase is not observed for PSI with Cl2NQ incorporated suggesting that Cl2NQ has been incorporated into these previously unoccupied or non-functional binding sites, and is functional. P700+A0− recombination can lead to 3P700 triplet state formation (35). The lack of a 50 ns decay phase would also then indicate little or no 3P700 triplet state formation. Thus the 3.8 and 139.5 μs phases observed in measurements at RT cannot be due to the decay of 3P700.

3.4.2 Comparison with previous studies

For PSI with Cl2NQ incorporated, at RT, absorption changes with time constants of 3.8 and 139.5 μs are obtained (Fig. 3.2). The 139.5 μs decay phase is in good agreement with that reported previously (47). The 3.8 μs decay phase was not reported previously, possibly because of insufficient time resolution. This latter hypothesis is supported by the fact that the amplitude of the ~140 μs decay phase reported previously was 83% of the total signal amplitude, which is roughly the same as the ratio of the amplitude of the slow phase to non-decaying component reported here (Table 3.1).

For PSI with Cl2NQ incorporated, at 77 K, decay phases with time constants of 2.3 and 78.2 μs are observed (Fig. 3.3). The latter time constant is considerably shorter than the 200 μs time constant reported previously from time-resolved W-band EPR measurements at 120 K. This discrepancy is unresolved at present but it is of note that the same time constant from two independent types of spectroscopic experiments are obtained (Table 3.1).
3.4.3 Origin of the observed decay phases

For PSI with Cl$_2$NQ incorporated at RT, the 3.8 and 139.5 µs decay phases are observed at 800, 703 and 487 nm. Absorption changes at 800, 703 and 487 nm are associated with P700$^+$, P700 and A$_1^-$, respectively (22, 28, 93, 112-114). The fact that the same two lifetimes are found at all three wavelengths indicates that both time constants are associated with P700$^+$A$_1^-$ charge recombination. In addition, a 130 µs decay phase is observed in measurements at several IR wavelengths, that are specifically associated with P700 (1697 cm$^{-1}$), P700$^+$ (1697 cm$^{-1}$) and Cl$_2$NQ$^-$ (1509 cm$^{-1}$) (90, 101, 115, 116). Again, since the same lifetime is found at all IR wavelengths, some which are very specific to P700$^+$ and A$_1^-$ it is likely that the ~130 µs decay phase is associated with P700$^+$A$_1^-$ charge recombination.

Previously, biphasic decay processes in PSI with PhQ or 2-methyl-1,4-naphthoquinone (2MNQ) incorporated (at RT) were associated with ET on the A and B branches (20, 30). Biphasic decay processes in PSI with PQ$_9$ incorporated were also associated with ET on the A and B branches (7). In line with these proposals it is suggest that the two phases observed here at both RT and LT are associated with P700$^+$A$_1^-$/P700$^+$A$_1^-$ recombination reactions that involve the A and B branches. Furthermore, the fast/slow phases at both RT and LT are associated with P700$^+$A$_{1B^-}$/P700$^+$A$_{1A^-}$ recombination, respectively. Several results support this conclusion. Firstly, ESEEM measurements on branch-specific site-directed mutants indicated that fast and slow decay phases are due to P700$^+$A$_{1B^-}$ and P700$^+$A$_{1A^-}$ states, respectively (23). Secondly, EPR measurements for PSI with Cl$_2$NQ incorporated indicate that the major decay phase at 77 K (the slow phase) is due to P700$^+$A$_{1A^-}$ recombination (47). Thirdly, kinetic modeling based on the ET theory suggests that the charge recombination reaction tends to favor the A-branch (37).
Given that the fast/slow kinetic phase is due to B/A branch ET, respectively, the fractional utilization of the two branches can be estimated from the decay amplitudes of each phase. At 77 K, the amplitude ratio of the fast and slow phases indicates an A/B branching ratio of ~95:5 (Table 3.3). The branching ratio is presumably determined upon initial charge separation at P700, but may be modified by the fast equilibration between P700^+A_{0A}^- and P700^+A_{0B}^- (37, 39, 119). The equilibration between the primary radical pair states, however, is predicted to occur at a much faster rate than the formation of P700^+A_1^- (119). Therefore, the directionality of ET in PSI does not depend on the nature of the quinone in the A_1 binding site, so the branching ratio calculated here should be the same for PSI with PhQ or Cl_2NQ in the A_1 binding site. That is, A-branch ET dominates (~95%) in cyanobacterial PSI particles at 77 K. This conclusion is in line with the EPR studies of Mula et al. (47), but not the EPR studies of Santabarbara et al. (23).

**Table 3.3** Comparison of time constants and decay amplitudes extracted from fitting the 298 and 77 K kinetic data. % acceleration indicates by how much the time constant decreases from 298 K to 77 K.

<table>
<thead>
<tr>
<th></th>
<th>(\tau) [(\mu)s] ((\Delta A_{800}, \Delta A_{703}) [a.u.])</th>
<th>(\tau_{slow}/\tau_{fast})</th>
<th>(\Delta A_{slow}: \Delta A_{fast})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\tau_{slow})</td>
<td>(\Delta A_{800}) ((1.79, -11.78))</td>
<td>(3.8) ((0.53, -3.4))</td>
<td>36.8</td>
</tr>
<tr>
<td>(\tau_{fast})</td>
<td>(\Delta A_{703}) ((1.38, -9.57))</td>
<td>(2.3) ((0.08, -0.46))</td>
<td>34</td>
</tr>
<tr>
<td>(%) accel.</td>
<td>43.9</td>
<td>39.5</td>
<td>-</td>
</tr>
</tbody>
</table>

At 298 K, the fraction of the fast phase is 15–20% of the total signal amplitude, compared to only ~5% at 77 K (Table 3.1). For PSI with Cl_2NQ incorporated at RT it is not clear if the amplitude of the fast and slow phases can be directly assigned to B and A branch radical pair recombination, as biphasic kinetics related to P700^+A_{1A}^- charge recombination may arise because of ET from A_{1B} to A_{1A} via FX (106). However, irrespective of the kinetic model, the amplitude ratio of the two phases at RT gives a lower bound estimate of the ET branching ratio, which is ~23:77. So at least 23% of the time, ET occurs down the B branch in cyanobacterial PSI at RT.
This estimate is in keeping with previous transient absorption studies of PSI particles with modified carotenoid composition (22).

3.4.4  *Heterogeneous ET in PSI at LT*

For PSI with Cl₂NQ, 2MNQ and PhQ incorporated, the relative fractions of the different states formed at 77 K, derived from identical sets of measurements, are listed in Table 3.2. The fraction of PSI particles in which P700⁺Fₓ⁻ recombination occurs is 13/6/3% for PSI with PhQ/2MNQ/Cl₂NQ incorporated, respectively (Table 3.2). The midpoint potential of 2MNQ in aprotic solvent is between PhQ and Cl₂NQ (40, 47). It appears therefore that the decrease in the fractional contribution of the P700⁺Fₓ⁻ state can be correlated with a positive shift in the midpoint potential of the quinone in the A₁ binding site. This positive shift lowers the equilibrium constant between A₁ and Fₓ leading to a decrease in the fractional contribution from the P700⁺Fₓ⁻ state.

For PSI with Cl₂NQ incorporated, the quantum yield for irreversible P700⁺Fₓ⁻A/B⁻ formation is considerably smaller than that for PSI with PhQ incorporated (Fig. 3.5). However, the maximal irreversible fraction does not appear to be impacted by the quinone in the binding site, and hence the midpoint potential of the quinone in the binding site.

Previously, the fraction of PSI particles undergoing irreversible charge separation at LT was found to be independent of the occupancy of the A₁ binding site (120). In a more recent review the irreversibility of P700⁺Fₓ⁻A/B⁻ state was proposed to involve a distribution of different radical pair conformational sub-states, as well as frozen-in nuclear coordinates of the surrounding medium. This latter proposal focuses on the freezing effect in and around predominantly Fₓ and Fₓ (14). The observations made here indicate that the irreversible fraction is independent of the quinone in the binding site and its degree of functionality, and support these previous hypotheses.
3.5 Conclusions

1. P700\(^{+}A_{1A}^{-}\) and P700\(^{+}A_{1B}^{-}\) radical pair recombination has been observed in PSI with Cl\(_2\)NQ incorporated, at both 298 and 77 K. At both temperatures P700\(^{+}A_{1B}^{-}\) recombination is \(\sim 40\) times faster than P700\(^{+}A_{1A}^{-}\) recombination.

2. In cyanobacterial PSI at 298 K the B-branch to A-branch ET branching ratio is 23:77.

3. B-branch ET is diminished to \(\sim 5\%\) at 77 K in cyanobacterial PSI.

4. The fractional extent of irreversible charge separation in cyanobacterial PSI at 77 K is \(\sim 45\%\), and is independent of the quinone in the A\(_1\) binding site.
4 MODULATING ELECTRON TRANSFER IN PHOTOSYSTEM I BY INCORPORATING NON-NATIVE QUINONES INTO THE A₁ BINDING SITE

4.1 Introduction

Time-resolved visible and infrared absorption difference spectroscopy at both 298 and 77 K has been used to study cyanobacterial photosystem I (PSI) particles from menB⁻ mutant Synechocystis sp. PCC6803 (S6803) with several non-native quinones incorporated into the A₁ binding site. Including the native phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone, PhQ), 2-bromo-1,4-naphthoquinone (2BrNQ); 2-chloro-1,4-naphthoquinone (2ClNQ); 2-methyl-1,4-napththoquione (2MNQ); 2,3-dibromo-1,4-naphthoquinone (Br₂NQ); 2,3-dichloro-1,4-naphthoquinone (Cl₂NQ); and 9,10-anthraquinone (AQ) were also incorporated. Transient absorption changes were measured at 487, 703, and 800 nm in the visible spectral range, and 1950–1100 cm⁻¹ in the infrared region. Time constants of the kinetic traces were obtained using multi-stretched exponential curve fitting, and the time constants obtained from the infrared and visible data are in good agreement. From knowledge of which ET processes contribute at the various probe wavelengths time constants for the ET processes for each of the incorporated quinones could be determined. The assignments and associated time constants are crucial for the development of an appropriate kinetic model describing ET processes in PSI (48).

4.2 Materials and Methods

Trimeric PSI particles from menB⁻ mutant cells from S6803 were isolated and stored as described previously (44). All chemicals, including the series of quinones (AQ, PhQ, 2MNQ, ClNQ, BrNQ, Cl₂NQ, and Br₂NQ) incorporated into the A₁ binding site, were obtained from Sigma-Aldrich (St. Louis, MO) and were used as received. To incorporate non-native quinones into the A₁ binding site, quinones dissolved in either ethanol or dimethyl sulfoxide were added to
a suspension of $\text{menB}^-$ PSI particles at ~500x molar excess. Concentrations of ethanol or dimethyl sulfoxide were kept below 2% of the total volume. The mixture was incubated at 277 K in the dark with continuous stirring for 24 h. The incubated mixture was pelleted by ultracentrifugation (408000g for 3 h). Sodium ascorbate (20 mM) and phenazine methosulfate (10 μM) were added to the pelleted mixture for rapid reduction of P700$^+$. For a preparation of the concentrated thin-film samples, the pelleted samples were squeezed between two windows as described previously (30). For a preparation of standard dilute samples, the pelleted samples were re-suspended in Tris buffer (pH 8.0) with 0.04% $n$-dodecyl-$\beta$-D-maltoside in a 1 cm path-length spectroscopic cuvette as described previously (30). All the samples were prepared free of cryoprotectants. The concentrated thin-film samples were measured at 703 nm at 298 K and 77 K. The standard dilute samples were measured at 703 nm and 487 nm at 298 K. For measurements at 77 K, the samples were mounted in a Model ND1110H liquid nitrogen cooled cryostat (Cryo Industries of America In., Manchester, NH).

Nanosecond to millisecond time-resolved visible absorption difference spectroscopy was undertaken using an LP920 flash photolysis spectrometer (Edinburgh Instruments, Livingston, UK) as described previously (21, 30). A saturating pump beam was provided by a 532 nm laser pulse of 5-7 ns duration from a Minilite or Surelite III Nd:YAG laser operating at 10 Hz repetition rate (Continuum, San Jose, CA). A xenon arc lamp was used as probe light source in either CW mode (for measurements over 10 ms timescale) or pulsed mode (for measurements on nanosecond to millisecond timescales). A 1 cm water cell was placed between the probe light source and the sample to reduce heating effects on the sample. The probe wavelength was selected by a monochromator (Bentham Instruments TMc 300) placed between the sample and the detector. The probe light was detected by Hamamatsu R928 photomultiplier tube. 10 nm FWHM
interference filters were placed in front of the sample to reduce the actinic effects on the sample. Optical filters were placed in front of the entrance slit of monochromator to attenuate scattered photons from the laser pump beam.

Microsecond time-resolved step-scan FTIR absorption difference spectroscopy was undertaken using Bruker Vertex80 (Bruker Optics Inc., Billerica, MA), as described previously (21, 30). The same source of pump beam was used as the visible absorption difference spectroscopy. Data were collected in the 1950–1100 cm\(^{-1}\) region at 4 cm\(^{-1}\) spectral resolution. 2000–1000 cm\(^{-1}\) bandpass filters were placed between the IR light source and the sample, and between the sample and the detector. All the samples were prepared on calcium fluoride windows.

Flash-induced absorption changes associated to forward ET are fitted to one or more exponential functions and a constant. Flash-induced absorption changes associated to charge recombination kinetics are fitted to a sum of stretched exponential functions and a constant. All function fittings were performed using the Levenberg-Marquardt algorithm implemented within Origin 7.5 (OriginLab Corporation, Northampton, MA).

### 4.3 Time-Resolved Visible Absorption Difference Spectroscopy

Transient absorption changes in the visible region were obtained for PSI with eight different quinones incorporated into the A\(_1\) binding site, at both 298 and 77 K. The flash-induced absorption changes were monitored at 487 nm and/or 703 nm at 298 K, and 703 nm at 77 K.

Fig. 4.1 shows 298 K flash-induced absorption changes at 487 nm for PSI with AQ, PhQ, 2MNQ, PQ\(_9\), 2ClNQ, 2BrNQ, and Cl\(_2\)NQ incorporated into the A\(_1\) binding site. At 487 nm, the flash-induced absorption changes are partially due to an electrochromic bandshift associated with carotenoid and chlorophyll pigments near the reduced quinones. The kinetics are therefore related to electron transfer from A\(_1^-\) to F\(_X\).
Figure 4.1 298 K flash-induced absorption changes at 487 nm for PSI with (A) AQ, (B) PhQ, (C) 2MNQ, (D) 2ClNQ, (E) 2BrNQ, and (F) Cl₂NQ incorporated into the A₁ binding site. The data associated with forward ET (A–C) are fitted to a sum of exponential functions plus a constant. The data associated with charge recombination (D–F) are fitted to a sum of stretched exponential functions plus a constant. The fitted functions are also shown (red). The initial signal amplitudes were scaled. The timescales are selected to highlight the most prominent decay phases. The time constants obtained from fitting the data are listed on Table 4.1. The time constants of minor phase associated with P700⁺A₁⁻ charge recombination (see Ch. 3) are not included.

Fig. 4.2 shows the 298 K flash-induced absorption changes at 703 nm for PSI with 2MNQ, PQ₉, 2ClNQ, 2BrNQ, Cl₂NQ, and Br₂NQ incorporated. The absorption changes are due to P700 ground state depletion (due to P700⁺ formation) and recovery. Identical kinetics are observed at 487 nm and 703 nm for PSI with high-potential quinones incorporated (2ClNQ, 2BrNQ, Cl₂NQ, and Br₂NQ), indicating that PSI undergoes P700⁺A₁⁻ charge recombination with these quinones incorporated. Identical observations were made using time-resolved infrared absorption difference spectroscopy (see below).
Figure 4.2 298 K flash-induced absorption changes at 703 nm for PSI with (A) 2ClNQ, (B) 2BrNQ, (C) Cl$_2$NQ, and (D) Br$_2$NQ incorporated into the A$_1$ binding site. The data are fitted to a stretched exponential function plus a constant. The fitted functions are also shown (red). The initial signal amplitudes have been scaled. The obtained time constant are listed on Table 4.1.

At cryogenic temperatures (77 K), the major reversible decay pathway in PSI is via P700$^+$A$_1^-$ radical pair recombination. Fig. 4.3 shows 77 K flash-induced absorption changes at 703 nm for PSI with AQ, PhQ, 2MNQ, 2ClNQ, 2BrNQ, Cl$_2$NQ, and Br$_2$NQ incorporated. For PSI with PQ$_9$ incorporated, a decay phase with time constant of ~200–250 µs was reported (30, 100). This decay phase is assigned primarily with the decay of $^3$P700.
Figure 4.3  77 K flash-induced absorption changes at 703 nm for PSI with (A) AQ, (B) PhQ, (C) 2MNQ, (D) PQ5, (E) 2CINQ, (F) 2BrNQ, (G) Cl2NQ and (H) Br2NQ incorporated. Fitted functions are shown in red and the calculated time constants are listed in Table 4.1.

4.4 Time-Resolved Infrared Absorption Difference Spectroscopy

Time-resolved step-scan (TRSS) FTIR difference spectroscopy was performed at 298 K for PSI with the high-potential quinones (2CINQ, 2BrNQ, Cl2NQ, and Br2NQ) incorporated. Kinetic traces of the select wavenumbers are shown in Fig. 4.4. Identical decay kinetics observed in bands associated with P700+/P700 (1754, 1742, 1717, 1697, 1686 cm⁻¹) and bands that are often associated with A1⁻ (1500 – 1510 cm⁻¹) indicate that with these high-potential quinones incorporated, PSI undergoes P700⁺A1⁻ charge recombination at 298 K. Similar conclusion has been drawn from time-resolved visible absorption spectroscopy (see above) and from EPR studies (47). The time constants obtained from fitting the FTIR data at the shown wavelengths (for PSI with each quinone incorporated) to a sum stretched exponential function fittings are similar to those obtained from fitting the data in the visible spectral region (Fig. 4.2).
Figure 4.4 298 K time-resolved absorption changes at several infrared wavelengths (wavenumbers) for PSI with (A) 2ClNQ, (B) 2BrNQ, (C) Cl₂NQ, and (D) Br₂NQ incorporated. In each caption the shown wavelengths were fitted simultaneously to a stretched exponential function and a constant (red). The calculated time constants are listed in Table 4.1.

Table 4.1 summarizes the experimentally obtained time constants at the respective probe wavelengths, and at the two temperatures.
Table 4.1  Time constants obtained from fitting the experimental data in Fig. 4.1–4.4, for PSI with eight different quinones incorporated into the A₁ binding site, at 298 and 77 K. Q refers to the species of the incorporated quinone. a-d From Refs (22)a, (30)b, (99)c, and (82)d.

<table>
<thead>
<tr>
<th>Q</th>
<th>298 K</th>
<th>77 K</th>
<th>703 nm</th>
<th>IR</th>
<th>703 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>487 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQ</td>
<td>50 ns</td>
<td>&gt; 100 ms c</td>
<td></td>
<td></td>
<td>797 µs</td>
</tr>
<tr>
<td>PhQ</td>
<td>25 ns a / 310 ns</td>
<td>50-100 ms d</td>
<td></td>
<td></td>
<td>366 µs</td>
</tr>
<tr>
<td>2MNQ</td>
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<td>14.4 ms b</td>
<td></td>
<td></td>
<td>239 µs</td>
</tr>
<tr>
<td>PQ9</td>
<td>13.9 µs b / 202 µs b</td>
<td>3.2 ms b</td>
<td></td>
<td></td>
<td>250 µs</td>
</tr>
<tr>
<td>2ClNQ</td>
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<td>187 µs</td>
<td>185 µs</td>
<td></td>
<td>114 µs</td>
</tr>
<tr>
<td>2BrNQ</td>
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<td>165 µs</td>
<td>181 µs</td>
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<td>140 µs</td>
<td>137 µs</td>
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<td>78 µs</td>
</tr>
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<td>Br₂NQ</td>
<td>124 µs</td>
<td></td>
<td>117 µs</td>
<td></td>
<td>70 µs</td>
</tr>
</tbody>
</table>
5 MODELING ELECTRON TRANSFER IN PHOTOSYSTEM I

5.1 Introduction

In oxygen evolving photosynthetic organisms, two photosystems called photosystems I and II (PSI and PSII) harness sunlight to make and break molecular bonds, resulting in both oxidizing and reducing products (2). PSII uses sunlight to produce a highly oxidizing species that ultimately leads to water splitting and the liberation of molecular oxygen into the biosphere (8). PSI uses light to produce reducing products that are eventually used to assimilate carbon dioxide into more complex organic molecules via the Calvin–Benson–Bassham cycle (9).

In both photosystems, light induces the transfer of electrons from a chlorophyll-\(a\) (Chl-\(a\)) donor species (P680 or P700), via a series of protein bound pigments, across a biological membrane (thylakoid membrane). This light induced transfer of electrons across a biological membrane is the basic mechanism underlying solar energy capture in all photosynthetic organisms. The molecular basis for such biological ET processes has long been a subject of interest.

In this chapter the focus is on ET processes in isolated cyanobacterial PSI particles from *Synechocystis* sp. PCC 6803 (S6803). The architecture of the protein bound ET cofactors in cyanobacterial PSI is very similar in PSI from higher plants (121) and green algae, and thus cyanobacterial PSI is considered as a template that is applicable to PSI in plants and algae.

In PSI the ET cofactors are bound to two intertwined membrane-spanning protein subunits called PsaA and PsaB. The organization of the bound cofactors is outlined in Fig. 5.1. Following light excitation of a special Chl-\(a\) species within the PSI core an electron is transferred rapidly to a primary Chl-\(a\) acceptor and then on to a secondary electron acceptor which is often termed A\(_1\) (Fig. 5.1). The true primary electron donor species has been a subject of some debate recently (15, 16, 38). However, within ~ 50 ps the light that impinges on PSI has induced the formation of the
secondary radical pair state, $P700^+ A_1^-$ (17-19). $P700$ is a Chl-$a$/Chl-$a'$ heterodimer (10), while $A_1$ is a phyloquinone (PhQ) molecule (14, 82). The photo-generated radical pair state is further stabilized by forward ET from $A_1^-$ to $F_X$, and then onto the terminal acceptors termed $F_A$ and $F_B$. $F_X$, $F_A$, and $F_B$, are iron–sulfur complexes (4Fe–4S) (83, 84).

![Figure 5.1](image)

**Figure 5.1** Arrangement of the ET cofactors in PSI from *Thermosynechococcus elongatus* (PDB file 1JB0, (10)). Edge-to-edge distances (in Å) between cofactors is represented by the dotted vertical lines. The established redox potentials for $P700/P700^+$ (36), $F_X/F_X^-$ (7), $F_A/F_A^-$ and $F_B/F_B^-$ (39) are shown. The kinetic model used to simulate the light-induced population evolution of radical states in PSI at both 298 and 77 K is outlined. The terminal iron-sulfur clusters ($F_A$ and $F_B$) were included for simulations at 298 K but not at 77 K. ET from $A_0^-$ to $A_1$ is assumed to be much faster than ET from $A_1^-$ to the other cofactors, and thus only the population dynamics of the cofactors following $P700^+A_1^-$ formation are considered. The initial population of $A_{1A}^-/A_{1B}^-$ was assumed to be 50/50 at 298 K and 95/5 at 77 K. *Inset:* Simplified kinetic model used previously to model only the kinetics of forward ET from $A_1^-$ to $F_X$ at 298 K.

In PSI the ET cofactors form two nearly symmetric branches, termed the A and B branches, (Fig. 5.1) extending from P700 and terminating at $F_X$. The A and B nomenclature used in Fig. 5.1
refers to the branch and not the main protein subunit (PsaA or B) which binds the cofactors (although the quinones occupying the $A_{1A}$ and $A_{1B}$ binding sites are bound to PsaA and PsaB, respectively). $F_X$ is bound at the juncture between PsaA and B proteins, while $F_A$ and $F_B$ are bound to the stromal protein subunit termed PsaC (10).

As indicated above, following light excitation the $P700^+ A_{1}^-$ state forms in $\sim 50$ ps, and ET from $A_{1}^-$ to $F_X$ proceeds biphasically with time constants of 10–25 and 260–340 ns (20, 26-30). These two lifetimes are due to ET down the B and A branches, respectively (20). While the exact fractional utilization of the two branches appears to be species dependent, it has been demonstrated recently from optical spectroscopy measurements that the relative use of the B/A-branch in cyanobacterial PSI is at least 23/77% at room temperature (RT, $\sim 298$ K), respectively (21). Depending on the kinetic model used, this ratio could be (considerably) more symmetrical.

Forward ET from $F_X^-$ to $F_A$ and $F_B$ also occurs on a nanosecond timescale at RT (31). In isolated cyanobacterial PSI particles at RT, the reduced terminal iron–sulfur clusters ($F_{A/B}^-$) recombine with $P700^+$ in $\sim 100$ ms (34, 98). The distance between $P700$ and $F_A$ or $F_B$ is greater than 17 Å (Fig. 5.1), which is much too large for direct ET from $F_{A/B}^-$ to $P700^+$ to occur in $\sim 100$ ms, and it is widely accepted that $P700^+ F_{A/B}^-$ recombination at RT occurs via repopulation of $A_{1}^-$. The fact that changing the quinone in the $A_1$ binding site modifies the rate of recombination supports this notion.

At 77 K, ET in PSI is heterogeneous. $P700^+ A_{1}^-$ and $P700^+ F_X^-$ radical pair recombination reactions occur in 35–38% and 13–25% of the particles, respectively, while the $P700^+ F_{A/B}^-$ state is irreversibly formed in 40–49% of the PSI particles (26, 30). At 77 K, in cyanobacterial PSI particles from S6803 the $P700^+ A_{1}^-$ state recombines with a lifetime ($\tau_{1/e}$) of $\sim 340$ μs (30), while the $P700^+ F_X^-$ state recombines in $\sim 1–5$ ms (26). The fractional utilization of the B-branch is
greatly reduced (~5%) at cryogenic temperature (21), and P700\(^+\) A\(_1\)^− recombination occurs almost exclusively on the A-branch (47). That is, the observed ~340 \(\mu\)s lifetime is due to P700\(^+\) A\(_{1A}\)^− recombination.

In recent years it has been proven possible to incorporate a variety of quinones into the A\(_1\) binding site in intact PSI particles. This was made possible through the use of mutant cells from S6803 in which the menB gene was inactivated. Inactivation of the menB gene inhibits the biosynthesis of PhQ, and it was subsequently found that plastoquinone-9 (PQ\(_9\)) is recruited into the A\(_1\) binding site in PSI from these menB null mutants (menB\(^−\) PSI) (44, 63). By incubating menB\(^−\) PSI with a large molar excess of the quinone of interest \textit{in vitro}, the loosely-bound PQ\(_9\) in the A\(_1\) binding site is displaced by the non-native quinone (30, 45, 47, 122). This incubation method therefore provides an elegant approach for incorporating non-native quinones into the A\(_1\) binding site in PSI with minimal disruption to the surrounding protein environment (123).

Much of the energetics of ET in PSI, especially those that involve A\(_1\), still remains incompletely understood. This is partly because the rates observed in time-resolved spectroscopy measurements do not correspond directly to the rates described in equations governing ET. Knowledge on the \textit{in situ} midpoint potential of the quinone occupying the A\(_1\) binding site is a crucial parameter in understanding ET in PSI. The \textit{in situ} midpoint potential of the A\(_{1A}\) PhQ, found through a variety of indirect approaches undertaken in the past, spans a wide range (−810 – −531 mV, see Refs. (38–43)), and a consensus value has not yet been reached. Much of this chapter is aimed at addressing this knowledge gap.

5.1.1 Marcus non-adiabatic ET theory

ET processes between weakly coupled (widely separated) donor and acceptor pigments in PSI is often analyzed within the context of non-adiabatic ET theory (66). ET in PSI is a quantum
mechanical tunneling process (59), and the fundamental equation governing the rate \( k_{et} \), in s\(^{-1}\) of non-adiabatic ET follows from Fermi's golden rule (Eq. 5.1).

\[
k_{et} = \frac{2\pi}{h} |V|^2 FC
\]

Equation 5.1

In Eq. 5.1, \( V \) is an electronic coupling term and \( FC \) is the Franck–Condon factor. In cases where the Franck–Condon term has been established, the factors that influence \( V \) can be investigated. It has been shown that for many intra-protein ET reactions that \( |V|^2 \propto e^{-\beta R} \) (62), where \( \beta \) is the medium-dependent coefficient that can be expressed in terms of the protein packing density, \( \rho \), and \( R \) is the edge-to-edge distance between electron donor and acceptor. From a consideration of several through-protein ET reactions a mean value of \( \beta = 1.4 \) Å\(^{-1} \) (or \( \rho = 0.76 \)) has been suggested (62).

In classical non-adiabatic ET theory, the Franck–Condon factor is considered as the overlap of harmonic oscillator wavefunctions. Within this context the rate of ET is expressed via Eq. 5.2.

\[
k_{et} = \frac{2\pi}{h} |V|^2 \frac{\sqrt{4\pi\lambda k_B T}}{\sqrt{\frac{(\Delta G^0 + \lambda)^2}{4\lambda k_B T}}} e^{\frac{-(\Delta G^0 + \lambda)^2}{4\lambda k_B T}}
\]

Equation 5.2

In Eq. 5.2, \( \lambda \) is the reorganization energy, \( k_B \) is Boltzmann's constant, \( T \) is the temperature, and \( \Delta G^0 \) is the standard free energy difference (\( -\Delta G^0 \) is the driving force). With appropriate quantum mechanical corrections (that are required to better simulate the temperature dependence of ET rates) a semi-classical expression for the ET rate can be derived (Eq. 5.3), where \( \omega \) is a characteristic mean vibrational frequency coupled to ET. More complex derivations involving all modes coupled to ET have also been presented (60, 64).

\[
k_{et} = \frac{2\pi}{h} \frac{|V|^2}{\sqrt{2\pi\lambda h\omega \coth(h\omega/2k_B T)}} e^{\frac{-(\Delta G^0 + \lambda)^2}{2\lambda h\omega \coth(h\omega/2k_B T)}}
\]

Equation 5.3

Through an extensive survey of biological ET processes, ET rates were shown to depend mainly on the edge-to-edge distance \( (R) \), the standard free energy difference \( (\Delta G^0) \), and the
reorganization energy ($\lambda$). This led Moser and Dutton (62) to develop the empirical relation outlined in Eq. 5.4.

$$\log k_{ET} = 13 - (1.2 - 0.8\rho)(R - 3.6) - 0.22 \left( \frac{(\Delta G^0 + \lambda)^2}{\lambda \hbar \omega \coth(\hbar \omega / 2k_B T)} \right)$$  \hspace{1cm} \text{Equation 5.4}

By choosing common values for the energy of the vibrational mode and the protein packing density ($\hbar \omega = 56$ meV and $\rho = 0.76$), at 298 K Eq. 5.4 reduces to Eq. 5.5. Eq. 5.5 has found wide utility in studies of biological ET.

$$\log k_{ET} = 15 - 0.6R - 3.1 \left( \frac{(\Delta G^0 + \lambda)^2}{\lambda} \right)$$  \hspace{1cm} \text{Equation 5.5}

In Eq. 5.5, with judicious choices of $R$ and $\lambda$, $\log k_{ET}$ depends parabolically on the standard free energy gap, $\Delta G^0$. In most applications of Eq. 5.5 to the study of ET reactions in PSI, the experimentally observed rates are taken as a direct measure of $k_{ET}$, which is then used to calculate $\Delta G^0$. Such an approach is necessarily oversimplified in PSI where it is known that ET is bidirectional, and in a recent study it has been shown that such an approach leads to unreasonable estimates of $\Delta G^0$ (30, 39). The experimentally observed rates can only be equated directly with $k_{ET}$ when ET is largely exothermic and unidirectional, a situation that usually does not apply to ET reactions in PSI. As shown previously, to find the rate constant ($k_{ET}$) that serves as input in Eq. 5.5 an appropriate kinetic model needs to be constructed, and the intrinsic rate constants associated with the model can then be extracted.

In previous time-resolved spectroscopy experiments time constants associated with forward ET from $A_1^-$ to $F_X$ at RT in PSI with PhQ, 2MNQ and PQ$_9$ incorporated have been obtained (30). A basic kinetic model was considered (Fig. 5.1, inset) and applied in combination with Eq. 5.5 to estimate the midpoint potential for the different quinones in the $A_1$ binding site that was compatible with the experimentally observed rates of forward ET (from $A_1^-$ to $F_X$ at RT).
Here this previous study is expanded by considering a more complete kinetic model (Fig. 5.1) for the simulation of time-resolved experimental data obtained for PSI particles with eight different quinones \([9,10\text{-anthraquinone} (AQ), \text{PhQ}, 2\text{MNQ}, \text{PQ}_9, 2\text{-chloro-1,4-naphthoquinone (2ClNQ)}, 2\text{-bromo-1,4-naphthoquinone (2BrNQ)}, 2,3\text{-dichloro-1,4-naphthoquinone (Cl}_2\text{NQ)}, \text{and 2,3-dibromo-1,4-naphthoquinone (Br}_2\text{NQ)})\] incorporated into the A\(_1\) binding site. The range of the reduction potentials of these quinones in solution (dimethylformamide, DMF) spans over 500 mV (Table 5.1), and allows for an exploration of greater range of the A\(_1\) midpoint potentials and its effect on the various ET reactions in PSI. In addition to the forward A\(_1\) to F\(_X\) ET at RT, the kinetic model developed also has to be compatible with the observed kinetics associated with P700\(^+\) A\(_1\)– radical pair recombination at both RT and LT (low temperature, 77 K). To be consistent with all of these types of experimental data requires quite stringent constraints on the possible range of parameters used to describe ET in Eq. 5.4. The simulations presented here are the first to consider ET reactions at both RT and LT within the framework of a single kinetic model.

**Table 5.1** Summary of the experimentally observed time constants associated with ET processes in PSI with different quinones incorporated into the A\(_1\) binding site, at 298 and 77 K. Q refers to the incorporated quinone. \(E_{1/2}\) refers to the midpoint potential of the quinones in DMF. \(^a\) From Refs (124)

<table>
<thead>
<tr>
<th>Q</th>
<th>(E_{1/2}) (mV)</th>
<th>(\tau_{A_1^-\rightarrow F_X})</th>
<th>(\tau_{P700^+(F_{A/B}/A_1^-)})</th>
<th>(\tau_{P700^+A_1^-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQ</td>
<td>-575(^a)</td>
<td>50 ns(^l)</td>
<td>&gt;100 ms(^h)</td>
<td>797 (\mu)s(^i)</td>
</tr>
<tr>
<td>PhQ</td>
<td>-470(^b)</td>
<td>25 ns(^d)/ 310 ns(^e)</td>
<td>50-100 ms(^f)</td>
<td>366 (\mu)s(^e)</td>
</tr>
<tr>
<td>2MNQ</td>
<td>-420(^a)</td>
<td>430 ns(^c)/ 3.1 (\mu)s(^c)</td>
<td>14.4 (\mu)s(^c)</td>
<td>239 (\mu)s(^c)</td>
</tr>
<tr>
<td>PQ(_9)</td>
<td>-369(^b)</td>
<td>13.9 (\mu)s(^c)/ 202 (\mu)s(^c)</td>
<td>3.2 (\mu)s(^c)</td>
<td>78 (\mu)s(^c)</td>
</tr>
<tr>
<td>2CINQ</td>
<td>-230(^c)</td>
<td>-</td>
<td>187 (\mu)s(^i)</td>
<td>114 (\mu)s(^i)</td>
</tr>
<tr>
<td>2BrNQ</td>
<td>-230(^c)</td>
<td>-</td>
<td>165 (\mu)s(^i)</td>
<td>94 (\mu)s(^i)</td>
</tr>
<tr>
<td>Cl(_2)NQ</td>
<td>-50(^a,b)</td>
<td>-</td>
<td>140 (\mu)s(^g)</td>
<td>78 (\mu)s(^g)</td>
</tr>
<tr>
<td>Br(_2)NQ</td>
<td>-50(^r)</td>
<td>-</td>
<td>124 (\mu)s(^i)</td>
<td>70 (\mu)s(^i)</td>
</tr>
</tbody>
</table>

\(^a\) From Refs (124)
\(^b\) From Refs (47)
\(^c\) From Refs (125)
\(^d\) From Refs (22)
\(^e\) From Refs (30)
\(^f\) From Refs (82)
\(^g\) From Refs (21)
\(^h\) From Refs (99)
\(^i\) From Refs (126)

\(\dagger\) For low potential quinones (AQ, PhQ, 2MNQ and PQ\(_9\)) incorporated P700\(^+\) A\(_1\)– charge recombination is observed on ms timescales at RT. For high potential quinones (2ClNQ, 2BrNQ, Cl\(_2\)NQ, and Br\(_2\)NQ) incorporated P700\(^+\)A\(_1\)– charge recombination is observed on \(\mu\)s timescales at RT. \(^\ddagger\) At 77 K, for PSI with PQ\(_9\) incorporated, a decay kinetics with a lifetime of \(\sim\)200 – 250 \(\mu\)s is observed that is due, at least in part, to the decay of \(3\)P700\(^3\) (30, 100). Therefore, the lifetime of P700\(^+\)A\(_1\)– charge recombination at 77 K for PSI with PQ\(_9\) incorporated is not listed.
5.2 Materials and Methods

Methods for preparation of PSI particles from menB− mutant cells from S6803, as well as methods for quinone incorporation into PSI are described in Ref. (126). Methods used for time-resolved spectroscopy measurements are also described in Ref. (126).

The calculated population evolution of A1A−, A1B−, FX−, FA−, FB− and P700 was obtained by solving a series of linear differential equations associated with the quasi-equilibrium kinetic model. At 77 K the population evolution of FA− and FB− was not considered. As outlined previously, the population of A1− was calculated as the sum of A1A− and A1B− populations. The differential equations were solved numerically using Mathematica 10.0 software (Wolfram Research, Champaign, IL).

5.3 Results

5.3.1 Time constants associated with flash induced absorption changes at 298 and 77 K

RT flash-induced absorption changes at 703 and 487 nm for PSI with PhQ, 2MNQ and PQ9 incorporated into the A1 binding site (30), as well as PSI with Cl2NQ incorporated (21) have been published. In addition, here PSI with 2CINQ, 2BrNQ, Br2NQ and AQ incorporated have also been studied. The flash induced absorption changes at various probe wavelengths, at both 298 and 77 K, for PSI with the different quinones incorporated have been presented (126). The time constants derived from fitting the transient absorption data are listed in Table 5.1.

At RT, for PSI with PhQ, 2MNQ, PQ9 and AQ incorporated (the low potential quinones), forward ET from A1− to FX occurs, and the time constants associated with the reaction can be obtained from the flash induced absorption changes at 487 nm (Table 4.1 and Fig. 4.1).

In addition, at RT for PSI with PhQ, 2MNQ, PQ9 and AQ incorporated, flash-induced absorption change at 703 nm can be used to establish time constants associated with P700+ FA/B−
charge recombination (Table 4.1 and Fig. 4.2). Obviously, the kinetics observed at 487 and 703 nm differ greatly in PSI particles where forward ET to F_{A/B} occurs.

At RT for PSI with the high-potential Cl_{2}NQ incorporated, flash-induced absorption changes at 703 and 487 nm display identical kinetics, indicating forward ET from A_{1} to F_{X} is inhibited, and P700^+ A_{1}^- charge recombination occurs instead (see (21, 126)). Similarly, with 2ClNQ, 2BrNQ, and Br_{2}NQ, which also have high midpoint potentials, forward ET from A_{1} to F_{X} is inhibited, and P700^+ A_{1}^- charge recombination is observed (see (126)). The time constants of the major decay phases are listed in Table 5.1.

At 77 K, ET in PSI becomes heterogeneous, as described above, and in repetitive flash experiments the major decay phase is associated with P700^+ A_{1A^-} recombination. Previously, in PSI with PhQ/2MNQ/Cl_{2}NQ incorporated at 77 K, a major decay phase with a time constant of 366/239/78 μs is observed (stretched exponential lifetime), respectively, and assigned to P700^+ A_{1A^-} charge recombination (Table 4.1 and Fig. 4.3). In PSI with AQ, 2ClNQ, 2BrNQ, and Br_{2}NQ incorporated into the A_{1} binding site, the major decay phases (at 77 K) are found with time constants of 797, 114, 94, and 70 μs, respectively (Table 4.1). These decay phases are also assigned to P700^+ A_{1A^-} charge recombination. For menB^- PSI with no foreign quinones added, PQ_{9} occupies a portion of the A_{1} binding sites (100) (in the original preparations PQ_{9} was shown to fully occupy the A_{1} binding site (89)), and the time constant of the major decay phase is ~200–250 μs (30, 100). From FTIR studies, these time constants have been at least in part associated with the decay of \(^3\)P700 (100). \(^3\)P700 forms in PSI particles in which the A_{1} binding site is empty, or the quinone in the site is not functional. However, as no other time constants are observed in menB^- PSI, the time constant may in part be associated with P700^+A_{1A^-} charge recombination.
5.3.2 Kinetic modeling

Fig. 5.1 outlines the kinetic model used to simulate the experimental data. This model advances from the relatively simple kinetic model (Fig. 5.1, inset) that was used previously to model forward ET from $A_1^-$ to $F_X$ at RT in PSI with PhQ, 2MNQ and PQ$_9$ incorporated (30). The kinetic scheme in the inset in Fig. 5.1 will be referred as the simple model while the kinetic scheme in Fig. 5.1 will be referred to as the more advanced model. In the simple model the $F_X^-$ to $F_A$ forward ET processes ($k_3$) was considered irreversible, and no attempt was made to model the observed $P700^+ F_X^-$ and $P700^+ F_{A/B}^-$ recombination reactions. The simple model also does not consider $P700^+ A_1^-$ recombination reactions at 77 K.

5.3.3 Application of non-adiabatic ET theory

Previously for the simple model, forward ET ($k_n$) rates were calculated using Eq. 5.5, and the reverse rates ($k_{-n}$) were calculated by invoking Boltzmann equilibration via the use of Eq. 5.6:

$$\ln \left[ \frac{k_n}{k_{-n}} \right] = -\frac{\Delta G^0}{k_B T}$$

Equation 5.6

The approach requires prior knowledge of the reorganization energy associated with the ET, and $\lambda = 0.7$ eV was used in this simple model. A series of coupled differential equations were then formulated in terms of these forward and reverse rate constants, and solved for the population dynamics of cofactors over time. A range of standard free energy gaps for the forward ET from $A_{1A}^-$ and $A_{1B}^-$ ($\Delta G_{A_{1A}^- F_X^0}$ and $\Delta G_{A_{1B}^- F_X^0}$) were tested for the simulation of the population evolution of the $A_1^-$ state. The population of $A_1^-$ was considered as the sum of the $A_{1A}^-$ and $A_{1B}^-$ populations, and the calculated population evolution was evaluated based on a weighted average time constant, $\tau_{AV}$. The calculated population evolution is obtained by solving a set of linear differential equations, with the solution being a sum of exponential functions, $\sum_i A_i \tau_i$, in which
the various time constants ($\tau_i$) are functions of the rate constants outlined in Fig. 5.1, *inset*. The weighted average time constant associated with population evolution is then calculated as

$$\tau_{Av} = \frac{\sum_i A_i \tau_i}{\sum_i A_i}$$

Equation 5.7

Calculations using the more advanced model progress similarly. To allow for the simulation of ET kinetics at both RT and LT, and also to allow for a finer specification of electron tunneling parameters, Eq. 5.4 is used for the calculation of forward ET rates ($k_n$). The reverse rates ($k_{-n}$) are still calculated using Eq. 5.6.

Assessment of forward ET from A$_1^-$ to F$_X$ is based on comparing the weighted average time constant of the multiphasic A$_1^-$ population dynamics (Eq. 5.7) with the experimentally obtained weighted average time constant. At the current level of simulation, the calculated data is expressed as a sum of unstretched exponential functions that assume single protein conformations. Experimentally, however, the data is fitted to a sum of stretched exponential functions.

The charge recombination reactions are assessed differently, and the time constant with the largest amplitude from the simulated dynamics is compared directly to the experimentally observed time constant. This approach is appropriate given that experimentally only the largest amplitude phase is generally associated with charge recombination. In these calculations presented here this distinction is not strictly necessary, however, as it is found that the time constant associated with the largest amplitude accounts for over 98% of the total amplitude for any quinone incorporated, and there are only negligible differences in the calculated recombination lifetime and the weighted time constant (for PSI with PhQ, the time constants are 96.67 ms *vs.* 96.95 ms).

5.3.4 Redox potentials of ET cofactors

For the simple model, no direct specification of cofactor midpoint potential was required. With only three cofactors involved ($A_{1A}$, $A_{1B}$, and $F_X$), the standard free energy gaps between $A_{1A}$,
$A_{1B}$ and $F_X$ could be calculated without assignment of any specific midpoint potentials. Of course, as the rate constant of ET from $F_X^-$ to $F_A$ ($k_3$) was fixed, a certain midpoint potential would be implied for $F_X$ given that the midpoint potential of $F_A$ is known to be $-526$ mV.

For calculations based on the more advanced model, which involves more cofactors, specification of the cofactor midpoint potentials becomes necessary. Srinivasan and Golbeck [24] have reviewed the literature detailing measurements that have been undertaken to establish the redox potential for $F_X$. A range of values have been obtained, but a consensus value of $-688$ mV was suggested. In keeping with this consensus estimate in the calculations presented here the midpoint potential of $F_X$ is fixed at $-680$ mV. From previous kinetic simulations (39) the midpoint potentials of $F_A$ and $F_B$ were calculated to be $-526$ mV and $-556$ mV, respectively. These values are similar to that obtained from experiment (127, 128), and are the values used in the current calculations. The midpoint potential of $P700$ ($P700/P700^+$) in PSI for S6803 is close to $+450$ mV (36), and this is the number used in calculations here. The midpoint potentials of the cofactors that were assigned are shown in Fig. 5.1.

5.3.5 Electron tunneling parameters

The use of Eq. 5.4 requires the specification of several parameters. In the presented calculations $\hbar\omega$ was initially set at 56 meV at 298 K and 70 meV at 77 K (62, 103). For PSI the protein packing densities between adjacent ET cofactors have been calculated from the x-ray crystal structure and presented previously (37). The values listed in reference (37) are also used here. For ET between cofactors where the packing density is not reported a value of $\rho = 0.76$ is used. The edge-to-edge distance between ET cofactors were also presented in reference (37), and are outlined in Fig. 5.1. Previous EPR studies have shown that the distance from $P700^+$ to $A_{1}^-$ is identical to that of native PhQ when non-native quinones are incorporated into the binding site,
and that the orientation also does not change (47, 122, 129). Therefore, the same edge-to-edge distances are assumed for all the quinones studied here, except for PQ₉ (see section 5.3.7).

Previously in the simple model, a conventional reorganization energy of $\lambda = 0.7$ eV was used. In the more advanced model, the ET reactions are divided into two groups, and assigned different reorganization energies: For the forward ET reactions at RT (from $A_{1A}^-$ to $F_X$, $A_{1B}^-$ to $F_X$, $F_X^-$ to $F_A$ and $F_A^-$ to $F_B$) the reorganization energy was assumed to be the same. For the $P700^+ A_{1A}^-$ and $P700^+ A_{1B}^-$ recombination reactions the reorganization energy was assumed to be the same, but not necessarily the same as that for the forward ET reactions. For the $P700^+ A_1^-$ recombination reactions, it was necessary to allow the reorganization energy of the reaction to vary slightly (< 0.1 eV) on going from RT to LT (see below).

The actual range in which the reorganization energy ($\lambda$) may fall is not entirely clear, and so the initially set values used in calculations was investigated. To this end calculations were undertaken in which the reorganization energies associated with forward ET at RT is set at 0.4, 0.7 and 1.0 eV. In the simple kinetic model studied previously, only 0.7 eV was used, and other possible solutions with different reorganization energies were not considered.

5.3.6 More parameters used in the advanced kinetic model

The more advanced kinetic model outlined in Fig. 5.1 allows the light-induced population dynamics of $A_{1A}^-$, $A_{1B}^-$, $F_X^-$, $F_A^-$, $F_B^-$, and $P700^+$ to be investigated.

As mentioned above, given the edge-to-edge distances between the iron–sulfur clusters and P700, recombination time constants in the ~12.5 to $2 \times 10^{15}$ s range are expected. Such large time constants do not agree with the experimentally determined time constant of ~100 ms (for PSI with PhQ incorporated). Therefore, re-reduction of $P700^+$ by charge recombination, whether from $F_X$,
F_A or F_B, is expected to occur through repopulation of A_1^-, and this is implicitly assumed in the model outlined in Fig. 5.1.

The kinetic model presented here considers the population dynamics of the cofactors following P700^+ A_1^- formation. Therefore, initial populations must be assigned to A_1A^- and A_1B^-.

At RT, a 50:50 branching ratio for A_1A^- and A_1B^- was chosen. This branching ratio is chosen for the following reasoning: 1) For eukaryotic PSI, the fractional usage of the two branches is ~ 50:50, [18] while for prokaryotic PSI the fractional usage ranges from ~ 15:85 to ~ 40:60 (22, 24, 119, 130). 2) These previously reported fractions were determined from the decay amplitude ratios of fast and slow kinetic phases. However, considering thermodynamic equilibration and a possible route of ET from A_1B^- to A_1A via F_X (106), the observed decay amplitudes of fast and slow phases do not necessarily correspond to the actual usage of two branches, but may indicate the usage of the branches shifted in favor of A-branch after quasi-equilibration. Through kinetic modeling it has been demonstrated that an asymmetry in decay amplitudes is achievable even with a symmetric initial population (39, 106). Interestingly, while an asymmetric branching ratio has been reported from transient absorption studies on intact PSI particles, similar studies on PSI core particles devoid of all iron–sulfur clusters (with no likelihood of ET from A_1B^- to A_1A via F_X) exhibit ~50:50 ratio in the decay amplitudes of the biphasic kinetics assigned to P700^+A_1B^- and P700^+A_1A^- charge recombination (98, 131).

In any case, it is found that changing this population ratio had little impact on the calculated population dynamics. For example, for the simulation of PSI with PhQ incorporated in the A_1 binding site, the weighted average time constant fluctuated by at most 6 ns by changing the initial population ratio from 50:50 to 20:80 (not shown).
Transient EPR and optical studies have both indicated that radical pair recombination in cyanobacterial PSI at 77 K occurs predominantly down the A-branch, so at LT, the initial $A_{1A}^-$ and $A_{1B}^-$ population ratio is set at 95:5.

At LT the $P700^+A_{1A}^-$ charge recombination is the main decay phase observed experimentally. $P700^+F_{A/B}^-$ is formed irreversibly at LT. In this chapter the interest is not in simulating these irreversible fractions. Therefore, for simulations at LT, the $F_A$ and $F_B$ cofactors are removed from the model. When these two cofactors are included in the simulations at LT, irreversible $F_A^-$ and $F_B^-$ states are accumulated, however (data not shown).

5.3.7 Simulations for PSI with PQ\textsubscript{9} incorporated

While the electron tunneling parameters and simulation conditions described above apply to PSI with seven of the quinones incorporated, simulations for PSI with PQ\textsubscript{9} incorporated requires additional considerations. PQ\textsubscript{9} is a substituted benzoquinone (BQ), while all of the other quinones incorporated are substituted NQs or AQ.

For PQ\textsubscript{9}, the lack of a secondary aromatic rings alters the edge-to-edge distance between the cofactors. Previous EPR studies have noted that PQ\textsubscript{9} is incorporated into the $A_1$ binding site in the same orientation as the native PhQ (89). This suggests that the distance between $A_1$ and P700 is the same for PQ\textsubscript{9} and PhQ in the $A_1$ binding site. This observation in turn suggests that the edge-to-edge distance between PQ\textsubscript{9} and $F_X$ is greater than 9.0 Å, which is the distance between PhQ and $F_X$. PQ\textsubscript{9} has two methyl groups instead of a secondary aromatic ring, and a PQ\textsubscript{9}–$F_X$ edge to edge distance of 10.05 Å is used. Since the exact distance cannot be verified, the simulation for PSI with PQ\textsubscript{9} incorporated focuses more on the recombination reactions at both RT and LT in which the edge-to-edge distances are the same for both PQ\textsubscript{9} and PhQ.
5.3.8 Calculating the temporal evolution of cofactor radicals

The temporal evolution of the various cofactors can be established by solving the series of coupled differential equations (Eq. 5.8) that are based on the kinetic model and associated rate constants outlined in Fig. 5.1. The forward ET rate constants \( k_n \) were calculated using Eq. 5.4, and the reverse ET rate constants \( k_n \) using Eq. 5.6. The population of \( A_1^- \) is the sum of \( A_{1A}^- \) and \( A_{1B}^- \) populations.

\[
\frac{d}{dt}[A_{1A}^-](t) = -(k_{1A} + k_{0A})[A_{1A}^-](t) + k_{-1A}[F_X^-](t) + k_{-0A}[P700](t)
\]
\[
\frac{d}{dt}[A_{1B}^-](t) = -(k_{1B} + k_{0B})[A_{1B}^-](t) + k_{-1B}[F_X^-](t) + k_{-0B}[P700](t)
\]
\[
\frac{d}{dt}[F_X^-](t) = -(k_2 + k_{-1A} + k_{-1B})[F_X^-](t) + k_{1A}[A_{1A}^-](t) + k_{1B}[A_{1B}^-](t) + k_{-2}[F_A^-](t)
\]
\[
\frac{d}{dt}[F_A^-](t) = -(k_{-2} + k_3)[F_A^-](t) + k_2[F_X^-](t) + k_{-3}[F_B^-](t)
\]
\[
\frac{d}{dt}[F_B^-](t) = -(k_{-3})[F_B^-](t) + k_3[F_A^-](t)
\]
\[
\frac{d}{dt}[P700](t) = -(k_{-0A} + k_{-0B})[P700](t) + k_{0A}[A_{1A}^-](t) + k_{0B}[A_{1B}^-](t)
\]

\[
[A_1^-](t) = [A_{1A}^-](t) + [A_{1B}^-](t)
\]

Equations 5.8

5.3.9 Simulation procedural details

A schematic outlining the simulation procedure is presented in Fig. 5.2. With the use of the model in Fig. 5.1, midpoint potentials for \( A_{1A} \) and \( A_{1B} \) \([E_m(A_{1A}) \) and \( E_m(A_{1B})\)] are assigned. For the assigned midpoint potentials to be acceptable, the model must produce weighted average time constants that agree with experiment for all ET reactions at both RT and LT. Additionally, the same model (within a narrow range of reorganization energies) must result in weighted average time constants that are in agreement with the data obtained for PSI with the different quinones incorporated at both RT and LT. The only adjustable parameters are the midpoint potentials of the quinones incorporated into the \( A_{1A} \) and \( A_{1B} \) binding sites.
Figure 5.2 Outline of the systematic procedures applied in simulations. The simulation starts with a kinetic modeling of PSI with PhQ incorporated into the A1 binding site (left column) and continues to the kinetic modeling of PSI with non-native quinones (right column).

All combinations of $E_m(A_{1A})$ and $E_m(A_{1B})$ within a relatively wide range have been tested systematically. A wide range of reorganization energies have also been tested. The detailed procedure of modeling, depicted in Fig. 5.2, is outlined in Appendix A. The steps employed in this systematic procedure can be separated into two parts: In the first part the energetics in PSI with PhQ incorporated are simulated. In the second part the energetics in PSI with non-native quinones incorporated are simulated.
In the first part three reorganization energies are set for the ET processes in PSI with PhQ incorporated: $\lambda_1$ for forward ET reactions at RT; $\lambda_2$ for $P700^+A_1^-$ recombination at RT; and $\lambda_3$ for $P700^+A_1^-$ recombination at LT. $\lambda_1$ is initially set at 0.7 eV. Calculations were also undertaken for $\lambda_1$ set at 0.4 and 1.0 eV, but it was found that these latter values could not accurately simulate all of the experimental data.

$E_m(A_{1A})$ and $E_m(A_{1B})$ are initially set at the values reported previously for PSI with PhQ incorporated. Previously the midpoint potential of PhQ in the $A_{1A}/A_{1B}$ binding site was calculated to be $+15/-10$ mV relative to that of $F_X$. If $E_m(F_X) = -680$ mV then $E_m(A_{1A})$ and $E_m(A_{1B})$ are initially set at $-665$ and $-690$ mV, respectively. $E_m(A_{1A})$ and $E_m(A_{1B})$ are then varied systematically in 15 mV increments over a 60 mV range. Once the best $E_m(A_{1A})$ and $E_m(A_{1B})$ have been established (the best pair is assessed in terms of producing a weighted averaged time constant that best agrees with experiment) further calculations are undertaken varying $E_m(A_{1A})$ and $E_m(A_{1B})$ in finer 5–10 mV steps (see Table SI1 in Ref. (48) for calculated values). Calculated weighted average time constants can be visualized based on its agreement to the experimental value. Fig. 5.3 shows a heat map of calculated agreement between theoretical and experimental weighted average time constants for different combinations of $E_m(A_{1A})$ and $E_m(A_{1B})$. 
Figure 5.3  A heat map of calculated agreement between theoretical and experimental weighted average time constants. Agreement is calculated as \(1-(t/e)/e\), where \(t/e\) is the theoretical/experimental weighted average time constant, respectively. The shaded area indicates the region where \(E_m(A_{1B})\) is more positive than \(E_m(A_{1A})\).

Upon establishment of \(E_m(A_{1A})\) and \(E_m(A_{1B})\) for PSI with PhQ incorporated, \(P700^+F_{AB}^-\) charge recombination is investigated by considering the population dynamics of \(P700\) using the same \(E_m(A_{1A})\) and \(E_m(A_{1B})\). In some cases the calculated recombination time constant is not in line with experiment. To improve the correspondence between calculation and experiment \(\lambda_2\) is varied. Alteration of \(\lambda_2\) modifies the simulated \(P700^+F_{AB}^-\) recombination time constant without impacting the already calculated weighted average time constant associated with the temporal evolution of \(A_1^-\). Finally, using the same \(E_m(A_{1A})\) and \(E_m(A_{1B})\) for PhQ in PSI obtained above, further simulations are undertaken using the LT kinetic model. Again, the reorganization energy associated with the \(P700^+A_1^-\) recombination, \(\lambda_3\), can be adjusted slightly. In no case was \(\lambda_2\) and \(\lambda_3\) adjusted by more than 0.1 eV between the different cases studied.
In the second part of the simulation procedure, the three reorganization energies set in the first part ($\lambda_1$, $\lambda_2$, and $\lambda_3$) are taken as the initial parameters for simulating ET in PSI with non-native quinones incorporated. The three reorganization energies are unaltered in the second part, except for $\lambda_2$ which is modified by less than 0.1 eV for the simulation of P700$^+$F$\text{A/B}$– radical pair recombination in PSI with low potential quinones incorporated (see below). Similar to the procedure in the first part, the $E_m(A_{1A})$ and $E_m(A_{1B})$ are set at a predetermined value and then varied systematically. The pair of $E_m(A_{1A})$ and $E_m(A_{1B})$ values that best agree with experimental data are then established. In addition to modeling reaction rates, the reaction pathway must be well modeled also. For example, for PSI with the high-potential quinones incorporated at RT, P700$^+$A$^{-}$ charge recombination must occur. P700$^+$A$^{-}$ recombination occurs when the A$^{-}$ population decays at the same rate as the population of P700 recovers.

At any point in this procedure, if the reaction rates or pathways are not consistent with experiment, the $E_m(A_{1A})$ and $E_m(A_{1B})$ pair are considered inadequate. This type of a systematic approach imposes strict limitations on possible values on $E_m(A_{1A})$ and $E_m(A_{1B})$. The main lifetimes ($\tau_i$), amplitudes ($A_i$), and the weighted average lifetime associated with the A$^{-}$ transient population (sum of A$^{-}_{1A}$ and A$^{-}_{1B}$), for the many different tested $E_m(A_{1A})$ and $E_m(A_{1B})$ pairs, are listed in the Tables in the supplementary information in Ref. (48).

Through the use of this systematic procedure, it was found that only $\lambda_1 = 0.7$ eV is capable of simulating ET kinetics for PSI with eight different quinones incorporated, at both RT and LT. Setting $\lambda_1 = 0.4$ or 1.0 eV leads to population evolutions that are not consistent with all of the data. The $E_m(A_{1A})$ and $E_m(A_{1B})$ listed in Table 5.2 for each quinone in the A$^{-}_{1A}$ and A$^{-}_{1B}$ binding sites represent the values that give a best match between the calculated and experimental weighted averaged time constants.
Table 5.2  Comparison of calculated and experimental averaged time constants for (A) forward ET and (B) charge recombination reactions at 298 K, and for (C) charge recombination at 77 K.  \( E_m \) is the quinone in situ midpoint potential. \(^a-d\) From Refs (30)\(^a\), (98)\(^b\), and (21)\(^c\). The average time constant for PhQ in the A\(_1\) site at RT follows from the fact that A\(_{1A}/A_{1B}^-\) decays in 310 ns/15 ns with amplitude ratio of 70/30, respectively. \(^\dagger\)As mentioned, for PSI with PQ\(_9\) incorporated, the major decay phase observed at 77 K occurs with a time constant of ~200–250 µs. However, because the kinetic phase is assigned primarily to the decay of \(^3\)P700, the time constant is not listed in Table 5.2C.

5.3.10 Temporal evolution of the radical cofactors at RT

Fig. 5.4 shows the temporal evolution of the A\(_{1A}^-, A_{1B}^-, A_1^- (A_{1A}^- + A_{1B}^-), F_X^-, F_A^-\), and F\(_B^-\) populations at 298 K, and the recovery of P700, for PSI with eight different quinones incorporated.
In PSI with AQ, PhQ, 2MNQ and PQ⁹ incorporated, \( A_1^- \) decays on ns to \( \mu s \) timescales (indicated by the blue dotted lines connecting the 50% population level of \( A_1^- \) in Fig. 5.4A–5.4D), while the population of P700 recovers on ms to s timescales (red dotted lines joining the 50% levels in Fig. 5.4A–5.4D). Such temporal profiles for the decay of \( A_1^- \) and the recovery of P700 are in excellent agreement with experiment (Table 5.2).
In contrast, in PSI with the higher potential quinones incorporated (2ClNQ, 2BrNQ, Cl₂NQ, and Br₂NQ in Fig. 5.4E–5.4H), identical temporal profiles are observed between the decay of \( A_1^- \) and the recovery of P700. In these latter simulations (Fig. 5.4E–5.4H) the population of reduced iron–sulfur clusters is very small. That is P700\(^+\) \( A_1^- \) charge recombination dominates in PSI particles with high potential quinones incorporated, as was found experimentally (21, 47).

Of particular note in Fig. 5.4B is the blue decay curve associated with \( A_1^- \). The ratio of the amplitudes of the fast and slow decay phases in this curve is \( \sim 15:85 \). This is in spite of the fact that the initial populations of \( A_{1A}^- \) and \( A_{1B}^- \) were set at 50:50. This is an important point that suggests that comparing amplitudes of fast and slow decay phases from experiment may not be an appropriate way to assess the fractional utilization of the A and B branches in PSI.

5.3.11 \( P700^+A_1^- \) radical pair recombination at 77 K

Fig. 5.5 shows the calculated population evolution of radical states at 77 K. Clearly, regardless of the quinone species incorporated into the A\(_1\) binding site, the decay of \( A_1^- \) and P700\(^+\) (recovery of P700) display identical kinetics, in line with that found experimentally (Table 5.2, (21, 126)). At both 298 and 77 K, the kinetic model adequately simulates the experimentally observed ET reactions in PSI with eight different quinones incorporated.
Figure 5.5  Population evolution of the radical states in PSI with eight different quinones incorporated (same order as in Fig. 5.4) at 77 K. The temporal profiles for $\text{A}_{1}^-$ (blue), $\text{F}_{X}^-$ (green) and P700 (red) are shown. At 77 K, for PSI with all of the different quinones incorporated, the population evolution of $\text{A}_{1B}^-$ is essentially complete within $10^{-5}$ s (not shown). Therefore, the population evolution curve shown for $\text{A}_{1}^-$ is essentially that of $\text{A}_{1A}^-$. For PSI with AQ incorporated $\text{A}_{1}^-$ and $\text{F}_{X}^-$ decay identically as P700 recovers. For PSI with PhQ or all the other quinones incorporated $\text{A}_{1}^-$ decays as P700 recovers (P700$^+$ decays). The gray dotted lines join the crossover points of the blue and red curves in each caption. Quinone structures are also shown (carbon, oxygen, chlorine, and bromine atoms are black, red, green, and purple, respectively).

As discussed earlier, the experimentally observed rates do not necessarily correspond to the intrinsic ET rates. The actual intrinsic rate constant of ET between donor and acceptor species will be discussed more fully in a future publication.
5.4 Discussion

From the calculations and modeling presented here, the midpoint potentials for PhQ in the A_{1A} and A_{1B} binding sites are found to be −635 and −690 mV, respectively. The midpoint potential of F_X was set at −680 mV, so forward ET from A_{1A} to F_X is slightly uphill in free energy (\(\Delta G^0 = −45\) meV), while ET from A_{1B} to F_X is slightly downhill (\(\Delta G^0 = 10\) meV). Previously \(−\Delta G^0 = −15/+10\) meV for the A/B branch ET was calculated using the simpler model outlined in Fig. 5.1, inset. The deviations are partly due to differences in the models, and partly due to differences in the choice of weighted average time constant. Previously, the weighted average time constant (\(\tau_{av}\)) for the A_{1}^{-} → F_X ET was taken to be in the 100–150 ns range, which resulted from using time constants of 5–20 and 150–300 ns, with equal weight. In the present work an average time constant of 221.5 ns is used. This time constant was calculated using decay time constants of 310 and 15 ns with the amplitude ratio of 70:30 (30). The choice of decay amplitude ratios may introduce some variance into the weighted average time constant, and based on the calculations spanning a broad range of quinone midpoints potentials (see Fig. 5.3 and Tables SI1-SI8 in Ref. (48)) \(E_m(A_{1A})/E_m(A_{1B})\) is estimated to be accurate to within ±10/±20 mV, respectively. This is a narrow range given that the range reported in the literature is over 200 mV (7, 14).

5.4.1 Correlation between in situ and in vitro midpoint potentials

A common approach to deciding or predicting what the relative ordering of in situ midpoint potentials (\(E_m\)s) might be for a range of quinones is to first consider the ordering of the potentials of the quinones in vitro (\(E_{1/2}\)s) (generally in aprotic solvents). This type of approach implicitly assumes an approximately linear correlation between \(E_m\) and \(E_{1/2}\).

Table 5.1 lists the \(E_{1/2}\)s and Table 5.2 lists the \(E_m\)s for the eight different quinones considered, and Fig. 5.6 shows a plot of \(E_m\) versus \(E_{1/2}\) for the eight quinones incorporated on both
the A and B branch. Fig. 5.6 demonstrates a linear correlation between $E_m$ and $E_{1/2}$ for quinones in the A$_1$ binding site. Linear equations governing this correlation for A and B branch quinones are also outlined in Fig. 5.6. There appears to be a somewhat greater degree of scatter in the $E_{ms}$ for the quinones on the B branch compared to the A branch.

![Figure 5.6](image)

**Figure 5.6** Plot comparing the $E_m$ for the eight quinones reported in Table 5.2 [A$_{1A}$ in black, A$_{1B}$ in red] with $E_{1/2}$ in aprotic solvent. The equations resulting from fitting the data to a linear function is also shown. The linear relationships obtained previously for solvent-extracted PSI are also shown for comparison. The equations governing the latter are $E_{m-A1}(V) = 0.720E_{1/2}(V) - 0.408$ (blue) and $E_{m-A1}(V) = 0.690E_{1/2}(V) - 0.433$ (green) (40).

By incubating intact PSI particles in the presence of organic solvents it is possible to remove the native PhQ ET cofactors (along with ~90% of the chlorophyll molecules and most of the carotenoids) (132, 133). In these so-called solvent-extracted PSI particles it is possible to incorporate foreign quinones back into the A$_1$ binding site (40, 78, 132, 134). From a series of investigation on solvent extracted PSI with different quinones incorporated it was suggested that the $E_{1/2}$ and $E_m$ are linearly correlated (40). This previously reported correlation for quinones incorporated into solvent-extracted PSI is also shown in Fig. 5.6. The bidirectional nature of ET in PSI had not yet been established for these previous studies on solvent-extracted PSI. However,
the $E_m$s for the intact PSI particles appear to be shifted to higher values compared to that found for the quinones in solvent extracted PSI.

It is important to point out that in the approach taken here to estimate the midpoint potentials of the quinones in the $A_1$ binding site, no assumptions (except those outlined in Fig. 5.1) were made as to what these potentials might be. The linear correlation demonstrated in Fig. 5.6 came naturally from the model developed, which may be an indicator of its appropriateness.

Woodbury et al. (135) have considered the correlation between $E_m$ and $E_{1/2}$ for a range of BQ, NQ and AQ derivatives incorporated into the QA binding site in purple bacterial RCs. In these studies it was found that the linear correlation was weak. The weaker correlation may relate to the broader range of quinones incorporated into the QA binding site in these previous studies.

In Fig. 5.6 the slope of lines through the data is 0.54 and 0.51. Previously, Iwaki and Itoh (40) have derived a slope of 0.69–0.72 for quinones incorporated into solvent extracted PSI particles. There is clearly a considerable modification of the quinone potential in situ, compared to in vitro. However, the rank ordering of the potentials in situ and in vitro is the same, at least for the quinones on the A branch. For PSI with 2ClNQ/2BrNQ and Cl$_2$NQ/Br$_2$NQ in the B branch this rule appears not to hold and the linear correlation is considerably weaker.

Iwaki and Itoh (40) used the concept of acceptor number to derive a relationship between in vitro and in situ redox potentials that appears to describe the observed relationship (shown in Fig. 5.6) very well. Exactly how the protein environment (specific amino acids and pigment-protein interactions) contributes to acceptor number is not well understood, however.

5.4.2 Possible range of reorganization energies and free energies

For forward ET at RT, a reorganization energy of $\lambda_1 = 0.7$ eV was used, and both the simple and more advanced models indicate that forward ET from $A_1^{-}$ to $F_X$ on the A/B-branch is slightly
exergonic/endergonic, respectively. If the reorganization energy is altered then for a given reaction rate this will also alter the driving force. In the simple model used previously (Fig. 5.1, inset) other possible values for the reorganization energy were not investigated, and the results obtained were not unique. For the advanced model, with more experimental data to simulate, it is possible to estimate more precisely what the possible range of reorganization energies (and associated driving forces) might be.

In previous studies two scenarios are often considered (7): 1) the midpoint potentials of both $A_{1A}$ and $A_{1B}$ are considerably lower than that of $F_X$ [$\Delta G_{A_1^- - F_X^0} \ll 0$]; 2) the midpoint potentials of both $A_{1A}$ and $A_{1B}$ are considerably higher than $F_X$ [$\Delta G_{A_1^- - F_X^0} \gg 0$].

In simulations where $\lambda_1 = 0.7 \text{ eV}$, both of these ranges are unreasonable (see Table SI1 in Ref. (48)). With a large driving force for forward ET from $A_{1A}^-$ to $F_X$ an averaged time constant that is much larger than expected is calculated. For example, for $E_m(A_{1A}) = -620 \text{ mV}$, the averaged time constant is 343–464 ns, depending on $E_m(A_{1B})$ (Table SI1 in Ref. (48)). This is considerably longer than the observed 225 ns. On the other hand, the average time constant is too short when the $A_{1A}^- \rightarrow F_X$ is taken to be largely endergonic [For example, for $E_m(A_{1A}) = -710 \text{ mV}$, the averaged time constant is 23–33 ns, for $E_m(A_{1B})$ in the $-735$ to $-705 \text{ mV}$ range (Table SI1 in Ref. (48))].

Since the rate of forward ET from $A_1^-$ to $F_X$ is dependent on the reorganization energy, simulations were also undertaken with $\lambda_1 = 1.0 \text{ eV}$ (Table SI7 in Ref. (48)) and $0.4 \text{ eV}$ (Table SI8 in Ref. (48)). In simulations with $\lambda_1 = 1.0 \text{ eV}$, the estimated range of midpoint potentials of $A_{1A}$ and $A_{1B}$ are approximately $-695$ to $-710 \text{ mV}$ and $-720$ to $-780 \text{ mV}$, respectively [see shaded values in Table SI7(A) in Ref. (48)]. These estimations indicate that both forward ET reactions are exergonic [$\Delta G_{A_{1A}^- - F_X^0} = -15$ to $-30 \text{ meV}$ and $\Delta G_{A_{1B}^- - F_X^0} = -40$ to $-100 \text{ meV}$]. In addition,
given these indicated midpoint potentials, charge recombination at RT with a lifetime of ~100 ms can be simulated using $\lambda_2 = 0.8$ eV [see Table SI7(B) in Ref. (48)]. However, for any value of $\lambda_3$ between 0.4 and 1.7 eV, the simulated ET kinetics are associated with $\text{P}700^+\text{F}_X^-$ radical pair recombination, and not $\text{P}700^+\text{A}_1^-$ recombination [see Table SI7(C) in Ref. (48)]. Therefore, the exergonic driving forces simulated with $\lambda_1 = 1.0$ eV can be ruled out.

In simulations with $\lambda_1 = 0.4$ eV, the calculated midpoint potentials indicate large endergonic free energies are associated with forward ET from $\text{A}_1^-$ to $\text{F}_X$ at RT on both the A and B branches [$E_m(\text{A}_1\text{A}) = -590$ mV, $E_m(\text{A}_1\text{B}) = -600$ mV] [see shaded values in Table SI8(A) in Ref. (48)]. Using these midpoint potentials radical pair recombination with a lifetime of ~ 100 ms at RT can be simulated using $\lambda_2 = 0.44$ or 0.45 eV [Table SI8(B) in Ref. (48)]. In addition, radical pair recombination at LT can be simulated using $\lambda_3 = 0.61$ eV [Table SI8(C) in Ref. (48)]. It would therefore appear that all ET reactions in PSI with PhQ incorporated at both RT and LT can be quite well simulated using $\lambda_1 = 0.4$ eV.

However, the simulated kinetics for PSI with 2MNQ incorporated cannot be reconciled with the experimental observations. For example, in PSI with 2MNQ incorporated, with $\lambda_1 = 0.4$ eV and $\lambda_2 = 0.45$ eV, the model used here suggests that $E_m(\text{A}_1\text{A})$ falls in the −485 to −500 mV range, while $E_m(\text{A}_1\text{B})$ falls in the ~ −525 to −600 mV range [Table SI8(D) in Ref. (48)]. However, forward ET from $\text{A}_1^-$ to $\text{F}_X$ at RT proceeds only in ~ 20% of the RCs [Table SI8(D) in Ref. (48)]. In about 80% of the reaction centers $\text{P}700^+\text{A}_1^-$ charge recombination occurs. This result is not in keeping with experiment. Therefore, $\lambda_1 = 0.4$ mV is ruled out as a viable possibility.

### 5.5 Conclusions

A detailed kinetic model was constructed and used to describe the transient evolution of radical pair states in PSI with eight different quinones incorporated into the A$_1$ binding site at both
298 and 77 K. All of the data could be well described only if the in situ midpoint potentials of the quinones fell in a tightly defined range of ±5–10 mV for the quinone on the A branch, and ±10–30 mV for the quinone on the B branch.

All of the data taken together could be well described if the reorganization energy associated with forward electron transfer from A1− to FX at RT is set at 700 meV. All of the data taken together could not be well described if the reorganization energy was set at 400 or 1000 meV. It is shown that even this level of discrimination is not possible by considering transient absorption data for PSI with only PhQ incorporated. Transient absorption data for PSI with several quinones incorporated, at both RT and LT, is required. In this first series of calculations no attempt was made to consider reorganization energies in the vicinity of 700 mV.

The modeling presented here shows that reorganization energies of 400/1000 meV are associated with forward ET processes that are largely endergonic/exergonic thermodynamically speaking, respectively, and the modeling of the experimental data presented here indicate that neither are possible. The modeling approach developed here indicates that forward ET from A1− to FX can only be slightly endergonic/exergonic on the A/B branch, respectively.

In the modeling undertaken here the midpoint potential of FX was set at −680 mV. It is doubtful that much higher or lower values could be appropriate but, this is a point of detail that can be investigated in future studies. The same statements also apply to the energy of the mean vibrational mode that is coupled to ET, which was set at 56/70 meV at RT/LT, respectively.

Through modeling of experimental data the in situ redox potentials for eight different quinones on both the A1A and A1B binding sites were calculated. Comparing this data to corresponding in vitro redox potentials for the quinones in solution, a linear correlation was found for the quinones on both branches.
6 INVERTED-REGION ELECTRON TRANSFER AS A MECHANISM FOR ENHANCING PHOTOSYNTHETIC SOLAR ENERGY CONVERSION EFFICIENCY

6.1 Introduction

In oxygen-evolving photosynthetic organisms (plants, algae and cyanobacteria), solar energy is captured and converted independently in two large membrane-spanning protein complexes called photosystem I and photosystem II (PSI and PSII) (2). In both systems light induces the transfer of electrons from a chlorophyll donor species, via a series of protein bound pigments, across a biological membrane. This transport of electrons across a biological membrane is the basic mechanism underlying solar energy capture and storage in all photosynthetic organisms. In PSI and PSII the photosynthetic electron transfer (ET) processes have a remarkably high quantum efficiency (136). In this chapter a mechanism that contributes to this high efficiency is explored.

In this chapter the focus is on ET processes that occur in isolated PSI photosynthetic reaction centers (RCs) from the cyanobacterium *Synechocystis* sp. PCC 6803 (S6803). The architecture of the protein bound ET cofactors (pigments) in cyanobacterial PSI is outlined in Fig. 6.1 (10, 137). The cofactor organization in PSI from plants is similar (121).
Figure 6.1  Arrangement of the ET cofactors (pigments) in PSI with the two possible routes of ET indicated. Figure was derived from the 2.5 Å crystal structure of PSI from *Thermosynechococcus elongatus* (10). Cofactor hydrocarbon tails have been truncated. A similar figure is obtained using the 2.8 Å crystal structure of PSI from S6803 (137). Edge-to-edge distances (in Å) between cofactors (dotted) as well as the cofactor redox potentials for P700 (36), A_{1A} and A_{1B} (48), F_X (138), F_A and F_B (14, 39) are shown. Arrows indicate the kinetic model that was used previously to analyze the light-induced dynamics of radical pair states in PSI (48). P700^+F_{A/B}^- and P700^+F_X^- radical pair recombination proceeds via repopulation of A_{1}^- (30), as indicated.

6.1.1  Bioenergetics in Isolated Photosystem I at 298 and 77 K

At room temperature (RT, ~298 K), within ~50 ps of light excitation, a secondary radical pair state P700^+A_1^- is formed (17, 18). P700 is a heterodimeric chlorophyll-α species (10, 139), while A_1 is a PhQ molecule (14, 82) (Fig. 6.1). The photo-generated radical pair state is further
stabilized by electron transfer (ET) from $A_1^-$ to $F_X$, and then from $F_X^-$ on to $F_A$ and $F_B$ (14). $F_X$, $F_A$, and $F_B$, are iron-sulfur clusters (82).

In PSI the ET cofactors form two nearly symmetric branches, termed the A and B branches, extending from P700 and terminating at $F_X$ (Fig. 6.1). At RT, ET from $A_1^-$ to $F_X$ proceeds biphasically, with time constants of ~25 and 300 ns (27). These lifetimes relate to ET down the B and A branch, respectively (21). The experimentally observed ET rates do not directly relate to the intrinsic ET rates because the observed rate is dependent on equilibration between the various radical states (Fig. 6.1) (30, 48).

In isolated PSI particles at RT, the reduced terminal iron-sulfur clusters ($F_{A/B}^-$) recombine with P700$^+$ in ~80 ms (82). The distance between P700 and $F_A$ or $F_B$ is greater than 17 Å (Fig. 6.1), which is much too large for direct ET from $F_{A/B}^-$ to P700$^+$ to occur in 80 ms, and it is widely accepted that P700$^+F_{A/B}^-$ recombination at RT occurs via repopulation of $A_1^-$ (30). This conclusion is consistent with the fact that the P700$^+F_{A/B}^-$ recombination is dependent on the quinone in the $A_1$ binding site (30, 48, 126).

In isolated PSI particles at low temperature (LT, ~77 K) forward ET from $A_1^-$ to $F_X$ is blocked in a portion of the PSI particles, and P700$^+A_1^-$ recombines to the ground state in ~300 µs (26, 30). This ~300 µs lifetime is due almost exclusively to ET down the A branch, and is therefore due to P700$^+A_{1A}^-$ recombination (21). Given the lack of equilibration between different states this observed decay rate at 77 K will be similar to the intrinsic ET rate.

In this article the focus is on the unproductive ($P700^+A_{1A}^- \rightarrow P700A_{1A}$) ET recombination reaction, with rate $k_{0A}$ (Fig. 6.1), which competes with the forward ET process. The ($P700^+A_{1A}^- \rightarrow P700A_{1A}$) is the dominant charge recombination pathway at both 298 and 77 K (7, 21). Conclusions drawn for the ($P700^+A_{1A}^- \rightarrow P700A_{1A}$) recombination reaction will also apply to
(P700\(^{+}\)A\(_1\)B\(^{-}\) → P700A\(_1\)B) ET recombination, with rate \(k_{0B}\), however. Often in the literature, the term A\(_1\) refers to the secondary electron acceptor, which is a PhQ molecule in native PSI.

6.1.2 Inverted-Region Electron Transfer

Marcus ET theory, in the classical or semiclassical limit, predicts a Gaussian dependence between ET rate and driving force (48). The ET rate increases with driving force until the reactions' reorganization energy matches the driving force. Further increase in the driving force leads to a nonintuitive decrease in ET rate, in the so-called inverted region where the reactions' reorganization energy is less than the driving force.

Forward ET in PSI is highly efficient, with a quantum yield approaching unity (136). Such a high yield indicates a near-complete suppression of wasteful recombination reactions. One mechanism that might contribute to this suppression is to tune the ET energetics so that recombination occurs in the inverted region. Since the free energy is likely much larger than the reorganization energy, the nonproductive ET reaction occurs in the inverted region with considerably reduced rate, allowing the productive forward ET to dominate. In his Nobel lecture Marcus proposed that this inverted-region mechanism could be a factor contributing to the high quantum efficiency associated with solar energy conversion in photosynthetic systems (69). This idea has been further discussed by others (73).

The inverted-region effect has been confirmed experimentally, most notably by Miller and colleagues (73) and Miller et al. (67) for studies on synthetic systems. However, despite the recognition that inverted-region ET may play a role in promoting the high quantum yields associated with photosynthetic ET, there has never been any direct demonstration of an inverted-region ET process in any native photosynthetic RC under physiological conditions.
6.1.3 PSI Cofactor Modification

In recent years it has proven possible to incorporate a variety of quinones into the A₁ binding site in PSI particles with minimal disruption to the surrounding protein (30, 45-48), and here P700⁺A₁⁻ recombination in PSI with 10 different quinones incorporated are considered (Table 6.1). Incorporation of different quinones allows easy modification of the driving force associated with ET. By establishing ET rates and associated driving forces in PSI with the different quinones incorporated, a Marcus curve can be constructed, revealing where on the curve ET in the native system occurs.

Table 6.1 In vitro \( (E_{1/2}) \) and in situ \( (E_m) \) midpoint potentials (in mV) for ten different quinones incorporated into PSI. \( E_{1/2} \) values are taken from (124, 140) and \( E_m(A_{1A}) \) are taken from (48) (except for AQS and DMNQ). The native quinone in PSI is PhQ (listed #4 in table). \( -\Delta G^0 = -e(E_m - 450) \) is the free energy associated with P700⁺A₁A⁻ recombination, in meV. The observed time constants \( \tau_{obs} \) (in \( \mu s \)) are obtained from fitting transient absorption data at 77 K.

<table>
<thead>
<tr>
<th>Q</th>
<th>( E_{1/2} )</th>
<th>( E_m ) (A₁A)</th>
<th>( \Delta G^0 )</th>
<th>( \tau_{[P700⁺A₁A⁻→P700A₁A]} )</th>
<th>( \tau )</th>
<th>( \tau )</th>
</tr>
</thead>
<tbody>
<tr>
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<td>-675</td>
<td>-1125</td>
<td>797</td>
<td>540</td>
</tr>
<tr>
<td>2</td>
<td>AQS</td>
<td>-569</td>
<td>-665</td>
<td>-1115</td>
<td>538</td>
<td>488</td>
</tr>
<tr>
<td>3</td>
<td>DMNQ</td>
<td>-501</td>
<td>-640</td>
<td>-1090</td>
<td>391</td>
<td>383</td>
</tr>
<tr>
<td>4</td>
<td>PhQ</td>
<td>-465</td>
<td>-635</td>
<td>-1085</td>
<td>366</td>
<td>365</td>
</tr>
<tr>
<td>5</td>
<td>2MNQ</td>
<td>-418</td>
<td>-580</td>
<td>-1030</td>
<td>239</td>
<td>226</td>
</tr>
<tr>
<td>6</td>
<td>PQ₉</td>
<td>-369</td>
<td>-560</td>
<td>-1010</td>
<td>200</td>
<td>193</td>
</tr>
<tr>
<td>7</td>
<td>2CINQ</td>
<td>-225</td>
<td>-475</td>
<td>-925</td>
<td>114</td>
<td>109</td>
</tr>
<tr>
<td>8</td>
<td>2BrNQ</td>
<td>-225</td>
<td>-460</td>
<td>-910</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>Cl₂NQ</td>
<td>-60</td>
<td>-415</td>
<td>-865</td>
<td>78</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>Br₂NQ</td>
<td>-60</td>
<td>-395</td>
<td>-845</td>
<td>70</td>
<td>73</td>
</tr>
</tbody>
</table>

6.1.4 ET in PSI with Foreign Quinones Incorporated

In previous chapters, time-resolved visible and infrared spectroscopy have been used to study the bioenergetics of ET in PSI with eight different quinones incorporated, at both 298 and 77 K (48, 126). With the experimental data, kinetic modeling in combination with non-adiabatic ET theory was used to estimate the in situ midpoint potentials \( (E_m) \) for the different quinones (48,
Here these studies are extended to consider two additional quinones, AQS and DMNQ (2 and 3 in Table 6.1, 6.2), incorporated into PSI.

Table 6.2  Free energy associated with P700$^+$A$_{1A}^-$ recombination at 298 K (in meV) for PSI with ten different quinones incorporated into the A$_{1A}$ binding site. Data was calculated using $-\Delta G^0 = -e(E_m - 450)$, using previously estimated $E_m$’s (48). The time constants $\tau_{obs}$ (in ms), are obtained from fitting the experimentally observed absorption changes at 703 nm, at 298 K, and are taken from (126) (except for AQS and DMNQ). The intrinsic time constants $\tau_{int}$ (in $\mu$s) were calculated as outlined in section 6.2.1.

<table>
<thead>
<tr>
<th>Q</th>
<th>$\Delta G^0$</th>
<th>$\tau_{obs}$</th>
<th>$\tau_{int}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AQ</td>
<td>-1125</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>2</td>
<td>AQS</td>
<td>-1115</td>
<td>551</td>
</tr>
<tr>
<td>3</td>
<td>DMNQ</td>
<td>-1090</td>
<td>~100</td>
</tr>
<tr>
<td>4</td>
<td>PhQ</td>
<td>-1085</td>
<td>~100</td>
</tr>
<tr>
<td>5</td>
<td>2MNQ</td>
<td>-1030</td>
<td>14.4</td>
</tr>
<tr>
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<td>PQ$_9$</td>
<td>-1010</td>
<td>3.2</td>
</tr>
<tr>
<td>7</td>
<td>2CINQ</td>
<td>-925</td>
<td>0.187</td>
</tr>
<tr>
<td>8</td>
<td>2BrNQ</td>
<td>-910</td>
<td>0.165</td>
</tr>
<tr>
<td>9</td>
<td>Cl$_2$NQ</td>
<td>-865</td>
<td>0.140</td>
</tr>
<tr>
<td>10</td>
<td>Br$_2$NQ</td>
<td>-845</td>
<td>0.124</td>
</tr>
</tbody>
</table>

With the estimated midpoint potentials for the incorporated quinones the free energy associated with each ET process can be calculated. In addition, the kinetic simulations allow for the calculation of intrinsic ET rates, which because of equilibration between states, can differ significantly from the observed rates (30, 39). In this chapter, both the free energy and intrinsic ET rates for ET from $A_{1A}^-$ to P700$^+$ at both 298 and 77 K are calculated. Using these parameters, Marcus curves is constructed and ET associated with unproductive radical pair recombination is shown to occur in the inverted region for all quinones incorporated. In particular, ET occurs well into the inverted region in native PSI at room temperature (RT). Following on from this, calculations are undertaken to demonstrate that inverted-region ET is an important mechanism for greatly increasing photosynthetic efficiency in PSI in situ.
6.2 Materials and Methods

Growth of *menB*– mutant cyanobacterial cells from S6803, and preparation of trimeric PSI particles from the cells, are undertaken as described previously (44). Methods used for foreign quinone incorporation into the A_1 binding site in photosystem I (PSI) is also as described previously (21, 30, 48, 126). Room temperature (~298 K) and low temperature (~77 K) transient absorption data for *menB*– PSI with the different quinones incorporated were undertaken as described previously (48, 126). Here data for PSI with AQS and DMNQ incorporated into the A_1 binding site are included.

Previously, kinetic modeling in combination with non-adiabatic electron transfer theory was used to calculate time constants associated with the population evolution of radical pair states (τ_{av}), for PSI with eight different quinones incorporated. By comparing the observed time constants (τ_{obs}) with the calculated time constants (τ_{av}), estimates of the midpoint potential (E_m) of the different quinones incorporated into the protein binding site were made (48). The same procedures were applied here to estimate the midpoint potentials of AQS and DMNQ incorporated into the A_1 binding site in PSI.

6.2.1 Calculation of Intrinsic ET Rates from Kinetic Modeling

In previous chapters, a kinetic model to describe the experimental transient absorption spectroscopic data was developed (48). The main objective in that study was to estimate the *in situ* midpoint potentials of the different quinones incorporated into the A_1 binding site in PSI (48). In those previous simulations the (intrinsic) forward and reverse ET rate constants (k_n and k_{-n} in Fig. 6.1) were calculated using Eqs. 6.1 and 6.2 for the forward/reverse rate (k_n/k_{-n}), respectively (48).

\[
\log k_{ET} = 13 - (1.2 - 0.8\rho)(R - 3.6) - 0.22\left(\frac{(\Delta G^0 + \lambda)^2}{\lambda \hbar \omega \coth(\hbar \omega / 2k_B T)}\right)
\]

Equation 6.1
\[
\ln \left( \frac{k_n}{k_{-n}} \right) = -\frac{\Delta G^0}{k_B T} \quad \text{Equation 6.2}
\]

In the previously described modeling, intrinsic ET rates were calculated for many hundreds of values of \( \lambda \) and \( \Delta G^0 \). For each pair of values of \( \lambda \) and \( \Delta G^0 \) the calculated \( k_{oS} \) were used as input in a set of coupled differential equations that allowed a calculation of the population evolution of each of the radical states. From these kinetic simulations, the values of \( \Delta G^0 \) and \( \lambda \) that best simulate the observed ET rates (1/\( \tau_{obs} \)) were determined. As the kinetic modeling was focused on simulating the observed rates, the intrinsic rates (\( k_{oS} \)) were not discussed or presented. These rate constants are required for the current discussion, however, as they allow the construction of a Marcus curve.

To calculate the intrinsic ET rate associated with P700\(^+\)A\(_{1A^-}\) charge recombination (\( k_{0A} \)), the parameters derived for ET between A\(_{1A^-}\) and P700\(^+\) that best simulated the observed time constant \( \tau_{obs} \) are reapplied using Eq. 6.1 and \( k_n \) is recalculated. For example, for PSI with PhQ incorporated at 298 K the parameters used to calculate \( k_{0A} \) are \( R = 18.3 \ \text{Å}, \rho = 0.76, \Delta G^0 = -1.085 \ \text{eV}, \lambda = 0.58 \ \text{eV}, \) and \( \hbar \omega = 0.056 \ \text{eV} \). This gives \( k_{0A} = 833.41 \ \text{s}^{-1} \) and \( k_{-0A} = 3.73 \times 10^{-16} \ \text{s}^{-1} \). The intrinsic time constant (1/\( k_{0A} = 1.199 \ \mu s \)) at 298 K is listed in Table 6.2.

This procedure was undertaken for PSI with all 10 quinones incorporated, at both 298 and 77 K, and the results are summarized in Tables 6.1 and 6.2.

In the presented modeling Eq. 6.2 was used to calculate the reverse rates. Ideally the reverse rate is calculated using a separate Eq. 6.1 with the driving force of opposite sign (i.e. for a forward reaction with \( \Delta G^0 \), a reverse rate will have a driving force of \(-\Delta G^0 \)). Under the approximations used in the experiments and calculations here, it is shown that the use of Eq. 6.2 is equivalent to using a separate Marcus or Moser-Dutton equation for the reverse reaction. The ratio of the forward and reverse ET rates is, starting from Eq. 6.3

\[
\frac{k_n}{k_{-n}} = \exp \left[ -\frac{(\Delta G^0 + \lambda)^2}{2\hbar \omega \coth(\hbar \omega / 2k_B T)} + \frac{(-\Delta G^0 + \lambda)^2}{2\hbar \omega \coth(\hbar \omega / 2k_B T)} \right] \quad \text{Equation 6.3}
\]
\[
\frac{k_n}{k_{-n}} = \exp \left[ -\frac{2\Delta G^0}{\hbar \omega \coth(\hbar \omega/2k_B T)} \right]
\]

Equation 6.4

At room temperature, for \( \hbar \omega < 2k_B T \), \( \coth(\hbar \omega/2k_B T) \approx 2k_B T/\hbar \omega \), so

\[
\frac{k_n}{k_{-n}} = \exp \left[ -\frac{\Delta G^0}{k_B T} \right]
\]

Equation 6.5

Eq. 6.5 is equation 6.2, and is valid at RT. At low temperature, \( \coth(\hbar \omega/2k_B T) \approx 1 \), and

\[
\frac{k_n}{k_{-n}} = \exp \left[ -\frac{2\Delta G^0}{\hbar \omega} \right]
\]

Equation 6.6

For radical pair recombination at 77 K, \( -\Delta G^0 >> \hbar \omega \), so \( k_{-n} << k_n \), as expected. By using Eq. 6.2 and 6.6 at 77 K, a difference in \( \Delta G^0 \) is calculated to be less than 12 meV, which is well below the \(~15–40\) mV level of uncertainty that was estimated previously for PSI with the different quinones incorporated (48).

Eq. 6.5 and 6.6 were obtained using the Marcus equation (Eq. 1.6). Eq.6.7 and 6.8 also follow from using a Moser-Dutton relation (Eq. 6.1):

\[
\frac{k_n}{k_{-n}} = \exp \left[ -\frac{1.013 \Delta G^0}{k_B T} \right]
\]

Equation 6.7

\[
\frac{k_n}{k_{-n}} = \exp \left[ -\frac{2.02 \Delta G^0}{\hbar \omega} \right]
\]

Equation 6.8

Equations 6.7 and 6.8 are essentially the same as equations 6.5 and 6.6, respectively, and lead to calculated results that are also well within the level of accuracy estimated previously for the calculated driving forces (15–45 mV) (48).

6.3 Results

In this chapter AQS and DMNQ are incorporated into PSI. Fig. 6.2A shows transient absorption changes at 703 nm obtained using PSI with AQS and DMNQ incorporated at 77 K, along with corresponding data obtained previously using PSI with seven other quinones incorporated (126). Observed time constants (\( \tau_{\text{obs}} \)) obtained from fitting the transient absorption
data at 77 K are listed in Table 6.1. At 703 nm it is well known that the absorption change is due to the loss of P700 ground-state absorption (due to P700\(^{+}\) formation) (35, 82), and that the temporal profiles of the absorption changes in Fig. 6.2A are associated with P700\(^{+}\)A\(_{1A}^{-}\) → P700A\(_{1A}\) recombination (7). The change in the recombination rate with the different quinones incorporated into PSI is obvious in Fig. 6.2A.

Figure 6.2  (A) 77 K flash induced absorption changes at 703 nm \((inverted)\) for PSI with ten different quinones incorporated. Fitted functions are also shown (solid lines). (B) Plot of the observed P700\(^{+}\)A\(_{1A}^{-}\) recombination rate at 77 K \textit{versus} the reaction free energy calculated using the quinone \textit{in vitro} midpoint potentials \([-\Delta G^{0} = -e(E_{1/2} - 450)]\). Data is fit to a parabolic function \((dotted)\) and is numbered according to Table 6.1.

Recently a kinetic modeling study, using the observed time constants calculated from transient absorption data as input, was undertaken in order to estimate the \textit{in situ} midpoint potential \((E_{m})\) for eight different quinones incorporated into the A\(_{1}\) binding site in PSI (48). Table 6.1 lists these \textit{in situ} midpoint potentials along with new data for PSI with AQS and DMNQ incorporated. In addition, Table 6.1 lists the \textit{in vitro} midpoint potentials \((E_{1/2})\) for the different quinones (in DMF vs SHE). These \(E_{1/2}\)'s span a range of over 500 mV (Table 1) while the \(E_{m}\)'s span a range of 280 mV (Table 6.1).
Based on the in situ midpoint potentials listed in Table 6.1, and taking into account a P700⁺/P700 midpoint potential of +450 mV (36), the driving force associated with unproductive radical pair recombination reaction (P700⁺A₁⁻ → P700A₁) can be calculated. These calculated driving forces (−ΔG⁰) are listed in Table 6.1.

In a first simple analysis of the data listed in Table 6.1, a plot of the experimentally observed ET rates at 77 K versus the driving force calculated directly from the quinone in vitro midpoint potentials (Fig. 6.2B) is considered. The plot in Fig. 6.2B makes no assumptions concerning any type of theory or kinetic model that may be appropriate. However, the data in Fig. 6.2B are well described by a parabolic function, suggesting that a theory that predicts such a parabolic dependence might be appropriate.

Previously the kinetic model outlined in Fig. 6.1 was used to calculate the population evolution of the various radical states (48). By comparing the time constant associated with this population evolution to the experimentally observed time constant, the in situ midpoint potentials of the quinones incorporated into PSI were calculated (48). These calculations required the forward and backward intrinsic ET rates (kₚ and k₋ in Fig. 6.1) as input. However, these intrinsic rates were never reported as the focus was on estimating in situ redox potentials. The intrinsic time constants, τₜₚ, are listed in Tables 6.1 and 6.2.

The two time constants (τₒ𝑏ₛ and τₜₚ) are similar for PSI with all of the different quinones incorporated at 77 K, except for AQ and AQS (Table 6.1). This similarity is expected because forward ET from A₁A⁻ to Fₓ is thermodynamically uphill (and less likely to occur at 77 K), so P700⁺A₁A⁻ recombination will occur directly without equilibration between P700⁺A₁A⁻ and P700⁺Fₓ⁻ states. Differences in τₒ𝑏ₛ and τₜₚ are expected for PSI with AQ and AQS incorporated, because these quinones have low potentials, and A₁A⁻ to Fₓ ET is thermodynamically favorable,
so recombination will involve thermodynamically uphill ET from $F_X^-$ back to $A_1$. For these reasons $\tau_{\text{obs}}$ and $\tau_{\text{int}}$ at 77 K are expected to differ for PSI with AQ and AQS incorporated.

6.3.1 Marcus Plot Associated with Radical Pair Recombination at 77 K

Fig. 6.3A shows a Marcus plot using the 77 K intrinsic ET rates and driving forces listed in Table 6.1. The data points in Fig. 6.3A lie on the fitted function because nonadiabatic ET theory was used to derive the rates and in situ potentials for the quinones incorporated (48). As outlined previously, for $\text{P}700^+A_{1A}^-$ recombination at 77 K, a reorganization energy of 660 meV was used ($\lambda_3$ in Ref. (48)), so the parabolic curve peaks at 660 meV, where the reorganization energy matches the driving force. It was previously shown that the ET processes in PSI could not be adequately simulated if the reorganization energy was as high as 0.1 eV or as low as 0.4 eV (48).
Figure 6.3  (A) Marcus plot of $P700^+A_{1A}^-$ intrinsic ($\tau_{\text{int}}$, ■) and observed ($\tau_{\text{obs}}$, ×) recombination rates at 77 K versus the reaction free energy [$-\Delta G^0 = -e(E_m - 450)$] for ten different quinones incorporated into PSI. Numbering is according to Table 6.1. The parabolic fitted function is based on ET parameters derived previously (48), along with the intrinsic time constants listed in Table 6.1. The horizontal error bars (items 5 and 10) are estimates of the in situ potential based on a possible ±0.2 Å error in the edge-to-edge distance between P700 and A1. (B) Marcus plot detailing $P700^+A_{1A}^-$ intrinsic recombination rates at 298 K versus the reaction free energy for ten different quinones incorporated. The parabolic fitted function is based on ET parameters derived previously (48) along with the intrinsic time constants listed in Table 6.2. A reorganization energy of 580 meV and a mean vibrational mode with energy of 56 meV was used.

$P700^+F_{A/B}^-$ recombination is characterized by a time constant of $\sim$80 ms for PSI with PhQ incorporated at RT. This recombination occurs via repopulation of the $A_1^-$ state. The actual time constant associated with $P700^+A_{1A}^-$ recombination at RT can be derived from the experimentally observed time constant only with the aid of kinetic modeling. The time constants derived from
modeling of RT data are listed in Table 6.2. For PSI with high potential quinones incorporated (quinones 7–10 in the tables) forward ET from $A_1^-$ to $F_X$ does not occur, and $P700^+A_1^-$ recombination occurs instead (21). So, for quinones 7–10 in Table 6.2 $\tau_{\text{obs}}$ and $\tau_{\text{int}}$ are similar. Fig. 6.3B shows a Marcus plot associated with $P700^+A_{1A}^-$ radical pair recombination at RT.

6.3.2 Modeling ET from $F_B^-$ to the Mobile Fd Electron Acceptor

In this section an extent of contribution by inverted-region ET to the high quantum efficiency of solar energy conversion in PSI in situ (PSI in the thylakoid membrane) is examined. In the main text inverted-region ET is demonstrated to occur in detergent isolated PSI particles that have been stripped from the thylakoid membrane. However, for native PSI in the thylakoid membrane, light excitation results in the transfer of electrons through PSI, to a mobile (membrane-diffusible) Fd that docks near $F_B$ (32, 141). Although inverted-region ET occurs in isolated PSI particles, it is not possible to assess to what extent this inverted-region ET mechanism contributes to the very high quantum yields observed for solar energy conversion in PSI embedded in the thylakoid membrane.

With this in mind, kinetic models are developed to estimate solar energy conversion efficiency (also loosely termed “photosynthetic efficiency”) in PSI in the presence of membrane-diffusible donors and acceptors, when the recombination ET process occurs in the inverted region, and when it is optimized ($-\Delta G = \lambda$). As indicated in the main text, solar energy conversion efficiency (photosynthetic efficiency) is estimated through calculation of the extent of $P700^+$ rereduction that occurs via unproductive charge recombination (ET from $A_1^-$ back to $P700^+$).

In previous kinetic models, only ET in isolated PSI particles is studied. In this case only ET to the terminal electron acceptors $F_A$ and $F_B$ and associated recombination reactions were considered (48). For membrane-bound PSI, electrons drain out of PSI via a diffusible Fd cofactor
To account for this process, here an extended kinetic model, in which ET from $F_B^-$ to Fd can occur (Fig. 6.4), is considered. The coupled differential equations associated with the kinetic model are listed (Eq. 6.9) and solved after calculation and input of the relevant ET parameters and rate constants, as described in above.

\[
\begin{align*}
\frac{d}{dt}[A_{1A}^-](t) &= -(k_{1A} + k_{0A})[A_{1A}^-](t) + k_{-1A}[F_X^-](t) + k_{-0A}[P700](t) \\
\frac{d}{dt}[A_{1B}^-](t) &= -(k_{1B} + k_{0B})[A_{1B}^-](t) + k_{-1B}[F_X^-](t) + k_{-0B}[P700](t) \\
\frac{d}{dt}[F_X^-](t) &= -(k_2 + k_{-1A} + k_{-1B})[F_X^-](t) + k_{1A}[A_{1A}^-](t) + k_{1B}[A_{1B}^-](t) + k_{-2}[F_A^-](t) \\
\frac{d}{dt}[F_A^-](t) &= -(k_{-2} + k_3)[F_A^-](t) + k_2[F_X^-](t) + k_{-3}[F_B^-](t) \\
\frac{d}{dt}[F_B^-](t) &= -(k_{-3})[F_B^-](t) + k_3[F_A^-](t) - k_{Fd}[F_B^-](t) \\
\frac{d}{dt}[Fd^-](t) &= k_{Fd}[F_B^-](t) \\
\frac{d}{dt}[P700](t) &= -(k_{0A} + k_{0B})[P700](t) + k_{0A}[A_{1A}^-](t) + k_{0B}[A_{1B}^-](t) \\
[A_1^-](t) &= [A_{1A}^-](t) + [A_{1B}^-](t)
\end{align*}
\]

Equations 6.9

For PSI in thylakoid membranes, electrons are transferred from $F_B$ to the mobile electron carrier Fd (32, 141). The midpoint potentials of $F_B$ and Fd are approximately $-556$ (39) and $-420$
(32) mV, and ET from Fd\(^{-}\) back to F\(_{B}\) is unlikely. This idea is incorporated into the kinetic model in Fig. 6.4.

ET from F\(_{B}\)\(^{-}\) to Fd has been studied extensively, and in cyanobacterial systems is characterized by three first order time constants (\(\tau/\hbar\)) of 721 ns (0.4), 23.8 \(\mu s\) (0.4), and 102.5 \(\mu s\) (0.2) (32, 141). The relative amplitudes of the three phases are indicated in parenthesis.

The extent of P700\(^+\) rereduction for each of the three quoted lifetimes were simulated separately. The simulations were undertaken for PSI with PhQ incorporated at RT (i.e., the native system under physiological conditions), using the parameters (reorganization energies, midpoint potentials, and time constants) calculated previously (48). The relative fraction of P700\(^+\) rereduction calculated is 0.02\%, 0.12\%, and 0.47\% for the 721-ns, 23.8-\(\mu s\), and 102.5-\(\mu s\) phases of Fd reduction, respectively (Table 6.3). The average extent of P700\(^+\) rereduction, found from a weighted average calculated using the relative amplitudes of the three phases, is 0.15\%.

### Table 6.3

A summary of calculated fractions undergoing charge recombination. The term “PSI/Fd, Inverted” refers to the kinetic model with Fd (Fig. 6.4) and the unaltered reorganization energies calculated previously (48). The term “PSI/Fd, Optimal” refers to the kinetic model with Fd and the altered reorganization energies that achieve optimal ET rates from A\(_{1}\)\(^{-}\) to P700\(^+\). Total recombination percentage is calculated by taking the average of fractions of each component weighted by the decay amplitude. The first-order and second-order phases each composed 50\% of the total fractions, and the first-order phases were composed of three phases (40\%, 40\%, and 20\% components).

<table>
<thead>
<tr>
<th>Model</th>
<th>Total Recombination %</th>
<th>First-order (50%)</th>
<th>Second-order (50%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>721 ns (40%)</td>
<td>23.8 (\mu s) (40%)</td>
</tr>
<tr>
<td>Isolated PSI</td>
<td>100.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PSI/Fd, Inverted</td>
<td>2.01</td>
<td>0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>PSI/Fd, Optimal</td>
<td>28.00</td>
<td>0.62</td>
<td>3.27</td>
</tr>
</tbody>
</table>

For PSI in the presence of Fd, a phase with time constant that is dependent on the Fd concentration is also observed (32, 141). This is referred to as a second order phase (142) related to diffusion-limited binding of Fd to the PSI complex. For the kinetic simulation presented here,
the relative fraction of the second-order phase is 50% (142), which means that the first order phases are associated with only 50% of the PSI complexes where Fd is “pre-bound”. A lower limit of the lifetime ($\tau_{1/e}$) of this second order phase is 908.9 $\mu$s (142). With the quoted lifetime and amplitude of the second order phase $P700^+$ re-reduction occurs in 3.87% of the PSI particles. Since this kinetic model accounts for 50% of the total PSI population, and the other 50% undergoes first order reactions, the weighted average fraction undergoing charge recombination is calculated to be 2%. The efficiency of solar energy conversion in PSI is therefore 98%. The dotted curves in Fig. 6.5 show the (weighted) population evolution of $A_1^-$ (blue), $F_X^-$ (red), $F_A^-$ (green), $F_B^-$ (orange) and $P700$ (dark blue) when inverted region ET occurs. The dotted dark blue curve clearly demonstrates the $\sim 2\%$ of centers undergoing radical pair recombination, to (re)form neutral $P700$.

![Figure 6.5](image.png)

**Figure 6.5** Population dynamics of ($A_1A^-+A_1B^-$) (cyan), $F_X^-$ (red), $F_A^-$ (green), $F_B^-$ (orange) and $P700$ (black) simulated using the first and second-order time constants and amplitudes associated with ET from $F_B^-$ to Fd. Dotted lines are for PSI where $P700^+A_1A^-$ and $P700^+A_1B^-$ radical pair recombination occur in the inverted region. Solid lines are for PSI where radical pair recombination is optimized by modifying the reorganization energy to match the free energy. In the inverted region the extent of recovery of $P700$ remains low (black, dotted) while it is considerably increased for the situation where the rate is optimized ($-\Delta G^0 = \lambda$) (black, solid).

To assess the importance of the inverted-region ET mechanism in modulating photosynthetic efficiency, a situation is considered in which the ET from $A_1^-$ to $P700^+$ is optimal.
The optimal ET rate is achieved when $-\Delta G^0 = \lambda$. This requires setting the reorganization energy ($\lambda$) at 1.085 eV (Table 6.2).

With this reorganization energy, for isolated PSI in the absence of Fd, the overall $\text{P700}^+\text{F}_{\text{A/B}^-}$ charge recombination reaction is characterized by a time constant of 3.60 ms and $\text{A}_1^- \rightarrow \text{P700}^+$ proceeds with a time constant of 50.4 $\mu$s. Such time constants are not in keeping with experiment. Fig. 5 (solid lines) shows the population evolution of the various radical states in this model where $-\Delta G^0 = \lambda$.

For the first-order phases the relative extent of $\text{P700}^+$ rereduction is 0.62%, 3.27%, and 11.33% for the 721-ns, 23.8-$\mu$s, and 102.5-$\mu$s phases, respectively. For the second-order phase, the relative fraction is 52.18% (Table 6.3). The weighted average of the four fractions that therefore lead to rereduction of $\text{P700}^+$ is 28.00%. That is, the efficiency of ET in PSI is reduced from 98% to 72.00% when the $\text{A}_1^- \rightarrow \text{P700}^+$ ET process is optimal ($-\Delta G^0 = \lambda$). The various time constants, amplitudes, and fractions undergoing recombination are outlined in Table 6.3.

To summarize, unproductive charge recombination is $\sim$2% where inverted-region ET occurs. This fraction increases to 28% when ET is taken to be optimal. The situation could be considerably worse than this, as only a “best-case” scenario where second-order phases (with large time constants) are limited to 50% is considered. These calculations suggest that $\text{A}_1^- \rightarrow \text{P700}^+$ ET for the native system has to occur in the inverted region for highly efficient solar energy conversion in PSI to occur.

6.3.3 Kinetic Models Including Membrane-Diffusible Electron Donors

In the sections above it was demonstrated that unproductive ET from $\text{A}_1^-$ to $\text{P700}^+$ in isolated PSI particles occurs in the Marcus inverted region, and it was proposed that this recombination reaction contributes to the high quantum efficiency observed for solar energy
conversion in PSI in the thylakoid membrane in the presence of membrane-diffusible donors and acceptors. This hypothesis was tested by constructing a kinetic model that included ET from $F_B^-$ to the mobile electron carrier Fd ($F_B^-\rightarrow Fd$). The simulation showed that photosynthetic efficiency dropped from $\sim98\%$ when inverted-region ET occurred, to $\sim72\%$ if the corresponding ET process was optimal. In these calculations photosynthetic efficiency is equated with the extent of unproductive $P700^+A_1^-\rightarrow P700 A_1$ recombination.

The calculations above, however, did not consider donor-side ET to $P700^+$ that could compete with charge recombination, and in so doing led to an increase in the extent of productive forward ET (charge recombination cannot occur if $P700^+$ has been rereduced).

Fig. 6.6 shows the kinetic model considered in this section, to investigate what effect donor-side ET may have on photosynthetic efficiency in the presence and absence of inverted-region ET.

![Figure 6.6 Kinetic model that includes both a mobile donor (Pc) and acceptor (Fd) species. Mobile donor/acceptor is red/blue, respectively. This model extends on the model outlined in Fig. S4 by including a Pc cofactor that can re-reduce $P700^+$. Numbers in brackets indicate the initial populations of the various states in the simulations.](image)

For membrane-bound PSI in plants and algae, $P700^+$ is rereduced by plastocyanin (Pc), while in some strains of cyanobacteria the donor is cytochrome $c_6$ (cyt-$c_6$). In this section the donor will simply be referred as Pc.
In the kinetic models, solar energy conversion efficiency (or photosynthetic efficiency) is assessed by comparing the fractions of productive forward ET and unproductive charge recombination that leads to P700$^+$ rereduction. As in the previous section, ET from F$_B^-$ to Fd represents a unidirectional drain.

For membrane-bound PSI, as considered in the context of the model outlined in Fig. 6.6, rereduction of P700$^+$ can occur via charge recombination, and also by ET from Pc.

In the model developed here Pc is added as a “source,” and is assigned an arbitrary initial population of 100%, along with 50% each for A$_{1A}$ and A$_{1B}$. That is, the Pc/PSI ratio is 1.0. The linear differential equations to be solved are listed in Eq. 6.10:

\[
\begin{align*}
\frac{d}{dt} [A^-_{1A}](t) &= -(k_{1A} + k_{0A})[A^-_{1A}](t) + k_{-1A}[F^-_X](t) + k_{-0A}[P700](t) \\
\frac{d}{dt} [A^-_{1B}](t) &= -(k_{1B} + k_{0B})[A^-_{1B}](t) + k_{-1B}[F^-_X](t) + k_{-0B}[P700](t) \\
\frac{d}{dt} [F^-_X](t) &= -(k_2 + k_{-1A} + k_{-1B})[F^-_X](t) + k_{1A}[A^-_{1A}](t) + k_{1B}[A^-_{1B}](t) + k_{-2}[F^-_A](t) \\
\frac{d}{dt} [F^-_A](t) &= -(k_{-2} + k_{3})[F^-_A](t) + k_{2}[F^-_X](t) + k_{-3}[F^-_B](t) \\
\frac{d}{dt} [F^-_B](t) &= -(k_{-3})[F^-_B](t) + k_{3}[F^-_A](t) - k_{Fd}[F^-_F](t) \\
\frac{d}{dt} [Fd^-](t) &= k_{Fd}[F^-_F](t) \\
\frac{d}{dt} [P700](t) &= -(k_{-0A} + k_{-0B})[P700](t) + k_{0A}[A^-_{1A}](t) + k_{0B}[A^-_{1B}](t) + k_{Pc}[Pc](t) \\
\frac{d}{dt} [Pc](t) &= -k_{Pc}[Pc](t) \\
[A^-_r](t) &= [A^-_{1A}](t) + [A^-_{1B}](t)
\end{align*}
\]

Equations 6.10

There are now two separate ET processes, but the maximum population any PSI radical cofactor can achieve is obviously 100%. Initially, upon light excitation, P700 is oxidized in 100% of the PSI particles. The model simulation is run and the temporal evolution of ET radical states
is calculated. An example of these population evolutions is shown in Fig. 6.7. Once the P700 population fully recovers (P700 population reaches 100%) then all of the remaining radical states must decay via ET through Fd. This is the productive ET process and so the photosynthetic efficiency can be estimated by the population of radical states present when P700+ is fully (re)reduced. The temporal evolution of the population of radical states is outlined in Fig. 6.7.

Figure 6.7 Example indicating how solar energy conversion efficiency is calculated in the Pc-PSI-Fd kinetic model shown in Fig. 6.6. Population dynamics for \((A_{1A}^+ + A_{1B}^-)\) (blue), \(F_X^-\) (red), \(F_A^-\) (purple), \(F_B^-\) (brown), P700 (yellow) and Fd (blue). Population of \(A_{1}^-\), \(F_X^-\), \(F_A^-\), \(F_B^-\) and Fd remaining when P700 has fully recovered are indicated by dotted lines. These radicals account for ~99% of the total population. Dotted lines are for PSI where P700\(^+\)\(A_1^-\) radical pair recombination occurs in the inverted region. Solid lines are for PSI where P700\(^+\)\(A_1^-\) radical pair recombination is optimized by modifying the reorganization energy to match the reaction free energy.

Pc is a diffusible cofactor and ET from Pc to P700\(^+\) is multi-phasic (33, 143). A fast first order phase results from the fraction of PSI in which Pc is pre-bound. Slower second-order phases follow from the unbound fraction (33, 144). The fast phase occurs on a µsec timescale, while the slow phases occur on 100 µs to 10 ms timescales. However, the lifetimes and associated fractions of the phases are highly species-dependent (33). Observed lifetimes and relative fractions of the fast first-order phase for PSI from different species are summarized in Table 6.4.

Table 6.4 Reported lifetimes and fractions for first-order ET from Pc to P700\(^+\) for PSI from different species. The previously reported half-lives were converted to exponential lifetimes. For Thermosynechococcus (T.) elongatus and S6803, a fast phase is not observed. For Anabaena (A.)
variabilis, a fast phase is observed and the fraction is reported, but the lifetime was not mentioned.  

$^{a-d}$ From Refs (144)$^a$, (145)$^b$, (146)$^c$, and (147)$^d$.

<table>
<thead>
<tr>
<th>Species</th>
<th>Lifetime (µs)</th>
<th>Fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thermosynechococcus elongatus</em>$^a$</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td><em>Synechocystis</em> sp. PCC 6803$^{a,b}$</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td><em>Synechococcus</em> sp. PCC 7002$^a$</td>
<td>4.32</td>
<td>~70</td>
</tr>
<tr>
<td><em>Anabaena variabilis</em>$^a$</td>
<td>–</td>
<td>~85</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardtii</em>$^c$</td>
<td>5.77</td>
<td>60–70</td>
</tr>
<tr>
<td><em>Spinacia oleracea</em>$^d$</td>
<td>17.31</td>
<td>90–95</td>
</tr>
</tbody>
</table>

As indicated in Table 6.4, no fast phase is observed in some species of cyanobacteria, possibly indicating difficulty in forming a cyt-c₆ – PSI complex at high enough concentration. In *T. elongatus*, the fast phase is observed *in vitro* only when the cyt-c₆ concentration is increased (144). Some cyanobacteria therefore are structurally capable of forming a pre-bound complex, yet other factors in the organisms (such as the relative concentration of cyt-c₆) do not lead to the occurrence of a fast phase *in vivo*. In both green algae and plants, the fast first-order phases are always observed. For *Synechocystis* sp. PCC 6803, which lacks the first-order phase, two slow second-order phases are observed, with lifetimes are 216 – 288.5 µs and 2.88 ms and relative fractions are 50% each (145).

At RT, the fast and the slow phases of ET to P700$^+$ from Pc are considerably faster than radical pair recombination reactions. Therefore, P700$^+$ is rereduced almost exclusively by Pc. This can be seen in Fig. 6.7, where P700 recovers in a few tens of microseconds.

In the kinetic model outlined in Fig. 6.6, P700$^+$ is rereduced almost exclusively via ET from Pc. Given this, unproductive charge recombination cannot occur, and photosynthetic efficiency will automatically be high. In such a scenario, rereduction of P700$^+$ by charge recombination is small, even if the ET process does not occur in the inverted region.
Under continuous illumination the population of reduced Pc capable of ET to P700\(^+\) will depend on other factors, such as the re-reduction of oxidized Pc in the b\(_{0}\)f complex (148). This more realistic type of model will be considered in the next section.

In intact photosynthetic systems (whole leaves) under continuous illumination P700\(^+\) is photo-accumulated (149, 150), and P700\(^+\) re-reduction occurs on a much longer timescale than Fd reduction. Thus a more detailed kinetic model for donor side ET to P700\(^+\) is clearly required. Such a model is described in the next section. Given that P700\(^+\) is photo-accumulated in intact photosynthetic systems (whole leaves or cells) the suggestion is that donor side ET is somehow limiting.

### 6.3.4 Kinetic Models Including Secondary Electron Donors

Under continuous illumination of thylakoid membranes or even intact cells or whole leaves, P700\(^+\) is photo-accumulated. This indicates that re-reduction of Pc\(^+\) is rate limiting. In intact thylakoids or whole cells there are several steps leading to re-reduction of Pc\(^+\) but it is widely accepted that plastoquinol (PQH\(_2\)) oxidation is the rate-limiting step, taking place in 14–28 ms depending on the species (148, 151).

While Pc\(^+\) does not directly oxidize PQH\(_2\) (several steps of ET exists between PQH\(_2\) oxidation and Pc\(^+\) reduction), in a first step to develop a kinetic model that accounts for Pc\(^+\) re-reduction the kinetic model shown in Fig. 6.8 is considered, where a PQH\(_2\) → Pc\(^+\) process is included as a single step with a lifetime of 14.43 ms. Such a model is appropriate if PQH\(_2\) reduction is the rate limiting step in Pc\(^+\) re-reduction.
Figure 6.8  Extended kinetic model that includes Pc$^+$ re-reduction. In this PQH$_2$-Pc-PSI-Fd model, an initial population (indicated in gray) is assigned to $A_1$ (50 each to $A_{1A}$ and $A_{1B}$) and PQH$_2$ (100). The relative position of the cofactors (on a vertical scale) is arbitrary, and does not reflect differences in cofactor redox potentials.

In the model outlined in Fig. 6.8, PQH$_2$ is a source of electrons for rereduction of P700$^+$, and Pc acts as an intermediate between PQH$_2$ and P700. The same series of reaction rates that were considered in the previous sections was also used for the model in Fig. 6.8. The procedure used to calculate the overall photosynthetic efficiency is similar to that illustrated in Fig. 6.7 of the previous section. The linear differential equations to be solved are listed in Eq. 6.10 and the calculated photosynthetic efficiencies for $S7002$ and spinach PSI are summarized in Table 6.5.

\[
\frac{d}{dt}[A_{1A}^-](t) = -(k_{1A} + k_{0A})[A_{1A}^-](t) + k_{-1A}[F_X^-](t) + k_{0A}[P700](t)
\]

\[
\frac{d}{dt}[A_{1B}^-](t) = -(k_{1B} + k_{0B})[A_{1B}^-](t) + k_{-1B}[F_X^-](t) + k_{0B}[P700](t)
\]

\[
\frac{d}{dt}[F_X^-](t) = -(k_2 + k_{-1A} + k_{-1B})[F_X^-](t) + k_{1A}[A_{1A}^-](t) + k_{1B}[A_{1B}^-](t) + k_{-2}[F_A^-](t)
\]

\[
\frac{d}{dt}[F_A^-](t) = -(k_{-2} + k_3)[F_A^-](t) + k_2[F_X^-](t) + k_{-3}[F_B^-](t)
\]

\[
\frac{d}{dt}[F_B^-](t) = -(k_{-3})[F_B^-](t) + k_3[F_A^-](t) - k_{Fd}[F_B^-](t)
\]

\[
\frac{d}{dt}[Fd^-](t) = k_{Fd}[F_B^-](t)
\]

\[
\frac{d}{dt}[P700](t) = -(k_{-0A} + k_{-0B})[P700](t) + k_{0A}[A_{1A}^-](t) + k_{0B}[A_{1B}^-](t) + k_{Pc}[Pc](t)
\]

\[
\frac{d}{dt}[Pc](t) = -k_{Pc}[Pc](t) + k_{PQH_2}[PQH_2]
\]
\[
\frac{d}{dt} [PQH_2](t) = -k_{PQH_2} [PQH_2]
\]

\[
[A^-_1](t) = [A^-_{1A}](t) + [A^-_{1B}](t)
\]

Equations. 6.10

The calculated overall efficiencies listed in Table 6.5 are the same (within 0.15%) as that calculated using the model in Fig. 6.4, confirming the expectation that slow ET to P700+ will have an negligible impact on calculated photosynthetic efficiencies in the absence of an electron donor.

**Table 6.5** Summary of the ET efficiencies (%) calculated using the “PQH_2-Pc-PSI-Fd” model outlined in Fig. 6.8, for S7002 and spinach PSI. The Pc to P700+ ET lifetimes are listed in Table 6.4. ET from PQH_2 to Pc^+ is set as 14.43 ms. Opt refers to ET recombination in PSI with \(-\Delta G^0 = \lambda\). Inv refers to the situation where ET recombination in PSI occurs in the inverted region. The final column to the right lists the overall photosynthetic efficiency given the lifetimes of ET to Fd and the ET reactions from PQH2 to Pc and then to P700^+.

<table>
<thead>
<tr>
<th></th>
<th>Fd first-order</th>
<th>Fd second-order</th>
<th>Pre-bound efficiency</th>
<th>Pre-bound fraction</th>
<th>Overall efficiency</th>
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<tbody>
<tr>
<td></td>
<td>721ns (40%)</td>
<td>23.8us (40%)</td>
<td>102.5us (20%)</td>
<td>908.9us (100%)</td>
<td></td>
</tr>
<tr>
<td>Opt</td>
<td>99.38</td>
<td>96.73</td>
<td>88.67</td>
<td>48.15</td>
<td>72.16</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>72.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>99.73</td>
<td>95.3</td>
<td>96.13</td>
<td>97.99</td>
<td>97.99</td>
</tr>
<tr>
<td>Inv</td>
<td>99.98</td>
<td>99.88</td>
<td>99.53</td>
<td>96.13</td>
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<td>S7002</td>
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<td>Spinach</td>
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The kinetic models developed here confirm the overall conclusion, that charge recombination in the inverted region is indeed an important mechanism contributing to a considerable decrease in unproductive charge recombination, even for PSI in thylakoid membranes in the presence of diffusible donors and acceptors.

**6.4 Discussion**

In the past, radical pair recombination in photosynthetic RCs has often been suggested to occur in the inverted region (14, 40, 59, 66, 69, 73). This notion arises from considering the large free energy associated with such a recombination, which will likely be larger than the reorganization energy. That is, because radical pair recombination occurs in the inverted region,
the thermodynamically downhill charge recombination pathway is effectively slowed, in turn ensuring the promotion of the less thermodynamically favorable forward ET pathway.

ET reactions in the purple bacterial photosynthetic reaction centers (pbRC) have been by far the most widely studied (62), and it is primarily in connection with this system that the idea of inverted region ET is considered (69, 73). However, in pbRC the primary radical pair recombination reaction (so-called P*H_L− recombination), at least for the native or wild type (WT) system, is known not to occur in the inverted region (152-155). In fact the unproductive P*H_L− recombination reaction in WT pbRC is nearly activationless (65). In pbRC it appears to be the case that mechanisms related to protein relaxations in response to ET are (more) important in maximizing photosynthetic efficiency (152-155). At the time of the study, at least for native photosynthetic systems under physiological conditions, no unproductive photosynthetic ET process has ever been demonstrated to occur in the inverted region.

In the past, quinones have been incorporated into (very) harshly chemically pre-treated PSI particles. This harsh procedure strips about 90% of the chlorophyll, lipids and carotenoid molecules from the protein (132), leaving some doubts as to the integrity of the binding site surrounding the incorporated quinones. Nonetheless, for these harshly treated systems, it was demonstrated that the observed rate of the P700∗F_A/B− charge recombination at RT increases when higher potential quinones were incorporated (which decrease the reaction free energy associated with the P700∗A_1− recombination reaction) (40). At that time it was not known that ET in PSI was bidirectional, and kinetic modeling was not undertaken to establish the true (intrinsic) ET rates associated with P700∗A_1− recombination. Therefore, it could not be established if recombination in the native system occurred in the inverted region in these harshly treated photosynthetic proteins. Furthermore, in order to extract *in situ* redox potentials for the incorporated quinones in
these harshly treated PSI particles, the data obtained was modeled assuming a fixed P700+*A_1^- recombinatio
n rate for all of the different incorporated quinones. This assumption was necessary given the limited amount of experimental data available and the large number of parameters that had to be estimated in the kinetic model. However, this assumption implies that the inverted region electron transfer mechanism does not apply to the P700+*A_1^- recombination reaction.

Here unambiguous evidence is provided to (Fig. 6.3) support the notion that the unproductive P700+*A_1^- radical pair recombination reaction in PSI, at both RT and LT, occurs in the inverted region. One could possibly argue about the precise values of the parameters involved in modeling the ET reactions outlined in Fig. 6.3. However, there is no reasonable scenario in which the parameters could be sufficiently altered that the data points (particularly data point 4 for PhQ, which represents the native system) in Fig. 6.3 could somehow be transferred from the right to the left side in the Marcus plots. The data plotted in Fig. 6.2 make no assumptions about any kind of applicable ET theory, and indicate that whatever theory may be appropriate, it will likely involve an inverted parabolic relation between the (logarithm of the) ET rate and reaction free energy, with all of the experimental data being to the right of the curve’s peak. Nonadiabatic ET theory predicts exactly that.

6.4.1 Inverted-Region ET is Necessary for High Photosynthetic Efficiency

The data presented here provide an unambiguous example of an unproductive ET recombination process in a native photosynthetic system under physiological conditions occurring in the inverted region (data point 4 in Fig. 6.3B). In the presented analysis of the bioenergetics in isolated PSI particles (48) only ET to the iron–sulfur clusters and associated recombination reactions are necessarily considered. In the thylakoid membranes of plants and bacteria, however, membrane-diffusible electron donors and acceptors are present that could impact radical pair
recombination, and subsequently photosynthetic efficiency. An important question to address therefore is, to what extent inverted region ET could contribute to the high quantum efficiency of solar energy conversion observed for PSI in the thylakoid membrane, in the presence of diffusible electron donors and acceptors?

The data in Fig. 6.5 indicate that P700$^+$ rereduction occurs in 28% of the PSI particles when the P700$^+$A$_{1A}^-$ radical pair recombination is optimized. That is, the quantum efficiency for solar conversion in PSI drops to 72%. For reasons discussed above, this is likely to be a best-case scenario. The results summarized in Fig. 6.5 demonstrate that A$_1^-$ to P700$^+$ ET (for native PSI at RT in the presence of electron acceptors) has to occur in the inverted region for highly efficient solar energy conversion in PSI to occur. Kinetic models that are extended to include diffusible electron donors to P700$^+$ are also considered (Fig. 6.8). The overall result, however, is that these extended models do not significantly alter the calculated photosynthetic efficiency obtained using the model outlined in Fig. 6.4 (Tables 6.3 and 6.5). Therefore, inverted-region ET from A$_1^-$ to P700$^+$ (for native PSI at RT) is required for highly efficient solar energy conversion in PSI in the presence of diffusible electron donors and acceptors.

6.4.2 Comparison of ET Processes in Other Photosystem

The bioenergetic ET scheme for native PSI at RT shows that forward ET from A$_{1A}^-$ to F$_X$ is slightly endergonic, while forward ET from A$_{1B}^-$ to F$_X$ is slightly exergonic. The recombination reaction is very highly exergonic. The corresponding ET scheme is very different in pbRC, however, where forward ET from H$_A^-$ to Q$_A$ is highly exergonic (65) (see Fig. 1 in (132) for a comparison of the bioenergetics in pbRC and PSI). These differences in the energetics between PSI and pbRC arise because the PSI ET cofactors are by necessity highly reducing. That is, the
different mechanisms involved in promoting high photosynthetic efficiency in PSI compared with pbRC may simply be a consequence of the requirement that PSI generate highly reducing species.
7 STRONG H-BOND TO PHYLLOSEMIQUINONE IN THE A₁ BINDING SITE
STUDIED USING TIME-RESOLVED FTIR DIFFERENCE SPECTROSCOPY

7.1 Introduction

In photosynthetic oxygen evolving organisms, solar energy is captured and converted in two separate membrane-bound protein complexes called photosystem I (PSI) and photosystem II. In PSI light initiates the transfer of electrons from a chlorophyll species called P700, via a series of acceptors, to a terminal set of 4Fe-4S clusters termed F₄₅₄ (82). The electron transfer (ET) cofactors, termed P700, A₀, and A₁, are bound to the membrane spanning protein subunits called PsaA and PsaB. The terminal 4Fe-4S clusters are bound to the PsaC protein subunit (82). In PSI the ET cofactors are arranged in two near identical C₂ symmetrical branches (Fig. 7.1).

Figure 7.1  (A) Arrangement of the ET cofactors in PSI, generated using the 2.5 Å X-ray crystal structure of PSI from T. elongatus (PDB 1JB0) (10). (B) View of PhQ in the A₁ₐ binding site. Possible H-bonding interactions are shown (dotted). (C) Molecular model for neutral PhQ in the A₁ binding site. Nitrogen/oxygen/sulfur/carbon atoms are colored blue/red/yellow/grey, respectively. High QM layer shown in ball and stick representation. Low QM layer shown in stick representation. MM layer shown as thin sticks. (D) Model demonstrating bond lengths and angles calculated for neutral and reduced PhQ. Black/Red/Blue: Neutral/Reduced/x-ray.
Following light excitation of P700, and electron is transferred from P700 via A₀ (a chlorophyll-α molecule) to A₁ within ~50 ps (35). A₁ is a phylloquinone (PhQ) molecule (82). PhQ is a 2-methyl-3-phytyl-1,4-naphthoquinone (also known as vitamin K₁). The structure and numbering of PhQ, and its relative orientation with respect to several nearby amino acids in the A₁ binding site, is outlined in Fig. 7.1B.

The main goal in this chapter is to undertake spectroscopic studies of PSI particles with different quinones incorporated into the A₁ binding site. In pioneering early studies foreign quinone incorporation into the A₁ binding site in PSI relied on first chemically extracting the native quinone from the binding site (156). The downside of this approach was that chemical extraction also led to the extraction of most of the chlorophyll pigments, and possibly to considerable alteration of the binding site itself (14).

More recently methods for quinone incorporation into PSI have relied on the use of mutant cyanobacterial cells from Synechocystis sp. PCC 6803 (S6803) in which the menB gene has been inactivated (44, 89). In mutant cells where the menB gene has been inactivated PhQ biosynthesis is inhibited. In PSI particles isolated from the mutant cells, plastoquinone-9 (PQ₉) has been shown to be recruited into the A₁ binding site instead of PhQ (44, 63, 89). In this chapter PSI particles from these mutant cells will be referred to as menB⁻ PSI particles. In menB⁻ PSI foreign quinones can replace PQ₉ in the A₁ binding site simply by incubating the particles in the presence of the quinone of interest (7, 46, 123, 157). This approach for incorporating non-native quinones into PSI involves minimal disruption of the protein binding pocket and is thus preferable to the chemical extraction method.

In the previous chapter, it was shown that both PhQ and 2-methyl-1,4-naphthoquinone (2MNQ) can be incorporated nearly quantitatively into the A₁ binding site in menB⁻ mutant PSI
simply by incubating the PSI particles in the presence of the quinone of interest (30). The 
incorporated quinones are fully functional. These PSI particles will simply be referred as PSI with 
PhQ or 2MNQ incorporated. The structure and numbering of PhQ is outlined in Fig. 7.1b. PhQ 
and 2MNQ differ only in that the phytyl chain of PhQ is replaced with a hydrogen (H) atom. 

For wild-type (WT) cyanobacterial PSI from S6803 at room temperature (RT, ~298 K), 
forward ET from $A_1^-$ to $F_X$ proceeds in a bi-directional fashion down both the A and B branches 
with time constants of ~310 and 20 ns, respectively (20, 27, 28, 30). ET in WT PSI at 77 K is 
heterogeneous, however, with the $P700^+A_1^-$ state recombining in ~45% of the particles, the 
$P700^+F_X^-$ state recombining in ~20% of the PSI particles, and in ~35% of the PSI particles ET is 
irreversible (26). Similar percentages are found for PSI with 2MNQ incorporated into the $A_1$ 
binding site (30). For PSI with PhQ/2MNQ in the $A_1$ binding site at 77K, the $P700^+A_1^-$ state 
recombines in ~360/240 $\mu$s, respectively (30). This time constant is associated with ET on the A 
branch, as the $P700^+A_1^-$ charge recombination occurs almost exclusively through the A branch at 
cryogenic temperature (23, 27, 47). 

Of interest in this chapter is the production of time-resolved (TR) infrared (IR) difference 
spectra (DS) associated with the $P700^+A_1^-$ state. For time-resolved step-scan (TRSS) FTIR 
measurements with microsecond ($\mu$s) time resolution, spectra with very high signal to noise ratio 
can be obtained (115, 158, 159). Since $P700^+A_1^-$ recombines in ~360/240 $\mu$s at 77 K, time-
resolved FTIR experiments aimed at probing the quinone in the $A_1$ binding site are usually 
conducted at 77 K, and in this chapter $\mu$s TRSS FTIR DS experiments at 77 K on PSI particles 
with either PhQ or 2MNQ incorporated into the $A_1$ binding site is described. 

The PhQ molecule occupying the $A_1$ binding site in PSI has a midpoint potential close to -700 mV (see (7) for a review), making it one of the most reducing quinones in biology. This
unprecedented redox potential is in part a result of interactions of PhQ with the surrounding protein environment. A detailed view of (neutral) PhQ and some of the surrounding amino acids in the A₁ binding site is shown in Fig. 7.1b. PhQ is asymmetrically H-bonded, with the C₁=O group free from H-bonding, and the C₄=O group is H-bonded to the backbone NH group of LeuA722 (T. elongatus numbering scheme). The N- O distance is 2.67 Å. The precise details of this H-bond (for neutral PhQ) are not easily delineated from the crystal structure. FTIR DS is sensitive to small changes in H-bonding that are well beyond crystallographic structural resolution, in both the neutral and reduced quinone states, and in this chapter one of the goals is to probe the nature of this H-bond for both the neutral and reduced states of incorporated quinones.

A₁/A₁ TRSS FTIR DS have been obtained previously. To aid in spectral band interpretation and assignment in these previous studies, density functional theory (DFT) based vibrational frequency calculations for PhQ in the gas phase (91), or PhQ that is asymmetrically H-bonded (92, 115), were undertaken. These calculations were useful but do not take into account the protein environment surrounding the quinone in the A₁ binding site. More recently, ONIOM type (160) QM/MM calculations have been undertaken in order to help in gaining a better understanding of the bands appearing in QA-/QA FTIR DS that had been obtained using purple bacterial photosynthetic reaction centers (161, 162). It was clearly shown that these QM/MM calculations provided a much more accurate simulation of the experimental spectra than that obtained using calculations associated with quinones molecules in the gas phase or solvent.

In this chapter μs TRSS FTIR DS at 77 K is used to study PSI particles with PhQ or 2MNQ incorporated. To aid in interpretation and assignment of the bands in the spectra obtained, three-layer ONIOM-type QM:QM:MM calculations were also undertaken.
7.2 Materials and Methods

All quinones and solvents used, as well as all other chemicals for buffers and growth media, were obtained from Sigma-Aldrich (Sigma-Aldrich Inc. St Louis, Mo.) and used as received.

7.2.1 FTIR Absorption Spectra of Quinones in Solution

FTIR absorption spectra at 4 cm\(^{-1}\) resolution were measured using a Bruker IFS66 FTIR spectrometer (Bruker Optics, Billerica, MA) with a Graseby mercury cadmium telluride detector. PhQ and 2MNQ were dissolved in tetrahydrofuran (THF), and placed between a pair of calcium fluoride windows separated by a \(~25\ \mu m\) spacer. The IR spectrum of pure THF was subtracted from the sample spectrum in order to eliminate the well-known THF bands from the spectrum.

7.2.2 Preparation of PS I Particles with Different Quinones Incorporated

Trimeric PSI particles were isolated from menB\(^-\) mutant cells from Synechocystis sp. PCC 6803 (S6803) and then stored as described previously (44). MenB\(^-\) PSI particles were incubated in the presence of \(~500X\) molar excess of the quinone of interest (PhQ or 2MNQ). Quinones were dissolved in ethanol and added to a suspension of PSI particles in such a way that the volume of the solvent in the mixture never exceeds 2% of the total volume. The PS I particle/quinone mixture was incubated at 277 K in the dark for \(~24\) hours with stirring.

7.2.3 Sample Preparation for FTIR DS

PSI particles were washed in buffer (50mM Tris buffer with 0.04% β-DM detergent), then ultra-centrifuged to produce a soft pellet. Sodium ascorbate (20 mM) and phenazine methosulfate (20 \(\mu M\)) were added and the soft pellet (0.1 \(\mu L\) each), which was then squeezed between two circular calcium fluoride (CaF\(_2\)) windows. The sample thickness (spacing between the two windows) was adjusted so that the peak of the amide I band (at \(~1654\ \text{cm}^{-1}\)) had an optical density
less than 1.0. For measurements at 77 K the sample was loaded into a liquid nitrogen cooled cryostat (Cryo Industries of America Inc., Manchester, NH).

Before 77 K TRSS FTIR DS measurements were undertaken, photo-accumulated [P700+ – P700] FTIR DS were recorded at 77K. For photo-accumulation measurements, a 20 mW helium neon laser, expanded to a spot size of ~ 1cm at the sample, was used for light excitation. [P700+ – P700] light minus dark FTIR DS were constructed as described previously (163).

7.2.4 Microsecond TRSS FTIR Difference Spectroscopy at 77K

TRSS FTIR DS, with 6 µs time resolution at 77 K, were undertaken in a manner similar to that described previously (88, 90), using a Bruker Vertex 80 FTIR spectrometer. Data were collected in the 1950-1100 cm⁻¹ region at 4 cm⁻¹ spectral resolution. Long pass filters were used to block light above 1950 cm⁻¹, and CaF₂ windows blocked light below 1100 cm⁻¹. For each PS I sample, measurements are repeated 3 times and then averaged. The standard error of the spectra is taken as a measure of experimental variability (noise level) in the measurements.

Global analysis of the TRSS FTIR DS were undertaken using Glotaran (164). The TRSS FTIR DS were fitted globally to multi-exponential functions and decay-associated spectra (DAS) were constructed. Previously, kinetics at selected wavenumbers have been analyzed along with the transient absorption kinetics in the visible spectral range, and the reaction lifetimes for the P700+Au⁻ charge recombination reaction have been determined for PSI with PhQ and 2MNQ incorporated (30). [P700+Au⁻ – P700Au] FTIR DAS were obtained with similar reaction lifetimes.

7.2.5 Three Layer ONIOM QM/QM/MM Calculations

All calculations were undertaken using Gaussian09 software (165) (Gaussian Inc. Wallingford, CT). Molecular models used in DFT calculations were constructed starting from the PSI crystal structure of PSI from T. elongatus (PDB entry 1JB0) (10). Near identical starting
structures can be obtained using the crystal structure of PSI from S6803 (137). PhQ in the A$_{1A}$ binding site was used. In all cases, the phytol tail of PhQ was truncated to only a 5-carbon unit [CH$_2$CHC(CH$_3$)$_2$]. Hydrogen atoms were added to molecular models using GaussView 5 software (Gaussian Inc. Wallingford, CT).

To account for the protein environment, three-layer ONIOM-type calculations were undertaken. The quinone atoms are treated at the highest level of theory (QM-high) using the B3LYP functional and the 6-31+G(d) basis set. It was shown previously that this level of theory was appropriate for the calculation of the vibrational properties of neutral and reduced quinones (91). For 2MNQ in the A$_1$ binding site, the truncated phytol tail of PhQ is simply replaced by a hydrogen (H) atom. This particular orientation is based on previous EPR studies that showed 2MNQ is incorporated in the same position and orientation as that of native PhQ, with the methyl group positioned meta to the H-bonded carbonyl (123, 166).

Atoms of important bonding amino acids (LeuA722, TrpA697, PheA689, SerA692, and MetA688) are included at the next level of theory (QM-low) using the B3LYP functional and the 6-31G(d) basis set. The MM layer includes atoms of residues PheA685 to LeuA700 and ArgA720 to IleA725. MM layer atoms are treated using UFF (167). These particular residues and atoms were considered here mainly because they are essentially the same as that considered previously in a two-layer ONIOM approach undertaken in order to calculate magnetic spectroscopic parameters of PhQ in PSI (168). One of the goals in these calculations is to be able to connect to these previous calculations.

Standard protonation states were used for all amino acids. To consider the electrostatic interaction between the MM and both QM layers, electronic embedding (169) was employed. The QM:QM:MM model used here is shown in Fig. 7.1c.
For geometry optimization in ONIOM calculations all backbone heavy atoms of amino acids are constrained. The heavy atoms of amino acid side-chains and the quinone, as well as all hydrogen atoms, are unconstrained. For PhQ$^-$ and 2MNQ$^-$, ONIOM calculations were undertaken by specifying the total charge of the QM layer as $-1$.

The QM-low layer was connected to the MM layer using the link atom approach (170). One of the advantages of the three-layer ONIOM approach is that the link atoms are distant from the pigment of interest, minimizing possible complications that may arise due to electrostatic interactions associated with the link atoms. A second advantage of the three-layer ONIOM approach is that the optimized quinone (the high QM layer) can be considered separately from amino acids in vibrational frequency calculations. This is an important advantage as it allows the easy calculation of normal mode potential energy distributions (PEDs) (see below).

Following geometry optimization of the three-layer model system, the quinone molecule was extracted and considered separately in DFT based vibrational frequency calculations using the B3LYP functional and the 6-31+G(d) basis set.

A general assignment of calculated vibrational frequencies to molecular groups is based on visual identification of the molecular groups that most prominently contribute to the vibration. This visual identification is carried out using GaussView 5, in which the atomic motions associated with each of the vibrational modes is animated (see animations of the vibrations in the supplementary materials). In addition to these animations, PEDs associated with the normal modes are also calculated using VEDA (171). PEDs allow an estimate of how much a molecular group vibration contributes to a normal mode.

In the work reported here all normal mode frequencies and intensities are calculated. With both the frequency and intensity information IR stick spectra can be constructed. By convolving
these stick spectra with a Gaussian function of 4 cm\textsuperscript{-1} half-width more realistic-looking spectra are constructed. These convolved stick spectra are referred as absorption spectra.

7.3 Results

7.3.1 FTIR Absorption Spectra of Quinones in THF

Fig. 7.2 shows FTIR absorption spectra obtained for PhQ and 2MNQ in THF. For PhQ, the bands at 1462 and 1377 cm\textsuperscript{-1} have been assigned to δCH\textsubscript{2} and δCH\textsubscript{3} modes of the phytol chain (91). In agreement with this assignment, these two bands disappear in the 2MNQ IR spectrum.

For neutral PhQ in THF, the band at 1662 cm\textsuperscript{-1} is due to the antisymmetric stretching vibration of both C=O groups (91). Replacement of the phytol tail with an H atom (as in 2MNQ) leads to a 4 cm\textsuperscript{-1} upshift in frequency of this antisymmetric coupled C=O vibration (Fig. 7.2). The band at 1619 cm\textsuperscript{-1} in the PhQ absorption spectrum is due mainly to the C\textsubscript{2}=C\textsubscript{3} stretching vibration [\nu(C\textsubscript{2}=C\textsubscript{3})] (91). This band upshifts 8 cm\textsuperscript{-1} to 1627 cm\textsuperscript{-1} for 2MNQ. The band at 1597 cm\textsuperscript{-1} in both the PhQ and 2MNQ spectra is due to C=C stretching of the aromatic ring of the NQ (91).

![FTIR absorption spectra for (A) PhQ and (B) 2MNQ in tetrahydrofuran (THF). The spectra were scaled so that the intensities of the bands at 1662 and 1666 cm\textsuperscript{-1} are similar.](image)
7.3.2  \([P700^+A_1^- – P700A_1]\) FTIR DAS

For the \textit{menB}^- PSI particles used here, it was previously shown that 2MNQ and PhQ are incorporated nearly quantitatively into the A$_1$ binding site (30). The DAS for PSI with PhQ/2MNQ incorporated have lifetimes of \(\sim 360/240\) \(\mu\)s. These lifetimes are identical to that found from visible transient absorption spectroscopy, and are associated with \(P700^+A_1^-\) radical pair recombination in PSI with PhQ/2MNQ incorporated, respectively. Kinetics at select infrared frequencies are outlined in Fig. 7.3, and the spectral time-slices are shown in Fig. 7.4.

Fig. 7.5a and 7.5b show \([P700^+A_1^- – P700A_1]\) FTIR DAS obtained at 77 K for PSI with PhQ and 2MNQ incorporated, respectively. The DAS shown in Fig. 7.5a are compared to static photo-accumulated \([P700^+ – P700]\) FTIR DS in Fig. 7.6.

**Figure 7.3** Kinetics of absorption changes at several infrared wavelengths obtained at 77 K following 532 nm laser flash excitation of PSI particles with PhQ (left) and 2MNQ (right) incorporated. Time resolution was 6 \(\mu\)s. Spectral resolution was 4 cm$^{-1}$. 
Figure 7.4  Time-resolved [P700$^{+}$A$_{1}$$^{-}$ − P700A$_{1}$] FTIR DS in the 1800-1390 cm$^{-1}$ region, obtained following laser flash excitation of menB$^{-}$ PSI particles with PhQ ($a$) and 2MNQ ($c$) incorporated. Spectra at $t = 0$, 12, 54, 102, 150, 204, 252, 300, 248, 402, 450, 498, 552, 600, 648, 702, and 750 µs after the laser flash are shown. Except for the spectrum shown at $t = 0$ µs (green), the spectra shown are the average of three spectra centered at the indicated time point. From global analysis, three DAS are obtained for PSI with PhQ ($b$) and 2MNQ ($d$) incorporated. A ~15 µs phase ($red$) is associated with a heating artifact related to laser flash excitation. A ~360/240 µs phase for PSI with PhQ/2MNQ is due to P700$^{+}$A$_{1}$$^{-}$ charge recombination. A DAS associated with the non-decaying component is shown in $black$. 
**Figure 7.5** [P700⁺A₁⁻ – P700A₁] TR FTIR DAS for menB⁺ PSI particles with PhQ (a) and 2MNQ (b) incorporated into the A₁ binding site. The spectra shown are the average of 3 separate experiments. The standard error spectra are also shown (dotted). Corresponding [P700⁺ – P700] FTIR DS are shown in Fig. 7.4. (c) (2MNQ – PhQ) FTIR DDS obtained by subtracting spectrum a from b. Propagated standard error is overlaid to the DDS (shaded).
Figure 7.6  Static, photo-accumulated [P700$^+$ – P700] FTIR DS for menB$^-$ PSI with PhQ (a), and 2MNQ (c) incorporated into the A$_1$ binding site. Corresponding [P700$^+$A$_1^-$ – P700A$_1$] TR FTIR DAS (from Fig 7.3a) are also shown (b, d). The static and time-resolved spectra are scaled so that the 1717/1697 cm$^{-1}$ difference band is of similar intensity. The standard error associated with the static spectra are also shown (dotted). The spectrum resulting from the subtraction of the two [P700$^+$ – P700] FTIR DS (c – a) is also shown (e), along with the propagated standard error spectrum (f). This spectrum suggests only very small alterations in P700 (or P700$^+$) resulting from changes in the tail associated with the incorporated naphthoquinone. [A$_1^-$ – A$_1$] FTIR DS for menB$^-$ PSI with PhQ (g) and 2MNQ (h) incorporated into the A$_1$ binding site. [A$_1^-$ – A$_1$] FTIR DS are calculated by subtracting the [P700$^+$ – P700] FTIR DS from [P700$^+$A$_1^-$ – P700A$_1$] TR FTIR DAS (b – a and d – c).
[\text{[A}_1^- - \text{A}_1\text{]} \text{ FTIR DS}] that results from subtracting [P700^+ - P700] FTIR DS from [P700^+\text{A}_1^- - P700\text{A}_1\text{]} FTIR DAS are also outlined in Fig. 7.6. This method for producing [\text{[A}_1^- - \text{A}_1\text{]} \text{ FTIR DS}] has been described previously (91).

Fig. 7.5c displays the result of directly subtracting the PhQ (Fig. 7.5a) from the 2MNQ spectrum (Fig. 7.5b). This spectrum will be referred to as a [2MNQ – PhQ] FTIR double difference spectrum (DDS). A [2MNQ – PhQ] FTIR DDS can also be constructed using [\text{[A}_1^- - \text{A}_1\text{]} \text{ FTIR DS}] obtained using PSI with 2MNQ and PhQ incorporated. This DDS is presented in Fig. 7.7, where it is compared to the DDS in Fig. 7.5c.

![Figure 7.7](image)

**Figure 7.7** Comparison of [2MNQ – PhQ] FTIR DDS obtained by subtracting [P700^+\text{A}_1^- - P700\text{A}_1\text{]} TR FTIR DAS (blue), and by subtracting the [\text{[A}_1^- - \text{A}_1\text{]} \text{ FTIR DS}] (red). The extent of the shading is the actual standard error in the measurement at that wavelength.

A similar DDS to that shown in Fig. 7.5c has been presented previously (115). However, the considerably higher signal to noise ratio established for the data presented here, along with the removal of features associated with a laser pulse heating artefact lead to some changes in the spectra, especially in the 1750-1550 cm\(^{-1}\) region.
7.3.3 ONIOM Calculated Optimized Geometry

DFT based vibrational frequency calculations for PhQ and 2MNQ molecules in the gas phase have been undertaken previously (91, 101), as well as for PhQ and 2MNQ molecules in the presence of a truncated leucine residue where the N–H backbone is H-bonded to the C₄=O group of PhQ (115). To more fully account for any effects that surrounding amino acids may have on the vibrational properties of the quinone in the A₁ binding site QM:MM calculations that include the protein environment is considered, using a three-layer ONIOM method. Fig. 7.1c shows the calculated optimized geometry for neutral PhQ in the A₁A binding site. The PhQ is extracted from this structure and used in vibrational frequency calculations. The A₁A binding site was chosen for the following reasons: (i) it is known that P700⁺A₁⁻ recombination at 77 K occurs almost entirely down the A branch (23, 27, 47). (ii) Although the crystal structure shows that the A₁B binding site is very similar to the A₁A binding site, functionally the two binding sites are significantly different, and recent studies also suggest a conformational difference for the anion state (172).

Table 7.1 ONIOM calculated bond lengths (in Å) and angles (in degrees) for neutral and reduced PhQ and 2MNQ in the A₁ binding site, obtained from ONIOM optimized molecular models, one of which is outlined in Fig. 7.1c. The nitrogen atom in question is that of the Leu backbone.

<table>
<thead>
<tr>
<th>Bond Length</th>
<th>X-ray</th>
<th>PhQ</th>
<th>PhQ⁻</th>
<th>2MNQ</th>
<th>2MNQ⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁=O</td>
<td>1.416</td>
<td>1.230</td>
<td>1.270</td>
<td>1.229</td>
<td>1.270</td>
</tr>
<tr>
<td>C₄=O</td>
<td>1.424</td>
<td>1.236</td>
<td>1.284</td>
<td>1.238</td>
<td>1.283</td>
</tr>
<tr>
<td>NH - - O₄</td>
<td></td>
<td>1.844</td>
<td>1.682</td>
<td>1.851</td>
<td>1.688</td>
</tr>
<tr>
<td>N - - O₄</td>
<td>2.694</td>
<td>2.859</td>
<td>2.713</td>
<td>2.867</td>
<td>2.717</td>
</tr>
<tr>
<td>N–H</td>
<td></td>
<td>1.016</td>
<td>1.030</td>
<td>1.016</td>
<td>1.032</td>
</tr>
<tr>
<td>∠N–O₄–C₄</td>
<td>145.8</td>
<td>147.8</td>
<td>149.9</td>
<td>147.8</td>
<td>149.3</td>
</tr>
<tr>
<td>∠N–H–O₄</td>
<td></td>
<td>176.4</td>
<td>179.9</td>
<td>177.7</td>
<td>174.4</td>
</tr>
<tr>
<td>∠N–O₄–C₄–C₃</td>
<td>142.9</td>
<td>138.0</td>
<td>145.3</td>
<td>151.6</td>
<td>151.5</td>
</tr>
</tbody>
</table>
Key bond lengths and angles obtained from the optimized geometry are listed in Table 7.1, and are outlined in Fig. 7.1d. Several points are noteworthy:

1. Both C=O bonds are shorter in the neutral state, compared to the reduced state. For PhQ in the A1 binding site, the C1=O/C4=O bond lengthens by 0.040/0.048 Å upon anion formation.

2. The H-bond length, or more specifically the distance between the backbone nitrogen atom of LeuA722 and the C4=O carbonyl oxygen atom of PhQ is 2.859 Å in the neutral state and 2.713 Å in the reduced state. Thus the H-bond length is slightly longer for both neutral and anion states (0.165 and 0.019 Å) compared to that found in the crystal structure.

3. Asymmetric H-bonding also leads to a lengthening of the C4=O bond compared to the C1=O bond in both the neutral and reduced states. The C4=O bond is calculated to be 0.006/0.014 Å longer than the C1=O bond for neutral/reduced PhQ in the A1 binding site respectively (Table 7.1). The C4=O bond is 0.008 Å longer than the C1=O bond in the X-ray structure, which is only for the neutral state.

4. Similar bond lengths and angles are calculated for PhQ and 2MNQ in the A1 binding site. Thus the hydrocarbon chain attached at C3 appears to have only a minor effect on the overall orientation of the NQ ring in the A1 binding site.

5. For PhQ the N-O distance decreases by 0.146 Å upon reduction, while the H-O distance decreases by 0.162 Å. The N-H bond length increases by 0.014 Å upon reduction, however.

6. In both the neutral and anion states, the calculated C=O bond lengths are shorter than that found in the PSI X-ray structure (Fig. 7.1d and Table 7.1). These shortened C=O
bond lengths could contribute to the calculated increase in the N - O\textsubscript{4} distance compared to that found in the PSI X-ray structure. In previous computational studies shorter C=O bond lengths (compared to X-ray structures) were also calculated (168, 173). In addition, in PSI crystal structures from S6803 and *Pisum sativum* the quinone C=O bond lengths are found to be 1.25 Å (121, 137), which is closer to that found in the current and previous computational studies. It should be pointed out, however, that the crystal structures of PSI from *S6803* and *Pisum sativum* are at a lower spatial resolution than the structure for PSI from *T. elongatus*. In a somewhat simplistic view it could be suggested that a calculated underestimate of the quinone C=O bond lengths will lead to an overestimate of the H-bond length, and hence an underestimate of the H-bond strength. This seems unlikely (see below). At present it cannot be ruled out that the rather long C=O bond lengths obtained from the PSI crystal structure from *T. elongatus* may simply be due to some uncertainty in the atomic coordinates.

### 7.3.4 ONIOM Calculated Double Difference Spectra

Calculated [A\textsubscript{1} - A\textsubscript{1}] DS for PhQ (*a*) and 2MNQ (*b*) are shown in Fig. 7.8. [2MNQ – PhQ] DDS are also shown (*c*), and can be compared to the corresponding experimental DDS in Fig. 7.5c. The experimental DDS may display features associated with protein vibrations that will not be captured in the calculated DDS since frequencies were only calculated for the quinones.
Figure 7.8  Calculated \([A_1^- - A_1]\) DS for PhQ (a) and 2MNQ (b) in the protein binding site. The calculated frequencies are scaled by 0.968/0.977 for the neutral/anion state, respectively. (c) Calculated [2MNQ – PhQ] DDS, obtained by subtracting spectrum a from b.

7.3.5  Calculated Vibrational Mode Frequencies and Assignments

Table 7.2 lists the calculated vibrational mode frequencies and intensities for neutral and reduced PhQ and 2MNQ that give rise to the prominent bands in the spectra in Fig. 7.8. Table 7.2 also lists the relative contribution that molecular groups make to the vibrational modes as expressed through calculated PEDs.
Table 7.2  
Calculated vibrational frequencies (in cm\(^{-1}\)) and intensities (in km/mol) for neutral and reduced PhQ and 2MNQ in the A\(_1\) binding site. Frequencies are scaled by 0.968/0.977 for the neutral/reduced state, respectively. Mode assignments and associated PED (in parenthesis, in %) are also shown. Only molecular groups that contribute above 10% are listed.

<table>
<thead>
<tr>
<th>Frequency (cm(^{-1}))</th>
<th>Intensity (km/mol)</th>
<th>Mode Assignment</th>
<th>Frequency (cm(^{-1}))</th>
<th>Intensity (km/mol)</th>
<th>Mode Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhQ</td>
<td>1656</td>
<td>229.8</td>
<td>(v(C_1=O)) (83)</td>
<td>1668</td>
<td>153.9</td>
</tr>
<tr>
<td></td>
<td>1630</td>
<td>112.5</td>
<td>(v(C_4=O)) (68)</td>
<td>1646</td>
<td>189.5</td>
</tr>
<tr>
<td></td>
<td>1614</td>
<td>68.7</td>
<td>(v(C_2=C_3)) (67)</td>
<td>1619</td>
<td>90.2</td>
</tr>
<tr>
<td></td>
<td>1582</td>
<td>73.3</td>
<td>(v(C=C)) (53) + (\delta(CH)) (12)</td>
<td>1580</td>
<td>80.7</td>
</tr>
</tbody>
</table>

| 2MNQ                     | 1494              | 232.9          | \(v(C_1\ldots=O)\) (57) | 1505              | 290.4          | \(v(C_1\ldots=O)\) (42) + \(v(C_2\ldots=C_3)\) (17) |
|                          | 1426              | 92.8           | \(v(C_4\ldots=O)\) (40) | 1426              | 213.8          | \(v(C_4\ldots=O)\) (40) |
|                          | 1393              | 112.4          | \(\delta(C_2C_3C_4H)\) (10) |                   |                | \(\delta(C_2C_3C_4H)\) (10) + \(\delta(CH_3)\) (11) |

For neutral PhQ an intense mode is calculated at 1656 cm\(^{-1}\) (Fig. 7.8a) that is due mainly to the C\(_1\)=O stretching vibration (83%). The corresponding mode for neutral 2MNQ is 12 cm\(^{-1}\) higher in frequency, at 1668 cm\(^{-1}\) (73%). Thus the C\(_1\)=O mode of neutral PhQ and 2MNQ gives rise to the 1668(−)/1656(+) cm\(^{-1}\) difference band in the [2MNQ − PhQ] DDS in Fig. 7.8c.

From DFT based vibrational frequency calculations for neutral PhQ and 2MNQ in the gas phase, or in solvent (THF), the C\(_1\)=O and C\(_4\)=O modes are strongly coupled, but the coupled C=O mode for 2MNQ is still 7 cm\(^{-1}\) higher in frequency than that for PhQ (91).

From ONIOM calculations for PhQ\(^-\) in the A\(_1\) binding site, an intense band is observed at 1494 cm\(^{-1}\). This band is due mainly to a C\(_1\)=O stretching vibration (57%). The corresponding mode for 2MNQ\(^-\) is 11 cm\(^{-1}\) higher, at 1505 cm\(^{-1}\) (42%). These modes give rise to the 1505(+)/1494(−) cm\(^{-1}\) feature in the DDS in Fig. 7.8c.
For neutral PhQ a normal mode is calculated at 1630 cm\(^{-1}\), that is due predominantly (68\%) to a C\(_4\)=O stretching vibration. For neutral 2MNQ the corresponding mode is upshifted 16 cm\(^{-1}\), to 1646 cm\(^{-1}\). For neutral 2MNQ however, the mode composition is more complex and of greater intensity than that for neutral PhQ. The modes for neutral 2MNQ and PhQ give rise to the 1646(−)/1630(+) cm\(^{-1}\) feature in the DDS in Fig. 7.8c. The C\(_4\)=O is downshifted 26/22 cm\(^{-1}\) compared to the C\(_1\)=O mode for neutral PhQ/2MNQ, respectively. Thus there is a clear asymmetry in H-bonding to the C=O groups, but the H-bond to the C\(_4\)=O group does not appear to be unusually strong, as judged by the relatively small downshift in frequency of the C\(_4\)=O mode.

For PhQ\(^{-}\) an intense normal mode is calculated at 1426 cm\(^{-1}\). This mode contains a 40\% contribution from a C\(_4\)=O stretching vibration, but is also mixed with vibrations from other molecular groups (Table 7.2). Similarly for 2MNQ\(^{-}\), a mixed C\(_4\)=O vibration is also observed at 1426 cm\(^{-1}\), but with considerably increased intensity. This intensity difference leads to a positive feature at 1426 cm\(^{-1}\) in the DDS in Fig. 7.8c.

For PhQ\(^{-}\)/2MNQ\(^{-}\) the calculations indicate that the C\(_4\)=O vibration is downshifted 68/79 cm\(^{-1}\) relative to the C\(_1\)=O vibration, respectively. The normal mode at 1426 cm\(^{-1}\) is far from a pure C\(_4\)=O vibration and it is difficult to gauge exactly how such a large downshift may translate into the strength of the H-bond to the C\(_4\)=O group. On a somewhat simplistic level it would seem that H-bonding is calculated to be stronger for the anion species compared to the neutral species.

For PhQ\(^{-}\) an intense band is calculated at 1393 cm\(^{-1}\). This band is primarily associated with CH bending vibrations of the methylene groups of the hydrocarbon tail of PhQ (53\%), and of the methyl group at C\(_2\) (11\%) (Table 7.2). Since 2MNQ lacks the hydrocarbon tail, the absence of such a band in the 2MNQ\(^{-}\) spectrum is expected.
7.4 Discussion

7.4.1 Semiquinone Vibrations

Neutral quinone species display intense absorptions associated with the C=C and C=O modes in the ~1670-1580 cm\(^{-1}\) region (Fig. 7.2). Semiquinones display intense absorption at considerably lower frequency, in the ~1550-1380 cm\(^{-1}\) region. In the DDS in Fig. 7.5c, in the semiquinone spectral region, negative bands are due to PhQ\(^{-}\) while positive bands are due to 2MNQ\(^{-}\). Thus the 1494\((-\)) and 1414\((-\)) cm\(^{-1}\) bands are due to PhQ\(^{-}\), while the 1504\((+\)) and 1430\((+\)) cm\(^{-1}\) bands are due to 2MNQ\(^{-}\).

The previous FTIR studies have established that PhQ\(^{-}\) gives rise to a band at 1494 cm\(^{-1}\) in [A\(_{1}^{-}\) – A\(_{1}\)] FTIR DS (88, 90). The calculations outlined in Fig. 7.8 and Table 7.2 indicate that this band is due to a relatively pure C\(_{1}\)^\(\equiv\)O mode of PhQ\(^{-}\), and that the corresponding mode for 2MNQ\(^{-}\) is 11 cm\(^{-1}\) higher in frequency. Therefore, there is little doubt that the 1504\((+\))/1494\((-\)) cm\(^{-1}\) feature in the experimental DDS (Fig 7.5c) is due to the C\(_{1}\)^\(\equiv\)O vibration of 2MNQ\(^{-}\)/PhQ\(^{-}\), respectively.

The calculations presented here are less definitive concerning the origin of the 1430\((+\))/1414\((-\)) cm\(^{-1}\) band in the experimental DDS in Fig. 7.5c. The C\(_{4}\)^\(\equiv\)O group of both 2MNQ\(^{-}\) and PhQ\(^{-}\) is calculated to contribute to a normal mode at 1426 cm\(^{-1}\), with the mode for 2MNQ\(^{-}\) having considerably higher intensity, thus giving rise to a positive feature near 1426 cm\(^{-1}\) (Fig. 7.8c). One possibility is that the 1426 cm\(^{-1}\) feature in the calculated DDS corresponds to the 1430 cm\(^{-1}\) feature in the experimental DDS. However, it seems unlikely that a predominantly C\(_{4}\)^\(\equiv\)O vibration of both 2MNQ\(^{-}\) and PhQ\(^{-}\) will have the same frequency. Notice also that comparison of calculated and experimental spectra would suggest that the 1414 cm\(^{-1}\) experimental band corresponds to the 1394 cm\(^{-1}\) calculated band. Given the relatively large discrepancy in
frequency it does seem that the calculations do not model this aspect of the experimental spectra particularly well.

At present, the suggestion is that the 1430/1414 cm\(^{-1}\) feature in the experimental DDS is associated at least in part with the C\(_4\)-\(\overline{\equiv}\)O group of 2MNQ\(^-\)/PhQ\(^-\), respectively. Therefore, the bands at 1504/1430 cm\(^{-1}\) in Fig. 7.5b are associated with C\(_1\)-\(\overline{\equiv}\)O/C\(_4\)-\(\overline{\equiv}\)O modes of 2MNQ\(^-\), while bands at 1494/1414 cm\(^{-1}\) in Fig. 7.5b are associated with C\(_1\)-\(\overline{\equiv}\)O/C\(_4\)-\(\overline{\equiv}\)O modes of PhQ\(^-\), respectively. So, replacing the C\(_3\) hydrocarbon tail of PhQ\(^-\) with a hydrogen atom causes the 1494/1414 cm\(^{-1}\) band to upshift 10/16 cm\(^{-1}\), respectively.

In conclusion, in the anion region, the experimental DDS are very clear, and the spectral data are the same no matter which method is used to construct the DDS (Fig. 7.7). In addition, the calculations for the most part agree well with the experimental data.

The vibrations calculated at 1426 cm\(^{-1}\) for both PhQ\(^-\) and 2MNQ\(^-\) are due predominantly to a C\(_4\)-\(\overline{\equiv}\)O stretching vibration coupled to various types of C–H bending vibrations. The calculations clearly indicate that the C\(_1\)-\(\overline{\equiv}\)O and C\(_4\)-\(\overline{\equiv}\)O modes are uncoupled from each other. They also indicate that the C\(_1\)-\(\overline{\equiv}\)O and C\(_4\)-\(\overline{\equiv}\)O modes are separated by 68-78 cm\(^{-1}\). Such a large downshift of the C\(_4\)-\(\overline{\equiv}\)O mode suggests a very strong H-bond to the C\(_4\)-\(\overline{\equiv}\)O group for both PhQ\(^-\) and 2MNQ\(^-\). However, the C\(_1\)-\(\overline{\equiv}\)O vibration is a relatively pure vibration, which is not the case for the C\(_4\)-\(\overline{\equiv}\)O vibration.

It seems unwise to attempt to consider isolated group vibrations, especially for vibrations involving the C\(_4\)-\(\overline{\equiv}\)O group. That being said, it could be because of a very strong H-bonding that leads to a considerable weakening of the C\(_4\)-\(\overline{\equiv}\)O bond, decreasing the frequency sufficiently to allow it to couple more strongly with other molecular groups that are close in frequency.
Finally, the ONIOM calculations undertaken here, at least in terms of the selection of residues for the molecular model, is very similar to that used previously in calculations of the EPR parameters of PhQ in the A₁ binding site (168). This was the initial motivation for choosing the model discussed here.

7.4.2 Neutral State Quinone Vibrations

In the neutral state spectral region (1670-1550 cm⁻¹), negative bands are due to 2MNQ while positive bands are due to PhQ. In the past identification of bands associated with neutral state quinone vibrations has been notoriously difficult, in part because of the reduced signal to noise ratio of the spectra in this region, and in part due to the many intense overlapping spectroscopic features of protein modes, and of the P700 (and possibly A₀) pigments. Given the very high signal to noise ratio spectra presented here, and also given the new approach to disentangling artefactual heat-induced spectroscopic features, it is now possible to analyze features in the neutral region.

Neutral PhQ/2MNQ in THF give rise to an absorption band at 1662/1666 cm⁻¹, respectively (Fig. 7.2). Calculations show that this band is due to the antisymmetric vibration of both C=O groups (91). In a protein environment, if the C₄=O group is H-bonded, the C=O modes are no longer coupled, and distinct vibrations result (92). It is calculated that the C=O group that is free from H-bonding has a vibrational frequency that is similar to that of the antisymmetrically coupled C=O vibration for the non-H bonded molecule (92). One might therefore expect that a C₁=O vibrational mode for neutral PhQ in PSI will appear near 1660 cm⁻¹, with a corresponding mode for 2MNQ at slightly higher frequency (Fig. 7.2).

The ONIOM calculated FTIR DDS in Fig 7.8 displays a difference feature at 1668(−)/1656(+) cm⁻¹. The calculations show that this feature is due to a pure C₁=O mode of
neutral PhQ/2MNQ, respectively (Table 7.2). An intense difference feature is observed at 1668(−)/1657(+) cm\(^{-1}\) in the experimental FTIR DDS, and accordingly this band is suggested to be due to a C\(_1\)=O vibration of the neutral PhQ/2MNQ in the A\(_1\) binding site, respectively. Thus for neutral 2MNQ in PSI, the C\(_1\)=O vibration is 11 cm\(^{-1}\) higher in frequency than the corresponding mode for neutral PhQ, in excellent agreement with calculations (12 cm\(^{-1}\)). Furthermore, the relative intensities of the differential signals associated with the neutral and anion quinone C\(_1\)=O modes are similar in both the experimental and calculated DDS. Therefore, at least as far as the neutral quinone C\(_1\)=O vibration is concerned, the calculated and experimental spectra are in excellent agreement.

In the neutral quinone region, the only other intense difference feature in the calculated DDS are at 1646(−)/1630(+) and 1620(−)/1614(+) cm\(^{-1}\), suggesting bands of neutral PhQ at 1630/1614 cm\(^{-1}\) that upshift 16/6 cm\(^{-1}\) for 2MNQ, respectively. The calculations indicate (Table 2) that the positive band at 1630 cm\(^{-1}\) is due predominantly to the PhQ C\(_4\)=O stretching vibration while the negative band at 1646 cm\(^{-1}\) is due to a predominantly to a coupled C\(_4\)=O and C\(_3\)=C\(_2\) vibration of 2MNQ.

Putting aside the complications of the normal mode composition, the calculations demonstrate a clear splitting of the C\(_1\)=O and C\(_4\)=O modes due to H-bonding. The C\(_4\)=O vibration of PhQ/2MNQ is downshifted 26/22 cm\(^{-1}\) relative to the C\(_1\)=O vibration, respectively. Such a downshift is small compared to that predicted for the anion state. Again, further computational work is underway to more fully assess these calculated predictions.

The 1620(−)/1614(+) cm\(^{-1}\) difference band feature in the computed DDS is due to a coupled C\(_4\)=O/C\(_2\)=C\(_3\) vibration of 2MNQ/PhQ, respectively (see animations in the supplementary information). This calculated feature could correspond to the ~1627(−)/1621(+) cm\(^{-1}\) feature in
the experimental DDS. The reduced intensity of this feature (relative to the 1668(-)/1657(+) cm⁻¹ feature) in the experimental DDS is in good agreement with the calculated DDS. This hypothesis then leaves as an open question the origin of the 1647(+) cm⁻¹ band in the experimental DDS.

In Fig. 7.7 two methods for producing a [2MNQ – PhQ] DDS are demonstrated. The similarity in the spectra demonstrate the method of their production is not important. The bands of major interest that are discussed in this chapter are found at the same frequencies, and differences in relative intensities are within the noise level. These observations indicate that contributions to the spectra from P700⁺/P700 are similar for PSI with both PhQ and 2MNQ incorporated, and any spectral effects cancel out when a DDS is constructed. The difference in the [P700⁺ – P700] DS for PSI with PhQ and 2MNQ incorporated is shown in Fig. 7.6e. Given this similarity in the spectra the preferred method of producing a DDS is to directly calculate the difference in the [P700⁺A⁻ – P700A₁] DAS, bypassing the need to first subtract [P700⁺ – P700] DS from [P700⁺A⁻ – P700A₁] DAS and eliminating a source of noise in the resultant DDS.

Another technical feature introduced in this chapter relates to the method of producing the initial [P700⁺A₁⁻ – P700A₁] FTIR DS. Previously, the FTIR DS were produced as an average of ten spectra collected every 5 µs over a ~50 µs interval following a laser flash. This method averages any laser flash heating artifacts into the resulting FTIR DS. By applying a global analysis procedure to produce the [P700⁺A₁⁻ – P700A₁] DAS, heating induced spectral artefacts can be isolated and removed from the spectral data of interest. The global analysis requires very high signal-to-noise ratio kinetics, which was achieved in the presented spectra (Fig. 7.3 and 7.4).

7.4.3 Effect of the asymmetric H-bond

The experimental and calculated FTIR DS support the idea that the quinone in the A₁A binding site is asymmetrically H-bonded. The calculations indicate asymmetric H-bonding for
both the neutral and anion states. However, H-bonding is considerably stronger for the semiquinone. Although asymmetric H-bonding to the A₁ pigment has been reported previously, what role this H-bond may play in modulating ET has not been resolved (see (7) for a review). From a purely structural perspective, it has been shown that PhQ is able to retain its native orientation even in a mutated A₁ binding site where H-bonding has been considerably altered (174). So H-bonding does not appear to be an issue in promoting a specific pigment orientation.

Thermodynamically, H-bonding is expected to stabilize the semiquinone state, making the quinone’s midpoint potential more positive (175). This might be expected to be disadvantageous for ET in PSI, which requires the A₁ pigment to operate at a very low redox potential. However, the previous kinetic simulations have shown that the driving force associated with the A₁A⁻ → Fₓ ET process is necessarily slightly positive (thermodynamically “uphill”), while the corresponding process on the B-branch is negative (39, 48). It could be that H-bonding to the quinone on the A branch is a factor that leads to an increase of the quinone’s redox potential relative to that on the B-branch. This idea implies that there is little or altered H-bonding to the A₁B quinone, however.

There is EPR data available supporting this idea (172), although there is little evidence for this from x-ray crystallography. X-ray crystallographic data probes only the neutral state, however, and here it is shown that there are large changes in H-bonding on going from the neutral to the anion state. In summary, the proposal is that for efficient ET in PSI to occur, the A₁B quinone has to have a more negative potential than the A₁A quinone, and that this difference in quinone potential on each branch may result from differences in H-bonding.

7.5 Conclusions

PhQ and 2MNQ were incorporated into the A₁ binding site in menB⁻ mutant PS I particles. Using these PSI particles, highly sensitive time-resolved FTIR DS associated with radical pair
recombination at 77 K have been produced. Global analysis of the experimental data has produced
(P700\(^+\)A\(_1^-\) - P700A\(_1\)) FTIR DAS that are free from spectral artefacts associated with sample
heating that have in the past obscured absorption changes particularly in the amide I spectral
region. From the collected (P700\(^+\)A\(_1^-\) - P700A\(_1\)) FTIR DAS, (2MNQ – PhQ) FTIR DDS was then
constructed. This was undertaken in order to help identify and distinguish quinone bands from
protein bands, and from bands of P700 in the FTIR DS. To further aid in spectral band assignment
three-layer ONIOM-type QM/MM vibrational mode frequency calculations were undertaken.
Upon detailed consideration of the calculated and experimental data it was found:

1. Absorption bands for neutral/reduced PhQ are found at 1657/1494 cm\(^{-1}\), while absorption
   bands for neutral/reduced 2MNQ are found at 1668/1504 cm\(^{-1}\), respectively. ONIOM
calculations indicate that these bands are due to the C\(_1\)=O/C\(_1\)–O groups that are essentially
   free from H-bonding.

2. ONIOM calculations predict that a band in the experimental spectrum at 1414/1430 cm\(^{-1}\)
is due in part to a C\(_4\)–O mode of PhQ\(^-\)/2MNQ\(^-\), respectively. Given that the frequency is
   ~80 cm\(^{-1}\) lower than that of the corresponding C\(_1\)–O mode, the calculations predict that
   the C\(_4\)–O mode of PhQ\(^-\) and 2MNQ\(^-\) is very strongly H-bonded in the A\(_1\) binding site.

3. The calculations indicate that the C\(_4\)=O mode of neutral PhQ/2MNQ in the A\(_1\) binding site
   is H-bonded (although less strongly compared to the anion state), resulting in a downshift
   (relative to the non-H-bonded C\(_1\)=O group) of ~20-40 cm\(^{-1}\).
8 PHOTOSYSTEM I WITH BENZOQUINONE ANALOGUES INCORPORATED INTO THE A₁ BINDING SITE

8.1 Introduction

In oxygen evolving organisms two photosystems, called photosystem I and II (PSI and PSII), capture and convert solar energy independently but cooperatively. The solar conversion reactions occur in a centralized pigment-protein unit called a reaction center (RC). In the RC, light is used to drive electrons from a chlorophyll donor species, via a series of acceptors, across a biological membrane (the thylakoid membrane). This light-induced electron transfer (ET) across a biological membrane is the basic mechanism underlying solar energy capture in all photosynthetic organisms.

Here the focus will be on ET processes in cyanobacterial PSI particles from *Synechocystis* sp. PCC 6803 (S6803). The architecture of the protein-bound ET cofactors is very similar in PSI from higher plants (13) and from green algae, so cyanobacterial PSI can be considered a template that is widely applicable.

In PSI the ET cofactors are bound to two membrane-spanning protein subunits called PsaA and PsaB. The organization of the bound cofactors is outlined in Fig. 8.1 (10). In PSI there are two cofactor branches, labeled the A and B branch. Here the A branch refers to the side in which the pigment in the A₁ binding site is bound to the PsaA subunit (A₁A in Fig. 8.1). At room temperature (~298 K, RT) both branches are utilized in ET in PSI but at 77 K (LT) it is predominantly (~95 %) the A-side (20, 21).
Figure 8.1  Arrangement of ET cofactors in PSI. Structure was derived from the 2.5 Å X-ray crystal structure of PSI from *Thermosynechococcus elongatus* (PDB ID 1JB0) (10). Fₓ, Fₐ, and Fₐ are iron-sulfur clusters, A₀ is chlorophyll-α, and A₁ is PhQ. Pigment hydrocarbon chains are removed for clarity.

In recent years it has been shown that a variety of different quinones can be incorporated into the A₁ binding site in menB⁻ mutant cells that cannot synthesize phylloquinone (PhQ). In PSI particles isolated from menB⁻ mutant cells plastoquinone-9 (PQ) was found to be incorporated into the A₁ binding site (44). PQ is a 1,4-benzoquinone (BQ) analogue that is loosely bound in PSI, and it has been shown that it can be displaced by a variety of 1,4-naphthoquinones (NQ) simply by incubation of menB⁻ PSI in a large molar excess of the NQ of interest (30, 45, 47, 123). No studies have been reported for menB⁻ PSI with other BQ analogues incorporated. In the work
reported here, spectroscopic data for PSI particles with three BQ analogues incorporated into the A1 binding site in PSI particles from S6803 are obtained.

Previously, electron paramagnetic resonance (EPR) spectroscopy has shown that the position and orientation (relative to P700) of PQ in the A1 binding site is identical, within error, to that of PhQ (47, 89). That is, the C=O groups of PhQ and PQ are oriented and positioned identically. There is disagreement between EPR and FTIR spectroscopic data, however, on the functionality of PQ in the A1 binding site at cryogenic temperature. EPR studies indicate light-induced P700+A1– radical formation (89), while time-resolved (TR) FTIR difference spectroscopy (DS) indicates P700 triplet state (3P700) formation (100). This light-induced 3P700 formation has stymied progress on using FTIR DS to study PSI with PQ incorporated into the A1 binding site.

In this chapter TR FTIR DS has been used to study PSI particles with PQ and two other BQ analogues incorporated into the A1 binding site. These studies resolve discrepancies between reported EPR and FTIR spectroscopic studies on menB– PSI samples.

8.2 Materials and Methods

Trimeric PSI particles from menB– mutant cells from S6803 were isolated as described previously (44). Incorporation of quinones into PSI, and preparation of samples for FTIR experiments at 77 K has also been described (88). MenB– PSI particles devoid of FAB (PSI–FX particles) were prepared as described previously (176, 177).

Transient absorption spectroscopy measurements probing at 703 nm were undertaken as described previously (126). Photochemistry was initiated using 1 mJ, 5 ns, 532 nm laser pulses from Nd:YAG laser (Continuum, San Jose, CA) operating at 10 Hz.

TR and static (photo-accumulated) FTIR DS experiments were undertaken using a Bruker Vertex80 (Bruker Optics, Billerica, MA) FTIR spectrometer, as described previously (21, 30, 163).

Transient absorption changes in the visible spectral region are fitted to exponential functions using the Levenberg-Marquardt algorithm implemented within Origin 7.5 (OriginLab, Northampton, MA) or Glotaran (164). Using Glotaran TR FTIR DS were fitted globally (at hundreds of frequencies) to multi-exponential functions to construct decay-associated spectra (DAS). The TR FTIR DAS produced are representative of (P700+A1– – P700A1) and (3P700 – P700) FTIR DS.
8.3 Results

In previous studies of PSI with different NQ’s incorporated, TR spectroscopy measurements in the visible, at RT, were undertaken prior to measurements on the same samples at 77 K (LT) (30). Using the same sample for measurements at 298 and 77 K is highly advantageous in assessing the extent of heterogeneous ET processes that occur in PSI as the temperature is lowered. However, for PSI with PQ incorporated in the A\textsubscript{1} binding site, differences were observed in the light-induced absorption changes at 77 K depending on whether the samples were subjected to laser flash illumination at RT prior to being cooled to 77 K. Flash illumination at RT typically consists of thousands of laser flashes at 10 Hz. Hereafter the two experimental RT pre-treatments will simply be referred as “pre-flashed” or “non-flashed”. Note that this behavior depending on pre-flash illumination at RT is not observed for PSI with a variety of NQ analogues incorporated.

For samples that were pre-flashed at RT, the lifetime and decay amplitude calculated from kinetic data collected at 77 K are similar to that reported previously (126). For non-flashed samples the lifetime/signal amplitude at 77 K is considerably longer 732 \textmu s/\textit{5x10}^{-3}.

8.3.1 \textit{TR (P700}\textsuperscript{+}A\textsubscript{1}A\textsubscript{1}– P700A\textsubscript{1}A\textsubscript{1}) FTIR DS for PSI with PQ incorporated

For all TR FTIR DS measurements, data was collected in the 2106-1065 cm\textsuperscript{-1} region, in 2 cm\textsuperscript{-1} increments. The data in the 1900-1250 cm\textsuperscript{-1} region, at 364 frequencies, was globally analyzed by fitting the data to a sum of two exponential components and a constant. A fast phase with lifetime \textsim 15 \textmu s was found, and is associated with a laser pulse induced thermal artifact, and has been considered previously (178). A slower phase with lifetime of 253/920 \textmu s is also found for pre-flashed/non-flashed PSI with PQ incorporated, respectively. These lifetimes agree well with the visible TR data. Fig. 8.2A/B \textit{(solid)} shows the DAS for the 253/920 \textmu s decay phases, respectively. The two spectra are very different. For pre-flashed samples the 253 \textmu s DAS is identical to the (\textit{3}P700 – P700) FTIR DS reported previously (100, 179) with intense bands at 1636(–), 1609(–), 1594(+) and 1585(+) cm\textsuperscript{-1}.
For non-flashed PSI, all IR spectral features of $^3$P700 are absent. Instead, features that are generally found in (P700$^+ – P700$) FTIR DS are observed (particularly in the 1700–1650 cm$^{-1}$ range) (Fig. 8.2B, solid). In addition, positive bands in 1550–1400 cm$^{-1}$ region are observed, which are associated with semiquinones (115). For these reasons, and taking into account that P700$^+ A_1^–$ radical pair recombination occurs predominantly down the A-branch at 77 K (21), the 920 µs DAS in Fig. 8.2B (solid) will be referred as a (P700$^+ A_{1A}^– – P700 A_{1A}$) FTIR DS.

Light induced $^3$P700 formation usually occurs in PSI samples when the quinone occupying the A$_1$ binding site is nonfunctional, or when the binding site is empty (14). The observation of $^3$P700 FTIR DS in PSI samples that have been subjected to actinic illumination at RT prior to freezing, but not in samples that are frozen without pre-illumination, suggests that the actinic illumination has somehow inactivated or led to the removal of PQ that was present in the A$_1$ binding site. Formation of inoperative A$_1$ by a series of actinic flashes at 298 K has been suggested previously for studies of menB$^–$ PSI, and for menD1 PSI from *Chlamydomonas reinhardtii* (97).
In these studies, it was clearly shown that PQ in the A₁ binding site lost its functionality due to double protonation of PQ in the binding site (plastoquinol (PQH₂) formation). PQH₂ formation likely explains the observations presented here, with the caveat that plastoquinol formation via flash illumination of PSI at 77 K does not occur, even in a small fraction (Fig. 8.2B).

The mechanism underlying repetitive flash-induced PQH₂ formation in PSI with PQ incorporated is not well understood but one proposed mechanisms suggests that the terminal acceptors Fₐ and Fₐ may participate in the double reduction of PQ (97). To address this possibility experiments were undertaken using menB⁻ PSI particles with Fₐ and Fₐ removed, hereafter called PSI–Fₓ particles. Fig. 8.2 shows DAS generated from TR FTIR DS at 77 K, obtained using pre-flashed (A) and non-flashed (B) PSI–Fₓ samples (dotted lines). Absorption changes at several frequencies are shown for pre-flashed (C) and non-flashed (D) PSI–Fₓ samples. Fig. 8.2 demonstrates very similar spectra and time constants for the two types of PSI samples under both sets of light treatments.

8.3.2 TR (P700⁺A₁⁻ – P700A₁⁺) FTIR DS for PSI with different BQ’s incorporated

In previous studies using menB⁻ PSI the quinone incorporated into the A₁ binding site was a NQ analogue (21, 30, 46-48, 123, 180), and no work has been undertaken using PSI with BQ analogues incorporated, other than studies of PSI with PQ incorporated (100). For a detailed understanding of PSI with PQ incorporated it would be very useful to also study PSI with a variety of BQ analogues incorporated. As a first step in this direction 2,6-dimethyl-BQ (DMBQ) and 2,3,5,6-tetrachloro-BQ (Cl₄BQ) are incorporated into the A₁ binding site in PSI. These BQs were chosen because of the considerable difference in their (in vitro) midpoint potentials, and because their FTIR DS are likely very different. For DMBQ and Cl₄BQ the in vitro midpoint potentials (E₁/₂) (in DMF versus SHE) are –317 and +380 mV, respectively. For comparison, for PQ and PhQ the corresponding midpoint potentials are –369 and –465 mV, respectively (47, 124, 140).

For pre-flashed PSI with DMBQ and Cl₄BQ incorporated, a (P700⁺-P700) FTIR DS identical to that in Fig. 8.2A was obtained at 77 K (not shown). Under non-flashed conditions a (P700⁺A₁⁻ – P700A₁⁺) FTIR DS was obtained. Fig. 8.3A/B shows (P700⁺A₁⁻ – P700A₁⁺) DAS for PSI with DMBQ/Cl₄BQ incorporated. Absorption changes at 1697 cm⁻¹ for PSI with the three different BQs incorporated are shown in Fig. 8.3B. The lifetimes for PSI with DMBQ and Cl₄BQ incorporated (382 and 629 µs) are considerably shorter than the 920 µs obtained for PSI with PQ
incorporated. Differences in the DAS in Fig. 8.3 and 8.2B are observed, and are particularly obvious in the 1500–1400 cm⁻¹ semiquinone spectral region. These spectral differences, along with the lifetime differences, indicate that the non-native BQs have displaced PQ, and have been successfully incorporated into the A₁ binding site, and are functional in ET. Spectral and lifetime differences for PSI with different BQs incorporated will be the subject of a more in depth and detailed study.

![Figure 8.3](image)

**Figure 8.3** TR (P700⁺A₁⁻ – P700A₁A) FTIR DAS obtained for PSI with (A) DMBQ (*blue*) and (B) Cl₄BQ (*green*) incorporated. Signal amplitudes were scaled so that the amplitude of the 1717(+)/1697(-) cm⁻¹ difference band is similar. (C) Absorption changes at 1697 cm⁻¹ for PSI with PQ (*black*), DMBQ (*blue*) and Cl₄BQ (*green*) incorporated. The fitted functions are shown (*red*) and calculated time constants indicated.

### 8.4 Discussion

Previously, EPR and FTIR spectroscopic studies have been undertaken using *menB⁻* PSI with PQ incorporated at LT (89, 100). In the FTIR studies a loss of functionality of PQ as an ET cofactor was observed (100). Loss of functionality of PQ in the A₁ site manifests itself as ³P700 formation, presumably resulting from P700⁺A₀⁻ charge recombination (35, 97), which occurred because ET from A₀⁻ to A₁ was not possible, or was inhibited. In *menD1* mutant cells from *C. reinhardtii*, after a period of anaerobiosis (> 60 min), a ~36 ns kinetic phase (~25 ns half-time)
was observed at 430 nm, that was assigned to P700$^\text{+}A_0^-$ charge recombination (96). In isolated menB$^{-}$ PSI, a nanosecond kinetic phase ($\tau = 23 - 107$ ns) was observed at 800 nm and 703 nm, and tentatively assigned to $^3P700$ formation (30). This phase was absent in native PSI with PhQ incorporated, and in menB$^{-}$ PSI with several NQ’s incorporated (48, 126, 181).

In spin-polarized transient EPR studies of PSI with PQ incorporated at 77 K, the PQ anion radical state is observed, while in TR FTIR DS measurements the $^3P700$ state has been observed (89, 100). In this work it is shown that both states can be generated and observed using TR FTIR DS at 77 K. The $^3P700$ spectrum and the P700$^+A_{1A}^-\text{spectrum can be generated under identical experimental conditions at 77 K, with the only difference being exposure to actinic flashes at RT prior to cooling to 77 K. In the previous FTIR studies repetitive actinic illumination at RT was indeed used prior to cooling, and so spectra associated with the $^3P700$ state were generated.

If light-induced plastoquinol formation (termed “photo-inactivation” in (97)) explains the data presented here then it has to also be pointed out that such a mechanism is only active at RT. For samples cooled to 77 K without pre-flashing at RT, no $^3P700$ spectral features were detected, even after prolonged (>30 h) exposure to actinic laser flashes at 10 Hz.

8.4.1 Mechanism of plastoquinol formation

Quinol formation in PSI has been achieved in a number of different experiments (42, 96, 97, 174, 182), and mechanisms have been proposed, but the issue is far from resolved. Quinol formation is an important topic in photosynthesis and respiration, and gaining a deeper understanding of this process is of value. PSI has evolved to prevent quinol formation, and understanding this evolution will contribute to an understanding of mechanisms underlying quinol formation in general.

In PSI research an often hotly debated question is the role of the strong asymmetric H-bonding to the quinone in A$_1$ binding site (7, 174). There is some speculation that the H-bond may be a structure that has evolved to prevent quinol formation in PSI, and so studies of quinol formation in PSI can and does contribute to this debate (see below).

Recent photo-inactivation studies of PSI with PQ incorporated prove unambiguously that when PQH$_2$ is formed in PSI it stays in the A$_1$ binding site (97), negating the possibility of a disproportionation reaction outside the binding site. Two mechanisms of protonation in the A$_1$ binding site have been proposed (97). One mechanism resembles the QA protonation process (183), and one involves the iron-sulfur clusters F$_A$ and F$_B$ (42). The first mechanism was not
favored as it requires the generation of a P700$^+$A$_0^-$A$^-$ state, which is unlikely given the short lifetime of P700$^+$A$_1^-$ (30, 63) and current models of charge separation (15, 16, 184). However, no mechanisms for the fast protonation of A$_1^-$ were considered. For the second mechanism involving F$_A$ and F$_B$, two scenarios were proposed. In one scenario, two successive actinic flashes initiate ET down each branch, generating P700$^+$A$_{1A}^-A_{1B}^-$. Inter-quinone ET via F$_X$ then creates PQH$_2$ on one of the branches. In the second scenario, the first ET proceeds all the way to the terminal acceptors, and the second ET generates P700$^+$A$^-F_{A/B}^-$. A partial back reaction from the terminal acceptors generate PQH$_2$. The first scenario is unlikely, again because it requires the charge to reside on A$_1$ for a long period, and by the fact that the same mechanism cannot be applied to the second branch after the first branch is inactivated. For these reasons, the mechanism involving the terminal electron acceptors F$_{A/B}$ has been the favored mechanism for PQH$_2$ formation in PSI (97).

The data presented here demonstrate that for pre-flashed PSI–FX samples signals associated with $^3$P700 are observed, confirming the presence of PQH$_2$ in the A$_1$ binding site. Therefore, the terminal acceptors F$_{A/B}$ are not necessary for PQH$_2$ formation. So this previously preferred mechanism of PQH$_2$ formation in PSI is not correct.

Previously proposed mechanisms that do not involve F$_{A/B}$ all require the charge to reside on A$_1$ until the second electron transfer occurs. Although the A$_1^-$ state is more stable for PSI with PQ ($\tau_{A_1^-} = \sim13$ $\mu$s) than with PhQ ($\tau_{A_1^-} \sim300$ ns), the state is still short-lived. However, a key observation is that the $^3$P700 state is only observed (at 77 K) for PSI with BQ analogues incorporated. It is not observed for PSI with NQ and AQ analogues incorporated (126). This is in spite of the fact with some of the high potential NQs extend the lifetime of the A$_1^-$ state to $\sim140$ $\mu$s at room temperature (21). These observations suggest that quinol formation (photo-inactivation) is more likely to be associated with the structural properties of the BQs rather than the operating midpoint potential or lifetime of semiquinone radical.

Unlike in PSI, double reduction and protonation of quinone is an integral part of the ET process in type II RCs. The mechanisms of the Q$_B$ quinone protonation process are well-studied and characterized for purple bacterial (pb) and PSII RCs. In pbRC, the amino acid residues Asp-M17, Asp-L213, and Ser-L223 are involved in the first protonation event, and Glu-L212 in the second event (185). Computational studies have revealed that in PSII, the first proton is donated from D1-Ser264 and is accompanied by proton transfer from D1-His252 to D1-Ser264. The second proton transfer is from D1-His215, which forms an ionic H-bond with Q$_B$H$^-$ (186). In
contrast to these $Q_B$ binding sites, the $A_1$ binding site of PSI does not possess residues in the vicinity of quinone that could donate protons to the two carbonyls. Even with such residues around, the proton transfer from the residues must somehow selectively occur only to BQs and not to NQs or AQs. The selectivity could be accomplished if the binding site itself is modified with a BQ incorporated, yet there is no spectroscopic evidence to support such a modification.

While the binding site lacks proton donating amino acid residues, a pocket for four water molecules exists close to the quinone in the $A_1$ site (10). These water molecules are found in cyanobacterial (10, 137) and plant (13) PSI crystal structures, and are outlined in Fig. 8.4. The water molecules are positioned between the quinone and $F_X$, and therefore face the aromatic ring of PhQ. In cyanobacterial PSI the shortest edge-to-edge distance between the NQ’s aromatic ring and the oxygen atom of the water molecules is $3.50 \, \text{Å}$. Within 5 Å of the quinone head-group, no water molecules are observed for PQ in the $Q_B$ site of PSII (50). Two water molecules exist for ubiquinone in the $Q_B$ site of pbRC, but are postulated to be a part of the proton transfer pathway and not a direct proton donor (51, 185).

EPR studies have indicated that non-native NQs and AQs in the $A_1$ binding site take the same position and orientation as PhQ (47, 122, 129). So for any incorporated NQ or AQ analogue, the quinone aromatic ring exists at the same location as that of the native PhQ. For BQs, however, this aromatic ring is absent and an additional space is created between $A_1$ and $F_X$, opening up a potential pathway for water molecules to more directly access the BQs carbonyl groups. Hence the hypothesize is that the water molecule(s) between $A_1$ and $F_X$ (observed in all PSI crystal structures) can act as proton donors specifically to BQs in the $A_1$ binding site in PSI. This $PQH_2$ formation hypothesis does not involve $F_{A/B}$ and is therefore supported by the observation that protonation occurs also in PSI--$F_X$ particles.

A $PQH_2$ formation mechanism that involves inter-quinone ET via $F_X$ cannot be entirely ruled out by the data presented here, as PSI-$F_X$ samples retain $F_X$. Yet, as pointed out previously, this inter-quinone ET mechanism fails to explain the double reduction of quinones on both branches. With water molecules present near the BQs on both branches, it is possible to have $PQH_2$ formation on both branches independently. So the proposed mechanism can naturally explain $PQH_2$ formation on both ET branches in PSI.
Figure 8.4 Positions of four water molecules relative to PhQ in the A_{1A} binding site. Distances between water molecules and to PhQ (in Å), are listed. The asymmetric H-bonding between the backbone of Leu722 and the C=O group is also shown.

The two-electron reduction potential for many quinones is extremely low, enough to make the A_{0} → A_{1} ET process thermodynamically unfavorable (140) and inactivate ET. Additionally, the NQ and BQ incorporation experiments demonstrate that the lifetime of A_{1} is not the leading cause of PQH_{2} formation in PSI. Therefore, it is likely that PQH_{2} formation in PSI does not involve two consecutive reduction reactions, but rather a stabilization of the BQ^{-} state by single protonation before the second ET takes place. An immediate protonation of BQ^{-} to BQH circumvents the problems listed above, and the presence of a proton donating source that is only accessible to BQs fits the scenario as well.

In summary, benzoquinol formation in PSI is proposed to proceed as follows: (i) the first charge separation creates P700^{+}BQ^{-}, (ii) BQ^{-} is protonated by a water molecule to BQH before BQ^{-} → F_{x} forward ET occurs, (iii) the second charge separation process [P700^{+}A_{0} BQH → P700^{+}A_{0}BQH^{+}] proceeds, and (iv) the second protonation follows, producing BQH_{2}. Since the overall efficiency of quinol formation in PSI is low (97), step ii (or any step for that matter) may also have low efficiency. Note that this working model could also incorporate an alternative
pathway that involves inter-quinone ET \[ \text{P}^700^+ \text{BQ}_A^- \text{BQ}_B \text{H} \text{F}_X \rightarrow \text{P}^700^+ \text{BQ}_A \text{BQ}_B \text{H} \text{F}_X^- \rightarrow \text{P}^700^+ \text{BQ}_A \text{BQ}_B \text{H}^- \text{F}_X \] but it is not necessary.

It has been suggested that one of the functions of the H-bond to the quinone in the A\textsubscript{1} binding site (backbone NH of Leucine residue H-bonds to quinone C\textsubscript{4}=O, see Fig. 8.4) is to prevent quinol formation, which would deactivate A\textsubscript{1} as the electron transfer cofactor (174). While different NQs, AQs and BQs all retain this H-bond in the A\textsubscript{1} binding site, the double protonation was only observed for BQs in the binding site. In the substitution mutant PsaA-L722W, which breaks or weakens this H-bond, an increase in the \(3^P700\) state population was observed in the thylakoids under illumination, but the population only accounted for 9 – 12 % of total PSI population (2 – 4 % in WT) (187). These findings indicate that while the H-bond may contribute somewhat to the prevention of quinol formation, this effect is minor and is not the primary role of the asymmetric H-bond.

8.5 Conclusions

For PSI with a series of BQ analogues incorporated, including PQ, exposure to actinic flashes at 298 K short circuits ET through A\textsubscript{1}, likely because of protonation of the incorporated BQ. Exposure to actinic flashes at 77 K did not lead to such a short circuiting of ET. Benzoquinol formation does not require the involvement of F\textsubscript{A/B}. Quinol formation is PSI is not possible for NQ and AQ in PSI because the quinone aromatic ring prevents access to proton donating species which are most likely the nearby water molecules between A\textsubscript{1} and F\textsubscript{X} observed in all PSI crystal structures.
9 TIME-RESOLVED FTIR DIFFERENCE SPECTROSCOPY FOR THE STUDY OF PHOTOSYSTEM I WITH DIFFERENT BENZOQUINONES INCORPORATED INTO THE A₁ BINDING SITE

9.1 Introduction

In all photosynthetic organisms, solar energy is absorbed and used to initiate the transfer of electrons via a series of pigments bound in a membrane spanning protein complex. This protein complex is called a photosystem. In oxygen-evolving photosynthetic organisms, there are two photosystems called photosystem I and II (PSI and PSII) that function independently but cooperatively in harnessing solar energy (2). In both photosystems, light is absorbed by a chlorophyll donor species (P700 and P680 in PSI and PSII, respectively), initiating electron transfer (ET) via a series of protein-bound cofactors, across a biological membrane (8, 9).

The ET cofactors are arranged in two nearly symmetric branches in the reaction centers (RCs). The architecture of the cofactors in cyanobacterial PSI RC is outlined in Fig. 9.1. In PSII, electrons are directed down only one branch of cofactors, while in PSI, both branches (termed A- and B-branches) are utilized. In PSII, ET occurs only along only the D1 branch (188) resulting in reduction of a quinone in the QA binding site. From QA⁻ an electron is transferred to QB. Upon successive electron transfer from QA⁻ and two proton transfer reactions, a quinone in the QB binding site can be doubly reduced to form a quinol, which is then released from the binding site. In PSI, within ~50 ps of light excitation, an electron has been transferred from P700 to a quinone molecule occupying the A₁ binding site, forming the P700⁺A₁⁻ charge separated state (17, 18, 189). Both branches are utilized, and to further stabilize the radical pair state, an electron is transferred from the quinone to FX, an iron-sulfur cluster. (20, 27, 28). From FX⁻ an electron is further transferred to FA and FB in a few hundred nanoseconds at room temperature (83, 84).
Figure 9.1  Arrangement of ET cofactors in PSI RC. Structure was derived from the 2.5 Å X-ray crystal structure of PSI from *Thermosynechococcus elongatus* (*T. elongatus*) (pdb 1JB0). Hydrocarbon chains of PhQ and chlorophyll molecules are truncated for clarity. Subscripts A and B for A\textsubscript{1} and A\textsubscript{0} refers to the ET branch.

In both PSI and PSII, quinone molecules serve as one of the ET cofactors. In PSI, the secondary electron acceptor A\textsubscript{1} is a phylloquinone (PhQ) molecule (or a close analogue of PhQ), while in PSII the terminal electron acceptors Q\textsubscript{A} and Q\textsubscript{B} are plastoquinone-9 (PQ-9) molecules (see Fig. 9.2 for molecular structure and atom numbering) (11, 50). Structurally, the crystal structures of two RCs reveal that carbonyl groups of both quinones are subjected to H-bonding by their respective binding site amino acids. A number of H-bonding interactions provided to a quinone varies by the binding site. In PSII, PQ-9 in the Q\textsubscript{A} binding site appears to form two H-bonds (one each to C\textsubscript{1}=O and C\textsubscript{4}=O), while PQ-9 in the Q\textsubscript{B} binding site seems to be in a position for three H-bonds (two to C\textsubscript{1}=O and one to C\textsubscript{4}=O) (50). In PSI, however, PhQs in both the A\textsubscript{1A}
and A1B binding sites are subjected to only one H-bonding to C4=O (10). Recently, the FTIR studies aided by density functional theory (DFT) based vibrational frequency calculations have shown that this H-bond is especially strong when the quinone is reduced (178). Much of the molecular details of the quinones and their interactions with the binding site including the exact role of this asymmetric H-bonding, however, is still far from being fully understood.

One approach to investigate the molecular properties of quinones in the binding site is to replace the cofactors with non-native quinones. For studies of quinones in the A1 binding site in PSI, use of mutant cells that cannot synthesize PhQ has been advantageous. In cyanobacterial cells from Synechocystis sp. PCC6803 (S6803) in which the menB gene is inactivated, PhQ synthesis is prohibited, and a PQ-9 molecule is found to occupy the A1 binding site instead (44). It has subsequently been found that in PSI isolated from these mutant cells (hereafter menB– PSI), PQ-9 in the A1 binding site can be replaced by a variety of non-native naphthoquinones (NQs) and anthraquinones (AQs) by incubation of menB– PSI in a large molar excess of the quinone of interest (45, 122). Using this minimally invasive method, a variety of spectroscopic studies have been conducted on menB– PSI with a series of non-native NQs and AQs in the recent past (47, 123, 126, 178). Properties of PQ-9 in the A1 binding site have also been investigated previously (63, 89, 97). One of the notable properties of PQ-9 is its ability to undergo double reduction in PSI, a feature not observable for NQs and AQs (97). Doubly-reduced quinones do not function as an ET cofactor, and as a result induces formation of the P700 triplet (3P700) state. This unique property which generates the 3P700 state has hindered the FTIR studies on PSI with PQ-9 in the A1 binding site for many years, but in the most recent studies it was shown that the double reduction of PQ-9 in PSI only takes place at room temperature and not at cryogenic temperatures (190). By controlling the exposure to actinic flashes at room temperature, the double reduction reaction was
prevented and PQ-9 in the A₁ binding site remained active as an ET cofactor at cryogenic temperature. The method has allowed for the incorporation of other benzoquinones (BQs) as an ET cofactor in the A₁ binding site of PSI for the first time (190).

In this study, the focus is on the construction and analyses of the \((A_1^- - A_1)\) FTIR difference spectra (DS) for PSI with PQ-9 incorporated into the A₁ binding site. More specifically, the focus is on the interpretation of the vibrational frequencies of anionic PQ-9⁻ in the A₁ binding site. Two other benzoquinones, 2,6-dimethyl-1,4-benzoquinone (DMBQ) and 2,3,5,6-tetrachloro-1,4-benzoquinone (Cl₄BQ) (Fig. 9.2) are investigated, and the \((A_1^- - A_1)\) FTIR DS for three BQs are compared against those for recently studied PhQ and 2-methyl-1,4-naphthoquinone (2MNQ) (178). To aid in the spectral interpretation, computational models that consider asymmetric H-bonding to quinones are developed, and density functional theory (DFT) based vibrational frequency calculations are undertaken. Studies on identical quinones in two different RCs presents an interesting case which allows for the direct comparison of the binding site properties.

**Figure 9.2** Structure and numbering scheme for (A) PhQ, (B) PQ-9, (C) DMBQ, and (D) Cl₄BQ. In the A₁ binding site, for PhQ (A) and PQ-9 (B), the carbonyl group on C₄ position (C₄=O) is subjected to H-bonding by the binding site residue. In the Q+H₂O and Q+Leu models, H-bonding was provided to C₄=O. For DMBQ, a model with H-bonding to C₁=O was also considered. For Cl₄BQ, H-bonded carbonyl group will be referred as C₄=O.
9.2 Materials and Methods

9.2.1 Sample preparation

Trimeric PSI particles from menB\(^-\) mutant cells from S6803 were isolated and stored until use, as described previously (44). All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) and were used as received. For a preparation of a thin-film sample, menB\(^-\) PSI particles suspended in Tris buffer (pH 8.0) with 0.04% \(n\)-dodecyl-\(\beta\)-D-maltoside was ultra-centrifuged (4081000 g for 3 h) to produce a soft pellet of PSI particles. 0.1 µL each of 200 mM sodium ascorbate and 3 mM phenazine methosulfate were added directly to the soft pellet. The pellet was squeezed between two circular calcium fluoride (CaF\(_2\)) windows as described previously, so that the OD of amide I band (1658 cm\(^{-1}\)) remained less than 1.0.

For measurements at cryogenic temperature, the sample was loaded into a Model ND 110H liquid nitrogen cooled cryostat (Cryo Industries of America Inc., Macheester, NH), and the temperature was stabilized at 77 K.

9.2.2 Incorporation of quinones into the A\(_1\) binding site

Incorporation of non-native quinones into the A\(_1\) binding site was performed as described previously (126). Quinones dissolved in dimethyl sulfoxide were added to a suspension of menB\(^-\) PSI particles at 500x molar excess, while keeping the v:v concentration of dimethyl sulfoxide below 2% of the total volume. The mixture was stirred for 24 h at 277 K in the dark.

9.2.3 Static photoaccumulation FTIR DS

Static FTIR absorption DS was applied as described previously using a Bruker IFS66 or Vertex80 (Bruker Optics Inc., Billerica, MA) to generate photoaccumulated (P700\(^+\) – P700) DS at 77 K (163). The P700\(^+\) state was generated by a steady-state illumination using a 20-mW helium neon laser, expanded to a spot size of ~1 cm at the sample. 64 spectra were collected at 80 kHz.
before, during, and after illumination. The dark-light-dark cycle was repeated 100 – 300 times and averaged. The spectra collected during and after the illumination were ratioed against the spectra before the illumination. To generate \((P700^+ - P700)\) DS, the spectra collected after the illumination were scaled and subtracted from the spectra collected during the illumination so that well-known \(CO_2\) features in the 2370 – 2310 cm\(^{-1}\) spectral region cancelled out.

9.2.4 *Time-Resolved Step-Scan (TRSS) FTIR DS*

TRSS FTIR absorption DS with 6 \(\mu s\) temporal resolution was undertaken as described previously using a Bruker Vertex80 (Bruker Optics Inc., Billerica, MA) (21, 30). Briefly, data were collected in the 1950 – 1100 cm\(^{-1}\) spectral region at 4 cm\(^{-1}\) spectral resolution on a ~3.5 ms timescale. Long-pass optical filters were placed between the probe light source and the sample, and between the sample and the detector, to block light above 1950 cm\(^{-1}\). the CaF\(_2\) window of the sample cell and the cryostat blocked light below 1100 cm\(^{-1}\). An actinic light was provided by saturating 5 ns pulses at 532 nm from a Minilite Nd:YAG laser operating at 10 Hz repetition rate (Continuum, San Jose, CA). An interferogram was divided into 946 steps, and 20 coadditions were made at each step. This process was repeated 50 – 60 times and averaged. The time-resolved kinetics were analyzed globally as described below, and \((P700^+A_1^- - P700A_1)\) or \((^3P700 - P700)\) DS were produced as a decay associated spectrum (DAS). From \((P700^+A_1^- - P700A_1)\) DS, \((A_1^- - A_1)\) DS was constructed by subtracting \((P700^+ - P700)\) DS scaled at 1718(+)/1697(–) cm\(^{-1}\).

9.2.5 *Spectral and kinetic data analysis*

The data obtained from the TRSS FTIR experiments are analyzed globally using Glotaran (164). All of the TR spectra were fitted globally to multi-exponential functions to construct decay-associated spectra (DAS). Heating induced artifact with a lifetime of ~15\(\mu\)s was separated as discussed previously (178).
9.2.6 DFT-based vibrational frequency calculations

Molecular geometry optimizations and harmonic vibrational frequency calculations of quinones were undertaken using hybrid density functional theory (DFT) methods, employing the B3LYP functional and the 6-31+G(d) method within Gaussian 09 (Gaussian Inc., Wallingford, CT) (191). For neutral state quinone, the overall charge was assigned as 0. For reduced semiquinone (anion state), the overall charge was defined as –1. The calculated stick spectra (intensity vs. frequency) were convolved with a Gaussian function of 4 cm\(^{-1}\) HWHM. Calculated frequencies were not scaled. The difference spectrum was constructed by subtracting the convolved neutral spectrum from the anion spectrum. An assignment of calculated frequencies to molecular groups was based on visual inspection of the calculated atomic displacements associated with the normal modes. The atomic displacements were visualized on GaussView 5 (Gaussian Inc., Wallingford, CT). The hydrocarbon tail of PQ-9 was truncated to 1 unit (PQ-1) in the computational model. The phytol tail of PhQ was also truncated to a 5-carbon unit \([\text{CH}_2\text{CHC(CH}_3)_2]\) which is identical to the single unit of the truncated PQ tail.

9.3 Results

Recent studies on men\(B^-\) PSI with BQs in the A\(_1\) binding site have shown that by limiting exposure to actinic flashes at room temperature prior to freezing, the quinones remain functional as ET cofactors at cryogenic temperature (190). Therefore, to construct the \((\text{P700}^+\text{A}_1^- – \text{P700A}_1)\) FTIR DS, men\(B^\cdot\) PSI with BQs analogues in the A\(_1\) binding site was cooled to 77 K without exposure to actinic flashes at room temperature.

9.3.1 TRSS FTIR DS

TRSS FTIR DS were collected for men\(B^-\) PSI with the three BQs analogues incorporated at 77 K. Three BQs studied here are PQ-9, DMBQ, and Cl\(_4\)BQ. The two non-native BQs were
chosen in part because of potential differences in their FTIR spectra relative to PQ-9, as estimated from the calculated gas phase infrared absorption spectra (see section 9.3.3). TR FTIR data was collected in the 2000-1000 cm\(^{-1}\) region at 4 cm\(^{-1}\) spectral resolution (kinetics every 2 cm\(^{-1}\)). Temporal resolution was 6 \(\mu\)s. The temporal profile of absorption changes at select frequencies for PSI with the BQ analogues incorporated were presented previously (190), and from global analysis of the TR FTIR data blocks, lifetimes of the major decay phases were found to be 920/382/629 \(\mu\)s for PSI with PQ-9/DMBQ/Cl\(_4\)BQ incorporated, respectively.

TR FTIR DS were analyzed globally to construct decay associated spectra (DAS). Fig. 9.3A shows the DAS for the major decay phase that correspond to \((P700^+A_1^- - P700A_1)\) FTIR DS for PSI samples with the three BQ analogues incorporated. For comparison, the DAS obtained for PSI with PhQ and 2MNQ incorporated, and the static \((P700^+ - P700)\) DS are also shown. Spectra for three BQs have been reproduced from Ref. (190), and 2MNQ from Ref. (178). The spectrum for PhQ was updated from the data presented in Ref. (178). Importantly, the DAS display no spectral features found in \((^3P700 - P700)\) FTIR DS. The spectra in Fig. 9.3A are typical of \((P700^+A_1^- - P700A_1)\) FTIR DS, indicating that all the quinones incorporated into the A\(_1\) binding site remained functional as ET cofactors at 77 K. By subtracting \((P700^+ - P700)\) DS collected by static photoaccumulation measurement from \((P700^+A_1^- - P700A_1)\) DS in Fig. 9.3A, \((A_1^- - A_1)\) FTIR DS can be constructed. One advantage of this approach is that it will remove any contributions that P700\(^+\)/P700 might make to the DS that could potentially interfere with interpretation of bands associated with the quinones. \((A_1^- - A_1)\) FTIR DS obtained using PSI with the three BQs and two NQs incorporated are shown in Fig. 9.3B.
Figure 9.3  (A) DAS corresponding to \((P700^+A_1^- - P700A_1^-)\) FTIR DS for PSI with PhQ (black), 2MNQ (orange), PQ-9 (green), DMBQ (red), and Cl₄BQ (blue) incorporated into the A₁ binding site. \((P700^+ - P700)\) DS collected by static photoaccumulation measurement is also included (gray). (B) \((A_1^- - A_1)\) DS constructed by subtracting the static \((P700^+ - P700)\) DS from the \((P700^+A_1^- - P700A_1^-)\) DS, in the anion region.

9.3.2  FTIR Double Difference Spectra (DDS)

To better visualize bands associated with quinones in the spectra in Fig. 9.3, FTIR DDS are calculated by subtracting the spectrum obtained for PSI with PhQ incorporated from the spectra for PSI with non-native quinones incorporated. These FTIR DDS are shown in Fig. 9.4. The DDS are constructed using \((P700^+A_1^- - P700A_1^-)\) DS (Fig. 9.3A). Similar DDS can be obtained by using \((A_1^- - A_1)\) DS (Fig. 9.3B), but with reduced signal-to-noise ratio (178). In this study, the focus is on the features of anionic semiquinones in the A₁ binding site, and spectral region was selected accordingly. In the FTIR DDS, in the anion spectral region (1540-1380 cm⁻¹), PhQ⁻ bands are negative and non-native quinone bands are positive. In regions where the different quinones have bands in common, no feature in the DDS is expected.
9.3.3 DFT Calculations of Gas Phase, $Q+H_2O$, and $Q+Leu$ Models

To aid in the spectral analyses and band assignments, DFT-based vibrational frequency calculations of neutral and anion state quinones were undertaken. Previously, EPR spectroscopy has shown that the position and orientation (relative to P700) of PQ-9 and other non-native NQs and AQs in the $A_1$ binding site is identical, within error, to that of PhQ (47, 63). That is, the C=O groups of the quinone are oriented and positioned identically. Additionally, the asymmetric spin density distribution found for PQ-9 in PSI suggests that the H-bonding is provided to $C_4=O$ (89). Following this result, it will be assumed that non-native BQs are also oriented and positioned identically in the $A_1$ binding site, and that the hydrocarbon tail of PQ-9 is in the same position relative to the H-bonding as that for the phytol tail of PhQ. To confirm the orientation of PQ-9, models which provides H-bonding to $C_1=O$ were also constructed.

The quinone-protein interaction of interest in this study is the asymmetric H-bonding to the C=O group of quinone. To consider the effect of this binding site interaction on quinones,
different levels of computational models were constructed. One model considers a quinone in gas phase, and two models introduce asymmetric H-bonding to a quinone. In the gas phase model, a quinone is simply considered in gas phase in absence of any other molecules. The models that provide asymmetric H-bonding to the quinone were constructed from the 2.5-Å X-ray crystal structure of PSI RC isolated from *T. elongatus* (pdb ID 1JB0). To construct BQs in these models, PhQ in the A$_{1A}$ binding site was modified without altering the position and orientation of the quinonic ring. The truncated hydrocarbon tail of PQ-9 described above assumes the exact position as that of PhQ in the crystal structure. A model which uses a water molecule as the H-bond provider (hereafter Q+H$_2$O model) was constructed by replacing the nitrogen atom of the LeuA722 backbone by an oxygen atom. A model that considers the original amino acid residues as the provider of asymmetric H-bonding (hereafter Q+Leu model) included the following in the structure: the backbone of LeuA722 and one carbon of its sidechain, the adjacent nitrogen atom of SerA723 (its valence was satisfied by converting to NH$_2$), and the backbone of AlaA721 and its sidechain. The starting structures of both Q+H$_2$O and Q+Leu for PhQ are depicted in Fig. 9.5.

Infrared absorption spectra were calculated for PhQ, PQ, DMBQ, and Cl$_4$BQ in the gas phase, Q+H$_2$O, and Q+Leu models, in anion and neutral state. The calculated frequencies, signal intensities, and assignments of vibrational mode for bands associated with C=O/C≡O are summarized in Table S1/Table 9.1, respectively. A general assignment of calculated frequencies to molecular groups was based on visual inspection of the molecular groups that most prominently contribute to the animated vibration. Calculated frequencies were not scaled.
Figure 9.5  Computational models for the vibrational frequency calculations. Asymmetrical H-bonding is provided to a carbonyl group of quinone (PhQ in these illustrations) by a water molecule in (A) Q+H$_2$O model and by amino acid residues cutout from the A$_{1A}$ binding site in (B) Q+Leu model. Parameters for the optimized geometry (bond lengths and angles) are listed in Table S2.

Fig. 9.6A/B.1 shows the calculated anion/neutral spectra for four quinones in the gas phase, respectively. For PhQ in the gas phase, the 1720 cm$^{-1}$ band is due to the antisymmetric stretching of both C=O groups (91). The corresponding band is shifted 8 cm$^{-1}$ lower for PQ, and 4/37 cm$^{-1}$ higher for DMBQ/Cl$_4$BQ, respectively. For PhQ$^-$ the main C=O antisymmetric stretching band appears at 1533 cm$^{-1}$, and is 14 cm$^{-1}$ lower for PQ$^-$ and 3/27 cm$^{-1}$ higher for DMBQ$^-$/Cl$_4$NQ$^-$, respectively. Similar shifts in C=O/C=O band had been calculated between PhQ/PhQ$^-$ and PQ/PQ$^-$ in the previous study, which model differed only by the length of hydrocarbon tail on PhQ (the previous model contained 4 carbons as opposed to 5 carbons in the current model) (100).

Two computational models were constructed for the calculation of vibrational frequency for a quinone with asymmetric H-bonding to its carbonyl group. Quinones were asymmetrically H-bonded to a water molecule in the first model (Q+H$_2$O model), and to truncated amino acid residues from the A$_{1A}$ binding site in the second model (Q+Leu model). For DMBQ, two
orientations are considered: the first orientation provides the H-bonding to C₄=O and directs the methyl groups ortho to the H-bond (ortho model), and the second orientation provides the H-bonding to C₁=O and the methyl groups are meta to the H-bond (meta model). Similar models were also constructed for PQ. For Cl₄BQ, H-bonded carbonyl group is numbered as C₄=O. Key bond lengths and angles obtained from the optimized geometry are listed in Table S2. The calculated (unscaled) anion spectra for PhQ⁻, PQ⁻, DMBQ⁻, and Cl₄BQ⁻ in Q+H₂O and Q+Leu models are shown in Fig. 9.6B and 9.6C. The neutral spectra for four quinones are shown in Fig. B.1B and B.1C. The spectra for PQ with an H-bond to C₁=O, and comparison to the PQ H-bonded at C₄=O are in Fig. B.2. Table B.1/9.1 summarizes the calculated vibrational mode frequencies and intensities for C=O/C⁻=O of four quinones.

With an asymmetric H-bonding to C₄=O, antisymmetric stretching mode of C=O groups of PhQ are decoupled, and the C₄=O vibrational mode is downshifted 14 and 27 cm⁻¹ relative to the C₁=O mode in PhQ+H₂O and PhQ+Leu models, respectively. Likewise, for PhQ⁻ the C⁻=O mode is decoupled and results in downshifting of the C₄⁻=O mode by 60 – 67 cm⁻¹ depending on the model.
Figure 9.6  Calculated anion spectra for PhQ\(^-\) (black), PQ\(^-\) (green), DMBQ\(^-\) (red), and Cl\(_4\)BQ\(^-\) (blue), in the gas phase model (A), Q+H\(_2\)O model (B), and Q+Leu (C) model. Relative shift from the C\(^\equiv\)O or C\(_1\)\(^\equiv\)O mode PhQ\(^-\) to the major C\(^\equiv\)O mode are labeled for each quinone. Dashed spectra in the Q+H\(_2\)O and Q+Leu model calculations correspond to the meta model of DMBQ\(^-\).

The asymmetric H-bonding induces decoupling of C=O/C\(^\equiv\)O modes for the BQ series as well. For neutral state PQ, in Q+H\(_2\)O model, C=O modes are decoupled to C\(_1\)=O (1720 cm\(^{-1}\)), antisymmetric C=O (1706 cm\(^{-1}\)), and C\(_4\)=O modes (1680 cm\(^{-1}\)). The C=O modes are also split into three major bands in Q+Leu model, but the antisymmetric C=O mode at 1702 cm\(^{-1}\) now contains more contribution from C\(_1\)=O. In the anion state, in Q+H\(_2\)O model, C\(_4\)\(^\equiv\)O modes are decoupled into multiple bands at 1502, 1490, and 1483 cm\(^{-1}\), all with a small amplitude, but C\(_1\)\(^\equiv\)O...
mode is not decoupled from the antisymmetric C=O mode at 1523 cm\(^{-1}\). In Q+Leu model, however, decoupled C\(_1\)-O band is observed at 1549 cm\(^{-1}\), along with antisymmetric C=O mode at 1534 cm\(^{-1}\) and decoupled C\(_4\)=O mode at 1492 cm\(^{-1}\).

Table 9.1  Summary of calculated vibrational frequencies associated with C\(^{\equiv}\)-O modes for PhQ\(^{-}\), PQ\(^{-}\), DMBQ\(^{-}\), and Cl\(_4\)BQ\(^{-}\) in the gas phase, Q+H\(_2\)O, and Q+Leu models. Italicized vibrational mode and frequencies are for meta model DMBQ\(^{-}\). For Cl\(_4\)BQ\(^{-}\), H-bonded carbonyl group is numbered as C\(_4\)-O. H\(_2\)O and Leu refer to vibrational modes associated with molecular bonds in H\(_2\)O and truncated amino acid that supplied H-bonding. The frequencies are in cm\(^{-1}\). Intensities in km/mol are listed in parentheses. Frequencies are not scaled.

<table>
<thead>
<tr>
<th></th>
<th>PhQ(^{-})</th>
<th>PQ(^{-})</th>
<th>DMBQ(^{-})</th>
<th>Cl(_4)BQ(^{-})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gas phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v(C(^{\equiv})-O)(_{as}), (\delta(C-H))</td>
<td>1533 ((392))</td>
<td>1519 ((365))</td>
<td>1536 ((418))</td>
<td>1560 ((369))</td>
</tr>
<tr>
<td>v(C(^{\equiv})-O)(_{as}), (\delta(C-H))</td>
<td>1483 ((111))</td>
<td>1472 ((282))</td>
<td>1475 ((339))</td>
<td></td>
</tr>
<tr>
<td><strong>Q+H(_2)O</strong></td>
<td>v(C(_1)-O), (\delta(C-H))</td>
<td>1536 ((314))</td>
<td>1544 ((89))</td>
<td>1536 ((531))</td>
</tr>
<tr>
<td>v(C(_1)-O), (\delta(C-H))</td>
<td>1477 ((186))</td>
<td>1502 ((69))</td>
<td>1539 ((474))</td>
<td></td>
</tr>
<tr>
<td>v(C(_4)-O), (\delta(C-H))</td>
<td>1490 ((47))</td>
<td>1505 ((108))</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Q+Leu</strong></td>
<td>v(C(_1)-O), (\delta(C-H))</td>
<td>1539 ((378))</td>
<td>1549 ((109))</td>
<td>1535 ((596))</td>
</tr>
<tr>
<td>v(C(_4)-O), (\delta(C-H))</td>
<td>1472 ((282))</td>
<td>1499 ((105))</td>
<td>1539 ((520))</td>
<td></td>
</tr>
<tr>
<td>v(C(_4)-O), (\delta(C-H))</td>
<td>1492 ((70))</td>
<td>1495 ((339))</td>
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9.4 Discussion

9.4.1 Vibrational frequencies of anionic quinones in PSI

Neutral state \(p\)-quinones and anion state \(p\)-semiquinones exhibit intense infrared bands due to the C=O groups and C\(^{\equiv}\)-O groups, respectively (91, 192). In organic solvent and in protein
environment such as the photosynthetic RCs, the neutral state C=O quinone bands are expected in ~1700 – 1600 cm\(^{-1}\) range and the anion C\(\equiv\)O bands in ~1550 – 1400 cm\(^{-1}\) range. In PSI, the neutral state region is obscured by intense protein mode and P700\(^+\)/P700 bands (Fig. 9.3), and isolation of quinone bands in this region is complicated (115). The anion region is less impacted by the protein and P700\(^+\)/P700 bands, and in this chapter the focus is mainly on the identification of anionic semiquinone bands.

In recent studies, for PSI with PhQ incorporated in the A\(_1\) binding site, an intense band at 1494 cm\(^{-1}\) is identified as (relatively pure) C\(\equiv\)O stretching mode (178). Furthermore, the vibrational frequency calculations have predicted that a minor band at 1414 cm\(^{-1}\) contains contributions from C\(\equiv\)O mode that is downshifted ~80 cm\(^{-1}\) due to strong asymmetric H-bonding. Similar bands in the anion region can be identified in the (A\(_1^-\) – A\(_1\)) DS for PSI with BQs incorporated into the A\(_1\) binding site. In (A\(_1^-\) – A\(_1\)) DS for PSI with PQ-9 incorporated in the A\(_1\) binding site, an intense band is observed at 1489 cm\(^{-1}\), and a minor band at 1443 cm\(^{-1}\). The bands for PSI with DMBQ incorporated are observed at similar wavenumbers as those for PSI with PhQ incorporated: a major band is observed at 1494 cm\(^{-1}\), and a minor band at 1414 cm\(^{-1}\). The spectral features of PSI with Cl\(_4\)BQ incorporated are more complicated, however, with multiple minor bands observed in the 1530 – 1485 cm\(^{-1}\) range. Additional minor bands are observed at 1467 cm\(^{-1}\) and 1444 cm\(^{-1}\).

Bands associated with quinones are better visualized in the DDS. Here, the DDS were constructed using (P700\(^+\)A\(_1^-\) – P700A\(_1\)) DS, and with respect to PSI with PhQ incorporated in the A\(_1\) binding site. In the anion region, the negative bands correspond to the bands due to PhQ\(^-\). In (2MNQ – PhQ), (PQ-9 – PhQ), and (Cl\(_4\)BQ – PhQ) DDS, a prominent negative band is observed at 1495 – 1494 cm\(^{-1}\). These results confirm the previous assignment of 1494 cm\(^{-1}\) band as the band
due to PhQ\(^-\). Similarly, a negative band at 1414 cm\(^-1\) is observed in the same three set of DDS, and the assignment to the PhQ\(^-\) is reconfirmed. In addition to these two bands, the three DDS also exhibit a common negative band at 1475 cm\(^-1\), which was previously unrecognized due to less pronounced intensity in (2MNQ – PhQ) DDS. The appearance of the bands in three DDS, however, indicate that this band at 1475 cm\(^-1\) is also due to PhQ\(^-\). In (DMBQ – PhQ) DDS, none of the 1495, 1475, or 1414 cm\(^-1\) bands are observed, because both species exhibit bands at these same positions in their (P700\(^+\)A\(_1^-\) – P700A\(_1\)) DS.

In (PQ-9 – PhQ) DDS, a prominent positive band is observed at 1487 cm\(^-1\), which forms a difference feature with the 1495 cm\(^-1\) of PhQ\(^-\). Minor positive bands are also found at 1526 cm\(^-1\) and 1444 cm\(^-1\). While 1526 cm\(^-1\) is rather too high for the band to be assigned to PQ-9\(^-\), both the 1487 cm\(^-1\) and 1444 cm\(^-1\) bands can tentatively be assigned to PQ-9\(^-\). This assignment indicates that the major band for PQ-9\(^-\) is downshifted ~8 cm\(^-1\) relative to PhQ\(^-\), while the minor band is upshifted 29 cm\(^-1\). No major difference features are observed in (DMBQ – PhQ) DDS. As mentioned earlier, in the anion region the (P700\(^+\)A\(_1^-\) – P700A\(_1\)) DS are very similar for PhQ and DMBQ. Lack of difference features in (DMBQ – PhQ) DDS reiterates this point. 1495 and 1414 cm\(^-1\) bands, therefore, are assigned DMBQ\(^-\). (Cl\(_4\)BQ – PhQ) DDS, on the other hand, exhibits multiple difference features. Positive bands are observed at 1526, 1518, 1487, 1466, and 1444 cm\(^-1\). As was the case for PQ-9\(^-\), the bands besides 1526 cm\(^-1\) (1518, 1487, 1466, and 1444 cm\(^-1\)) can tentatively be assigned to Cl\(_4\)BQ\(^-\). Several band positions, including 1526 cm\(^-1\) coincide with those assigned for PQ-9\(^-\). These overlap of the band position with PQ-9\(^-\) could arise if the binding sites are incompletely replaced by Cl\(_4\)BQ, resulting in partial occupation of the binding site by PQ-9. Although a change in reaction kinetics indicate such a scenario is unlikely, if these bands are due to unreplaced PQ-9, then only the bands at 1518 and 1466 cm\(^-1\) can be assigned to Cl\(_4\)BQ\(^-\). These
assignments indicate that the higher band for Cl₄BQ⁻ is upshifted by ~23 cm⁻¹ relative to the major band for PhQ⁻. In summary, the position of the prominent band in the anion region relative to that for PhQ is upshifted for PQ⁻/9, unaffected for DMBQ, and downshifted for Cl₄BQ.

9.4.2 Calculated vibrational mode frequencies and assignments of anionic quinone bands

9.4.2.1 Gas phase model

For interpretation of the experimental spectra computational vibrational frequency calculations were employed. Here, three different computational models were developed and considered. In the simplest calculation using the gas phase model, the resulting spectra are composed mainly of the antisymmetric C=O mode. Relative to PhQ, the C=O mode for PQ⁻/DMBQ⁻/Cl₄BQ⁻ is shifted by −14/+2/+27 cm⁻¹, respectively. Such shifts are in agreement with the assignment of anionic quinone bands in the experimental spectra (−8/+0/+23 cm⁻¹ for PQ⁻/DMBQ⁻/Cl₄BQ⁻ relative to PhQ⁻). From the previous studies, it is known that the 1494 cm⁻¹ band for PhQ⁻ is due to a relatively pure C₁=O mode, and that the gas phase calculation does not sufficiently explain this feature of the experimental spectra. The gas phase calculation, however, serves as an initial inspection of the assignment, and the agreement between the experimental and calculated spectra suggests that the band assignments are reasonable.

9.4.2.2 Q+H₂O model

Two computational models were developed to consider the effect of asymmetric H-bond. In the Q+H₂O model, a water molecule is the source of H-bonding. C=O mode is often decoupled in this model, and the H-bonded carbonyl group is downshifted relative to the non-H-bonded one. Comparing the most intense band produced by this calculation in the anion region, PQ⁻/DMBQ⁻/Cl₄BQ⁻ are shifted −13/+0/+28 cm⁻¹. For DMBQ⁻, if an H-bonding is made to C₁=O, the shift is +3 cm⁻¹. These resulting shifts for the Q+H₂O model agree with the experimentally observed
shifts, and are similar to the ones calculated by the gas phase model. Compositions of the bands, however, are different from the previous model. For PhQ⁻, the band at 1536 cm⁻¹ has contributions from C₁=O, while for PQ⁻/DMBQ⁻/Cl₄BQ⁻, the corresponding major bands are due to antisymmetric C=C=O. Decoupling of C₄=O modes, however, are observed in all four quinones, and vibrational mode containing C₄=O contributions are observed at 1477 cm⁻¹ for PhQ⁻, 1502 and 1490 cm⁻¹ for PQ⁻, 1491 cm⁻¹ for DMBQ⁻, and 1549 cm⁻¹ for Cl₄BQ⁻.

9.4.2.3 Q+Leu model

In the Q+Leu model, decoupling of C=C=O mode is even more pronounced. In this model, the shift of the major band relative to PhQ⁻ is -15/-4/+26 cm⁻¹ for PQ⁻/DMBQ⁻/Cl₄BQ⁻. For DMBQ⁻, the shift is +0 cm⁻¹ if the asymmetric H-bonding is made to C₃=O. The band shifts, again, are in agreement with the experimentally observed shifts. For PhQ⁻, 1539 cm⁻¹ band is mainly composed of C₁=O mode contribution, and 1472 cm⁻¹ is of C₄=O mode contribution. A minor C₄=O mode contributions is also observed in 1520 cm⁻¹ band. The calculated separation between C₁=O and C₄=O modes is ~67 cm⁻¹, and is closer to the experimentally observed ~80 cm⁻¹ than the separation predicted by the Q+H₂O model (59 cm⁻¹). Furthermore, the Q+Leu model for PhQ predicts a band at 1520 cm⁻¹, which could explain the newly recognized 1475 cm⁻¹ band for PhQ⁻ in the experimental spectra. A minor band is observed in the Q+H₂O model for PhQ⁻ as well, but the separation between the main C=C=O mode is again in better agreement with the Q+Leu model (19 cm⁻¹ experimentally, 13 cm⁻¹ with the Q+H₂O model, and 19 cm⁻¹ with the Q+Leu model). Compositions of 1520 cm⁻¹ band calculated for PhQ⁻ by the Q+Leu model includes C-H and C₄=O modes.

For PQ⁻, contributions from C=C=O mode is observed in three bands: 1549 cm⁻¹ with C₁=O, 1534 cm⁻¹ with C=C=O, and 1492 cm⁻¹ with C₄=O mode contributions. In addition to these C=C=O modes.
mode, multiple bands exhibit relatively high intensities in the 1530 – 1480 cm\(^{-1}\) region. Of these bands, 1499 and 1483 cm\(^{-1}\) are due to vibrational modes of C-H groups in PQ\(^{-}\). Experimentally, two major bands identified are at 1487 cm\(^{-1}\) and 1444 cm\(^{-1}\). Given the relative shift from the PhQ\(^{-}\) peaks, 1487 cm\(^{-1}\) in the experimental spectra for PQ-9\(^{-}\) is predicted to contain C=O contributions. This prediction can be supported by both the Q+H\(_2\)O and Q+Leu model calculations. Although calculations of the two models predict multiple bands below the C=O band, in all cases these bands are at higher wavenumber than the C=O mode calculated for PhQ\(^{-}\). This is also in agreement with the experimental spectra, as 1444 cm\(^{-1}\) band for PQ-9\(^{-}\) is at considerably higher wavenumber than PhQ\(^{-}\)'s 1414 cm\(^{-1}\) band. Following this, it is likely that 1444 cm\(^{-1}\) band for PQ-9\(^{-}\) is at least partially due to the C=O mode.

For DMBQ\(^{-}\), C=O modes are observed at 1535 cm\(^{-1}\), or at 1539 cm\(^{-1}\) if the H-bonding is made to C=O. If C=O group is H-bonded, the C=O mode is calculated at 1487 cm\(^{-1}\). If C=O group is H-bonded, the downshifted C=O mode is at 1495 cm\(^{-1}\). Experimentally, no major difference features were observed in the DDS between DMBQ and PhQ. The calculated major band is in agreement with this result as the position is within 4 cm\(^{-1}\) of the PhQ\(^{-}\)'s. This band for DMBQ\(^{-}\) is therefore assigned to the C=O vibrational mode. However, in the calculated spectra the downshifted C=O modes for DMBQ\(^{-}\) do not align with that for PhQ\(^{-}\). For DMBQ\(^{-}\), in both the Q+H\(_2\)O and Q+Leu models, the split C=O mode is at higher wavenumber than the C=O mode of PhQ\(^{-}\). This feature is more pronounced in the meta model (where the H-bonding is provided to C=O), with both Q+H\(_2\)O and Q+Leu calculations predicting a smaller downshift. Given this condition, it is likely that the orientation of DMBQ in the A\(_1\) binding site is such that the asymmetric H-bonding is provided to C=O.
For Cl₂BQ⁻, the Q+Leu model results in a significant decoupling of C\=O mode which was not observed in the gas phase or Q+H₂O model. In the Q+Leu model, the band containing C\=O vibrational mode is observed at three wavenumbers, all three with comparable intensities. Presence of three C\=O bands may explain multiple positive bands observed in the experimental (Cl₂BQ – PhQ) DDS. However, if some of the bands are due to PQ-9⁻ remaining in the binding site, then only the 1518 cm⁻¹ can be assigned confidently. Given the relative upshift from PhQ⁻, the experimental 1518 cm⁻¹ most likely corresponds to the 1565 cm⁻¹ band calculated by the Q+Leu model. The band composition includes antisymmetric C\=O mode with more contributions from C₁\=O.

In any case, the Q+H₂O and Q+Leu models have simulated the experimental spectra more accurately than the simple gas phase calculations. That is, splitting of C\=O modes could only be simulated adequately in the presence of asymmetric H-bonding. Additionally, for PhQ, PQ, and Cl₂BQ, a presence of truncated amino acid residues resulted in better simulation of the experimental spectra than a water molecule. These results indicate that the BQs, including PQ-9, are asymmetrically H-bonded in the A₁ binding site much like the native PhQ.

Comparing the simulated anion spectra for PQ with H-bonding provided to C₄=O and to C₁=O, the spectra for PQ with H-bonding to C₁=O predicts the split C\=O band to be more intense than for PQ with H-bonding to C₄=O (Fig. B2). The feature is especially notable in the Q+Leu model, in which the intensity of the split band is comparable to the main C\=O band (172 km/mol at 1493 cm⁻¹ vs. 247 km/mol at 1526 cm⁻¹). In the experimental spectra, the band assigned for the main C\=O mode is clearly more intense than the minor 1444 cm⁻¹ band. The calculations for PQ with H-bonding to C₁=O, therefore, does not simulate the experimental spectra accurately. These
calculations suggest that the H-bonding to PQ-9 is provided to C₄=O, which is in line with the previous EPR results that suggested PQ-9 is H-bonded at C₄=O in the A₁ binding site (89).

9.4.3 Vibrational mode frequencies of anionic PQ-9 in the A₁ binding site

From the experimental DDS and the computational vibrational frequency calculations, the 1489 cm⁻¹ is assigned to the C\textsuperscript{=}O mode of PQ⁻. The assignment indicates a 5 cm⁻¹ downshift relative to the C\textsuperscript{=}O mode of PhQ⁻ (at 1494 cm⁻¹). Previously, it was suggested, based on relatively low signal to noise ratio data, that PQ⁻ in the A₁ binding site in PSI displays a C\textsuperscript{=}O stretching vibration at 1487 cm⁻¹, 8 cm⁻¹ lower than the corresponding band of PhQ⁻ (100). The observations in the previous study are verified in this study, which clearly shows an intense band at 1489 cm⁻¹. The result is supported by the vibrational frequency calculations, which suggests that the C\textsuperscript{=}O mode of PQ⁻ downshift by 15-13 cm⁻¹, depending on the computational model used. All calculations presented here, however, overestimates the separation. In the experimental spectra, a minor band is observed at 1444 cm⁻¹ for PQ⁻. Such features are simulated by the Q+H₂O and Q+Leu models, which suggest that the band is associated with the C\textsuperscript{=}O mode.

The assignment of the 1489 cm⁻¹ band signifies a 10 cm⁻¹ shift in the C\textsuperscript{=}O mode of PQ⁻ between A₁ of PSI and QA of PSII (193, 194). Although the number of H-bonds made to the quinone is one of the major differences between the two binding sites that could impact the vibrational frequency, computational calculations have shown that a different number of H-bonds does not affect the position of the C\textsuperscript{=}O mode drastically (195). A similar type of shift in the quinone bands has been observed previously for PhQ in different RCs as well: 1494 cm⁻¹ band assigned to the C\textsuperscript{=}O mode in the A₁ binding site is upshifted by (at least) 17 cm⁻¹ from that in the QA binding site of pbRC (115, 196). Again, such a shift cannot simply be accounted for by a difference in the number of H-bonds. In both cases, however, the anionic quinone bands are
upshifted in the A\textsubscript{1} binding site of PSI relative to the respective binding sites in type II RCs. The observations reinforce the idea that the accurate simulation and interpretation of FTIR DS of quinones in photosynthetic RCs require consideration of the surrounding protein environment.

9.5 Conclusions

TRSS FTIR DS was applied to investigate PSI with PQ-9 and two other BQs incorporated in the A\textsubscript{1} binding site. Using non-photoinactivated menB\textsuperscript{-} PSI, (A\textsubscript{1}\textsuperscript{−} − A\textsubscript{1}) FTIR DS for PQ-9 in the A\textsubscript{1} binding site was produced for the first time. Comparison of the similar DS for PSI with PhQ and other BQs suggests that the C=C=O mode of anionic plastosemiquinone is at 1489 cm\textsuperscript{-1}, a 5 cm\textsuperscript{-1} downshift from that of PhQ\textsuperscript{−}, and a \textasciitilde 10 cm\textsuperscript{-1} upshift relative to PQ-9\textsuperscript{−} in the QA binding site of PSII. Frequency calculations suggest that BQs are also asymmetrically H-bonded in the A\textsubscript{1} binding site, and PQ-9 is H-bonded at C\textsubscript{4}=O. In addition, construction of DDS with BQs revealed a band for PhQ\textsuperscript{−} at 1475 cm\textsuperscript{-1}, which was assigned to \(\delta\)(C−H) and \(\nu\)(C=O) mode of PhQ\textsuperscript{−}. 
10. SUMMARY, CONCLUSIONS, AND OUTLOOK

10.1 Summary and Conclusions

The work presented in this dissertation is based on a series of spectroscopic studies undertaken on PSI RC. Understanding the bioenergetics of a specific electron acceptor in a megadalton protein complex requires an establishment of spectroscopic techniques with high temporal and spectral resolutions. In this dissertation, two types of spectroscopic methods were exploited for the investigation of energetics and molecular mechanisms involving the secondary acceptor $A_1$; with time-resolved visible absorption difference spectroscopy, the rates and pathways of the ET in PSI were studied, and with time-resolved step-scan FTIR difference spectroscopy, molecular details of the quinone in the $A_1$ binding site were studied. As methods to study biomolecular systems, the signal-to-noise ratios attained on kinetics and spectra presented here demonstrate that these techniques are applicable for the study of specific molecules or molecular interactions in multi-subunit protein complexes. This chapter is intended to summarize and conclude the work introduced in the previous chapters regarding the bioenergetics of PSI. The summary of the current work is followed by a proposal of the future work, along with preliminary data to support this proposal.

In the first half of the dissertation, the work pertaining to the kinetics and energetics of ET in PSI was organized. This part of the research was mainly conducted using time-resolved visible absorption difference spectroscopy and directly followed the work summarized in the Master’s thesis in 2012 (49). In the thesis in 2012, the fundamentals of flash-induced transient absorption spectroscopy and instrumentation of the spectrometer for the study of kinetics in PSI RC were discussed. To test the capability of the instrument, the transient absorption kinetics of PSI isolated from the wildtype (WT) and $menB$ deletion mutant of *Synechocystis* sp. PCC6803 were measured.
The measurements of the P700$^+$F$\alpha$$\beta^-$ charge recombination and forward A$_1^-$→F$_X$ ET kinetics for both the WT and menB$^-$ PSI, which correctly reproduced the previously reported reaction lifetimes, demonstrated that the setup of the spectrometer is appropriate for the study of ET kinetics in PSI. Additionally, by restoring PhQ into menB$^-$ PSI and comparing its transient absorption kinetics with those of the WT PSI, the method of quinone incorporation was established. A ~50 ns phase was monitored for menB$^-$ PSI probed at 800 nm, but not for WT PSI or menB$^-$ PSI with PhQ incorporated. At the time of the study, the origin of the phase was undetermined but was hypothesized to be due to the formation of $^3$P700 state.

Following the work presented in the 2012 thesis, in Ch. 2 of this dissertation, the first non-native quinone (2MNQ) was incorporated into the PSI RC. The ~50 ns phase at 800 nm, which hypothetical origin is due to the $^3$P700 state formation, was used as an indicator for successful incorporation of quinones into the A$_1$ binding site, as the phase disappeared upon recruitment of PhQ and 2MNQ into the A$_1$ binding site. In this study, the kinetics of PSI with PhQ, 2MNQ, and PQ$_9$ incorporated were measured in both the visible and infrared spectral ranges, at room and cryogenic temperatures. To measure the kinetics in two spectral regions and at two different temperature ranges under the identical sample conditions, a new sample preparation method was developed. This method allowed for the visible and infrared spectroscopic measurements at both room and cryogenic temperatures to be made on the identical sample conditions, if not on the same exact sample.

In an attempt to relate the observed ET kinetics to the in situ midpoint potentials of A$_1$, the rate was initially analyzed using the empirical Marcus equation (Moser-Dutton’s ruler) to solve for the driving force. The analysis, however, quickly revealed that this traditionally-accepted method of applying the Marcus equation results in unreasonable driving forces, as it inherently
assumes that all ET, including the forward ET from $A_1^-$ to $F_X$, are unidirectional. A kinetic analysis, instead, needs to consider the possibility of forward and reverse reactions between each donor/acceptor pair, or the quasi-equilibration of ET processes between each cofactor. To incorporate this quasi-equilibration aspects into the kinetic analysis, a kinetic simulation model based on the Marcus theory was developed. In the initial model that was introduced in Ch. 2, three cofactors ($A_{1A}$, $A_{1B}$, and $F_X$) were considered to simulate the biphasic forward electron transfer kinetics. The redox potentials estimated from the model were more reasonable than the values predicted by the direct employment of the Marcus equation. However, inspection of the initial version of the model also suggested that the finer estimation of the redox potentials would require finer specifications of the ET parameters used in the simulation.

One approach to define the ET parameters more accurately is to impose more conditions on the kinetic simulation model, so that the parameters are only allowed to fluctuate in ranges that satisfy such imposed conditions. Therefore, the model can be improved if it allowed for the simulation of not just the forward ET for three quinones, but the charge recombination kinetics and the kinetics at cryogenic temperatures as well, for quinones spanning a wide range of midpoint potentials. The studies in Ch. 3 and 4 were intended to explore such additional conditions. In Ch. 3, a quinone with a high midpoint potential ($\text{Cl}_2\text{NQ}$) was incorporated into PSI and its ET kinetics were studied using the transient absorption spectroscopy in the visible and infrared spectral regions. With a high potential quinone in the $A_1$ binding site, the dominant process observed is the $\text{P700}^+A_1^-$ charge recombination reaction at both 298 and 77 K. Using this property, the fractional usage of A- and B-branches in prokaryotic PSI at 77 K was determined. The fractional use of the two ET chains calculated from this study was implemented into the later version of the kinetic simulation model as one of the initial parameters (the initial population assigned to $A_{1A}^-$
and $A_{1B^-}$). In Ch. 4, including the previously introduced PhQ, PQ$_9$, 2MNQ, and Cl$_2$NQ, total of 8 different quinones were incorporated into the $A_1$ binding site and studied for the ET kinetics, both at room and cryogenic temperatures. The experimental data from Ch. 2, 3, and 4 were then used to construct the kinetic simulation models, which details were organized in Ch. 5.

While several methods of kinetic simulation existed in the past, the model developed in this dissertation was unique for its attempt to simulate the kinetics of PSI with 10 different quinones in the $A_1$ binding site, both the forward and backward (charge recombination) kinetics, and at 298 and 77 K. Initially developed as the extension of the model presented in Ch. 2, the calculation was carried out to simulate the forward ET kinetics, the charge recombination kinetics at 298 K, and the recombination kinetics at 77 K, in the presented order, for all 10 quinones incorporated into PSI. If, at any stage of the simulation, the kinetics could not be sufficiently simulated, the process was halted and re-started from the beginning with adjusted ET parameters. This process was repeated until the kinetics for all 10 quinones at two temperatures were successfully simulated. Compared to the previous version of the model presented in Ch. 2, the stringent conditions imposed in this model had allowed for the estimation of in situ midpoint potentials as well as some of the ET parameters such as the reorganization energies within tight ranges. From this kinetic model, the in situ midpoint potentials of 10 quinones (8 quinones studied in Ch. 5 + 2 quinones introduced in Ch. 6) were estimated, and a linear relationship was revealed between the in situ and in vitro potentials of quinones.

The studies presented in the thesis in 2012 and in this dissertation up to Ch. 5 revolved around the experimental observation of ET kinetics in PSI and theoretical estimation of the cofactor midpoint potentials. The knowledge gained from these studies essentially led to the work in Ch. 6 to address the long-standing question regarding the involvement of the Marcus inverted
region effect in the recombination reaction in photosynthetic RCs. To address this question, the kinetic simulation model presented in Ch. 5 was used to extract the intrinsic rate of ET for A$_{1A}^-$ to P700, which corresponds to the dominant recombination reaction pathway. Comparison of the intrinsic rates and the estimated midpoint potentials demonstrated that, at both room and cryogenic temperatures, the charge recombination reaction in PSI occurs in the inverted region. Furthermore, comparison of the experimentally-observed reaction time constants at 77 K, which are expected to represent the unidirectional ET process between A$_{1A}^-$ and P700, to the in vitro midpoint potentials of the incorporated quinones, an analysis that is not influenced by any theoretical treatments or presumptions, also exhibited an inverted parabolic feature with all the data points residing to the right of the vertex. The relationship observed in this analysis showed that 1) the ET theory is appropriate for the interpretation of the recombination kinetics, and 2) the assignment of the recombination kinetics at 77 K to the inverted region can be justified regardless the simulation parameters used in the model. The profile of the Marcus curve and the exact positions of the data points predicted for the kinetics at 298 K are subject to the choice of parameters used in the kinetic simulation. No reasonable combinations of the ET parameters, however, would shift the points from the right side of the vertex to the left. The experimental results and theoretical analyses presented here are the first demonstration of the charge recombination reaction in the photosynthetic RC occurring in the Marcus inverted region. Additionally, an extent of contribution by the inverted region effect to the quantum yield of ET in PSI was assessed through the modified kinetic simulation model that includes the diffusible donor and acceptor. The simulation indicated that without the inverted region effect, the quantum yield of PSI ET would drop from 98% to 72%.

In conclusion of the first half of the dissertation, time-resolved visible absorption spectroscopy was applied to study the ET processes in PSI with a series of non-native quinones
incorporated into the A₁ binding site. The kinetics were monitored at 298 and 77 K, and the obtained reaction rates were used to develop the kinetic simulation model based on the Marcus theory. From the simulation, the recombination reaction, both at 298 and 77 K, were determined to occur in the Marcus inverted region, and that the inverted region effect contribute to the overall quantum yield of photosynthetic ET in PSI.

In the latter half of the dissertation, the molecular details of the quinone in the A₁ binding site were investigated using time-resolved FTIR spectroscopy. In Ch. 7, the (2MNQ – PhQ) DDS were produced at high signal-to-noise ratio and analyzed by the three-layered DFT-based vibrational frequency calculations. Much of the theoretical work in this chapter was undertaken by Nan Zhao and Leyla Rohani in our group. The initial development of the two-layer model was carried out by Nan Zhao, and the refinement and revision to the three-layer version was conducted by Leyla Rohani. The infrared bands due to PhQ⁻ and 2MNQ⁻ in the A₁ site were determined, and the computational analyses indicated that the two prominent bands were due to the carbonyl groups under the influence of the intense asymmetric H-bonding. Ch. 8 and 9 focused on the functions of PQ₉ in the A₁ binding site of PSI. In these studies, it was determined that PQ₉ and other BQs can be doubly protonated in the A₁ binding site by an exposure to repetitive actinic flash at room temperature, but not at cryogenic temperature. The P700⁺A₁⁻/P700A₁ FTIR DS for PSI with PQ₉ in the A₁ binding site was produced for the first time, and the previously-existed discrepancy between the EPR and FTIR measurements on menB⁻ PSI was resolved. These findings in Ch. 8 and 9 can be tied to the unknown kinetics initially presented in the thesis in 2012. The ~50 ns phase at 800 nm, which was observed in menB⁻ PSI but not when any of the NQ or AQ were incorporated, appeared when BQs were incorporated. Given the universal nature of BQs to be
doubly protonated by exposure to the repetitive actinic flash at room temperature, the origin of the phase can now be associated with the \(^3\)P\(_{700}\) state formation.

The types of work summarized in the second half of the dissertation (Ch. 7 – 9), which involved the use of time-resolved step-scan FTIR spectroscopy and construction of the DDS for the analysis of semiquinone bands, were previously presented in a dissertation and a review article from our group (115, 197). In the current work, both the experimental and theoretical methods were re-evaluated to make better use of the time-resolved spectra and to gain better agreement between the experimental and theoretical results. The spectra, and interpretations of the spectra, presented in Ch. 7 – 9 are therefore derived from completely different sets of experiments and calculations based on re-established methodologies. Since the focus of Ch. 7 through 9, and of this dissertation in general, were on the analyses of obtained data for understanding the functions/properties of ET and cofactors in PSI, and not on the instrumentation and the experimental details themselves, the details of improvements were not discussed in these chapters. Instead, the development and improvements on the spectroscopic techniques that enabled the current work are summarized in Appendix C.

10.2 Outlook

The spectroscopic studies undertaken and organized in this dissertation were intended to advance the research on the bioenergetics in PSI, with a special focus on the secondary electron acceptor A\(_1\). As summarized, the spectral methods developed and applied in the studies presented here allowed to determine some of the key aspects of ET energetics in PSI. At the same time, however, these studies introduced a series of new questions to be answered, some of which are already under investigation in our group. In this section, a direction for the future research is proposed along with some of the preliminary data to support the proposal.
10.2.1 *On the* $^3$P700 state and the 50-ns phase at 800 nm

While the 50-ns phase observed at 800 nm for *menB*– PSI at room temperature was never the main focus of this project, the phase has found its use since its initial observation in 2012 as the indicator for quinone incorporation (30, 49). More recently, the phase was characterized to occur with any BQ incorporated into the A1 binding site (Ch. 8), and given the nature of BQs to be doubly-protonated, its origin was finally associated with the $^3$P700 state formation. Although the kinetics involving the $^3$P700 state and the spectra associated with the $^3$P700 state have been observed by many different types of spectroscopic methods, generation of $^3$P700 has always required special (and in many cases very harsh) treatment to be applied to the PSI RC (179). In *menB*– PSI, it is now characterized that the triplet state can easily be induced by simply exposing the sample to a series of actinic flashes at room temperature, a treatment that does not affect the state of PSI other than PQ9 that is doubly-reduced in the binding site. The double reduction deactivates A1 as an ET cofactor, and reroutes the ET pathway to undertake P700$^+$/A0$^-$ charge recombination, which involves the formation of the $^3$P700. The feature can therefore be used as the simple and non-invasive method to generate the $^3$P700 state. With this simplified method, several studies are now possible.

Although the $^3$P700 state has been studied in the past, much of the studies were done below freezing/cryogenic temperatures (93, 179). Knowing that the 50-ns phase corresponds to the formation of the triplet state and ~10 – 15 µs to the decay of this triplet state, the DAS corresponding to the $^3$P700 state can be constructed at room temperature in the visible spectral region. Construction of the DAS corresponding to the ~10 – 15 µs phase in the same experimental condition as that for the measurement of P700$^+$/P700 allows for determination of the extinction coefficient of $^3$P700/P700 relative to P700$^+$/P700 at room temperature.
While the study on \(^3\)P700 is important for understanding the molecular mechanisms of the primary electron donor, the focus of this research has been on the electron acceptors. Therefore, a greater interest is on using the method of non-invasive deactivation of A\(_1\) for the construction of the (A\(_0^-\) – A\(_0\)) DS. While the spectrum has been reported by many groups previously, the position of the maximum bleaching fluctuates by several wavelengths by different studies due to congestions by the antenna Chl which transfers energy on the same ps timescale as the ET through A\(_0\) (17, 189, 198). The 50-ns phase in \(menB^-\) PSI which generates the \(^3\)P700 state is due to the P700\(^+\)A\(_0^-\) charge recombination. Therefore, by following the 50-ns phase, the DAS representing (P700\(^+\)A\(_0^-\) – \(^3\)P700A\(_0\)) DS is constructed. From the (\(^3\)P700 – P700) DS obtained in the process described above, and using the well-known (P700\(^+\) – P700) DS, (A\(_0^-\) – A\(_0\)) DS can be isolated. The procedure is summarized below.

1. Construct the DAS of the 50-ns phase (Spectrum 1). The DAS represents the (P700\(^+\)A\(_0^-\) – \(^3\)P700A\(_0\)) DS.

2. Construct the DAS of the ~10 – 15 µs phase (Spectrum 2). The DAS represents the (\(^3\)P700 – P700) DS.

3. Obtain the (P700\(^+\) – P700) DS (Spectrum 3) through the photoaccumulation measurement.

4. Subtract Spectrum 2 from Spectrum 3. The resulting DS (Spectrum 4) is (P700\(^+\) – \(^3\)P700) DS.

5. Subtract Spectrum 4 from Spectrum 1. The resulting DS is the (A\(_0^-\) – A\(_0\)) DS.

In almost all the previous studies, a detection of the A\(_0^-\) state had required the use of ultra-fast spectroscopy. A method to modify the rate of forward ET from A\(_0^-\) relative to the energy transfer rate have been reported previously, but even in these studies most of the slowed kinetics
still exhibit ps lifetimes (AQs substituted with two amino groups and non-quinone molecules such as fluorenone and anthraldehyde have produced kinetics on the ns timescales) (78, 199). With the method presented above, however, only a ns resolution is required to produce the \((A_0^- - A_0)\) DS on PSI with minimal modifications. Because the reactions measured here are all on the ns timescale or slower, the antenna energy transfer will not interfere with these measurements.

Deactivation of \(A_1\) by double protonation also allows for the investigation of the \(P700\) triplet state in the infrared region. While the previous protocol required complete removal of iron-sulfur clusters and/or addition of strong reducing agents (179), pre-flashing at room temperature is all that is required when using \(menB^-\) PSI. With this simplified measurement process, application of the additional treatments such as isotope labeling becomes feasible. Additionally, with sufficient temporal resolution, the \(^3P700\) state becomes observable at room temperature in the infrared region for the first time. Although the current resolution does not allow for the proper measurement of the \(^3P700\) phase, the preliminary data is presented to prove this point. The \((^3P700 – P700)\) DS produced using \(^{13}\)C-labeled PSI and the DAS corresponding to the \((^3P700 – P700)\) DS at room temperature are presented in Fig. 10.1.

![Figure 10.1](image)

**Figure 10.1** The \((^3P700 – P700)\) DS for \(^{13}\)C-labeled PSI at 77 K (A, bottom) and unlabeled PSI at 298 K (B, bottom). In both A and B, the spectrum on top corresponds to the \((^3P700 – P700)\) DS for unlabeled PSI at 77 K. The time constants associated with the spectra are \(~200 – 240\) µs for the DS at 77 K, and \(~14\) µs for the DS at 298 K.
10.2.2 Temperature dependence of ET in PSI

In the current studies, the kinetic simulation model incorporated the ET kinetics measured at two distinct temperatures: 298 and 77 K. The studies observing the temperature dependence of the ET kinetics had been previously presented by other groups, but only analyzed in the context of the unidirectional ET (26, 27, 187, 200). In addition, in these studies, the data were analyzed to extract the activation energy of the reaction. Given the relationship between the activation energy and the driving force (Eq. 1.3), any error associated with the derived activation energy (often calculated as a slope of the reaction rate constant vs. inverse of the temperature) is amplified when solved for the driving force. To make a better use of the temperature dependence for the estimation of the reaction driving force, the kinetics at each temperature must be analyzed assuming the quasi-equilibration. That is, the kinetics at each temperature needs to be analyzed using the kinetic simulation model, as shown in Ch. 5. The temperature dependence therefore serves as a new set of conditions for the simulation to satisfy. This temperature dependence can be measured for all the quinones investigated. As a part of the preliminary data, in Fig. 10.2, the temperature dependence of the ET kinetics in PSI with 2MNQ incorporated into the A1 binding site is presented and compared against the dependence seen in PSI with PhQ in the A1. The transient absorption kinetics at 487 nm at various temperature were measured by the time-resolved visible absorption spectroscopy as described in Ch. 2. The temperature was controlled using the K-4/R Lauda/Brinkman circulator, and the sample cuvette was exposed to continuous flow of gaseous nitrogen to prevent condensation near/below freezing temperature. From the linear fit of the temperature dependence, the activation energies for the two phases of forward ET from A1− to Fx are calculated. For PSI with 2MNQ incorporated, the activation energies were 257 and 436 meV for the fast and slow phases, respectively. The values translate to the driving force of 148 and 392...
meV when the reorganization energy is set to 700 meV. Assuming $E_m$ of F$_X$ to be –680 mV, the midpoint potentials of 2MNQ calculated from these driving forces are –532 and –288 mV for A$_{1B}$ and A$_{1A}$, respectively. The values, especially for A$_{1A}$, are widely different from the ones predicted by the kinetic simulation model, as this analysis inherently assumes the ET process to be unidirectional.

![Graph](image)

**Figure 10.2** Temperature dependence of the two phases of the $A_1^- \rightarrow F_X$ forward ET rate for PSI with 2MNQ in the A$_1$ binding site. The dashed line is the dependence of the slower phase for PSI with PhQ, derived in Ref. (26). From the linear fit, the activation energies calculated are 257 meV for the faster phase and 426 meV for the slower phase. The reported activation energies for the two phases of PhQ are 15 meV and 110 – 220 meV for the fast and slow phase, respectively (26, 27). If the reorganization energy of 700 meV can be assumed, these activation energies for the fast/slow phase correspond to 148/392 meV for 2MNQ, and –495/(-145 or 85) meV for PhQ.

10.2.3 The Marcus curve for PSI with BQ in the A$_1$ binding site

Initially, the high potential NQs were incorporated into PSI in an attempt to cross the optimal rate from the inverted region into the normal region. By having the experimental relationship that exhibits the both regions of the Marcus curve, the estimation of the ET parameters
becomes less theoretical and more empirical. The vertex of the curve predicts both the reorganization energy and the coupling element, and the width of the curve can be used to assess the vibrational frequency coupled to the ET. Even with an incorporation of Cl2NQ or Br2NQ, which exhibits one of the highest midpoint potentials as a NQ, the reaction rate still resided in the inverted region (Ch. 6).

BQs were initially incorporated into the A1 binding site to overcome this problem, as the range of in vitro midpoint potentials for BQs are more positive than that for NQs, and many BQs with midpoint potentials higher than Cl2NQ or Br2NQ are commercially available. However, the reaction rate for PQ9, which was reported in Ch. 8 of this dissertation, does not seem to follow the trend built by all the other NQs and AQs. Given the environmental difference that BQs experience in the A1 binding site (see Ch. 8 for the discussion), it is likely that the midpoint potentials of BQs are influenced differently than NQs and AQs are, and/or the ET parameters, such as the reorganization energy, are modified.

The reaction rates for the BQs does not seem to fit the relationship predicted by the other NQs and AQs. However, the rate’s dependence on the reaction driving force should still predict the inverted parabolic pattern if the reaction is governed by the ET theory. Additionally, the range of in vitro midpoint potentials of BQs that are commercially available spans over 800 mV, which is wider than the ~500 mV range investigated in the current study using NQs and AQs, and is shifted more in the positive direction than the range of NQs and AQs. Given these conditions, construction of the Marcus curve that includes both the normal and inverted regions may be possible with BQs in the A1 binding site. As a preliminary study, the P700+A1– charge recombination kinetics at 77 K for four BQs incorporated into the A1 binding site are compared to the driving force calculated using the in vitro midpoint potentials, and presented in Fig. 10.3.
Although the data consists only of four points, it is possible to explain the reaction rate dependence by an inverted parabolic curve. Furthermore, the plot indicates that it is possible to cross the optimal rate with the range of midpoint potentials displayed by the BQ analogues.

![Graph](image)

**Figure 10.3** Plot of the observed P700+A\(_1^-\) recombination rate at 77 K *versus* the reaction free energy calculated using the quinone *in vitro* midpoint potentials. The plots corresponding to NQs and AQs (*blue, circle*) are fitted to a parabolic function (*blue, dotted*). The plot for BQs (*black, diamond*) are not fitted.

**10.2.4 Future directions for the FTIR studies on PSI**

In this dissertation, the (P700\(^+\)A\(_1^-\) – P700A\(_1\)) DS for PSI with PhQ, 2MNQ, PQ\(_9\), DMBQ, and Cl\(_4\)BQ incorporated into the A\(_1\) binding site produced using time-resolved step-scan FTIR difference spectroscopy were analyzed, assisted by the computational vibrational frequency calculations, to understand the quinone-protein interaction in the A\(_1\) binding site. Many other quinones, other than the five listed above, were also incorporated into PSI and studied using TRSS FTIR spectroscopy through this research but was not discussed in the main chapters. As described previously, one method to identify the nature of an infrared absorption band is to induce a shift in
the frequency by modifying the system. The major advantage provided by menB– PSI is its ability to recruit non-native quinones as A₁ with minimal disturbance to the surrounding protein environment. To make use of this advantage and apply to the study of molecular details of quinone in the A₁ site, more than 25 different types of the (P700⁺A₁⁻ – P700A₁) DS were produced. In fact, although in Ch. 7 the bands associated with PhQ⁻ were identified by constructing the (2MNQ – PhQ) DDS and simulating such spectra by the three-layered ONIOM calculations, the experimental evidence displayed by these sets of the (P700⁺A₁⁻ – P700A₁) DS were sufficient to arrive at the same conclusion without any computational work as well. These spectra are presented in Appendix D, in which all the static and time-resolved FTIR DS that I have recorded are organized. These spectra, many of which are not discussed in this dissertation, are to serve as the foundation to the improved computational models which are already under development in our group. As was the case for the kinetic simulation model in Ch. 5, a model becomes more reliable when it is capable of simulating multiple situations. The model presented in Ch. 7 allowed for the simulation of 2MNQ and PhQ. Already, the improved version is developed in our group that is capable of simulating PhQ, 2MNQ, ¹⁸O-labeled PhQ, and DMNQ.

In the collection of spectra presented in Appendix D, some of the bands due to semiquinones are visually identifiable, without construction of the DDS. This is because the P700⁺/P700 states do not contribute to the absorption changes in the region where semiquinone absorption is expected. In the region where the neutral state quinone absorption is expected, that is not the case. In the 1700 – 1600 cm⁻¹ region, a presence of multiple intense bands, both negative and positive in amplitude, complicate the identification of the quinone bands. The region overlaps with the 1650 cm⁻¹ amide I band, and as such the intense bands are not removed completely even in the (A₁⁻ – A₁) DS. Initially, multiple quinones, including the isotope labeled ones, were
incorporated into the A₁ binding site to cancel these intense non-quinone bands and resolve the bands due to neutral state quinones in the DDS. However, the recent results have shown that this approach is most likely not going to resolve such bands. This conclusion is drawn from the (P700⁺A₁⁻ – P700A₁) DS of PSI with acequinocyl, PhQ, and 2MNQ incorporated. Acequinocyl, one of the non-native quinones incorporated into PSI but not discussed in the main chapters, was incorporated into the A₁ binding site with a specific purpose of observing the bands due to neutral state quinone. The substituent on this quinone contains an acetoxy group which gives rise to a C=O mode well above 1700 cm⁻¹. This band allows for the observation of the neutral state quinone, although the C=O mode observed here is not due to quinonic C₁=O or C₄=O. The spectra of acequinocyl in both the unlabeled PSI and ¹³C-labeled PSI are organized in Appendix D. From these spectra, a pair of absorption bands corresponding to the same vibrational mode (acetoxy C=O stretching mode) was observed for the neutral and anion quinone for the first time. These bands show that the intensity of the neutral band is similar to that of the corresponding anion band. However, In the most up-to-date (2MNQ – PhQ) DDS shown in Fig. 10.4, in which the spectral signal-to-noise ratio is by far the highest compared to any other DDS presented in this study, no difference feature with an intensity comparable to those of the anion features are observed in the 1800 – 1600 cm⁻¹ region. These observations indicate that the current methods would not lead to the identification of the neutral quinone bands.
Figure 10.4 (2MNQ – PhQ) FTIR DDS produced by taking the difference of the \((P700^+A_1^- – P700A_1)\) DS for PSI with 2MNQ and PhQ incorporated into the A1 binding site. The DS for 2MNQ is the average of 3 independent measurements, and the DS for PhQ is the average of 4 independent measurements. The DS for PhQ has been updated from the one reported in Ref. (178). The gray horizontal lines indicate the level of signal amplitude for 2MNQ and PhQ in the anion spectral region. The solid lines correspond to the amplitude of the C1=O band, and the dashed lines correspond to that of the C4=O band.

Observation of the bands due to quinone in the A1 binding site, both the neutral and anion, is complicated by the fact that the current method monitors both the P700+/P700 contributions and the A1-/A1 contributions simultaneously. Unlike in pbRC, in which direct photoaccumulation measurements of the \((QA^- – QA)\) or \((QB^- – QB)\) DS are possible, in PSI the \((A1^- – A1)\) DS can only be observed by the time-resolved approach. The current setup of the spectrometer has a temporal resolution of 6 µs, and thus the measurements were limited to the P700+A1- charge recombination reaction at 77 K. By measuring this kinetic phase, however, the absorption changes related to A1 or A1- are always accompanied by the changes related to P700 and P700+. The complication associated with the extracting the absorption changes related to A1 from this type of spectrum was explained already. To identify the vibrational modes associated A1, the absorption changes associated with A1/A1- must be detected without having the congestions by the P700+/P700
absorption changes. The only viable approach for such detection is to monitor the forward ET from $A_{1A}^- \rightarrow F_X$. In this forward ET process, the P700$^+$ state remains unaffected and does not contribute to the absorption changes. The reaction, however, occurs on the ns timescale ($\tau = \sim 300$ ns), and the detection of the reaction requires an implementation of the ns temporal resolution to the TRSS FTIR spectroscopy. The instrumentation of ns TRSS FTIR DS has been discussed previously (201-203). In addition to the synchronization of the actinic laser which must be controlled externally as well as different electronic coupling schemes to be employed for the analog-to-digital converter, the major difficulty of ns TRSS FTIR DS is provided by the increased noise level. The signal-to-noise ratio as well as the method of analysis for µs TRSS FTIR DS has been improved in last few years in our group (see Appendix C). The method of quinone incorporation is now well-established (Ch. 2), and the reaction kinetics are identified for over ten quinones (Ch. 4). With these improvements on TRSS FTIR and the fact that the overall signal size is nearly doubled at room temperature due to no irreversible state formation (although 30 – 40% of this amplified signal may be unobservable if this fraction takes the faster B-branch route), implementation of the ns time resolution to TRSS FTIR DS should enable the direct measurement of the $(A_1^- - A_1)$ FTIR DS.
REFERENCES


65. Gunner MR & Dutton PL (1989) Temperature and -.DELTA.G.degree. dependence of the electron transfer from BPh.cntdot.- to QA in reaction center protein from
Rhodobacter sphaeroides with different quinones as QA. *Journal of the American Chemical Society* 111(9):3400-3412.


Zhao N, Lamichhane HP, & Hastings G (2013) Comparison of calculated and experimental isotope edited FTIR difference spectra for purple bacterial photosynthetic
reaction centers with different quinones incorporated into the Q(A) binding site.


197. Zhao N (2014) Vibrational Properties of Quinones in Photosynthetic Reaction Centers. Doctor of Philosophy (Georgia State University, Georgia State University).


APPENDICES

Appendix A: Systematic Procedures of Kinetic Modeling

As outlined in section 5.3.9, in order to estimate the midpoint potential of quinones in the A\textsubscript{1A} and A\textsubscript{1B} binding sites (hereafter referred to as \(E_m(A_{1A})\) and \(E_m(A_{1A})\), respectively), the kinetic model has to first be able to simulate the temporal evolution of \(A_1^-\) and P700\(^+\) at RT for PSI with the low-potential quinones (AQ, PhQ, 2MNQ, and PQ\textsubscript{9}) incorporated, and then P700\(^+\) and A\textsubscript{1}\(^-\) at RT for PSI with the high-potential quinones (2ClNQ, 2BrNQ, Cl\textsubscript{2}NQ, and Br\textsubscript{2}NQ) incorporated.

An underlying assumption is that exchange of quinones in the A\textsubscript{1} binding site does not alter any of the ET parameters (such as the midpoint potentials of the other cofactors (particularly F\textsubscript{X}), the reorganization energies of the ET reactions, and the edge-to-edge distance between the cofactors [with an exception of PQ\textsubscript{9}]). Only the midpoint potential of the incorporated quinone is modified. That is, the only adjustable parameters are \(E_m(A_{1A})\) and \(E_m(A_{1B})\). In certain cases minor alteration to other ET parameters are considered and fully discussed.

Another assumption is that the same cofactor midpoint potentials that were calculated at 298 K are also used at 77 K. Determination of ET parameters, including three reorganization energies, and their associated range of values, are discussed in section 5.3.5.

The same value of the reorganization energy, \(\lambda_1\), was used for all forward ET processes at RT (A\textsubscript{1}\(^-\) to F\textsubscript{X}, F\textsubscript{X}\(^-\) to F\textsubscript{A} and F\textsubscript{A}\(^-\) to F\textsubscript{B} ET). Three values for \(\lambda_1\) were tested (0.4, 0.7, 1.0 eV). For the P700\(^+\)A\textsubscript{1}\(^-\) recombination reaction at RT/LT, a different value of reorganization energy \(\lambda_2/\lambda_3\) is assigned, respectively. This value may be modified slightly (< 0.1 eV) between simulations at 298 and 77 K. While the same \(\lambda_1\) is used for all eight quinones incorporated, \(\lambda_2\) and \(\lambda_3\) were allowed to vary slightly (< 0.1 eV) for the different quinones incorporated if required. Motivations for such adjustments are discussed in section 5.3.9.
Systematic simulations were undertaken as outlined in Fig. 5.2. Numbers in italics in the following description refer to the numbers in Fig. 5.2.

1. The simulation starts with the modeling of PSI with PhQ incorporated (1).

2. For PSI with PhQ incorporated, a value of $\lambda_1$ is chosen (2). Three possible values of $\lambda_1$ are considered here (0.4, 0.7, and 1.0 eV). In the previous simulation of forward ET from $A_{1}^{-}$ to $F_{X}$ at RT, only $\lambda_1 = 0.7$ eV was considered. Initially $\lambda_1$ was set at 0.7 eV.

3. The midpoint potentials of $A_{1A}$ and $A_{1B}$ [$E_m(A_{1A})$ and $E_m(A_{1B})$] are set (3). Initially, the values calculated previously were used (39). For PSI with PhQ incorporated $E_m(A_{1A}) = -665$ mV and $E_m(A_{1B}) = -690$ mV, with $E_m(F_X) = -680$ mV. From these initial values, the differential equations associated with the kinetic model are solved and the temporal evolution of the radical pair states is calculated. A weighted average time constant for forward ET from $A_{1}^{-}$ to $F_{X}$ ($\tau_{AV_{A_{1}^{-}}}^{-}$) is then calculated (4). If this calculation does not agree well with experiment, then this procedure is repeated for different $E_m(A_{1A})$ and $E_m(A_{1B})$ values. In actuality, weighted average time constants are calculated for all values of $E_m(A_{1A})$ and $E_m(A_{1B})$ over a broad range, modified first in 15 meV increments with finer increments being tested as necessary. $E_m(A_{1A})$ and $E_m(A_{1B})$ are varied until the weighted average time constants are in good agreement with experiment (3, 4).

4. Once the weighted average time constants for forward ET from $A_{1}^{-} \rightarrow F_{X}$ at RT are satisfied, the population dynamics of P700 are studied in order to evaluate the time constant of charge recombination ($\tau_{P700^+}$). For PhQ, the rate of decay of the $A_{1}^{-}$ and P700$^+$ population are significantly different. The decay of the $F_{A}^{-}$ and $F_{B}^{-}$ populations are identical to the decay of the P700$^+$ population, however, indicating that the simulated kinetics represent 700$^+$F$_{A/B}^{-}$ charge recombination (5). In many cases, for a best $E_m(A_{1A})$ and $E_m(A_{1B})$ found in step (4),
the time constant calculated for the recovery of P700 does not correspond well to the experimentally observed time constant. To achieve better correspondence, $\lambda_2$ could be slightly modified (6). Because the timescales of forward ET from $A_1^-$ to $F_X$ and $P700^+F_{A/B}^-$ charge recombination are significantly different, the effect of modifying $\lambda_2$ on the calculated time constant for forward ET from $A_1^-$ to $F_X$ is negligible.

5. To model the kinetics of $P700^+A_1^-$ charge recombination ($\tau_{P700^+A_1^-}$) at LT the kinetic model was modified by removing the $F_A$ and $F_B$ cofactors. This is reasonable as $F_A$ and $F_B$ do not contribute to reversible ET reactions in PSI at LT. The same $E_m(A_{1A})$ and $E_m(A_{1B})$ obtained from RT simulations are used for the simulation at LT. $\lambda_2$ is initially set at the value calculated for RT simulation (5, 6), but could be modified slightly ($<0.1$ eV) to produce a weighted average time constant that better simulates the experimentally obtained value (8). The simulated kinetics are considered to represent $P700^+A_1^-$ charge recombination only in situations where the $A_1^-$ and $P700^+$ populations evolve at the identical rates.

6. The kinetic modeling of PSI with PhQ in the $A_1$ binding site is completed upon successful simulations of forward ET from $A_1^-$ to $F_X$, $P700^+F_{A/B}^-$ charge recombination at RT, and $P700^+A_1^-$ charge recombination at LT (9). The same set of reorganization energies used for PSI with PhQ incorporated ($\lambda_1$, $\lambda_2$, and $\lambda_3$) will be applied to the kinetic simulations for PSI with non-native quinones incorporated (10).

7. Similarly to the simulations of PSI with PhQ incorporated, the initial midpoint potentials are assigned to $E_m(A_{1A})$ and $E_m(A_{1B})$ for the simulations of PSI with non-native quinones incorporated (11). For 2MNQ and PQ9, the initial parameters are taken as the values obtained from previous simulations (30). For AQ incorporated, the initial values are taken as those of PhQ. For the series of high-potential quinones, the initial values are taken as those of PQ9.
Weighted average time constants are calculated for a range of $E_m(A_{1A})$ and $E_m(A_{1B})$ values, in 15 mV increments. Finer increments are tested as required (11).

8. For a series of low potential quinones (AQ, 2MNQ, and PQ), the $E_m(A_{1A})$ and $E_m(A_{1B})$ are varied until the calculated weighted average time constant agrees with experiment.

9. Following the simulation of forward ET from $A_1^-$ to $F_X$, the P700$^+A_1^-$ charge recombination kinetics at LT is investigated (14). Again, the same $E_m(A_{1A})$ and $E_m(A_{1B})$ are used as those found in RT simulations.

10. Lastly for low-potential quinones, the P700$^+F_{A/B}^-$ charge recombination kinetics at RT is simulated (15). While $\lambda_2$ is initially set to the value found through the simulation for PSI with PhQ in the A$_1$ binding site, the value is allowed to vary by up to 0.1 eV to obtain better correspondence with the experimental observations (16).

11. For a series of high potential quinones, the midpoint potentials are varied until the calculated time constant agrees with the lifetime assigned to P700$^+A_1^-$ charge recombination at RT (17, 18). In addition, the population evolution of $A_1^-$ and P700 must occur at the same rate.

12. The P700$^+A_1^-$ charge recombination kinetics at LT are then simulated for PSI with high-potential quinones incorporated (19).

13. At any point in the simulation procedure, if the calculated kinetics or time constants for all of the quinones incorporated do not agree with experiment, then the parameters that are being utilized are considered invalid. For example, the $E_m(A_{1A})$ and $E_m(A_{1B})$ first calculated for PSI with PhQ incorporated are considered invalid if the associated $\lambda_1$, $\lambda_2$, and $\lambda_3$ cannot be used to adequately simulate the observed kinetics for PSI with non-native quinones incorporated.
The simulated results for $\lambda_1 = 0.7$ eV, including the weighted average time constants, individual time constants and associated amplitudes, and as well as all of the midpoint potentials investigated, are listed in Tables SI1–SI6. The simulation with $\lambda_1 = 0.4$ eV or 1.0 eV did not yield satisfactory results. The parameters from these simulations are listed in Tables SI7 and SI8. The weighted average time constant of forward ET from $A_1^-$ to $F_X$ for a PSI with PQ$_9$ in the $A_1$ binding site is not in good agreement with the experimental time constant (3.2 $\mu$s vs. 62.2 $\mu$s, for the simulated and experimental values, respectively). As mentioned in section 5.3.7, the variance is due to an uncertain edge-to-edge distance between PQ$_9$ and $F_X$. 
Table B.1 Summary of calculated vibrational frequencies associated with C=O modes for PhQ, PQ, DMBQ, and Cl₄BQ in the gas phase, Q+H₂O, and Q+Leu models. Italicized vibrational mode and frequencies are for meta model DMBQ. For Cl₄BQ, H-bonded carbonyl group is numbered as C₄=O. H₂O and Leu refer to vibrational modes associated with molecular bonds in H₂O and truncated amino acid that supplied H-bonding. The frequencies are in cm⁻¹. Intensities in km/mol are listed in parentheses. Frequencies are not scaled.

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Figure B.1 Calculated neutral spectra for PhQ (black), PQ (green), DMBQ (red), and Cl₄BQ (blue), in the gas phase model (A), Q+H₂O model (B), and Q+Leu (C) model. Bands associated with C=O vibrational mode, except for symmetric C=O mode, are marked. The frequencies of the marked bands can be identified in Table B.1. Dashed spectra in Q+H₂O and Q+Leu model calculations correspond to the meta model of DMBQ.

Figure B.2 Calculated spectra for PQ with H-bonding provided to C₁=O (red, solid) or C₄=O (black, dotted). The spectra were calculated in the Q+H₂O model (A, B) and in the Q+Leu model (C, D), with a quinone in the neutral (A, C) and anion (B, D) states.
Table B.2  Calculated bond lengths (in Å) and angles (in degrees) for neutral and anion PhQ, PQ, DMBQ, and Cl₄BQ, optimized in the gas phase, Q+H₂O, and Q+Leu models. For the gas phase optimization, no distinction is made between C₁=O and C₄=O for Cl₄BQ (the calculated bond lengths are identical). For the Q+H₂O and Q+Leu models, the H-bonded carbonyl group is referred as C₄=O for Cl₄BQ.

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Appendix C: Time-Resolved Step-Scan FTIR Difference Spectroscopic Methods for the Study of the Secondary Electron Acceptor in Photosystem I

Previously, to produce the \((A_1^- - A_1^-)\) DS for PSI with any quinone in the A_1 binding site, time-resolved step-scan FTIR spectroscopy was applied at 77 K using a helium gas-flow cryostat with a 5 µs temporal resolution, and the timescale of the measurement was ~1.35 ms (the timescale is approximate because different measurements had slightly different number of data points). The size of an aperture to control the spot size of incident IR beam was set to 6 mm. Approximately 60 to 80 measurements were made per sample and averaged. From the averaged time-resolved spectra, first 9 spectra following the actinic flash were extracted and averaged, to produce what is referred to as the “early 9” spectra. The procedure up to this point constituted a process required for one experiment. The experiment was then repeated ~10 times (i.e. on ~10 different samples), and the early 9 spectra were averaged to produce one \((P700^+A_1^- - P700A_1^-)\) DS. Spectral variations between experiments were calculated as standard error, and presented as a noise in this spectrum. The \((P700^+ - P700)\) DS, obtained through a static photoaccumulation measurements, were subtracted from the DS to produce \((A_1^- - A_1^-)\) DS. The DDS is produced either by taking a difference of two \((P700^+A_1^- - P700A_1^-)\) DS or of two \((A_1^- - A_1^-)\) DS. The standard error associated with each spectrum is propagated accordingly to the final DDS. Duration of the TRSS FTIR measurements are determined by number of steps and coadditions required at each step, as described in Ch. 1. If 80 measurements were made per experiment, and the experiments were repeated 10 times, a minimum of 420 hr are required to produce a single \((A_1^- - A_1^-)\) DS.

While this method successfully produced the \((2MNQ - PhQ)\) DDS that correctly predicted the bands of anionic 2MQN and PhQ in the A_1 binding site, the procedure required improvements due to 1) a large number of experiments required to produce each \((A_1^- - A_1^-)\) DS and 2) the fact
that procedure made use of only 9 spectra out of 300 time-resolved spectra that were obtained in each experiment, and did not fully utilize the three-dimensional property of the spectra. Essentially, in this procedure the kinetics obtained from the TRSS FTIR measurements served a little purpose because only the first 9 spectra were considered. The concept behind taking the average of the early 9 spectra as the representation of the \((P700^+A_1^- - P700A_1)\) DS was that the spectral contributions in those 9 spectra are only due to the \(P700^+A_1^-\) state which lifetime is \(~360\ \mu s\) (for PSI with PhQ in the \(A_1\)). Close inspection of the kinetics in the early timescale, however, shows that a faster kinetics can be observed at some wavenumbers (see the kinetics for \(1504\ \text{cm}^{-1}\) in Fig. 9C in the review (115), for example). If this faster phase is not due to the \(P700^+A_1^-\) state, then the early 9 spectra do not represent the pure \((P700^+A_1^- - P700A_1)\) DS but are contaminated by this unknown faster phase. Furthermore, even after extensive averaging, the kinetics at late timescales obtained through this setup were far less reliable than the same kinetics monitored in the visible spectral region by time-resolved visible absorption spectroscopy. The kinetics seemed to experience a significant “drift” in the baseline, and an extent and direction of the “drift” differed by wavenumbers as well. As a result, a determination of the reaction lifetime relied on time-resolved visible absorption difference spectroscopy conducted separately from the TRSS FTIR measurements, further increasing the time/sample required for the study.

A new method was developed to address these issues listed above. The method was designed to remove the “drift” in the kinetics, make use of the three-dimensional properties of the time-resolved spectra, separate/isolate the unknown faster phase from the \(P700^+A_1^-\) phase, and improve on the signal-to-noise ratio to reduce the time required to produce \((A_1^- - A_1)\) DS which exceeded 2 weeks in the previous method. The issues regarding the “drift” and the signal-to-noise
ratio were addressed by modifying the experimental setup, and the separation of phases was undertaken by recruiting a new analytical method.

The source of the “drift” was identified as the vibration caused by the backing pump to the diffusion pump, which was constantly operating to evacuate the sample compartment of the helium gas-flow cryostat during the measurement. Since the desired temperature was only 77 K, the cryostat was replaced by a pour-fill liquid nitrogen cryostat, which can be evacuated prior to the measurement and does not require constant evacuation during the measurement. The replacement removed the problematic “drift”, and the kinetics were measured accurately to the later part of the timescale. Following this modification, the measurement timescale (after the actinic flash) was increased from ~1.35 ms to 3.378 ms. This change was made to ensure that the entire decay kinetics as well as the non-decaying component fit inside the measurement window. The effect of increasing the measurement timescale by ~2 ms to the overall measurement duration is unnoticeable. Inspection of the single-beam spectra from the former measurements suggested that in many cases the intensity recorded at the peak of amide I position is above 0.100. Given the intensity recorded at 1800 cm\(^{-1}\), where little to no absorption is expected, is usually in the range of 0.300 to 0.400 with two 2000 – 1000 cm\(^{-1}\) IR filters and four CaF\(_2\) windows and a sample in the path of the probing IR beam, 0.100 corresponds to the absorption of 0.48 to 0.60 at 1654 cm\(^{-1}\). With an intensity of 0.150, the range becomes 0.3 to 0.43. During the sample preparation stage, the actual OD of the sample was adjusted so that the value at 1654 cm\(^{-1}\) is less than 1.0 and above 0.6. Therefore, an intensity higher than 0.100 at 1654 cm\(^{-1}\) in the single-beam spectrum indicates that the sample OD was recorded incorrectly in these measurements. The incorrect measurement in these past experiments are most likely due to the 6-mm aperture used the TRSS FTIR measurements, which is significantly larger than the 1.5-mm aperture used in the static FTIR
measurements. The larger beam spot produced by the 6-mm aperture likely did not capture all of the sample, and resulted in the lower and incorrect absorption. Because the recorded OD of the sample is significantly lower than the actual OD of the sample, the flash-induced ΔOD was also diminished in these measurements, worsening the signal-to-noise ratio. To prevent such a misalignment, the aperture size was reduced to 4 mm. By doing so, in all the TRSS FTIR measurements presented in this dissertation, the intensity at 1654 cm$^{-1}$ was kept at 0.100 or lower. Additionally, many of the averaged single-beam spectra in the previous experiments exhibited patterns of water vapor. The indication of this observation is that some measurements were made while the purging of the spectrometer was incomplete, and that these spectra were averaged into the final spectra. While the TRSS FTIR is intended to measure changes induced by the actinic flash and the actual profile of the single-beam spectrum is not what is being analyzed, the profile serves as the baseline for the induced changes. An incorrect single beam spectrum can lead to an incorrect absorption difference spectrum. The issue can easily be corrected by ensuring that the spectrometer is well-purged before the measurement is initiated, and/or discarding any measurement that shows the spectral pattern of water vapor. This process was implemented into the new method.

With the new experimental setup, similar or better signal-to-noise ratio as the previous setup was achieved by averaging only 40 measurements and three to four experiments, and the obtained kinetics were free of the “drift” artifact (see data in Ch. 7). The time required to produce the ($A_1^−$ – $A_1$) DS was reduced to ~84 hr (40 measurements per experiment, 4 experiments). Because the kinetics were measured reliably from the TRSS FTIR, reaction lifetimes could be determined directly from the TRSS FTIR measurements, without undertaking transient visible absorption difference spectroscopy (see Ch. 7 and 8).
Lastly, to fully utilize the kinetic information obtained through the time-resolved measurement, and also to isolate the faster artifact from the (P700\textsuperscript{+}A\textsubscript{1} – P700A\textsubscript{1}) DS, the early 9 method was abandoned and the global analysis of the time-resolved spectra was implemented. In this analysis, the spectra were globally fitted to a sum of exponential functions. The amplitude associated with each exponential phase at each wavenumber is plotted to construct decay-associated spectrum (DAS). By doing so, the spectra are separated based on the kinetic phases. Applying this method to the time-resolved spectra of PSI at 77 K, three different DAS are obtained; the faster phase, which lifetime was ~15 µs and corresponded to the artifact due to heating by the actinic laser, the 70 – 900 µs phase, which corresponded to the P700\textsuperscript{+}A\textsubscript{1} recombination phase, and the non-decaying phase with a lifetime that exceeds the measured timescale which corresponds to the long-living P700\textsuperscript{+} state. Through this method, the (P700\textsuperscript{+}A\textsubscript{1} – P700A\textsubscript{1}) DS free of the artifact was extracted. Comparison of the (2MNQ – PhQ) DDS or individual DS produced by the previous method (see Fig. 2.8 and 2.9A in Ref. (197) and Fig. 8B Ref. (115)) and by the current method (Fig. 7.5c and Fig. 10.4), and the spectral profile of the extracted artifact (Fig. 7.4b and 7.4d) shows many of the bands previously associated to the neutral or anionic quinones in the A\textsubscript{1} binding site are at least in part due to the heating artifact.
Appendix D: The Collection of the FTIR Difference Spectra

This section organizes the collection of FTIR DS that I have created using static, time-resolved rapid-scan, and time-resolved step-scan FTIR difference spectroscopy. Some of the spectra in this collection are discussed in detail in the main chapters. The collection includes many other spectra that are neither analyzed or even mentioned in the main text of this dissertation, partially because these spectra and the experimental details do not relate to the focus of this dissertation directly, and partially because the spectra are meant to serve as the building blocks to the new computational models which is still under development. All the DS obtained through TRSS FTIR are DAS calculated through the global analysis. All the measurements were done with 4 cm$^{-1}$ spectral resolution, except for two spectra which were measured with 8 cm$^{-1}$ spectral resolution. The FTIR DS for PSI were measured at 77 K unless noted otherwise. The DS noted as the “room temperature (RT)” spectra are mixture of the spectra at both 298 K and 283 K. PSI devoid of F$_{AB}$ (PSI-F$_X$ particles) were prepared as described previously (176, 177). For the DS of PSI with $^{18}$O-labeled PhQ, both the original DS spectrum and the DS corrected for the contributions from the unlabeled PhQ (the procedure of correction is detailed in Ref. (88)) are listed. The static DS noted as “QH$_2$” were obtained on the menB$^-$ PSI samples that were pre-flashed at 298 K (following the procedure described in Ch. 8) prior to the measurements. The FTIR DS on pbRC was measured on the RC isolated from \textit{Rb. sphaeroides}. FeRC refers to the RC with an iron atom between QA and QB, and ZnRC refers to the RC in which the iron atom is substituted by a zinc atom. The FTIR DS for pbRC were measured at 298 K, except for those noted as 100 K. To terminate the ET to QB at room temperature, o-phenanthroline was added. To re-reduce the primary donor, 2,3,5,6-tetramethyl-1,4-phenylenediamine and sodium ascorbate were added. To increase the level of QB occupation, pbRC was incubated with 5-fold molar excess
of ubiquinone-9 (UQ$_0$) for 3 hr at 277 K. The gas phase anion – neutral DS were calculated as described in Ch. 7, 8, and 9. Below, the collection of spectra is divided into 11 sets of figures. The contents of these sets are described in Table D.1. An index to this collection is also included at the end (Table D.2).
### Set 4

| 4a | PSI-Fx, (P700$^+$ – P700) DS     |
| 4b | PhQ, PSI-Fx, (P700$^+$A$_1^-$ – P700A$_1$) DS |
| 4c | 2MNQ, PSI-Fx, (P700$^+$A$_1^-$ – P700A$_1$) DS |
| 4d | PQ$_9$, PSI-Fx, (P700$^+$A$_1^-$ – P700A$_1$) DS |
| 4e | PhQ, PSI-Fx, S7002 (P700$^+$A$_1^-$ – P700A$_1$) DS |

### Set 5

| 5a | (P700$^+$ – P700) DS, RT |
| 5b | 2BrNQ, RT, (P700$^+$A$_1^-$ – P700A$_1$) DS |
| 5c | Br$_2$NQ, RT, (P700$^+$A$_1^-$ – P700A$_1$) DS |
| 5d | Cl$_2$NQ, RT, (P700$^+$A$_1^-$ – P700A$_1$) DS |
| 5e | Cl$_2$NQ, PSI-Fx, RT, (P700$^+$A$_1^-$ – P700A$_1$) DS |
| 5f | Cl$_2$NQ, 13C-PSI, RT, (P700$^+$A$_1^-$ – P700A$_1$) DS |

### Set 6

| 6a | (P700$^+$ – P700) DS |
| 6b | (P700$^+$ – P700) DS, QH$_2$ |
| 6c | PSI-Fx, (P700$^+$ – P700) DS |
| 6d | (P700$^+$ – P700) DS, 8cm$^{-1}$ |
| 6e | 13C-PSI, (P700$^+$ – P700) DS |
| 6f | 13C-PSI, (P700$^+$ – P700) DS, QH$_2$ |

### Set 7

| 7a | (P700$^+$ – P700) DS, RT |
| 7b | (P700$^+$ – P700) DS, QH$_2$, RT |
| 7c | PSI-Fx, (P700$^+$ – P700) DS, RT |
| 7d | 13C-PSI, (P700$^+$ – P700) DS, RT |
| 7e | 13C-PSI, (P700$^+$ – P700) DS, QH$_2$, RT |
| 7f | S7002, (P700$^+$ – P700) DS, RT |
| 7g | S7002, PSI-Fx, (P700$^+$ – P700) DS, RT |
Set 8
8a \((P700^+ – P700)\) DS
8b \((^3P700 – P700)\) DS
8c PSI-Fx, \((^3P700 – P700)\) DS
8d \(^{13}C\)-PSI, \((^3P700 – P700)\) DS
8e \((^3P700 – P700)\) DS, RT
8f \((P700^+ – P700)\) DS, RT

Set 9
9a \((P^*Q_A^– – PQ_A)\) DS, FeRC
9b \((P^*Q_A^– – PQ_A)\) DS, ZnRC
9c \((P^*Q_A^– – PQ_A)\) DS, 100 K, FeRC
9d \((P^*Q_A^– – PQ_A)\) DS, 100 K, ZnRC
9e \((P^*Q_B^– – PQ_B)\) DS, FeRC
9f \((P^*Q_B^– – PQ_B)\) DS, UQ\(_0\), FeRC
9g \((P^*Q_B^– – PQ_B)\) DS, UQ\(_0\), ZnRC

Set 10
10a \((Q_A^– – Q_A)\) DS, FeRC
10b \((Q_A^– – Q_A)\) DS, ZnRC
10c \((Q_B^– – Q_B)\) DS, FeRC
10d \((Q_B^– – Q_B)\) DS, UQ\(_0\), FeRC
10e \((Q_B^– – Q_B)\) DS, UQ\(_0\), ZnRC

Set 11
11a PhQ, gas phase (Ani – Neu) DS
11b DMNQ, gas phase (Ani – Neu) DS
11c 2MNQ, gas phase (Ani – Neu) DS
11d Cl\(_2\)NQ, gas phase (Ani – Neu) DS
11e Br\(_2\)NQ, gas phase (Ani – Neu) DS
11f 2ClNQ, gas phase (Ani – Neu) DS
11g 2BrNQ, gas phase (Ani – Neu) DS
11h Lapachol, gas phase (Ani – Neu) DS
11i 2OHNQ, gas phase (Ani – Neu) DS
11j MeONQ, gas phase (Ani – Neu) DS
11k AceQ, gas phase (Ani – Neu) DS
11l PQ\(_9\), gas phase (Ani – Neu) DS
11m DMBQ, gas phase (Ani – Neu) DS
11n Cl\(_4\)BQ, gas phase (Ani – Neu) DS
Figure D.1  Set 1 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.2  Set 2 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.3  Set 3 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.4  Set 4 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.5 Set 5 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.6  Set 6 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.7  Set 7 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.8  Set 8 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.9  Set 9 of the FTIR DS. See Table D.1 and D.2 for the details of the spectra.
Figure D.10  Set 10 of the FTIR DS.  See Table D.1 and D.2 for the details of the spectra.
Figure D.11  Set 11 of the DS. See Table D.1 and D.2 for the details of the spectra.
Table D.2  The index of the FTIR DS presented in Appendix D.

<table>
<thead>
<tr>
<th>PSI</th>
<th>(P700(^\text{+})A(<em>\text{1}^-) – P700(A</em>\text{1})) DS</th>
<th>(P700(^\text{+}) – P700) DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhQ</td>
<td>1b, 2b, 3b, 4b (dotted), 8a</td>
<td>1a, 2a, 3a, 4a, 6a, 6b-d (dotted)</td>
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<tr>
<td>(^{18}\text{O})-PhQ</td>
<td>1c (dotted)</td>
<td></td>
</tr>
<tr>
<td>(^{18}\text{O})-PhQ, corrected for unlabeled PhQ</td>
<td>1c</td>
<td></td>
</tr>
<tr>
<td>DMNQ</td>
<td>1d</td>
<td></td>
</tr>
<tr>
<td>2MNQ</td>
<td>1e, 4c (dotted)</td>
<td></td>
</tr>
<tr>
<td>Cl(_\text{2})NQ</td>
<td>1f</td>
<td></td>
</tr>
<tr>
<td>2CINQ</td>
<td>1g</td>
<td></td>
</tr>
<tr>
<td>Lapachol</td>
<td>2c</td>
<td></td>
</tr>
<tr>
<td>2OHNQ (2-hydroxy-1,4-NQ)</td>
<td>2d</td>
<td></td>
</tr>
<tr>
<td>MeONQ (2-methoxy-1,4-NQ)</td>
<td>2e</td>
<td></td>
</tr>
<tr>
<td>AceQ</td>
<td>2f</td>
<td></td>
</tr>
<tr>
<td>AceQ in (^{13}\text{C})-PSI</td>
<td>2g</td>
<td></td>
</tr>
<tr>
<td>PQ(_\text{9})</td>
<td>3c, 4d (dotted)</td>
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<tr>
<td>DMBQ</td>
<td>3d</td>
<td></td>
</tr>
<tr>
<td>Cl(_\text{2})BQ</td>
<td>3e</td>
<td></td>
</tr>
<tr>
<td>AQS</td>
<td>3f</td>
<td></td>
</tr>
<tr>
<td>PhQ, 8cm(^{-1})</td>
<td>3g</td>
<td></td>
</tr>
<tr>
<td>PhQ, S7002</td>
<td>3h, 4e (dotted)</td>
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</tr>
<tr>
<td>PhQ, PSI-F(_\text{X})</td>
<td>4b</td>
<td></td>
</tr>
<tr>
<td>2MNQ, PSI-F(_\text{X})</td>
<td>4c</td>
<td></td>
</tr>
<tr>
<td>PQ(<em>\text{9}), PSI-F(</em>\text{X})</td>
<td>4d</td>
<td></td>
</tr>
<tr>
<td>PhQ, S7002, PSI-F(_\text{X})</td>
<td>4e</td>
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</tr>
<tr>
<td>2BrNQ, RT</td>
<td>5b</td>
<td></td>
</tr>
<tr>
<td>Br(_\text{2})NQ, RT</td>
<td>5c</td>
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<tr>
<td>Cl(_\text{2})NQ, RT</td>
<td>5d</td>
<td></td>
</tr>
<tr>
<td>Cl(<em>\text{2})NQ, PSI-F(</em>\text{X}), RT</td>
<td>5e</td>
<td></td>
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<tr>
<td>Cl(_\text{2})NQ, (^{13}\text{C})-PSI, RT</td>
<td>5f</td>
<td></td>
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<tr>
<td>(P700(^\text{+}) – P700) DS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700</td>
<td>1a, 2a, 3a, 4a, 6a, 6b-d (dotted)</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, QH(_\text{2})</td>
<td>6b</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, PSI-F(_\text{X})</td>
<td>4a, 6c</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, 8 cm(^{-1})</td>
<td>3g (dotted), 6d</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, (^{13}\text{C})-PSI</td>
<td>2g (dotted), 6e, 6f (dotted)</td>
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<tr>
<td>P700(^\text{+}) – P700, (^{13}\text{C})-PSI, QH(_\text{2})</td>
<td>6f</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, S7002</td>
<td>3h (dotted), 6g, 6h (dotted)</td>
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</tr>
<tr>
<td>P700(^\text{+}) – P700, S7002, PSI-F(_\text{X})</td>
<td>6h</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, RT</td>
<td>5a, 7a, 8e</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, QH(_\text{2}), RT</td>
<td>7b</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, F(_\text{X}), RT</td>
<td>5e (dotted), 7c</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, (^{13}\text{C})-PSI, RT</td>
<td>5f (dotted), 7d</td>
<td></td>
</tr>
<tr>
<td>P700(^\text{+}) – P700, (^{13}\text{C})-PSI, QH(_\text{2}), RT</td>
<td>7e</td>
<td></td>
</tr>
</tbody>
</table>
P700$^+ – $P700, S7002, RT 7f
P700$^+ – $P700, S7002, PSI-F$\chi$, RT 7g

$({}^3P700 – P700)$ DS

$^2P700 – P700$ 8b
$^3P700 – P700, PSI-F$\chi$ 8c
$^3P700 – P700, ^13C$-PSI 8d
$^3P700 – P700, RT$ 8e

pbRC

(P$^+Q^– – PQ$) DS

P$^+Q_A^– – PQ_A, FeRC$ 9a
P$^+Q_A^– – PQ_A, ZnRC$ 9b
P$^+Q_A^– – PQ_A, 100 K, FeRC$ 9c
P$^+Q_A^– – PQ_A, 100 K, ZnRC$ 9d
P$^+Q_B^– – PQ_B, FeRC$ 9e
P$^+Q_B^– – PQ_B, UQ_0, FeRC$ 9f
P$^+Q_B^– – PQ_B, UQ_0, ZnRC$ 9g

(Q$^– – Q$) DS

Q$^{\tilde{A}}$ – Q_A, FeRC 10a
Q$^{\tilde{A}}$ – Q_A, ZnRC 10b
Q$^{\tilde{B}}$ – Q_B, FeRC 10c
Q$^{\tilde{B}}$ – Q_B, UQ_0, FeRC 10d
Q$^{\tilde{B}}$ – Q_B, UQ_0, ZnRC 10e

Gas phase

(Ani – Neu) DS

PhQ 11a
DMNQ 11b
2MNQ 11c
Cl$_2$NQ 11d
Br$_2$NQ 11e
2ClNQ 11f
2BrNQ 11g
Lapachol 11h
2OHNQ 11i
MeONQ 11j
AceQ 11k
PQ$_9$ 11l
DMBQ 11m
Cl$_4$BQ 11n