Near Infrared Electrogenerated Chemiluminescence and Related Electrochemical Properties of Gold Nanoclusters

Hedi Ma

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Near Infrared Electrogenerated Chemiluminescence and Related Electrochemical Properties of
Gold Nanoclusters

by

Hedi Ma

Under the Direction of Gangli Wang, PhD

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy
in the College of Arts and Sciences
Georgia State University
2022
ABSTRACT

This dissertation studies the fundamental principles and analysis applications of electrochemiluminescence (ECL) from atomically precise nanoclusters. The nanoclusters under study are composed of a metal core stabilized by a monolayer of covalently attached ligands. Chapter one describes the basics of ECL and an overview of gold nanoclusters including both fundamentals and applications. In chapter two, the kinetics of ECL generation are investigated where nanoclusters are either immobilized on the surface or free diffusing in solution. The ECL intensity-time profiles suggest that bimolecular or pseudo first order reactions limit the ECL generation immediately following the establishment of the applied potentials, while later ECL generation is governed by diffusion or mass transport displaying a Cottrell type decay over inverse square root time. Analytical equations are derived based on ECE reaction mechanism. Successful fitting to the experiments paves ways for generalized application. In chapter three, a ratiometric analysis strategy is developed based on the kinetics of charge transfer reactions. Absolute and ratiometric electrochemiluminescence signals are elucidated from single measurements for the detection of hydroxyzine and cetirizine as prototype drugs. The two compounds function as ECL coreactants to greatly enhance the near-infrared ECL from Au_{22}(LA)_{12} NCs on ITO electrodes. The kinetic profiles as signals not only improve the signal/noise ratio but also offer greater resolving power to differentiate analogue species and nonspecific interference. These case studies successfully detected and identified drug compounds in the sub nanomolar physiological range and confirmed the effectiveness of point-of-care applications. The fundamental multi-point kinetics-based ratiometric concept/strategy is not limited to a specific ECL system and generalizable to other detection systems. In chapter four, the energy band gap at the nonmetallic to metallic transitions are revealed with Au_{133}(TBBT)_{52},
Au_{144}(BM)_{60}, and Au_{279}(TBBT)_{84} (whereas TBBT is 4-tert-butylbenzenethiol and BM is benzyl mercaptan; abbreviated as Au_{133}, Au_{144}, and Au_{279}). Electrochemical experiments resolve different energy gaps for Au_{133} and Au_{144} at room temperature, but not for Au_{279} which is metallic. Spectroelectrochemistry features of Au_{133} and Au_{144} are compared with ultrafast spectroscopy to demonstrate a generalizable analysis approach to correlate steady-state and transient spectrum features. Insights on the factors affecting the energy band gap and quantized double-layer capacitance will guide future studies on improving ECL and photoluminescence properties of metal nanoclusters.

INDEX WORDS: Electrochemiluminescence, ECL kinetics, Gold nanoclusters, Ratiometric analysis, piperazine drugs
Near Infrared Electrogenerated Chemiluminescence and Related Electrochemical Properties of Gold Nanoclusters

by

Hedi Ma

Committee Chair: Gangli Wang
Committee: Shahab Shamsi
Suri Iyer

Electronic Version Approved:

Office of Graduate Services
College of Arts and Sciences
Georgia State University
December 2022
DEDICATION

I want to dedicate this dissertation to my parents. Without their love and support none of this would have been possible.
ACKNOWLEDGEMENTS

All the work was done under the guidance and support of Dr. Gangli Wang. His motivating support along with his many valuable suggestions along the way were highly beneficial to not just my research but life as a whole.

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<td>A</td>
<td>amperes or Amps</td>
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<tr>
<td>Au NPs</td>
<td>Gold Nanoparticles</td>
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<td>Au NCs</td>
<td>Gold Nanoclusters</td>
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<td>Differential Pulse Voltammetry</td>
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<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
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<td>LUMO</td>
<td>Lowest Unoccupied Molecular Orbital</td>
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<tr>
<td>QDL</td>
<td>Quantized Double-Layer Charging</td>
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<td>Abbreviation</td>
<td>Full Name</td>
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<tr>
<td>-------------</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<tr>
<td>HDZ</td>
<td>Hydroxyzine</td>
</tr>
<tr>
<td>CTZ</td>
<td>Cetirizine</td>
</tr>
<tr>
<td>TBBT</td>
<td>4-tert-butylbenzenethiol</td>
</tr>
<tr>
<td>BM</td>
<td>benzyl mercaptan</td>
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1 INTRODUCTION

1.1 Gold Nanoclusters (Au NCs): Characterizations and Fundamental Properties

Nanomaterials are an exciting class of materials with unique properties and numerous practical applications. The science of nanomaterials is a field of investigation at least one-dimension in "nanoscale," from 1 to 100 nm. The history of nanomaterial utilization is long-standing, and humans have utilized these materials for various purposes for centuries. Romans produced a dichroic cup called the “Lycurgus Cup” in the 4th century A.D. It varies in color depending on the incident light. These color variations are caused by the presence of Ag and Au nanoparticles (NPs). Currently, the field of nanomaterials research are evolving rapidly, and there are now powerful characterization tools and synthesis strategies for producing nanomaterials with more precisely controlled dimension, morphology, and corresponding physicochemical properties.

Gold as noble metal is inert and intrinsically suitable for biomedical applications in terms of toxicity concerns. Au nanoparticles (Au NPs) are developed from “colloid gold” about two decades ago amongst first and most studied plasmonic nanomaterials. Smaller Au nanoparticles are often referred as monolayer protected clusters (MPCs) in earlier literature and recently as “atomically precise gold nanoclusters (NCs)” Arguably the most fundamental distinctions from larger metallic NPs are the molecular-like or discrete energy bands in NCs. The significance to achieve atomic precision cannot be overstated, owing to the tremendous successes of solution phase synthesis and isolation. Definitive composition/structure-property correlation and quantitative reproducibility become possible, which pave ways for various applications. The atomically precise NCs consist of a few to hundreds of metal atoms with a specific ligand monolayer covalently attached, forming NCs or nanomolecules with a specific chemical
composition. Many NCs have their molecular formula characterized by mass spectrometry.\textsuperscript{6, 7} Also, with the aid of single crystal X-ray diffraction, it is now possible to determine the entire structures of these ultrasmall NPs if single crystals can be formed.\textsuperscript{8-10} These techniques help researchers to reveal the atomic structures of NCs from inner kernel structure to the metal-ligand interface, then to the outer ligand shell, and give researchers an opportunity to study every detail of the particle and interparticle interactions. Creating well-defined clusters with varying ligands and chemical properties has spawned many applications in chemistry, biology, materials science, and device fabrications.\textsuperscript{11-13} All these applications have substantially increased the value of gold to produce functional materials, in addition to its primary function as a store of wealth.

Various types of ligand-protected Au NCs have been successfully synthesized in recent years, including phosphine-metal NCs, carbonyl-metal NCs, thiolate-metal NCs and so on.\textsuperscript{14-16} Among all the metal NCs, the chemistry of thiolate-gold NCs (thiolate-Au NCs) has been investigated the most. The Brust-Schiffrin method, developed by Brust et al. for making small (1–5 nm) Au NPs by using organic thiols as ligands, was a very important step forward.\textsuperscript{17} Because this facile method allows the reproducible synthesis of stable particles with controllable quantities, it has been widely used by scientists, and it has also affected the development of thiolate-Au NCs with well-defined sizes and chemical compositions. The use of thiolates increased the stability of NPs due to the stronger Au-S intermediates. More importantly, the use of organic thiolates as the capping ligand gave rise to new research on nanoparticles because it improved their structural diversity. Figure 1.1 illustrates the Brust-Schiffrin procedure. Specifically, the synthesis of these gold nanoparticles starts with the transfer of gold (III) salt from the aqueous phase to the organic phase, such as toluene, then mixed with the selected thiols at certain mole ratio.\textsuperscript{17} The resulting Au(I)-SR intermediate is reduced by using a reducing agent
such as sodium borohydride (NaBH₄) to form the metallic NPs/NCs, so-called monolayer protected nanoclusters (MPC). Au NCs created by using this method can be varied easily using different thiolate ligand or via a typical modification called “place exchange (PE)” reaction. Typically, the ligand on the monolayer can be partially or totally exchanged by reacting with a higher mole ratio of another thiol.

![Chemical equation and diagram]

**Figure 1.1** The synthetic scheme of nonpolar gold nanoclusters with Brust-Schiffrin method.

Au NCs obtained by Brust-Schiffrin method are polydisperse in size and they require laborious and impractical post-synthetic separation. For example, in the later 1990s, Murray and his coworkers conducted extensive research to increase the monodispersity of thiolate-protected gold nanoparticles, such as etching treatment, chromatographic separation, and selective-size precipitation.¹⁸ They used Laser desorption ionization (LDZ) mass spectrometry to separate the initial mixture and got a series size of gold nanoparticles. PAGE (Polyacrylamide gel electrophoresis) is another common separation technique to separate water soluble Au NCs such as glutathione (SG)- gold nanoclusters.¹⁹ Tsukuda et al. used PAGE and high-resolution electrospray-ionization (ESI) mass spectroscopy to determine different types of Au-SG clusters.
including \( \text{Au}_{10}(\text{SG})_{10} \), \( \text{Au}_{15}(\text{SG})_{13} \), \( \text{Au}_{18}(\text{SG})_{14} \), \( \text{Au}_{22}(\text{SG})_{16/17} \), \( \text{Au}_{25}(\text{SG})_{18} \), \( \text{Au}_{29}(\text{SG})_{20} \), \( \text{Au}_{33}(\text{SG})_{22} \), and \( \text{Au}_{39}(\text{SG})_{24} \).\(^{20}\) However, the separation yield was relatively low, preventing in-depth studies and applications of gold nanoclusters.

Among the various synthetic modifications attempted to resolve the polydispersity issue, a unique size-focusing methodology introduced by Jin’s group in the late 2000s\(^3\), stimulated the explosive growth of metal NCs library. High-purity \( \text{Au}_n(\text{SR})_m \) NCs have been synthesized with decent yield without suffering the intricate steps of post-synthetic separations.\(^{21}\) Typically, there are two major steps for a “size-focusing” synthesis. In the first step, a mixture \( \text{Au}_n(\text{SR})_m \) NCs at certain size range were obtained. Next, by using aging/etching in a harsh environment, the most robust species can survive, and a single-sized product can be generated from the mixture of Au NCs. By shifting the initial size range, it is possible to obtain NCs of various sizes. In the past ten years, several representative atomically precise Au NCs with a relatively large quantity and high molecular purity were synthesized, such as \( \text{Au}_{25}(\text{SR})_{18} \), \( \text{Au}_{38}(\text{SR})_{24} \), \( \text{Au}_{40}(\text{SR})_{24} \), \( \text{Au}_{64}(\text{SR})_{32} \), \( \text{Au}_{99}(\text{SR})_{42} \), \( \text{Au}_{102}(\text{SR})_{44} \), \( \text{Au}_{130}(\text{SR})_{50} \), \( \text{Au}_{133}(\text{SR})_{52} \), and \( \text{Au}_{144}(\text{SR})_{60} \) NCs.

Among the reported well-defined \( \text{Au}_n(\text{SR})_m \) nanoclusters, \( \text{Au}_{25}(\text{SR})_{18} \) has been studied the most, likely due to its thermodynamic stability and prevalence in several successful synthesis methods. The reported high-yield synthetic procedures are size-conversion, two-phase, and one-phase methods. Jin’s group has expanded the one-phase method to produce the monodispersed \( \text{Au}_{25}(\text{SR})_{18} \) as the main product.\(^{22, 23}\) In this protocol, the reaction was carried out in tetrahydrofuran (THF) that can dissolve the reactants including gold salt, thiol and tetraoctylammonium bromide (TOABr). The reduction of Au(I)-SR polymer by NaBH\(_4\) generated a mixture of sizes, as indicated by the featureless UV-vis spectrum and the mixture of sizes in the mass spectrum in Figure1.2. Pure \( \text{Au}_{25}(\text{SR})_{18} \) was obtained after a spontaneous size-
focusing process on the presence of excess thiol, in which the initial featureless UV-vis spectrum gradually evolved into the characteristic spectrum of Au$_{25}$ (distinct absorbance peaks at $\sim$400 nm (3.11 eV), $\sim$450 nm (2.76 eV) and $\sim$670 nm (1.86 eV), with optical band gap of $\sim$1.33 eV) and all other sizes disappeared in the MALDI-MS.

**Figure 1.2** Size-focusing synthesis of Au$_{25}$(SC$_2$H$_4$Ph)$_{18}$ nanoclusters. (A) Evolution of UV-vis spectra during the size-focusing process. (B) Evolution of MALDI-MS spectra. Adapted with permission from Ref. 3,23. Copyright 2010 American Chemical Society and 2009 Royal Society of Chemistry.

Based on this protocol, several Au$_{25}$(SR)$_{18}$ clusters were synthesized by different protecting thiol ligands such as glutathione, 2-phenylethanethiol, and hexanethiolate. Notably, whatever type of ligand was used, if it adopted the same gold core structure, the UV-vis bears similar features. Interestingly, different charge states of the Au$_{25}$(SR)$_{18}$ with corresponding counterions can be synthesized. The absorbance features show subtle differences at different valence states. For example, in the presence of TOABr, the anionic species, Au$_{25}^-$ is the primary product with TOA as counterion and exhibits a broad shoulder at 800 nm. The neutral species, Au$_{25}^0$ does not have this shoulder in the absence of TOABr during synthesis. In addition, the solvent,$^{24}$ the ratio of gold to thiol, and the rate of reduction$^{25,26}$ all significantly impact the yield,
monodisperse, and size of gold nanoclusters. For example, when THF, acetone, or ethyl acetate are used as solvent, \( \text{Au}_{25}(\text{SR})_{18} \) is the dominant product, whereas methanol or acetonitrile favors forming \( \text{Au}_{144}(\text{SR})_{60} \) and larger nanoparticles.\(^{24}\) Also, the rate of the reducing agent addition changes the composition as reported for \( \text{Au}_{20}(\text{SR})_{16} \) and \( \text{Au}_{24}(\text{SR})_{20} \).\(^{3,26}\) Same synthesis protocols were used but the rate of reductant addition changed from drop wise (\( \text{Au}_{20} \)) to slow addition (\( \text{Au}_{24} \)).

\( \text{Au}_{144}(\text{SC}_2\text{H}_4\text{Ph})_{60} \) is another case of size focusing protocol for the facile and large-scale synthesis.\(^{27,28}\) By tuning the gold salt to thiol ratio (Au/SR) to 1:3, the initial size distribution range was controlled around 29 kDa, together with some \( \text{Au}_{25}(\text{SC}_2\text{H}_4\text{Ph})_{18} \) clusters. The crude polydispersed clusters were incubated in large amounts of thiol at 80 degrees for 24 hours during the size-focusing step. During this time, the initially present \( \text{Au}_{25} \) clusters were thermally decomposed, resulting in the production of pure \( \text{Au}_{144} \) clusters. After size focusing, the UV-vis spectrum of \( \text{Au}_{144} \) NCs showed a step-like bands at 510 and 700 nm. Also, ESI-MS characterized the precise weight and the \( \text{Au}_{144}(\text{SC}_2\text{H}_4\text{Ph})_{60} \) formula were determined.

Few groups including ours focus on the synthesis of aqueous soluble gold nanoclusters.\(^{13,14,29-31}\) Similar as Figure 1.1, gold salt was dissolved in pure water or a solvent mixture like methanol/acetic acid and then mixed with the aqueous soluble ligand such as lipoic acid (LA) or mercaptosuccinic acid (MSA). Sodium borohydride can be used to reduce this mixture into gold clusters further. An annealing process with more of the protective ligand or ligand exchange reaction can be done to change or tune their properties. Figure 1.3 shows an increase in photoluminescence (PL) after the annealing process of the crude Au-MSA NCs. In the presence of carboxyl or amine groups on the ligands, the coupling reactions can be performed to covalently attach compounds such as N,N-diethylethlenediamine (DEDA),
methoxypolyethylene glycol amine (PEG) into the ligand monolayer. The new molecular structures can enable or improve applications in cells imaging, electrogenerated chemiluminescence (ECL) detections and so on.\textsuperscript{32, 33}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{UV-visible absorbance and emission spectra before and after etching for Au-MSA. Adapted with permission from Ref. 31. Copyright 2014 Royal Society of Chemistry.}
\end{figure}

The sizes of Au NCs are in the size regime where the quantum confinement effect can be observed. Different from bulk gold, they exhibit novel optical, electronic, and magnetic properties, more specifically photoluminescence, HOMO-LUMO and other discrete energy band transitions, and intrinsic magnetism. As mentioned in the case of Au\textsubscript{25} nanoclusters, the UV-vis absorbance features are significant for most of nanoclusters and show distinct absorbance bands. Photoluminescence of Au NCs in the near infrared range has also been observed. A broad emission peak of Au\textsubscript{25} was observed at the range from 600 nm to 850 nm with a weak quantum yield, less than 1\%. Au\textsubscript{22} NCs with the protection of glutathione or lipoic acid ligand has a relatively higher quantum yield from 7\% to 10\% in the near IR range.\textsuperscript{14} The bimetallic cluster,
[Ag_{13}Au_{12}(PPh_3)_{10}(SC_2H_4Ph)_5Cl_2]^{2+}, has a quantum efficiency as high as 40% solubilized in nonaqueous solvent.\textsuperscript{34}

Advances in mass spectrometry (MS) make it a primary technique for determining the chemical composition of metal NCs. The most prevalent MS ionization techniques are ESI and MALDI. ESI is highly effective for multi-charged clusters, and MALDI is normally used to determine the single charged species. To visualize the AuNPs/ AuNCs, the most straightforward method is high resolution transmission electron microscopy (HR-TEM). Figure 1.4 shows the shape of Au_{333}(SR)_{79}, a giant nanocluster. TEM had been used to characterize metal clusters since 1990s.\textsuperscript{35, 36} An obvious limitation is that molecular formula cannot be determined because ligands are invisible under TEM. Another concern is that the high energy electron beam could alter the size/shape of the NCs.

\textbf{Figure 1.4} Size-focusing synthesis of Au_{333}(SC_2H_4Ph)_{79} nanoclusters. (A) TEM and (B) HR-TEM of the Au_{333}(SC_2H_4Ph)_{79}. Adapted from Ref. 36. Copyright 2014 Springer, Cham.

NMR is a useful technique to analyze the ligand structure of Au NCs. The NMR of Au NCs is not like their ligands with sharp peaks, their peaks are significantly broadened, known as
line-broadening effect.\textsuperscript{37} The extent of such effect is highly influenced by the shape and size, the type of ligand, the size dispersity of NCs and how close to the gold core the proton is. Two-dimensional (2D) NMR can also be used to provide valuable information for the analysis of different ligand compositions of the monolayer. In our recent works, NMR was used in determining the numbers of tertiary amine compound coupled on the lipoic acid ligand of Au\textsubscript{22} nanoclusters. Changes in chemical shift reveal the oxidation occurring in the monolayer of Au-LA clusters.

In addition to the mentioned techniques, many other techniques can be used to characterize gold nanoclusters e.g., electrochemistry, X-ray photoelectron spectroscopy (XPS), X-ray crystallography, electron microscopy and so on. Electrochemical analysis of gold clusters is the major method in our group and the details are discussed in the next section.

\subsection*{1.2 Electrochemical properties of Au NCs}

The electrochemical techniques, such as Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV), Square Wave Voltammetry (SWV), and Bulk Electrolysis (BE) can be used to understand the electrochemical properties of metal NCs, and they are extremely useful in unraveling their electronic structures combined with spectroscopic techniques. The electrochemistry of “molecule-like” gold in a size range of 1 to 2 nanometers in diameter with the core mass in the range of 8 to 38 kilodaltons (kDa) reported by Murry’s group was a significant accomplishment in the field of gold nanoparticle electrochemistry.\textsuperscript{38} They first announced that the gold nanoparticles have quantized charging properties, which can be multi-electron donor/acceptor reagents. The smaller gold NCs, such as Au\textsubscript{25}(SR)\textsubscript{18} showed a well-defined differential pulse voltammograms (DPVs) with a clear HOMO-LUMO gap (1.32 eV). Similarly, the HOMO-LUMO gaps of 0.99, and 0.61 eV were electrochemically estimated for
Au_{38}, and Au_{67} respectively.\textsuperscript{39} The larger gold NCs showed a multiple redox activity, such as Au_{144}(SR)_{60} that has at least 15 detectable charging peaks representing positive, neutral, and negative states for the cluster.\textsuperscript{40}

### 1.2.1 Quantized Double-Layer Charging

The quantized double layer (QDL) charging of the core of gold NCs occurs because of the effective capacitance ($C_{CLU}$) of the ligand protected Au NCs with a small core size (< 2 nm in diameter). The single electron changes in their core charges occur at palpably large voltage intervals ($\Delta V = e/C_{CLU}$, where $e =$ electron charge), even at room temperature. The changes in the core of nanoclusters charge electrostatically evoke a change in the ionic space charge in the surrounding electrolyte solution. Nanoparticles can be identified as nanoscopic electrodes so that the change in ionic space charge around nanoclusters is similar with what occurs when the potential of a macroscopic metal electrode coated with a self-assembled monolayer. The capacitance of nanoclusters can be modeled as a concentric sphere capacitor.

$$C_{CLU} = 4\pi \varepsilon \varepsilon_{0}(r/d)(r + d)$$

Where $\varepsilon_{0}$ is the permittivity of free space, $\varepsilon$ is the monolayer static dielectric constant, $r$ is the radius of the gold core, and $d$ is the length of the extended monolayer.\textsuperscript{41}

QDL charging behavior is prevalent for larger NCs. Figure 1.5.A shows that the evenly spaced current peaks are electrochemically observed for Au_{102}, Au_{144} and Au_{333},\textsuperscript{42} which suggests that these NCs act as quantum capacitors. The double layer capacitances of the larger NCs become so small that QDL charging behavior can be experimentally observed.

Our group synthesized the mixed-thiolate Au_{130} showing clear QDL features.\textsuperscript{43,44} This unique nanocluster has a protective monolayer that was composed of both the monothiolate (phenylethyl thiolate) and the dithiolate (Durene-DT). The optical features of this Au_{130} NCs are
four distinct UV-vis absorbance peaks were observed at 360, 493, 585, and 716 nm, and a low near-IR luminescence. The composition of Au$_{130}$(Durene-DT)$_{29}$(PET)$_{22}$ was characterized by using MALDI. DPVs of this cluster showed a clear QDL feature that is representative of clusters of this size. As Figure 1.5B to show, this cluster has eight QDL peaks in both oxidation and reduction scans, the average peak spacing is 0.19 V and an energy gap of 0.35 V was calculated revealing an energy state below the frontier states.$^{44}$

![Figure 1.5](image)

**Figure 1.5** (A) SWVs of Au$_{102}$, Au$_{144}$, and Au$_{333}$ clusters in CH$_2$Cl$_2$ containing 0.1 M Bu$_4$NPF$_6$. (B) Differential pulse voltammograms (DPVs) of the Au$_{130}$ nanoclusters in CH$_2$Cl$_2$. Adapted from Ref. 43,44. Copyright 2017, 2015 American Chemical Society.

Another Au$_{130}$(SR)$_{50}$ (SR: 4-Methylbenzenethiolate) NCs synthesized by Jin’s group$^{45}$ also observed an uniform QDL peaks with an average peak spacing of 0.21 V and a energy gap of 0.5 V.$^{46}$ Generally, energy gap increases with the decrease in the core size of nanoclusters. However, discrepancies can happen in the nonmetal to metal transition range. The largest nonmetallic nanocluster in the quantum size regime is Au$_{246}$(p-MBT)$_{80}$, but Au$_{329}$(PET)$_{84}$ has no obvious electrochemical gap and being metallic.$^{47-49}$ Our group electrochemically analyzed
another metallic cluster, Au$_{279}$(TBBT)$_{84}$, which has no clearly defined energy gap and will be discussed in the next chapter.

1.2.2 Spectroelectrochemistry of Au NCs

Spectroelectrochemistry (SEC) is an analytical technique that provides both spectroscopic and electrochemical information. The most common spectroscopic analysis since the advent of SEC is UV-vis absorbance SEC, due in part to its low cost, simple design, and ease of use.$^{50}$ For the spectroelectrochemistry of gold nanoclusters, electrochemistry enables the core and the ligands' redox states to be actively regulated. After electrolysis, the absorbance at different charge levels can be measured, and the energy states of the resulting electronic transitions can be correlated. It provides a new insight for the electronic transitions of metal clusters that was mainly obtained by ultrafast transient absorption spectroscopy. Compared to the technique of ultrafast absorption spectroscopy, without using a pump laser to modulate the population of electrons in different energy state, spectroelectrochemistry can selectively change the charge states of the sample by an electrode at appropriate potentials.

The initial absorbance spectra of gold nanoclusters in their neutral state should be used as a baseline for a spectroelectrochemistry experiment. Next, based on their unique voltammogram features, selected positive/negative potentials were held to change the charge states of nanoclusters. During the charging period, absorbance spectrums were taken at each potential so that a differential spectrum can be calculated. Notably, the spectrums’ differences caused by changing the charge state should be reversible if the potential did not cause a decomposition of gold nanoclusters.

Figure 1.6 shows a differential spectra of Au$_{130}$ after electrolysis under different potentials in CH$_2$Cl$_2$. The dash lines represent the peak values of absorption of Au$_{130}$ in CH$_2$Cl$_2$. This
figure shows that the differential reduction and oxidation spectra mirror each other in the most wavelength ranges. The additional and suppressed absorption transitions were positive and negative changes, respectively. By combining the ultrafast transition analysis with the specific features from differential spectra, such as the isosbestic point, bump values, valley values, the increasing and decreasing of the absorbance, an energy diagram can be proposed. This energy diagram successfully explained the major electronic transitions of the Au$_{130}$ NCs.

![Differential spectra after electrolysis under different potentials in CH$_2$Cl$_2$. Adapted from Ref. 46. Copyright 2017 American Chemical Society.](image)

**Figure 1.6** Differential spectra after electrolysis under different potentials in CH$_2$Cl$_2$. Adapted from Ref. 46. Copyright 2017 American Chemical Society.

### 1.3 AuNC Applications

Noble metal nanoclusters are ideally suited for numerous applications, including sensing, catalysis, optics, imaging, and biolabeling, due to their distinctive physiochemical, electrical, and optical characteristics.

#### 1.3.1 AuNCs Sensors for metal ions, small molecules, proteins, and amino acids

Optical properties, such as absorption and luminescence are critical for sensing applications. Due to their photoluminescence properties and resistance to photobleaching, some
useful AuNCs fluorescent probe were used for the detection of metal ions, small molecules, proteins, and polypeptides or amino acids.

Heavy metals, such as mercury, copper, and lead are harmful not only for the human body but also for the environment. Various types of ligands protected Au nanoclusters have been synthesized to detect these heavy metals by many research groups. Their detection limitation can be as low as sub-nanomolar range.\textsuperscript{51-53} Xie’s group synthesized bovine serum albumin (BSA) stabilized Au nanocluster to detect Hg ions.\textsuperscript{51} Based on the strong affinity of Au\textsuperscript{+} ions for Hg\textsuperscript{2+} ions on the surface of Au nanoclusters, which quenches the clusters' fluorescence. The detection limit of Hg\textsuperscript{2+} can reach to 0.5 nM, much less than the safe limit of the United States Environmental Protection Agency. Small molecules like glucose, dopamine, cholesterol, and ascorbic acid also can be detected by Au nanoclusters, most because of quenching fluorescence. Unlike previous fluorescence quenching strategies, cysteine, a type of amino acid, was absorbed with gold nanoclusters using BSA as the template to form Au cluster–cysteine complexes, which enhanced the fluorescence signal intensity. Some Au nanoclusters are made using protein templates. Adding certain enzymes will change the fluorescence signal intensity of Au clusters. With this approach, the detection of specific enzymes is possible.

\textit{1.3.2 Au NCs for bioimaging}

Because many of Au nanoclusters offer the benefits of strong stability, a large Stokes shift, good biocompatibility, and ease of modification, Au nanoclusters are a better candidate than organic dyes in cell targeting imaging and metabolism research. Our group synthesized a monothiolate protected aqueous soluble Au nanocluster, Au-MSA NCs, performing up to 5-10 \% quantum efficiency in near IR range, and its quantum efficiency is stable over a wide pH range. The emission maximum in 750-900 nm range makes it superior for bioimaging imaging. Our z-
stack confocal analysis clearly demonstrates Au-MSA nanoclusters present inside cell nucleus in single cell imaging.  

Chang et al. described how lipoic acid-protected Au nanoclusters can be successfully coupled to biomolecules such as BSA, avidin, streptavidin, and ethylene glycol (PEG) via EDC coupling and employed for biological imaging application. The streptavidin coupled Au-LA nanoclusters displayed endogenous labeling of biotin inside human hepatoma cells (HepG2). While the uncoupled Au-LA clusters showed a week emission from the cell, streptavidin coupled Au-LA clusters performed a high intensity in the biotin containing cells. A similar method was used by Pradeep’s group to image HepG2 cells using streptavidin coupled Au\(_{23}\) clusters.

Through modification of the surface of Au nanoclusters, the modified AuNCs can be used to image specific tumor cells. In certain cancerous cells, the positive expression of folic acid (FA) receptors can happen. For example, oral carcinoma KB cells were used to internalize and image folic acid coupled Au nanoclusters. Archana et al. synthesized folic acid coupled Au-BSA nanoclusters. The folate receptor on oral cancer cells was then used to attach the Au-BSA-FA fluorescent marker.

### 1.3.3 AuNCs for catalysis

Gold substance was barely concerned as the catalytic active metal until 1987 when a surprising activity in carbon monoxide oxidation at very low temperature was observed by making Au NPs casted on semiconducting transition-metal oxides. Atomically precise gold nanoclusters, such as Au\(_{25}\), Au\(_{38}\), Au\(_{144}\) etc. have shown extreme potential in catalysis due to their unique structure-property relationships. Compared to the polydisperse nanoclusters, gold nanoclusters with monodisperse/atomically precise are crucial for improving catalytic performance. Catalytic activities of nanocluster are highly dependent on their size, for example,
among the three gold nanoclusters, \( \text{Au}_{25} \), \( \text{Au}_{38} \), and \( \text{Au}_{144} \), the diameter is from 1 to 1.3 to 1.6 nm. They were found when utilizing for the selective oxidation of styrene the catalytic performance was in order, \( \text{Au}_{25} > \text{Au}_{38} > \text{Au}_{144} \) showing the size dependence in catalytic performance.\(^{57}\) A lot of catalytic behavior of gold clusters were reported for the reactions such as oxidation of styrene,\(^ {58, 59}\) benzyl alcohol,\(^ {60, 61}\) carbon monoxide,\(^ {62-64}\) cyclohexane,\(^ {65}\) and hydrogenation,\(^ {66}\) reduction of nitrophenol, and electrochemical reduction of oxygen. Some important catalytic behaviors will be described in detail.

CO oxidation attracts a lot of attention due to its simplicity and significance. Since it is the first catalytic activity that was discovered in gold, researchers lovely use this as prototypical reactions for understanding gold catalysis. It is worth mention to know the thiolate ligands have an important effect on the catalytic activities of gold nanocluster for CO oxidation. Wu’s group investigated the catalytic activity of \( \text{Au}_{25}(\text{SR})_{18} / \text{CeO}_2 \)-rod for CO oxidation reaction.\(^ {67}\) The initial \( \text{Au}_{25}(\text{SR})_{18} / \text{CeO}_2 \)-rod did not exhibit catalytic activity because Au was not exposed out for CO absorption. After partially removing thiolate ligand, CO was favored to absorb on the exposed Au atom and then reacted with CeO\(_2\) to generated CO\(_2\). It showed that the thiolate ligand on gold nanoclusters had a dual effect on the catalytic activity of CO oxidation, maintaining the structural integrity of the gold nanoclusters while simultaneously obstructing the catalytic sites for CO oxidation.

Reduction of CO\(_2\) is a hot topic with the increase of greenhouse effect. The catalytic properties between \( \text{Au}_{25}(\text{C}_2\text{H}_4\text{Ph})_{18} \) and CO\(_2\) were studied by Jin’s group.\(^ {56}\) They observed the oxidation of \( \text{Au}_{25} \) spectroscopically and electrochemically with a spontaneous and reversible electronic interaction. With the help of DFT calculation, the favorable binding sites and adsorption structures were determined at atomic scale. DFT results show the interaction of
oxygen atoms with three sulfur atoms of gold clusters during the reaction. The potential-dependent formation rate of CO and H₂ described the mechanism of reduction.

1.4 Electrochemiluminescence or Electrogenerated Chemiluminescence

Electrochemiluminescence (ECL), also known as electrogenerated chemiluminescence, is a light emission process where the ECL luminophore undergoes electron transfer (ET) reaction on electrode surface, followed by subsequent reaction/s to produce excited state species that emit light. ECL can be distinguished from chemiluminescence (CL) and photoluminescence (PL) studies. Figure 1.7 shows the details of these three processes. In PL process, a light source is needed to excite electrons from HOMO to LUMO to generate an excited state(s). In CL process, the emission of light occurs because of certain chemical reactions. Both ECL and CL do not need any external light.

![ECL, CL, and PL processes](image)

**Figure 1.7** The illustration of electrochemiluminescence processes, chemiluminescence processes and photoluminescence processes.

ECL studies were first detailed described by Hercules and Bard et al. in the mid-1960s. Anthracene and rubrene emitted blue and red light respectively that is possible because of the formation of radical cations and anions. An electron transfer from anionic to cationic species
generated an excited state. Later, Bard’s group studied a wide range of organic and inorganic compounds to investigate their ECL processes in organic and inorganic solvent.\textsuperscript{70,71} There are many advantages for ECL: (1) Not like PL process, the relaxation of excited state(s) to ground state in ECL process is direct. There is no thermal or vibrational relaxation. (2) The localized activation reaction/s, on electrode surface and in the electrical double layer (EDL) region, provide the foundation for temporal and spatial controls for higher resolution ECL signal generation and detection. (3) Differences in the energy input and the signal output eliminate cross-interference such as excitation photons in fluorescence or non-faradic charging current in amperometric sensors, making ECL arguably background-free signal-readout mechanism limited only by environmental factors and/or electronics noise. (3) ECL intensity can be varied based on the surface area/ materials of working electrode and the electrode potentials. (4) ECL emitters could be regenerated after the ECL emission.

As a subcategory of chemiluminescence, ECL is further divided into self-annihilation and co-reactant pathways based on whether the subsequent chemical reactions are between the (same) ECL luminophores or another reagent is added as coreactant to boost the ECL signals. Separately, ECL can also be referred to as either cathodic or anodic ECL depending on whether the emission is activated by electrode reduction or oxidation respectively.

\textbf{1.4.1 Ion annihilation route}

The early ECL studies originated with ion annihilation ECL. For ion annihilation route, as the below mechanism shows, both involved reactants (A and B) are directly formed during electrochemical oxidation and reduction. The excited state A* was generated by the reaction of the oxidant and reductant species, then relaxed to the ground state to emit light. A and B can be the same or different molecules.
\[ A - e \rightarrow A^+ \quad (1.1) \]
\[ B + e \rightarrow B^- \quad (1.2) \]
\[ A^+ + B^- \rightarrow A^* + B \quad (1.3) \]
\[ A^* \rightarrow A + h\nu \quad (1.4) \]

A formula describing the minimum energy to generate excited state was developed by Bard et al.:

\[-\Delta H_{\text{ann.}} = E_p \left( \frac{R}{R^{++}} \right) - E_p \left( \frac{R}{R^-} \right) - 0.16 \quad (1.5)\]

where \( -\Delta H_{\text{ann.}} \) (in eV) is the enthalpy for ion annihilation, \( E_p \) is the peak potential for electrochemical oxidation or reduction (in V), and 0.16 is the entropy approximation term \( (T\Delta S) \) at 25 °C (0.1 eV) with an addition of 0.057 eV that is the peak potential of reversible redox reactions.

The annihilation route, which requires potential steps or sweeping the potentials in two directions, paved a fundamental way for the research of coreactant ECL.

### 1.4.2 Coreactant route

It is essential to understand the mechanisms of coreactant ECL route since, nowadays, most of the commercially available ECL analytical tools are based on coreactant ECL pathway. Unlike annihilation ECL, coreactant ECL usually requires a one direction potential scanning at an electrode in a solution including both luminophore and a coreactant. The coreactant can be the radicals that react with oxidized or reduced luminophore to reach the excited state. Depending on the polarity of the applied potential, the mechanisms of coreactant ECL can be described to “oxidative-reduction” and “reductive-oxidation” pathways.

Persulfate \((\text{S}_2\text{O}_8^{2-})\) and hydrogen peroxide \((\text{H}_2\text{O}_2)\) are the efficient coreactant for the “reductive-oxidation” pathway. In this pathway, both luminophore and coreactant were reduced first. A strong oxidizing radical formed from the reduced coreactant and it further reacted with...
the reduced luminophore to generated the excited state. Take $\text{Ru(bpy)}_3^{2+}$, which is the commonly used luminophore, and persulfate ($\text{S}_2\text{O}_8^{2-}$) as an example. (eq. (1.6) - (1.12)).

$$\begin{align*}
S_2\text{O}_8^{2-} + e & \rightarrow S_2\text{O}_6^{3-} \quad (1.6) \\
\text{Ru(bpy)}_3^{2+} + e & \rightarrow \text{Ru(bpy)}_3^{3+} \quad (1.7) \\
\text{Ru(bpy)}_3^{3+} + S_2\text{O}_8^{2-} & \rightarrow \text{Ru(bpy)}_3^{2+} + \text{SO}_4^{2-} + \text{SO}_4^{2-} \quad (1.8) \\
\text{Ru(bpy)}_3^{3+} + \text{SO}_4^{2-} & \rightarrow \text{Ru(bpy)}_3^{2+} + \text{SO}_4^{2-} \quad (1.9) \\
\text{Ru(bpy)}_3^{2+} + \text{SO}_4^{2-} & \rightarrow \text{Ru(bpy)}_3^{3+} + \text{SO}_4^{2-} \quad (1.10) \\
\text{Ru(bpy)}_3^{2+} + \text{Ru(bpy)}_3^{3+} & \rightarrow \text{Ru(bpy)}_3^{2+} + \text{Ru(bpy)}_3^{3+} \quad (1.11) \\
\text{Ru(bpy)}_3^{2+} & \rightarrow \text{Ru(bpy)}_3^{3+} + h\nu \quad (1.12)
\end{align*}$$

The persulfate can be reduced and produced by a strong oxidizing intermediate $\text{SO}_4^{2-}$ that has a redox potential of $E^0 \geq 3.15$ V vs SCE.\textsuperscript{72} Also, $\text{Ru(bpy)}_3^{2+}$ is reduced to $\text{Ru(bpy)}_3^{3+}$, then participates in an electron reduction reaction with $\text{SO}_4^{2-}$ (eq. 1.10). $\text{Ru(bpy)}_3^{2+}$ forms and it relaxes to the ground state and emit light.

The other pathway, the “oxidation-reduction” pathway, is the one attracting more researchers’ attention. The most common oxidation-reduction system is the $\text{Ru(bpy)}_3^{2+}$/ tri-n-propylamine (TPrA) pair system. TPrA, which is a good coreactant candidate due to a good solubility in organic and aqueous phase, is a tertiary amine that can be oxidized electrochemically to TPrA$^+$ at around 0.8 V vs SCE forming a reactive radical intermediate TPrA$^-$:

$$\begin{align*}
\text{Ru(bpy)}_3^{2+} - e & \rightarrow \text{Ru(bpy)}_3^{3+} \quad (1.13) \\
\text{TPrA} - e & \rightarrow \text{TPrA}^+ \quad (1.14) \\
\text{TPrA}^+ & \rightarrow \text{TPrA} + H^+ \quad (1.15) \\
\text{Ru(bpy)}_3^{3+} + \text{TPrA}^- & \rightarrow \text{Ru(bpy)}_3^{2+} + \text{TPrA}^+ \quad (1.16) \\
\text{Ru(bpy)}_3^{2+} & \rightarrow \text{Ru(bpy)}_3^{3+} + h\nu \quad (1.17)
\end{align*}$$
Because the ECL reaction is complex, researchers spend much effort to investigate ECL mechanisms and explore different types of coreactant that can improve the ECL signal. Recently, our group studied the ECL mechanism of Au$_{22}$ NCs and Ag$_{13}$Au$_{12}$ NCs and found out several effective tertiary amine coreactants to enhance the near IR ECL of Au NCs, such as Ethylenediaminetetraacetic acid (EDTA) and N-(2-Hydroxyethyl)piperazine-N'- (2-ethanesulfonic acid (HEPES). Not only the fundamental research is important for ECL reactions but also the applications of ECL have been wildly applied in different fields.

### 1.4.3 ECL Applications

With the development over the past years, ECL techniques such as ECL detection and sensing, ECL immunoassay, and ECL microscopy (ECLM) imaging have received significant attention. Unlike the traditional single-response ECL detection method, which may be influenced by various intrinsic or extrinsic factors such as environmental conditions, electrode modification, and instrumental efficiency. The ratiometric ECL gives a more accurate analysis because it can eliminate interferences caused by the self-calibration of two emissions in a complex sample detection environment. Another prevalent ECL technique is the ECL of single entities. It helps us understand the underlying mechanisms of the individual responses from the bulk in many fields, such as catalysis and cellular biology. The individual “entities and processes” could be a molecule, a cell, a reaction, or a nanoparticle. ECL microscopy promotes imaging-based single-entity analysis because it inherits and keeps the advantages from both microscopy and ECL, such as low reagent consumption, high throughput, good spatiotemporal resolution, low background, and fewer interferences.

Ratiometric ECL analysis uses two or more ECL signals that respond to the target analytes differently. The main goal is to make more accurate analyses in complex sample
matrices, like biological samples, where interferences are hard to predict. Individual intensities cannot successfully normalize the electrode factor, detector efficiency, or other solution and measurement characteristics. Only the intensity ratio can do this. In general, there are two types of ratiometric ECL systems: one uses two different luminophores,\textsuperscript{83-85} and the other uses a single luminophore to produce both anodic and cathodic ECL signals.\textsuperscript{86,87} To the best of our knowledge, the signal specificity does not originate from ECL itself but almost entirely from other recognition components such as antibodies, nucleic acids, enzymes, etc. The ratiometric ECL can be applied for the analysis of proteins and nucleic acids and the detection of metal ions, small molecules, and cells.

Another important field for ECL applications is the single entity ECL and ECL microscopy. Unlike the traditional ECL approach that achieves averaged electrochemical quantities from a bunch of individual entities, single-entity ECL provides another perspective to elucidate the truth of nature. In 1995, Wightman's group reported the first single-molecule ECL reaction of 9,10-diphenylenanthracene (DPA) in acetonitrile using pulse voltammetry at an ultramicroelectrode (UME).\textsuperscript{88} The shorter time constant of an ultramicroelectrode makes it possible to improve temporal resolution to resolve stochastic signals. In the past few years, ECL has been used to separate many single things, such as single cells, single-entity catalysts, and single molecules or particles. These quantized and often random activities give us information that we cannot get from ensemble measurements. This is important for heterogeneous dynamic processes like catalytic reactions and in-vitro or in-vivo behavior. These insights go beyond the basic curiosity of reaching the ultimate detection limit. The underlying difference between monitoring the signal from single ECL luminophores versus a plurality of emitters exposing single entities is a less clear and occasionally perplexing issue for ordinary readers.
2 KINETICS OF THE NEAR INFRARED ELECTROCHEMILUMINESCENCE FROM METAL NANOCLUSTERS ON SURFACE AND IN SOLUTION

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The electronics structures of some metal nanoclusters enable strong photoluminescence in the near infrared spectrum range. Activation of the luminescence via electrode reactions, rather than light source, i.e., electrochemiluminescence (ECL), has received growing interests due to the various potential benefits, but has been mostly limited to steady-state behaviors such as overall emission intensity and materials optimizations. Here, the ECL kinetics in representative experiments where nanoclusters as luminophores are either immobilized on the surface or free diffusing in solution were investigated based on classic theory. An analytical equation derived under a sequential mass transport limit regime quantitates the experimental ECL kinetics features in a wide range of conditions. Deconvolution of non-faradic charging current from redox current provides the threshold in time ranges for the analysis of ECL kinetics. The ECL kinetics profiles suggest that bimolecular or pseudo first order reactions limit the ECL generation immediately following the establishment of the applied potentials, while later ECL generation is governed by diffusion or mass transport displaying a Cottrell type decay over inverse square root time. Physical meanings of key parameters as defined in classic theorem are
discussed in representative experimental systems for appropriate quantitation and evaluation of ECLs properties from different materials systems.

2.1 Introduction

Recent interests in the near infrared (near IR) electrochemiluminescence (ECL) from metal nanoclusters (NCs) align with the longstanding research efforts to develop better ECL luminophores and detection systems.\(^{73, 74, 90-94}\) The primary focus has been on the overall emission intensity or steady-state ECL behaviors, optimization in materials characteristics and syntheses, and sensing applications, whereas fundamental mechanism and kinetics studies remain rather limited.

ECL is a subcategory of chemiluminescence also referred as electrogenerated chemiluminescence. The excited state species are generated through annihilation reactions of oxidative and reductive radical species produced after electrode reactions, instead of a light source in photoluminescence (PL).\(^{68, 93, 95}\) Elimination of the interferences from excitation hardware/photons offers significant benefits for both fundamental studies and applications. Evaluation of the ECL efficiency, counterpart of the quantum yield in fluorescence, however, is complicated by the multi-step (side) reactions and their relative kinetics at the electrode-solution interfacial zone.\(^{96, 97}\) While comparisons to the ECL signal from standard materials system are common practice due to the challenges to determine absolute ECL efficiency (# photons per transferred electrons/charges), meaningful outcome requires adequate controls and understanding of the underlining assumptions and approximations.\(^{98}\) Further, the much less explored ECL kinetics could provide mechanistic insights for fundamental understanding and for better ECL based applications.
ECL can be generated through annihilation or coreactant pathways. In annihilation ECL, both oxidized and reduced radicals are generated by electrode reactions, often from the same original reactant species. This can be done either by controlling the potential on the same (working) electrode to drive the oxidation and reduction reactions sequentially/alternatively, or by driving opposite reactions on two separate but closely positioned electrodes. A coreactant species can be added to be oxidized or reduced together with the ECL luminophore in one electrode process. The oxidized (or reduced) coreactant species subsequently undergo a chemical reaction becoming a reductant (or oxidant), which in turn reacts with the electrode-activated ECL luminophore to generate the excited state species, which undergo radiative decay to generate coreactant ECL.68, 99

Basic ECL reaction mechanism and kinetics have been studied since the 1960s, and analytical and numeric simulations effectively describe the experimental results.97, 100-102 Most ECL studies, however, remain limited to steady-state behaviors or the apparent overall emission intensity. The approach is warranted for reasons96, 103, 104 such as 1. the rate limiting step/reaction and the intermediate radical species often vary in different experimental systems, which are nontrivial to characterize; 2. the kinetics descriptions in earlier work are often qualitative or semi-quantitative, and key parameters are not immediately available or applicable to different experiments. The advances in data collection and processing with modern instrumentation and computer software make digitized experimental ECL kinetics data readily accessible for more quantitative interpretations. In this communication, classic kinetics theory is invoked to describe several representative experimental ECL responses from metal NCs. The mechanism and kinetics interpretations are supported by high quality fittings which justify the applicability to other ECL luminophore system.
2.2 Theory

2.2.1 ECL reaction pathways and kinetics:

The essential reactions for the ECL generation from metal NCs can be expressed in general as 1-4 in annihilation pathways (oxidation as first step, oxidative-reduction). Notable competing reactions 5 - 7 are nonradiative decays of the excited state species, possible quenching, and the reversal electrode reduction of the oxidized NCs (or the oxidation of the reduced NCs if step 1 & 2 switches order) respectively.\(^{99}\) The reversal processes can be negligible compared to the forward ones under given experimental conditions and omitted for simplicity, though some do require considerations discussed accordingly.

\[ NC \xrightarrow{k_{ET-NC\,(Ox)}} NC^+ + e^- \quad (1) \]
\[ NC + e^- \xrightarrow{k_{ET-NC\,(Red)}} NC^- \quad (2) \]
\[ NC^+ + NC^- \xrightarrow{k_A} NC + NC^* \quad (3) \]
\[ NC^* \xrightarrow{k_r} NC + \text{hv} \quad (4) \]
\[ NC^* \xrightarrow{k_{nr}} NC + \text{heat} \quad (5) \]
\[ NC^* + Q \xrightarrow{k_Q} NC + Q + \text{heat} \quad (6) \]
\[ NC^{+\pi} \pm 1(\text{or } 2)e^- \xrightarrow{\text{Red}} NC \,(\text{or } NC^{++}) \quad (7) \]

where \(k_{ET}\) is the heterogeneous electron transfer (ET) constant, dependent of self-exchange rate constant \(k^0\) and the overpotentials (\(\eta = E_{\text{applied}} - E^{0'}\)) correlated in Butler-Volmer equation; \(k_A, k_r, k_{nr}\) and \(k_Q\) are bimolecular rate constant of the annihilation reaction, radiative and nonradical decay rate constants, and quenching by species Q (can be another NC,
impurity, or electrode surface etc.) respectively. Note if the same excited state species are involved in photoluminescence (PL) and ECL, 4-6 give the quantum yield expression of \( \phi = k_r/(k_r+k_{nr}) \) by ignoring quenching. The 2e process in reaction 7 is listed considering the electrochemical reversibility of the QDL charging behaviors in metal NCs.

When a coreactant (CoX) is oxidized in step 1 together with the ECL luminophores (NCs), step 2 & 3 will no longer be needed and be replaced by reactions 1’, 8 and 9 commonly expressed in ECL literature. An example is the oxidation of tripropylamine as CoX followed by fast deprotonation to produce a highly reductive radical (arguably large \( k_s \) can make this process invisible), which then reduces the oxidized NCs at the electrode-solution interfacial zone and becomes side products CoP. Reductive oxidation coreactant systems will occur together with 2 as 2’ and the reaction 1 & 3 replaced by 10 and 11 accordingly.

\[
\begin{align*}
\text{CoX} & \quad \text{ET-} \quad \text{CoX(Ox)} \quad \rightarrow \quad \text{CoX}^+ + e^- \quad (1') \\
\text{CoX} + e^- & \quad \text{ET-} \quad \text{CoX(}\text{Red}) \quad \rightarrow \quad \text{CoX}^{-} \quad (2') \\
\text{CoX}^+ & \quad \rightarrow \quad \text{CoX}^{-}H + \text{H}^+ \quad (8) \\
\text{CoX}^{-}H + \text{NC}^+ & \quad \rightarrow \quad \text{NC}^+ + \text{CoP} \quad (9) \\
\text{CoX}^- & \quad \rightarrow \quad \text{CoX}^{-}Y + \text{Y}^- \quad (10) \\
\text{CoX}^- + \text{NC}^- & \quad \rightarrow \quad \text{NC}^+ + \text{CoP} \quad (11)
\end{align*}
\]

However, the heterogeneous ET reaction can be sluggish depending on the coreactant species as well as electrode materials. The excited state species or reaction intermediates in metal NCs ECL studies, abbreviated as complex concentration \( C_{Comp} \) in equations, have not been directly characterized to the best of our knowledge. Based on various reported catalytic behaviors of NCs and earlier ECL studies where pre-assembly of NC-CoX is inferred,
alternate processes are proposed as steps 12-13 for systems composed of NCs assembled on planar electrodes as films with coreactants in solution (Figure 2.1).

\[
\text{CoX} + \text{NC}^+ \xrightarrow{k_{\text{CoX}}} \left[ \text{CoX} - \text{NC}^+ \right] \quad (12)
\]

\[
[\text{CoX} - \text{NC}^+] \xrightarrow{k_{\text{ET-CoX}, -H^+}} [\text{CoX}_{-H} - \text{NC}^+]^+ + e^- \quad (13)
\]

**Figure 2.1** ECL reaction processes with NCs assembled on electrode surface. Coreactant molecules (CoX) react with the NCs on the top layer of the film, supported by electron hopping/diffusion in the NCs film. Defects (pin holes) and CoX permeation into the film are omitted, while counterion transport in the film assumed sufficiently fast for the overall ET reactions. Key resistance and capacitance components at the bulk solution, film, and interfaces (electrical double layer, EDL) affecting potential distribution and non-faradic charging are listed on the right.

The theoretical analysis results are then demonstrated applicable to other experimental systems (with NCs free diffusing in solution under pseudo first order conditions) under similar straightforward approximations.
2.2.2 Kinetics in current and ECL

By assuming the nano-to-micro-seconds lifetime from PL studies, based on the similarities in the PL and ECL steady-state spectra, the reaction rate for excited state formation is approximated to limit the emission rate in common ECL experimental conditions. This is also consistent with the facts that to generate stronger ECL signals, higher rates of electron transfer (ET) reactions are generally preferred, by applying higher overpotential. The faster ET rate makes the current signal limited by mass transport. For macroscopic planar electrodes that are most frequently used, semi-infinite linear diffusion (to/away from electrode) dominates mass transport, given sufficient supporting electrolytes to suppress migration effect and ignoring convection/flow. Fick’s law of diffusion combined with the corresponding reaction kinetics at the electrode surface \(x = 0\) are:

\[
\frac{\partial(c_{CoX})}{\partial t} = D \frac{\partial^2(c_{CoX})}{\partial x^2} - k_{CoX}(A\Gamma_{NC}\alpha)c_{CoX} \quad \text{Eq. (1)}
\]

\[
\frac{\partial(c_{comp})}{\partial t} = D \frac{\partial^2(c_{comp})}{\partial x^2} + k_{CoX}(A\Gamma_{NC}\alpha)c_{CoX} = D \frac{\partial^2(c_{comp})}{\partial x^2} - k(c^*_{CoX} - c_{comp}) \quad \text{Eq. (2)}
\]

Where \(A, \Gamma_{NC}, \alpha\) are electrode surface area, NC surface concentration (mol/cm\(^2\)) and fraction of the activated (oxidized or reduced) NCs respectively. When on surface and under experimental conditions, those are combined into a general expression of pseudo first order rate constant \(k\) because coreactant is in large excess in most cases.

The boundary conditions and approximations based on experimental systems include:

1. all species in solution have equal diffusion coefficient
2. \(t = 0, x \geq 0 \& t \geq 0, x \to \infty:\) \(c_{CoX} = c^*_{CoX}, c_{comp} = C_{NC} = 0\)
3. \(t > 0, x = 0:\) \(c_{CoX} = C_{comp} = 0\)
4. \(t = 0, x = 0:\) \(c_{CoX} = C^*_{CoX}, C_{comp} = 0\)

This can be viewed as classic ECE reaction pathways, with the oxidation of NCs as the first E reaction instead.\(^{109,110}\) With coreactants in large excess, the current contributions from
NCs are ignored. The reaction rate (mol/s) is expressed in Eq. (3) by current at electrode surface solved by Laplace transform. More rigorous solution has been provided by considering the reaction (10) as reversible, which is helpful in understanding the first term in Eq. (2) by considering facile complex dissociation.\textsuperscript{109} The small differences (in final math outcome) and the complexity (eq. 11 in ref\textsuperscript{109}) makes Eq. (3) more favorable for analysis applications.

Correspondingly the ECL time course is written in Eq. (4):

\[\frac{i(t)}{F} = n_{CoX} AD \left( \frac{\partial C_{\text{comp}}}{\partial x} \right)_{x=0} = \frac{n_{CoX} A \sqrt{D} C_{CoX}}{\sqrt{\pi t}} (1 - e^{-kt}) \quad \text{Eq. (3)}\]

\[I_{ECL} = f \phi \ast i_d(t) (1 - e^{-kt}) \quad \text{Eq. (4)}\]

in which \(t\) is time, \(F\) is Faraday constant, \(n_{CoX}\) is the number of electron transferred for \(CoX\) (often =1), \(A\) is electrode area, \(D\) is diffusion coefficient, \(C^*\) stands for bulk concentration, \(f\) is the detector calibration factor, \(\phi\) is the quantum yield of the excited species, and the limiting current \(i_d(t)\) is described by Cottrell equation in a reversible (fast ET rate) redox system:

\[i_d(t) = \frac{n_{CoX} F A \sqrt{D} C_{CoX}}{\sqrt{\pi t}} \quad \text{Eq. (5)}\]

ECL is generated solely after the reactions between NCs and \(CoX\), and thus should be described by Eq. (4). The current, however, can be observed upon direct electrode reaction, regardless of the interaction with or catalytic effects of NCs. Therefore, Cottrell function in Eq. (5) (or the general form if lower overpotential is used in a reversible ET system) is more universally applicable and used. Further, the measured current will include both faradic current from all active redox species, plus non-faradic charging current (Eq. (6)). As illustrated in Figure 2.1, the solution resistance and electrical double layer capacitance defines the time constants (RC) for the electrical double layer (EDL) charging of any electrode-electrolyte interface. In the presence of NCs deposited on the electrode surface, at least one additional RC time constant corresponding to the film needs to be accounted for. The resistance term could include the
electron hopping rate as well as counterion permeation, while the specific capacitance depends on the film thickness and the relative permittivity (ligand molecular structures and ion/solvent permeation). The two RC terms can be models as in series, both following single exponential decay function over time under the applied potential $V$.

$$i_{\text{total}} = \sum_n (i_F)_n + i_{\text{non-}F} = \sum_n (i_F)_n + i_{R_{\text{EDL}}} + i_{R_{\text{Film}}}c_{\text{Film}} = \sum_n (i_F)_n + \sum_m (V/R_m e^{-t/\theta_m c_m})_m$$  \hspace{1cm} \text{Eq. (6)}$$

The ECL mechanism and kinetics equations are firstly evaluated under limiting conditions qualitatively. Prior to electrode reactions, none of the radicals exist and no ECL is produced. Initial generation of the radicals and intermediate complexes will be at high rate due to the high concentration gradient characterized by Cottrell current, causing a sharp increase in ECL intensity. The exponential function in Eqs. (3) and (4) describes the earlier kinetics features limited by the pseudo first order reaction. The exponential term becomes negligible quickly overtime, and subsequently the $t^{-\frac{1}{2}}$ decay governed by electrode reactions become prominent. If diffusion or mass transport is sufficiently fast (for example high excess $CoX$), steady-states ECL (constant intensity over time) will be observed limited by activated NCs only, analogous to enzymes or catalysts (NCs) saturated by substrates. It might worth emphasizing that the rate limiting step/s could vary in different time domains or experimental conditions, but generally only one or two of those processes dominate the reaction kinetics within a zone.

Next, the simplified equation is challenged with several drastically different experimental ECL systems. As those experiments have their respective goals rather than ECL kinetics analysis, the data quality and conditions vary and are not sufficiently systematic for the full evaluation of the fitting parameters which require future work. Regardless, the combined exponential and $t^{-\frac{1}{2}}$ components successfully describe the ECL kinetics features that cover the mostly used experimental conditions and should be readily applicable to other materials and
measurement systems. Equation (4) was modified into Eq. (7) to accommodate measurement uncertainties during fitting.

\[ I_{ECL} = A \left( 1 - e^{-k(t-\tau)} \right) (t - \tau)^{-\frac{1}{2}} + B \]  

Eq. (7)

\( \tau \) is a correction factor for the RC time constant from non-faradic charging current, non-ideal experimental conditions such as delays in synchronization (potentiostat and camera), limit in camera response time etc. B corrects stray light, dark current, or baseline drift during the data recording.

2.3 Validation with experimental results:

![Figure 2.2](image)

**Figure 2.2** Current (A) and ECL (B) time traces of Au-LA NCs filmed on ITO electrode with 0.5 mM cetirizine as coreactant in solution. Data recorded in 0.1 M PBS at pH 7.4 without degassing. The NCs were mixed with polyamines and spin coated on a ~ 8 mm by 15 mm ITO piece via precipitation. The ITO as working electrode, an Ag/AgCl wire quasi-reference and a Pt foil as counter electrode were positioned in a quartz cuvette, with the ITO surface facing the window of a CCD camera. The electrode potential was stepped from 0.00 V at 1.0 s to + 0.80 V and held for 1.5 s. Baseline current without Faradic processes (<1 s & >2.5 s) was not plotted due to the log scale used. ECL baseline was adjusted with respect to data prior to or long after the potential was applied. Redlines are the fittings of the experimental curves (in blue). The fitting of ECL data starts from 1.1 s to 2.5 s, while the current was fitted separately at the 0.1 s after the potential was stepped, with two exponential decay functions before and with \( t^{-\frac{1}{2}} \) function after. The data sampling rate was 1 ms for current and 15 ms for ECL.
The experimental results where lipoic acid stabilized Au$_{22}$ NCs are deposited on electrode surface are first evaluated. With the same Au$_{22}$LA NCs, solution ECL behaviors, metal ion sensing and intro-NC charge transfer mechanism for enhance ECL have been reported previously.\textsuperscript{32, 75,107} The analyses of both current and ECL are summarized in Figure 2.2 and 2.3.

\textbf{Figure 2.3} Residual plots of the current-time traces corresponding to Figure 2.1A. (A) double exponential fit of earlier current \( t < 0.1 \text{s} \) \((t_1 \sim 5 \text{ ms}; t_2 \sim 20 \text{ ms})\); (B) \( t^{-\frac{1}{2}} \) fitting of later current \( t > 0.1 \text{s} \); (C) fitting and (D) residual plot using combined single exponential (time constant \( \sim 6 \text{ ms} \)) and \( t^{-\frac{1}{2}} \) function.
The faradic current fits the \( t^{-\frac{1}{2}} \) function exceptionally well by excluding the earlier period (< 0.1s) when non-F charging is dominant. The high-fidelity fitting is confirmed by the highly randomized residuals plotted in Figure 2.3.B, with magnitudes much less than (< 0.1%) the original current and \( R^2 > 0.99 \). The cutoff of 0.1 s is determined from the fitting of the whole current curve with a simple single exponential decay combined with the \( t^{-\frac{1}{2}} \) function shown in Figure 2.3 C & D. Although \( R^2 > 0.99 \) is also achieved, the residual plot clearly shows appreciable deviations from theory predictions. Two exponential decay functions improve the earlier current kinetics fitting by an order of magnitude in residual current (2.3 A vs. D), corresponding to two charging processes with the RC time constant \( \tau_{RC} \) typically at about 5 and 20 milliseconds, assigned to EDL and NCs films respectively. The non-random patterns in the residual plot, albeit small in amplitude, are likely due to the omitted Faradic processes (for both NCs and coreactants) during this early period.

Eq. (7) accurately describes the ECL kinetics features: an initial sharp increase followed by a gradual decay as shown in Figure 2.2.B. Earlier data points (< 0.1 s) deviate from this trend because the applied potential has not been established due to the non-F charging process. A criteria of \( 3 \tau_{RC} \) will be adequate for most analysis to select the data range, which is conveniently determined from the current analysis. For example, with a \( \tau_{RC} \) at 20 ms, data after 60 (or 100 ms if \( 5\tau_{RC} \) criteria is used) will have e-3 (or e-5), about 5% (or 0.7%) distortion due to non-F charging effect (delays to establish the applied potential at the electrode-electrolyte interface).
Figure 2.4 Residual plots of the ECL traces by adjusting the time correction factor $\tau$ by +/- 15 ms (left) and +/- 30 ms (right). Data corresponding to Figure 2.2.

Considering the ECL data sampling rate at 15 ms, the residual plots with $\tau$ (time delay correction factor) adjusted by $\pm$ 15 ms and $\pm$ 30 ms are compared in Figure 2.4. The differences are only noticeable at the few earlier points. The fitting quality $R^2$ decreases slightly for the -15/-30 ms traces, but drastically for +15/+30 ms traces. In other words, the accuracy or time resolution of this kinetics analysis is limited by the camera response time. Analysis of faster kinetics will require data from detectors (sensitive to near-IR photons) with faster response time and experimental systems with faster time constants (such as microelectrodes) beyond the scope of this report.
**Figure 2.5** Current (A) and ECL (B) profiles of dissolved 50 μM Au-LA NCs with 25 mM HEPES at pH 7.4 as coreactant. A printed carbon strip (4 mm diameter, DRP-110 from DropSes) working electrode, an Ag/AgCl quarsi-reference and a Pt foil counter are used with 0.2 M NaClO4 as supporting electrolyte without degassing. The electrode potential was held at 0.0 V for 2 s, then stepped to 1.0 V and held for 6 s. No potential was applied after 8 s. Redlines are the fittings of the experimental curves (in blue). ECL curve fitting starts from 2.21 s to 8 s. The data sampling rate is 10 ms for current and 15 ms for ECL.

Next, the experiments where both NCs and coreactants are free diffusing in solution are analyzed in Figure 2.5. Because the coreactants are in large excess, as in most applications, pseudo-first order approximation can still be valid. The efficacy is attested by the high-quality residual plots compared in Figure 2.6 (A & B) for current and Figure 2.7 for ECL. A single $\tau_{RC}$ exponential decay combined with $t^{-\frac{1}{2}}$ functions is used to fit the whole current time trace in Figure 2.6 C & D.
Figure 2.6 Residual plots of the current-time traces corresponding to Figure 2.5.A. (A) single exponential fit of earlier current before 2.21 s; (B) $t^{-\frac{1}{2}}$ fitting of later current after 2.21 s; and the cutoff of 2.21 s is determined from the (C) fitting and (D) residual plot using combined single exponential and $t^{-\frac{1}{2}}$ function.
Figure 2.7 ECL data fittings with single $t^{-1/2}$ function (A & C) and their residual plots (B & D). The data range starts at 2.80 s (A), 2.21 s (C) and their corresponding residual plot (B and D). Data corresponding to Figure 2.5.B.

From the current residual plot, data points prior to the 0.21 s, albeit less than 5% of the corresponding current, display clear deviations from the simple model, suggesting dual exponential decays might still be necessary. Unfortunately, the data sampling rate was inadequate to accurately resolve any fast RC charging processes. A rather slow RC time constant at about 57 ms is revealed from the exponential fitting. Since there is no preassembled film on electrode surface, a possible origin is the adsorption on the rough surface of the carbon strips that requires further exploration. The better fitting quality by separating earlier and later kinetics is likely due to the baseline current drift during the measurements.

Unlike the surface ECL kinetics in Figure 2.2, the initial rising portion corresponding to the first term (exponential function) in Eq. (7) is much faster and fewer data points could be
captured. Although the initial increase is not obvious visually, bimolecular reaction kinetics impacts on the ECL kinetics can be elucidated from the fitting using Eq. (7). This concept is further illustrated by the fitting with $t^{-\frac{1}{2}}$ only in Figure 2.7. Decent fitting ($R^2 \sim 0.98$) can only be obtained by excluding data up to 0.80 seconds after the potential step Figure 2.7 (A & B), whereas fitting of whole kinetics data after the potential is established in Figure 2.7 (C & D, > 0.21 s based on current analysis) clearly deviates from experiments. In other words, data points between 0.21 - 0.80 s should be sufficient to reveal the bimolecular reaction kinetics, which can be conveniently adjusted within the pseudo first order conditions in other experimental systems. While the $t^{-\frac{1}{2}}$ function is adequate to analyze later kinetics in all experimental systems in this report, similar to steady-state responses, the benefit of simplicity in data treatment is associated with the tradeoff of losing kinetics information for mechanistic understanding and potentials to discriminate against interference signals in ECL based analysis.
Figure 2.8 Current (A) and ECL (B) profiles of bimetallic Ag$_{13}$Au$_{12}$ NCs on ITO surface and the corresponding fitting parameters coefficient $A$ in (C) and rate constant $k$ in (D). The organic soluble Au$_{12}$Ag$_{13}$ NCs were assembled on ITO electrode as thin films via dip-pull method. Reductive-oxidation coreactant ECL were generated in 0.01 M PBS to buffer pH at 7.5, 0.1 M KCl as supporting electrolyte, and 1 mM $K_2S_2O_8$ as coreactant. The electrode potential was stepped cyclically between -1.2 and 0.0 V every 3 s (five cycles shown). No potential was applied in the first and final 3 s. Current fitting was separately at 0.3s after the potential was stepped, with two exponential decay functions before and with double-step $t^{-\frac{1}{2}}$ function after. Redlines are the fittings of the experimental data (in blue). Trendlines in (C) and (D) were added with the fitting of peaks 2 - 5 using the function of $y = a + \frac{b}{1+x}$. Current sampling rate is 10 ms. ECL sampling rate is 15 ms.

Unlike aqueous soluble Au$_{22}$LA NCs that require polycations as co-precipitants for surface assembly, organic soluble Ag$_{13}$Au$_{12}$ NCs are directly deposited on ITO surface for aqueous applications. Of note, the solution ECL of the Ag$_{13}$Au$_{12}$ NCs in self-annihilation and oxidative-reduction coreactant pathway has been shown to be the orders magnitude stronger than Ru(bpy)$_3^{2+}$ standard. The reductive oxidation ECL features with persulfate as coreactant are analyzed in Figure 2.8. The multiple cycles are exemplary for the frequently employed approaches to evaluate the signal robustness in applications using either double-step potential waveform or square wave voltammetry. Using the same criteria of the fitting residuals from simple exponential and $t^{-\frac{1}{2}}$ decay function, a cutoff of 0.3 s is applied based on the results in Figure 2.9.
Figure 2.9 Current data fittings (A & C) and their residual plots (B & D) using combined single exponential (time constant ~400 ms) and $t^{-\frac{1}{2}}$ function. Data corresponding to Figure 2.8.A.

Consistent fittings of an earlier (6 - 12 s) and a later cycle (27 - 33 s) are shown. The charging current (< 0.3 s) requires double exponential functions, for EDL and film respectively, to achieve reasonable quality confirmed by the residual plots in Figure 2.10 (A & C). With limited data points due to the low data sampling rate, the faster EDL charging process with sub-ten ms time constant could not be evaluated. The longer RC time constant is about 100-150 ms. Correspondingly, at 0.3 s or about 2-3$\tau_{RC}$, the applied potential is almost completely established for faradic current and ECL analysis.
Figure 2.10 Residual plots of current fitting corresponding to Figure 2.8.A. Single exponential fit of earlier current before 6.3 s (A), 9.3 s (C); The $t^{-\frac{1}{2}}$ fitting of later current after 6.3 s (B), 9.3 s (D); and the cutoff of 6.3 s and 9.3 s is determined from Figure 2.9. Because the concentration profiles in the reversal period (i.e. at 0.0 V) are modified by the forward step (i.e. at −1.2 V), the current profiles in different steps (excluding the initial RC charging portion) are fitted with the $t^{-\frac{1}{2}}$ formula for the forward and reversal current equations in double-step chronocoulometry respectively.

Coreactant ECL is observed under sufficient reductive potentials (−1.2 V herein). The potential of 0.0 V is inadequate to oxidize the NCs to generate ECL. The current at 0.0 V, however, follows $t^{-\frac{1}{2}}$ decay nicely by excluding the RC charging portion (> 0.3 s), attributed to the oxidation of the reduced NCs from previous step. Observation of this faradic process over seconds indicate sluggish kinetics likely limited by the slow counterion permeation in the film. Again, Eq. (7) fits ECL-time traces well as plotted in Figure 2.8.B and residual plots in Figure 2.11.
Figure 2.11 ECL residuals of the three fitted peaks. (A) First peak from 3.3 to 6 s. (B) Second peak from 9.3 to 12 s. (C) Third peak from 27.3 to 30 s. Data corresponding to Figure 2.8.

The five consecutive cycles allow us to evaluate the coefficient A and rate constant k shown in Figure 2.8.C and 2.8.D. As stated earlier, surface NCs can be viewed as catalyst or enzymes in the reaction with ECL coreactants. The available NCs as unoccupied sites follow Langmuir isotherm type responses, expressed in fraction term as \[ \frac{\Gamma_{\text{total}} - \Gamma_{\text{occu}}}{\Gamma_{\text{total}}} = 1 - \alpha = \frac{1}{1 + K_a a_{\text{Cox}}} \]. Either a decrease of total sites (NCs decomposition, surface contamination, etc.) on the left, or a change in binding/association (i.e., changes in the activity/concentration of the reactants/intermediates) on the right would cause the ECL intensity to change, and accordingly the two parameters, A and k. The results from the first peak are not included in the fitting because the initial interfacial conditions are not actively controlled: it is a common practice to discard the first segment in cyclic voltammetry for the same reason. Another technical clarification is that the time correction factor \( \tau \) precedes the potential stepping rather than a delay in this system. Our speculation is these NCs are at mixed valance states or contain redox active impurities based on the consistent faradic current at zero volts. In either case, the time of potential switches will no longer be the sole factor to initial the reaction (time zero in ECL.
kinetics would shift). The hypothesis is supported by the decrease in $\tau$ from about 0.5s for peak 1 to 0.1s for peak 5 but requires further investigations.

2.4 Experimental Section

2.4.1 Surface treatment of ITO electrode and preparation of Au-LA films on ITO electrode

Indium Tin oxides (ITO) surface was cleaned by ultrasonicating sequentially in toluene, acetone, ethanol, and nanopure water for 15 mins each and stored in nanopure water. To make the ITO surface more hydrophilic for better assembly of aqueous samples, the cleaned and dried ITO was placed in a vacuum chamber and treated with $O_2$ plasma for 3 mins prior to usage. Layer-by-layer assembly of aqueous soluble Au-LA NCs was controlled by spin coating with a spin coater (Chemat Technology series KW-4A) in 1.0 CG aluminum vacuum chuck at 1000 RPM each step. First, a 10 µL drop of about 10 mM PAA solution was drop casted on the ITO surface and let sit for about 2 mins for adsorption, and then spined 1 minute for further spread. Next, a 10 µL drop of 0.25 mM Au-LA NCs solution was drop casted and waited for 20 seconds, and then spined for 1 minute. Multiple layers of AuNCs film on ITO surface were fabricated by repeating the above steps.

2.4.2 Preparation of assembly of Au$_{12}$Ag$_{13}$ and ITO electrode:

The synthesis and characterization of Au$_{22}$LA$_{18}$ and [Ag$_x$Au$_{25-x}$PPh$_3$)$_{10}$SC$_2$H$_4$Ph)$_3$Cl]$_2^{2+}$ ($x \leq 13$, i.e. Au$_{12}$Ag$_{13}$) follow our previous report.$^{73, 74}$ Organic soluble Au$_{12}$Ag$_{13}$ NCs are coated on ITO electrode for aqueous tests with a dip coating method using a home-built experiment setup. Briefly, ITO working electrode was fixed with a clamp that can be dragged with a certain uniform linear motion. ITO was firstly soaked into Au$_{12}$Ag$_{13}$ NCs methylene chloride solution
(~50 µM) and then pulled out at a velocity of about 1.7 cm/min, which are further used for ECL studies.

2.5 Conclusion

To summarize, simplified equations and strategies for the analysis of ECL kinetics are developed. The approach is demonstrated effective in representative experimental systems with different NCs, both on surface and in solution. Current-time traces provide key insights, such as appropriate time domains and reactant/intermediate concentrations, for the evaluation of the ECL intensity and reaction rates. Future experiments with systematically varied conditions such as reactant concentrations and greater time resolution are needed for the validation of the key parameters, in particular the coefficient \( A \) and rate constant \( k \). In addition to the commonly known factors such as electrode dimension/geometry and materials, systematic variation in the film thickness and relative permittivity when the NCs are assembled on surface, and the concentrations and stoichiometry when coreactants are used, will immediately reveal the reaction kinetics and mechanism under the respective conditions.

Several approximations are made, where simplicity in the analytical expression is prioritized to reduce the number of variables for ease of use and to avoid overinterpretations in data fitting. The reactions are treated as diffusion limited, whereas heterogenous electron transfer kinetics in some experiments will be slow (quasi- or irreversible) and affect the measured kinetics profile. Side reactions that vary in different experimental systems are not considered, whose impact on the kinetics can be mitigated by appropriate choice of data range (prior to significant side reaction occurs). While numeric solutions can be obtained with more rigorous boundary conditions and reaction pathways, those cannot be readily adopted for the analysis of experiments.
3 KINETICS-BASED RATIOOMETRIC ELECTROCHEMILUMINESCENCE ANALYSIS FOR SIGNAL SPECIFICITY: CASE STUDIES OF PIPERAZINE DRUG DISCRIMINATION WITH AU NANOCLESERS

(This chapter has been published as “Ma, H.; Yi, M.; Messinger, M.; Wang, G., Kinetics-Based Ratiometric Electrochemiluminescence Analysis for Signal Specificity: Case Studies of Piperazine Drug Discrimination with Au Nanoclusters. Analytical Chemistry 2022, 111 (34), 11760-11766.” Reprinted with permission from Ref 111. Copyright 2022, American Chemical Society.)

A multi-parameter calibration and analysis strategy has been developed based on the kinetics of charge transfer reactions. Absolute and ratiometric electrochemiluminescence signals are elucidated from single measurements for the detection of hydroxyzine and cetirizine as prototype drugs which greatly enhance the near-infrared electrochemiluminescence from atomically precise Au$_{22}$ nanoclusters stabilized with lipoic acid ligands on ITO electrodes. The signal-on sensing mechanism eliminates the need for recognition elements and highly excess co-reactants in conventional electrochemiluminescence practice. The rates of sequential charge transfer reactions render specificity in electrochemiluminescence intensity and kinetics toward the target molecular/electronic structures and are conveniently controlled/optimized by operation parameters. Signal kinetic profiles, in stark contrast to steady-state or single point recordings, not only improve the signal/noise ratio but also offer greater resolving power to differentiate
analogue species and nonspecific interference. The fundamental kinetics-based ratiometric concept/strategy is not limited to a specific luminophore or a co-reactant and is thus generalizable. The case studies successfully detect and discriminate drug compounds at sub-nanomolar physiological ranges, with efficacy validated using synthetic urine toward point-of-care applications in the therapeutic/abuse drug monitoring.

3.1 Introduction

Electrochemiluminescence or electrogenerated chemiluminescence (ECL) is a light emission process where the excited state species are generated through sequential electron transfer (ET) reactions. Eliminating the need for light sources, which are required in fluorescence, drastically reduces the interference from excitation photons and simplifies the analysis, which allows for the pursuit for lower signals in more challenging applications such as trace analysis in complex matrices and point-of-care settings. In the widespread applications in immunoassays, bioimaging, and sensing, the usage of ECL has been limited to primarily a signal readout mechanism and almost exclusively with highly excess co-reactant species for signal enhancement. The requirement of recognition elements such as antibodies or nucleic acid sequence/s for signal specificity constitutes fundamental limitations, with labeling/derivation, multi-step sample treatments, and cost intime/resources of practical obstacles. To achieve a high signal-to-noise/interference ratio, a major recent research effort is to develop better luminophores and to take advantages such as lower background interference and deeper penetration depth in the near infrared for biological imaging, sensing, and analysis applications.

The ECL reaction mechanism and signal kinetics are quite complex due to the highly reactive radical intermediates and side reactions. Using the widely adopted ECL standard of Rubpy$_3^{2+}$: TPrA (tris(2,2'-bipyridine)ruthenium : tri-n-propylamine) system as an example,
the oxidative reduction ECL generation starts from the oxidation of both Ru(bpy)$_3^{2+}$ and co-reactant TPrA under appropriate electrode potentials. Deprotonation of the oxidized tertiary amine in TPrA produces highly reductive radicals which react with the Ru(bpy)$_3^{3+}$ to generate the excited state species, Ru(bpy)$_3^{2+*}$. This main route, however, is among a variety of scenarios dependent on specific experimental conditions (relative abundance of the reactants, radical intermediates, impurities or environmental factors, electrode potentials, etc.). Numeric simulations are routinely adopted in ECL kinetics/mechanistic studies. While detections of single entities/events have been successful, either with ensemble ECL intensity or by incorporating multiple ECL luminophores into single entities, and recently super-resolution ECL imaging has been achieved, analysis based on ECL kinetics has not been demonstrated to the best of our knowledge.

![Figure 3.1](image)

**Figure 3.1** Basic setup and principles of ratiometric ECL analysis to differentiate similar compounds. HDZ, CTZ and HEPES contain common piperazine structures in which the tertiary amines with neighboring C-H groups are effective coreactants in oxidative reduction ECL pathway. $V_1$ and $V_2$ represent different overpotentials to control electron transfer rates and ultimately the ECL intensity/kinetics.
A new strategy to render signal specificity is to take advantage of the dependence of ECL intensity, and more broadly kinetics, on the relative rates of the multi-step heterogeneous and homogeneous charge transfer reactions.\textsuperscript{89} As described in the classic ET kinetics theorem, the ET rate and thus ECL intensity would vary among different co-reactant species under the same conditions and for the same co-reactant under different (over)potentials, that is, the applied potential over the standard/formal potential. As illustrated in Figure 3.1, a higher overpotential $V_2$ (vs $V_1$) will drive a faster ET rate and a stronger ECL intensity before approaching mass transport limitations (the decrease at very high potential and longer time). The dependence of ECL on electrode potentials, either constant or sweeping, is often qualitatively reminiscent of that of Faradaic current in classic amperometry or voltammetry.\textsuperscript{125} A key difference is that the ECL arises from subsequent reaction/s of the intermediate radicals formed by electrode reactions. The extra reaction step/s make the ECL signals highly sensitive to the factors affecting the kinetic interactions involving the ECL luminophore and specific co-reactant species an analyte either directly or indirectly affecting the reaction kinetics will be detected. Furthermore, the ratio in ECL intensity at different overpotentials distinguishes co-reactant species with subtle differences in molecular structures because of the exponential dependence in signal intensity (ECL intensity/ kinetics) with activation energy barriers (overpotentials and electronic/molecular structures). This fundamental principle is anticipated to be generalizable to other ECL luminophore–coreactant systems for broader applications. Note that the kinetics-based ratiometric analysis differs fundamentally from classic ECL ratiometric methods where (1) either multiple ECL luminophores and/or energy transfer reagents or both cathodic and anodic reactions are necessary; and (2) the ECL signals are again almost exclusively under equilibrium or steady states, and additional recognition elements are required.\textsuperscript{126-129}
In this report, lipoic acid (LA)-stabilized gold nanoclusters (NCs), described by the precise composition of Au$_{22}$L$_{12}$ in which 22 and 12 are the numbers of core atoms and ligands, respectively, are used as near-IR luminophores. Hydroxyzine (HDZ) and its metabolite, cetirizine (CTZ), the first and second-generation antihistamines for anxiety, allergic disorders, and so forth, are chosen to serve dual functions as effective ECL co-reactants and prototype target drugs. The ECL reactions in general involve steps 1−5 as modified based on the common ECL co-reactant TPrA and pH buffer HEPES (N-(2-hydroxyethyl)piperazine-N-(2-ethanesulfonic acid). Similar functional molecular structures and/or properties make them highly challenging interferents for one another, which demonstrates the superb ability to discriminate the target analyte against interference from this kinetics-based multi-parameter calibration and analysis. The generalizability and the resolving power of the new methodology are supported by (1) the common tertiary amine structures, which are effective in the enhancement of ECL generation for point-of-care applications in other treatment and abuse drugs; and (2) other atomically precise nanoclusters where the charge transfer rates will depend on their respective electronic/molecular structures.

\[
\text{NC} - e \rightarrow \text{NC}^{+} + e^- \quad (1) \\
\text{CoX} - e \rightarrow \text{CoX}^{+} + e^- \quad (2) \\
\text{CoX}^{+} \rightarrow \text{CoX}_{-H}^{+} + H^+ \quad (3) \\
\text{CoX}_{-H}^{+} + \text{NC}^{+} \rightarrow \text{NC}^* + \text{CoP} \quad (4) \\
\text{NC}^* \rightarrow A + h\nu \quad (5)
\]

3.2 Results and Discussion

The ECL intensity of Au−LA NC films on ITO electrode peaks at around +0.95 V during cyclic voltammetry (CV) scans (Figure 3.2.A) without a co-reactant in solution.
Figure 3.2 AuNCs film characterization and parameter optimization. (A) ECL-potential curves without and with 1 mM coreactant compounds (HDZ/CTZ/HEPES). Inset is the enlarged view of the much weaker signals without coreactant and with HEPES. (B) CV-ECL with 1 mM HDZ using narrower potential window. Inset highlights the more consistent ECL intensity from five consecutive CV scans. Data in A & B were collected in 0.1 M pH 7.4 PBS buffer under ambient conditions without degassing at 0.1 V s⁻¹ scan rate. (C) Electrochemical impedance spectroscopy (EIS) spectra collected with 1 mM ferrocene as redox probe with 0.1M TBAP in acetonitrile. The 1X, 4X and 8X refer to the repeated cycles in the layer-by-layer surface assembly of AuNCs with polyallylamine. (D) photoluminescence imaging and analysis of Au-LA NCs film on ITO electrodes. The emission intensity is integrated along y-direction within each color frame and plotted along x-direction (distance zero is the left edge).

$I_{\text{ECL}}$ increases by a factor of 1.5, 18, and 89 times with 1 mM HEPES, CTZ, and HDZ as co-reactants, respectively, at the same oxidation potential of +0.95 V. The current in the corresponding CVs (Figure 3.3.A) is only slightly higher in the presence of the coreactant.
species, and a defined anodic peak is not observed. By using higher co-reactant concentrations (HEPES at 20 mM, Figure 3.3.B), a weak irreversible anodic peak at around +0.9 V is resolved.

![Cyclic voltammograms (CV) of Au-LA NCs films w/ and w/o coreactants](image)

**Figure 3.3** Cyclic voltammograms (CV) of Au-LA NCs films w/ and w/o coreactants (corresponding to ECL results in Fig. 4.2). Data collected in 0.1 M PBS pH 7.4 under ambient conditions without degassing. Potential scan rate was 0.1 V s⁻¹. (A) CV curves w/wo 1 mM coreactant compounds (HDZ/CTZ/HEPES). (B) CV curves w/wo 20 mM HEPES. 4X refers to the repeated cycles in the layer-by-layer surface assembly of AuNCs with polyallylamine.

Interestingly, the co-reactant anodic current is much higher in the presence of NC film versus that of bare ITO, clearly demonstrating the catalytic effects of NCs. These current features are consistent with the sluggish ET kinetics in the oxidation of tertiary amines that are known to depend on electrode materials.¹⁰⁶,¹³²
Figure 3.4 Three consecutive ECL-Potential (A) and CV (B) curves from a 4X Au-LA NCs film. (red) 1st cycle, (blue) 2nd cycle, and (yellow) 3rd cycle of Au-LA NCs film in 0.1 M PBS pH 7.4 w/ and w/o 1 mM coreactant compounds (HDZ/CTZ/HEPES). Data collected under ambient conditions without degassing. Potential scan rate was 0.1 V s⁻¹.

Both ECL and current are found to decrease significantly over multiple scans (Figure 3.4), attributed to irreversible reactions at higher oxidation potentials. By limiting the potential range (Figure 3.2.B), more stable ECL signals can be generated that are favorable (but not required) for the ratiometric sensing method being developed. The self-annihilation ECL of Au−LA NCs (green curve without co-reactant) on the surface under a scanning potential, albeit weak, is significant in revealing strong ECL generation in these systems, whereas either co-reactants or potential steps for fast generation of ox/red species are needed to generate detectable self-annihilation ECL previously.
Figure 3.5 Cyclic voltammogram (top, left axis) and ECL-potential (bottom, right axis) curve of (A) PAA film with 1 mM HDZ without AuNCs. (B) 50 mM AuNCs with 0.1 mM PAA without HDZ. Data were collected in 0.1 M pH 7.4 PBS buffer with under ambient conditions without degassing at 0.1 V s⁻¹ scan rate.

Since PAAs do not have detectable ECL signals under the measurement conditions, as shown in Figure 3.5, the partial oxidation of the sulfur atoms at the core ligand interface, previously characterized and correlated with the enhancement of photoluminescence quantum efficiency,¹⁴ is believed to be the origin of pre-existing mixed redox states that enable this self-annihilation ECL, although impurities such as NCs at different charge states are always a possibility.

The charge transfer resistance (RCT) of three exemplary AuNC films is characterized by EIS in Figure 3.2.C. Without NC films, different bare ITO pieces give a consistent R_CT at about 250 Ω (per ~1cm²). The R_CT values increase linearly with the number of layers (0.66/2.5/4.5 × 104 Ω, for the 1×/4×/8×) in approximation, demonstrating the consistency in the NC film assembly. The increase is anticipated given that both the LA alkane chain portion and the PAA backbones would decrease the surface conductivity or increase the tunneling barrier. While a large R_CT will obviously decrease the current which is required to activate the ECL, an intermediate R_CT is believed beneficial to stabilize the radical intermediates and suppress the
annihilation caused by potential reversals/steps, that is, the electrode reduction of the oxidized intermediate, which would decrease the ECL.

The strong near-IR photoluminescence of AuNCs and the optical transparency of ITO electrodes allow for the evaluation of the AuNC film under ECL-relevant conditions (Figure 3.2.D). With all parameters consistent, the intensity profiles are relatively uniform across the film, and films with fewer NCs (1×) are consistently weaker than those with more (4×). The stronger intensity in the region of about 0.4 - 1 mm is caused by the accumulated NC aggregates during the spin-evaporation process. This region and the edge of the bare ITO are retained purposely to provide contrast to demonstrate the uniformity of the film by controlling the spin speed during the layer-by-layer assembly process. The film stability or data consistency is confirmed conveniently from the qualitatively consistent features before and after the ECL measurements by noninvasive PL imaging and EIS analysis (Figure 3.6).

![Image](image)

**Figure 3.6** Photoluminescence intensity (A) and electrochemical impedance spectroscopy (EIS) spectra (B) from Au-LA NCs film collected before and after ECL tests. 1X, 4X refer to the repeated cycles in the layer-by-layer surface assembly of AuNCs with polyallylamine.

The similarities in ECL curves and CVs, regardless of the potential range adopted, clearly indicate that conventional electroanalytical sensors or ECL detection systems would fail to
differentiate those similar compounds if both the molecular identity and concentration are unknown. Differences in ET kinetics from those compounds can be inferred from the differences in the slopes of the current or ECL during the anodic scan (i.e., around +0.8 V in Figure 3.3.A & Figure 3.1), which enable the ratiometric analysis based on ET kinetics and eliminate the needs of defined and well-separated anodic peaks to differentiate similar compounds in conventional sensors.

**Figure 3.7** ECL-time profiles of Au-LA NCs films under different potentials in (A) hydroxyzine and (C) cetirizine at representative concentrations; and the analysis in (B) and (D) with systematically varied concentrations (in log scale over 6-order magnitudes). Data recorded in 0.1 M PBS at pH 7.4 without degassing. The electrode potential was stepped to 0.70 V, 0.75, and 0.80 V and held for 1.5 s each, with 0 V in between for 1.0 s. The NCs films were conditioned by stepping potential between 0-0.8 V multiple cycles to lower the self-annihilation ECL and stabilize the ECL signals (i.e. the gray curve at the bottom as the baseline) prior to titration. The integrated ECL intensity in B & D is the sum of the intensity during each 1.5 s period, i.e. each area. Repeats from three separate films are included to show consistency. Camera exposure time is 15 ms.
The ECL-time traces in Figure 3.7.A and C are under three constant potentials from the titration of HDZ and CTZ (one as a prototype analyte and another as an analogue interferant). The total intensities are plotted as conventional calibration curves from the nanomolar to sub-millimolar range in panels B and D (+ 0.75 V data similar, not included for clarity). The non-parallel nature of the + 0.7 and + 0.8 V curves, which is the foundation for ratiometric analysis and multi-parameter calibration, arises from the differences in the signal generation kinetics. As explained in our recent ECL kinetics report, this can be understood by (1) faster ET rate at higher overpotentials; and the logarithm concentration dependence. (2) The time-dependent features within individual potential steps reveal the relative kinetics of consecutive electrode reactions and chemical reactions: the ECL intensity is stronger and increases sharply upon the application of higher oxidation under + 0.8 V, followed by a gradual decay overtime (t\(^{-1/2}\), as described by Cottrell’s equation in amperometry) versus a gradual increase toward a plateau under + 0.7 V. In other words, at lower overpotentials and/or at lower co-reactant concentrations, it takes a longer time to generate radical intermediates from NCs and co-reactants, which are needed in the chemical reactions to produce the excited state species for ECL. The intermediate potentials (between an onset potential at about + 0.5 V and a peak at + 0.95 V, Figure 3.2.A) are chosen to achieve (1) lower background signals, that is, a trace without co-reactants, generally at lower overpotentials; and (2) higher signals for higher sensitivity or lower limit of detection (LOD), normally requiring higher overpotentials without causing significant side reactions during the titration.

The total intensity (over 1.5 s herein) obviously offers the benefit of reducing random errors over the intensity at a single time point, which can arise from environmental/electronic noise and so forth. Given the noise level of the ECL-time traces (standard deviation \(\sigma\), about 70
a.u. at a 15 ms data sampling rate) using the threshold of 3 \( \sigma \) for qualitative and 10 \( \sigma \) for quantitative analysis, the detection limit at a nanomolar concentration range or lower for HDZ and CTZ is established. These classic calibration profiles, in particular the lower LOD, while adequate for the clinical therapeutic range for most drugs in this category, would not be able to differentiate HDZ and CTZ because of their similar responses/properties. It is worth mentioning that the differences among repeats (Figure 3.7.B and D) reflect the heterogeneity of the surface film/devices, which is a separate issue and does not affect the main goal of this report in the direct differentiation of similar compounds. A direct normalization would require an underlying assumption of linear dependence of related parameters against the tested variables, which is not always fulfilled, especially under the wide concentration range adopted and the complex reaction mechanism (rate-limiting step/s).\(^{89}\)
Figure 3.8 ECL-time profiles of Au-LA NCs films under different potentials in (A) TPrA and (C) HEPES at representative concentrations; and the analysis in (B) and (D) with systematically varied concentrations (in log scale over 6-order magnitudes). Data recorded in 0.1 M PBS at pH 7.4 without degassing. The electrode potential was stepped to 0.70 V, 0.75, and 0.80 V and held for 1.5 s each, with 0 V in between for 1.0 s. The NCs films were conditioned by stepping potential between 0-0.8 V multiple cycles to lower the self-annihilation ECL and stabilize the ECL signals prior to titration. The integrated ECL intensity in B & D is the sum of the intensity during each 1.5 s period, i.e. each area. Repeats from three separate films are included to show consistency. Camera exposure time is 15 ms.

For comparison and better association with the ECL literature, the ECL-time profiles and the corresponding analyses of a commonly used ECL co-reactant, TPrA, are included in Figure 3.8.A and B. The features are qualitatively similar to those of HDZ and CTZ. However, the ECL enhancement and concentration dependence are marginal if any when HEPES, another piperazine compound commonly used as pH buffer, is used as a co-reactant (Figure 3.8.C and D). This is somewhat surprising because HEPES has been shown to be effective for enhancing the ECL of solubilized NCs. The differences in the molecular structures, without the large aromatic portion in HDZ/CTZ, render less steric hindrance and hydrophobicity for the HEPES to intercalate into the NC film.

Figure 3.9 Differences in EIS spectrum of a 4X Au-LA NCs film induced by cetirizine and HEPES over incubation time. Electrochemical impedance spectroscopy (EIS) spectra collected with a 4X Au-LA NCs film dipped in (A) 1 mM HEPES solution and (B) 1 mM cetirizine solution.
at the listed incubation time, with 1 mM ferrocene as redox probe with 0.1M TBAP in acetonitrile.

Characterized by EIS spectra (Figure 3.9), the $R_{CT}$ increases significantly and continuously, indicating the adsorption of HEPES into NC films over time. In contrast, much less change is induced by CTZ during the same period at the same concentration. The much higher $R_{CT}$ leads to lower current or a less-activated radical intermediate, which explains the lower ECL enhancement. Note that the ECL intensity would not be sustained over time by those pre-adsorbed HEPES in the film, which might correspond to the minor sharper spike immediately after the application of the oxidation potential (at around 1 s in Figure 3.8.C) versus the parabolic curve shapes in other cases explained by reaction kinetics.89

Figure 3.10 Ratiometric and dual-parameter ECL calibration profiles for HDZ (A), CTZ (B), HEPES (C) and TPrA (D). Normalized ECL intensity (blue, right y-axis) is after the subtraction of the one without coreactant (bottom gray curve in Fig. 4.7 & 4.8). The ratios of the normalized ECL intensity (red) are calculated with the data at 0.7 V and 0.8 V in Fig. 4.7 & 4.8. Three sets of data of HDZ, CTZ, and TPrA are further compared in the heat map (E), and urine
effects on HDZ are illustrated in F. A pair of data (dumbbell shape) illustrates the analysis of an unknown.

The principles and approaches of ratiometric and dual parameter ECL analysis are demonstrated in Figure 3.10. For each compound, two parameters are elucidated from a single measurement (data shown in Figures 3.7 and 3.8): (1) the normalized total ECL intensity over time under a constant potential, $I_{+0.8\text{V}}$; and (2) the norm. intensity ratio at two different overpotentials, $I_{+0.8\text{V}}/I_{+0.7\text{V}}$. Unlike the norm intensity that depends (not necessarily linear) directly on the electrode size, reagent concentration and can be affected by nonspecific interference (such as surface adsorption) that is detrimental for most sensors and so forth, the intensity ratio is dimensionless and mainly governed by the relative kinetics. Two calibration curves are established for each compound in Figure 3.10. The two parameters of a hypothetical unknown (molecular identity and concentration) need to match both calibration curves simultaneously and thus greatly improve the analysis specificity. For example, the blue circle of the $I_{+0.8\text{V}}$ value can arise from about 10 nM of HDZ, 9 μM of CTZ, and 10 nM of TPrA or HEPES at a broad concentration range. By further considering the parameter of $I_{+0.8\text{V}}/I_{+0.7\text{V}}$ (red circles), the molecular identity of HDZ is immediately obvious. For another instance, because the $I_{+0.8\text{V}}/I_{+0.7\text{V}}$ in HDZ calibration is much higher, any value more than 3-3.5 immediately rejects the possibility of TPrA, HEPES, or CTZ over a wide concentration range. In the cases of ambiguity (blue/red/orange points overlapping or closely positioned in Figure 3.10.E), simple dilutions of the unknown will shift the ECL responses into clearly separated zones. The optimizations of surface film preparations and measurement parameters will further improve the resolution if needed.
Figure 3.11 Ratiometric analysis of hydroxyzine (A), cetirizine (B), HEPES (C), and TPrA (D) using three 4X Au-La NCs films for each analyte. The concentration dependence of integrated ECL signal ratio at +0.8 V versus +0.7 V is in red on the left y-axis, and the normalized ECL intensity at +0.8 V in blue on the right y-axis. Different symbols are results from different sensors.

It is worth mentioning that the trends and separation of different calibration curves are quite consistent and well defined, supported by three additional data sets for each molecule in Figure 3.11. The effective discrimination of these similar compounds/responses is further demonstrated in the heatmap by plotting the two y-axis data (left against the right). Over 6-7 orders of magnitude in analyte concentration, a minimum overlap is found until toward a very high concentration range in the repeated titrations. HEPES can be easily discriminated against and thus not compared in the heat map because the ECL intensity is low and independent of concentration. Toward the lower LOD, the same random noise would “amplify” the scattering of
repeated results when plotting the intensity ratio, which can be mitigated by taking the ratio of lower to higher intensity instead (reciprocal of the results in this report) if preferred.

\[ I_{ECL} \times (10^3 \text{ a.u.}) \]

\[ \text{Time (s)} \]

**Figure 3.12** Ratiometric ECL analysis of hydroxyzine in the presence of synthetic urine using a 4X Au-LA NCs film. (A) ECL-time profiles without and with hydroxyzine at different amount of urine under different potentials. (B) ECL-time profiles under different potentials in hydroxyzine at representative concentrations in the presence of urine.

In addition to the highly challenging task of discriminating against compounds with similar structures/properties, synthetic urine is evaluated for general nonspecific interference toward applications (Figures 3.10.F & Figure 3.12). Because this ECL signal is only enhanced by specific tertiary amine molecular structures, no false positive signal is observed as expected. The absolute ECL intensity is lower in the presence of 20% urine, presumably due to the presence of multivalent ions or other species that alter the interactions and reaction kinetics of radical intermediates that require further analysis. Importantly, the intensity ratio $I_{+0.8V}/I_{+0.7V}$ is less affected and even stronger, which again strongly attests to the benefit of the ratiometric and multi-parameter analysis. The lower LOD is still in the 5-60 nM range that obviously can be
further optimized by systematic variations of the experimental parameters toward future applications.

3.3 Experimental Section

3.3.1 Chemicals

Tetrachloroauric acid trihydrate (HAuCl$_4$·3H$_2$O, > 99.99%), lipoic acid (LA, ≥ 99%), sodium borohydride (NaBH$_4$, ≥ 99%), sodium hydroxide (NaOH, ≥ 97%), cetirizine hydrochloride (CTZ, 2-[4-[4-chlorophenyl]-phenylmethyl]piperazin-1-yl]ethoxy]acetic acid), Poly(allylamine) solution (PAA, Mw:~17000 Da, 20 wt.% in H$_2$O), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid or N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES, ≥ 99.5%), hydroxyzine (HDZ, 2-[2-[4-[4(chlorophenyl)-phenylmethyl]piperazin-1yl]ethoxy] ethanol), sodium phosphate monobasic (NaH$_2$PO$_4$, ≥ 99.5%) and sodium phosphate dibasic (Na$_2$HPO$_4$, ≥ 99%) were purchased from Sigma-Aldrich and used as received. Synthetic urine solution (ACS certified) is purchased from Grainger. ITO glass (25 mm × 8 mm × 1 mm, Rs = 4 – 10 Ω/cm$^2$) was purchased from Delta technologies Co., Ltd..

3.3.2 Synthesis of lipoic acid (LA) stabilized Au$_{22}$LA$_{12}$ nanoclusters

In a typical reaction, HAuCl$_4$·3H$_2$O (0.006 M) and lipoic acid (0.035M) were separately dissolved in nanopour water. The pH of the lipoic acid solution was adjusted with NaOH to basic to ensure solubility prior to mixing. Under vigorous stirring, the HAuCl$_4$ and lipoic acid solutions were mixed to reach a final Au:LA mole ratio of 1:3. After 4 h reaction when the color of the mixture solution changed from light yellow to colorless, NaBH$_4$ (0.7 equiv.) was added at room temperature. The reaction proceeded for about 17 h and checked periodically until the near IR photoluminescence emission reached maximum (the emission would decrease afterwards).
The solvent was then removed by rotovap. The final Au-LA NCs was dialyzed in cellulose dialysis tube (M.W.C.O. ~3000) in nanopure water over 4 days with periodic water replacements. The final products were collected after the removal of solvent with rotovap.

3.3.3 Absorbance, Photoluminescence Imaging, and Electrochemical Measurements.

UV–visible spectra were recorded using a Shimadzu UV-1700 spectrophotometer. A fluorescence microscope (Olympus IX73) was used for the imaging of AuNCs on the ITO surface. A 377 ± 50 nm excitation filter and a 647 nm long-pass emission filter were used to record photoluminescence with a 33 ms exposure time. The images were analyzed using ImageJ. Electrochemical impedance spectroscopy (EIS) was collected using the Gamry Reference 600 potentiostat with a Ag/AgCl quasi-reference electrode, a platinum (Pt) foil counter electrode, and an ITO working electrode.

3.3.4 AuNCs Assembly on ITO Electrodes via Spin Coating.

The ITO surface was cleaned following a general process by ultrasonicingating in toluene, acetone, ethanol, and nanopure water for 15 mins each sequentially and storing it in nanopure water before usage. To make the ITO surface more hydrophilic or better assembly of aqueous samples, the cleaned and dried ITO was placed in a vacuum chamber and treated with O2 plasma for 3 min.

A layer-by-layer assembly of AuNCs on an ITO electrode is carried out via electrostatic interactions with polyallylamines (PAAs). Spin coating of different solutions was controlled with a spin coater (Chemat Technology series KW-4A) in 1.0 CG aluminum vacuum chuck at 1000 rpm. First, a 10 μL drop of about 10 μM PAA solution was drop cast on the ITO surface, waited for 2 min, and then spinned for 1 min. Next, another 10 μL drop of 250 μM AuNC solution was drop cast and waited for 20 s, and then spinned for 1 min. The stoichiometry of the
AuNCs/PAAs is optimized based on UV−vis absorbance where absorbance intensity increase is observed, indicating the initiation of precipitation (Figure 3.13). Multiple layers of AuNC film on the ITO surface were fabricated by repeating the above steps. As demonstrated in Figure 3.1, the electrostatic interactions between the negatively charged carboxyl groups of Au−LA NCs and the positively charged protonated amines in PAA ensure the precipitation/assembly of Au−LA NCs on the ITO surface. As a control, PAA alone does not generate ECL or appreciable current under the test conditions (Figure 3.5.A).

![Absorbance vs Wavelength](image)

**Figure 3.13** Stoichiometry of AuNCs: PAA for surface assembly determined by absorbance spectra. Data collected in 50 μM Au_{22}LA_{12} NCs in 0.1M PBS pH 7.4 with the titration of polyallylamine solution. The stoichiometry ratio of AuNCs and PAA is estimated to be about 25:1 to reach precipitation based on the numbers of carboxyl and amine groups (using M.W. of ca. 17K Da of the commercial PAA at 20%).

### 3.3.5 ECL Measurements.

ECL was recorded in a 10 × 20 mm quartz cuvette. A 3-Dprinted spectrometer cuvette holder was used to hold the cuvette in front of the camera window at a fixed position. An ITO
electrode was fixed onto a cap on top of the cuvette to ensure consistent electrode-camera alignment. A quasi-reference Ag/AgCl wire reference and a Pt foil counter electrode were used. The supporting electrolyte was 0.1 M phosphate-buffered saline (PBS) at pH 7.4. For results to be directly relevant to real-life application settings, all measurements were performed under ambient conditions without degassing. The emission intensity was recorded with an Andor iDUS CCD camera (model DU401A-BR-DD). To synchronize the camera response and electrochemical measurements, the camera is externally triggered using potentiostat (Gamry Reference 600) at time zero when the potential is applied. The ECL intensity is the sum of photon counts from all pixels; the exposure time is 15 ms for step ECL measurement unless otherwise noted.

3.4 Conclusion

In summary, three unique properties are assembled to establish a generalizable multi-parameter ratiometric ECL analysis strategy based on signaling kinetics: (1) AuNCs as near-IR ECL luminophores; (2) piperazine drugs that enhance ECL as co-reactants for signal-on detection; and (3) ratiometric analysis based on reaction kinetics tailored by operation parameters and material characteristics. The signal-on mechanism, enabled by the enhancement in ECL only from suitable tertiary amine molecular structures, is highly immune to most nonspecific interference and thus reduces false positive responses. Ratiometric signal analysis renders signal specificity toward targets, combined with classic calibration curves in absolute signal intensity, for quantitative analysis over a large dynamic range without the need for additional recognition elements and multi-step analysis. A nanomolar detection limit and a large dynamic range are established with great resolving power against compounds containing different tertiary amine structures. Efficacy tested in synthetic urine promises future applications such as therapeutic
drug monitoring, especially the critical needs for those with narrow therapeutic reference ranges.\textsuperscript{134}

4 INHOMOGENEOUS QUANTIZED SINGLE-ELECTRON CHARGING AND ELECTROCHEMICAL-OPTICAL INSIGHTS ON TRANSITION-SIZED ATOMICALLY PRECISE GOLD NANOCoclusters\textsuperscript{135}


Small differences in electronic structures, such as an emerging energy band gaps or the splitting of degenerated orbitals, are very challenging to resolve but important for nanomaterials properties. A signature electrochemical property called quantized double layer charging, i.e., “continuous” one electron transfers (1e, ETs), in atomically precise $\text{Au}_{133}(\text{TBBT})_{52}$, $\text{Au}_{144}(\text{BM})_{60}$, and $\text{Au}_{279}(\text{TBBT})_{84}$ is analyzed to reveal the nonmetallic to metallic transitions (whereas TBBT is 4-tert-butylbenzenethiol and BM is benzyl mercaptan; abbreviated as $\text{Au}_{133}$, $\text{Au}_{144}$, and $\text{Au}_{279}$). Sub hundred milli-eV energy differences are resolved among the “often approximated uniform” peak spacings from multi pairs of reversible redox peaks in voltammetric analysis, with single ETs as internal standards for calibration and under temperature variations. Cyclic and differential pulse voltammetry experiments reveal a 0.15 eV energy gap for $\text{Au}_{133}$ and
a 0.17 eV gap for Au_{144} at 298 K. Au_{279} is confirmed metallic, displaying a “bulk-continuum”
charging response without an energy gap. The energy gaps and double layer capacitances of
Au_{133} and Au_{144} increase as the temperature decreases. The temperature dependences of charging
energies and HOMO–LUMO gaps of Au_{133} and Au_{144} are attributed to the counterion permeation
and the steric hindrance of ligand, as well as their molecular compositions. With the subtle
energy differences resolved, spectroelectrochemistry features of Au_{133} and Au_{144} are compared
with ultrafast spectroscopy to demonstrate a generalizable analysis approach to correlate steady-
state and transient energy diagram for the energy-in processes. Electrochemiluminescence
(ECL), one of the energy out processes after the charge transfer reactions, is reported for the
three samples. The ECL intensity of Au_{279} is negligible, whereas the ECLs of Au_{133} and Au_{144}
are relatively stronger and observable (but orders of magnitudes weaker than our recently
reported bimetallic Au_{12}Ag_{13}). Results from these atomically precise nanoclusters also
demonstrate that the combined voltammetric and spectroscopic analyses, together with
temperature variations, are powerful tools to reveal subtle differences and gain insights otherwise
inaccessible in other nanomaterials.

4.1 Introduction

Metal nanoclusters (NCs) differ from regular nanoparticles with ultrasmall sizes and thus
quantum effects, and with definitive atomic compositions and structures built on recent advances
in the syntheses and characterizations.\textsuperscript{7, 108} From the perspective of properties, one might argue
that the existence of an energy band gap would be a more fundamental and quantitative
descriptor for metal nanoclusters over larger nanomaterials of the same metal.\textsuperscript{136, 137} Whether a
band gap exists and how it is dependent on the composition/structure are fundamentally
significant and have broad implications to various electrochemical, optical and other properties.
However, determining small energy differences and how the energy states change under reaction conditions, especially in the transition-size range from nanoclusters to larger nanomaterials, is challenging experimentally.

Electrochemical techniques are intrinsically suitable to measure charge transfers and related energy states/orbitals by direct oxidation or reduction at a specific orbital/energy state via electrode reactions. Resolving small energy differences in transition-sized nanoclusters, however, is complicated by a signature electrochemical behavior called quantized double layer (QDL) charging initially observed from Au nanoclusters.\textsuperscript{40, 41} The Au nanoclusters are likely the most studied prototype, also referred as monolayer protected clusters (MPCs) in earlier literature, which are composed of an Au core stabilized by a monolayer of thiolate ligands.\textsuperscript{39, 136, 138} The QDL results from continuous or multiple one-electron transfer reactions (1e ET) when the nanoclusters are considered as nanosized capacitors. With the capacitance in the atto-Farad range depending on the core and ligands, the charging energy needed for 1e ETs would be constant (at about hundred/s milli-eV) assuming that the capacitance remains constant, and thus display ‘uniformly spaced’ peaks in current-potential plots.\textsuperscript{139-141} However, inhomogeneity in QDL peak spacing was noticed long ago, but the interpretation was limited by the previously inevitable sample polydispersity which causes the capacitance and thus the charging energy to vary.\textsuperscript{142-144}

Unlike pure metals or naked clusters, ligand effects on the overall energy diagram can no longer be ignored especially when ligands are covalently attached to metal cores in nanoclusters.\textsuperscript{10, 145-149} An exemplary case is the significant contributions from sulfur atomic orbitals to the energy states of Au-thiolate clusters revealed by superatom theory.\textsuperscript{150} In general, the energy gap would increase with the decrease in size, though it also depends on atomic structures. Currently, discrepancies in the literature dispute such a trend of monotonous
dependence in the non-metal to metal transition range. Au$_{246}$(p-MBT)$_{80}$ is shown to be the largest non-metallic nanocluster in the quantum size regime to date, whereas Au$_{329}$(PET)$_{84}$ are found to have no obvious electrochemical gap and being metallic.$^{47-49}$ Further, Au$_{279}$(TBBT)$_{84}$ is determined to be metallic by transient absorption spectroscopy whereas electrochemistry analysis is absent.$^{151,152}$ An abnormal case is the electrochemical properties of Au$_{133}$(TBBT)$_{52}$ reported by Dass and coworkers, in which no electrochemical energy gap in Au$_{133}$ was identified.$^{153}$ The same report analyzed Au$_{144}$(PET)$_{60}$ and showed a clear ~0.37 V peak spacing.$^{154}$ Theoretical analyses on Au$_{144}$ and similar-sized Au$_{146}$ also showed small gaps.$^{155,156}$ Further, the similar sized Au$_{130}$ exhibits a clear ~0.45 and 0.50 V voltammetric peak spacing with monothiol ligands$^{46,157}$ and ~0.35 V with mixed mono- and di-thiol ligands in earlier work.$^{44}$ With the increasing availability of atomic structures and compositions of nanoclusters, density function theory calculations provide energy diagrams with great details; meanwhile, experimental probing and confirmation of theory would be highly desirable, ideally in both energy levels and density of states, at least semiquantitatively.$^{158-160}$ Electrochemical characterizations at sub-hundred milli-eV, the energy range of electronic transitions overlapping with nuclear vibrations, remain scarce and challenging. The correlation and validation of energy diagram with structures in the nanocluster-nanoparticle transition would provide much needed guidance on the design and development of the fast-growing atomically precise nanoclusters and other relevant materials.

In this chapter, well-established Au$_{133}$, Au$_{144}$ and Au$_{279}$ NCs (atomic structures shown in Figure 4.1.A, steady-state UV–visible absorption spectra in Figure 4.1.B) are chosen to demonstrate how to achieve sub-hundred milli-eV resolution in electrochemical analysis, to reconcile the apparent discrepancy in the qualitative trend of the transition from molecular-like nanocluster with discrete orbitals to larger nanoparticles with band structure, and to demonstrate
a generalizable approach to correlate steady-state and transient spectroscopic features under related charge transfer conditions.

![Figure 4.1 Structures and UV-vis absorption features of Au133, Au144 and Au279 NCs](image)

**Figure 4.1** Structures and UV-vis absorption features of Au133, Au144 and Au279 NCs (A) The structures of Au133, Au144 and Au279 and size transition from nonmetallic molecule-like to metallic nanoparticles. (B) The steady-state UV-visible absorption spectra of Au133, Au144 and Au279 nanoclusters.

### 4.2 Results and Discussion

#### 4.2.1 Basic voltammetry features.

Electrochemical properties of the Au133, Au144 and Au279 NCs characterized by cyclic and differential pulse voltammetry (CV and DPV) are summarized in Figure 4.2. Twelve pairs of quantized double layer (QDL) charging peaks are identified in Au133. The consistent 59 mV potential difference between each pair of reduction and oxidation QDL peaks indicate facile and reversible electron transfer (ET) for the core charging/discharging activities as well as the chemical stability.
Figure 4.2 The CVs and DPVs of Au_{133}, Au_{144} and Au_{279}. The CVs (A-C) and DPVs (D-F) of Au_{133} (A and C, pink lines), Au_{144} (B and E, blue lines) and Au_{279} (C and F, green lines). The concentration of Au NCs is ca. ~1 mM in DCM with 0.1 M TBAP (tetra-n-butylammonium perchlorate). Potential scan rate in CV is 0.1 V/s. All experiments were performed at 298 K except for the DPV of Au_{279} that is at 232 K. Red arrows is oxidation scan and black arrows is reduction scan in DPVs. The dashed lines indicate the rest potentials of Au_{133}, Au_{144} and Au_{279} at -0.19 V, 0.01 V and 0.12 V, respectively.

The ET reversibility is further confirmed by the independence of peak positions at higher scan rates shown in Figure 4.3.A. In Au_{144}, about 13 pairs of QDL redox peaks are observed in the mid potential range. Toward more negative potentials, the cathodic CV current at above -1 V is much higher than the one-electron QDL current (about 8 times herein). Though under thorough purging and stringent controls for consistency in analyzing different samples, residual oxygen might still contribute to this current and affect the quantitation. Similar to the Au_{133}, the reversible ET of these QDL charging is confirmed by the consistent 60 mV peak separations of each redox pair that remain unchanged at higher scan rates (Figure 4.3.B). The CV of Au_{279} is smooth and featureless as opposed to the stepwise quantized one-electron QDL charging in the two smaller NCs. The two current branches are largely in parallel, which is characteristic of
normal capacitive charging, or “bulk-continuum” (double layer) capacitor behaviors. The ‘bulk’ nature of Au$_{279}$ is qualitatively consistent with concentric sphere model in which a large core or capacitance would cause the peak spacing or charging energy to decrease, and thus harder to resolve experimentally.

**Figure 4.3** The cyclic voltammograms of Au$_{133}$ and Au$_{144}$ NCs. (A) CVs of Au$_{133}$ at 0.1, 1.0 and 2.5 V/s scan rate. (B) CVs of Au$_{144}$ at 0.1, 1.0 and 2.5 V/s scan rate.

The stability of all three NCs during the voltammetric measurements is also supported by the insignificant changes in the UV–vis absorption spectra before and after the voltammetric measurements (Figure 4.4).
Figure 4.4 The UV-visible spectra of Au_{133}, Au_{144} and Au_{279} before and after voltammetry measurements. Solid lines are the original spectra, and dash lines are the spectra after measurements.

4.2.2 Differentiating charging energy from different energy states or shift/splitting.

The peak positions and minor irregularities among the peak spacing (ΔV) are better resolved in the corresponding DPV results. The ΔV values determined from both oxidation and reduction DPVs are highly consistent (within about ± 4 mV) listed in Table 2.1. For Au_{133}, a much larger 0.40 V gap is clearly discernable, between the oxidation and reduction peaks at 0.18 V (O1) and -0.21 V (R1), among the otherwise more uniform peak spacings. As for Au_{144}, two pairs of cathodic peaks can be seen at around -1.2 V and -0.8 V, separated by a 0.39 V gap from five highly uniform QDL peaks on the positive direction. QDL (10 pairs) features can only be resolved from the Au_{279} at lower temperature (232K) by DPV explained further below. The peak spacing ΔVs are highly uniform (ΔVs in the range of 0.16 - 0.17 V). Although single millivolt resolution can be read from the measured data, the subten mV difference is within the
broadening by Boltzmann distribution and further limited by the pulse height (50 mV). In other words, an energy gap (if any) of Au\textsubscript{279} cannot be determined from these electrochemical analyses.

Table 4.1 Peak spacings of Au\textsubscript{133}(TBBT)\textsubscript{52}, Au\textsubscript{144}(BM)\textsubscript{60} and Au\textsubscript{279}(TBBT)\textsubscript{52} from the oxidation and reduction DPVs.

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<td>0.18\textsubscript{6}</td>
<td>0.22\textsubscript{2}</td>
<td>0.18\textsubscript{4}</td>
<td>0.20\textsubscript{0}</td>
<td>0.16\textsubscript{0}</td>
<td>0.16\textsubscript{4}</td>
</tr>
<tr>
<td></td>
<td>Re.</td>
<td>0.16\textsubscript{6}</td>
<td>0.16\textsubscript{4}</td>
<td>0.29\textsubscript{2}</td>
<td>0.15\textsubscript{8}</td>
<td>0.41\textsubscript{4}</td>
<td>0.18\textsubscript{8}</td>
<td>0.22\textsubscript{0}</td>
<td>0.18\textsubscript{4}</td>
<td>0.20\textsubscript{0}</td>
<td>0.15\textsubscript{2}</td>
<td>0.16\textsubscript{4}</td>
</tr>
<tr>
<td>195 K</td>
<td>Ox.</td>
<td>0.16\textsubscript{4}</td>
<td>0.18\textsubscript{0}</td>
<td>0.31\textsubscript{0}</td>
<td>0.14\textsubscript{8}</td>
<td>0.41\textsubscript{4}</td>
<td>0.17\textsubscript{6}</td>
<td>0.21\textsubscript{2}</td>
<td>0.17\textsubscript{1}</td>
<td>0.20\textsubscript{0}</td>
<td>0.15\textsubscript{0}</td>
<td>0.15\textsubscript{0}</td>
</tr>
<tr>
<td></td>
<td>Re.</td>
<td>0.16\textsubscript{4}</td>
<td>0.18\textsubscript{0}</td>
<td>0.30\textsubscript{6}</td>
<td>0.14\textsubscript{8}</td>
<td>0.41\textsubscript{6}</td>
<td>0.18\textsubscript{0}</td>
<td>0.20\textsubscript{0}</td>
<td>0.17\textsubscript{6}</td>
<td>0.21\textsubscript{2}</td>
<td>0.15\textsubscript{2}</td>
<td>0.15\textsubscript{0}</td>
</tr>
<tr>
<td>Au\textsubscript{279}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>232 K</td>
<td>Ox.</td>
<td>-</td>
<td>0.14\textsubscript{4}</td>
<td>0.16\textsubscript{9}</td>
<td>0.16\textsubscript{4}</td>
<td>0.16\textsubscript{4}</td>
<td>0.17\textsubscript{4}</td>
<td>0.16\textsubscript{3}</td>
<td>0.16\textsubscript{4}</td>
<td>0.16\textsubscript{4}</td>
<td>0.13\textsubscript{0}</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Re.</td>
<td>-</td>
<td>0.14\textsubscript{4}</td>
<td>0.16\textsubscript{9}</td>
<td>0.16\textsubscript{4}</td>
<td>0.16\textsubscript{4}</td>
<td>0.17\textsubscript{5}</td>
<td>0.16\textsubscript{4}</td>
<td>0.16\textsubscript{9}</td>
<td>0.16\textsubscript{4}</td>
<td>0.14\textsubscript{0}</td>
<td>-</td>
</tr>
</tbody>
</table>

Next, we scrutinize the irregularities in the peaking spacing ΔVs to resolve different energy states. By identifying more uniform ΔV or charging energy as internal standard, a resolution of lower tens milli-eV can be achieved to differentiate energy states/orbitals because thermal agitation and other measurement limitations are the same within a data set. The DPVs of Au\textsubscript{133} and Au\textsubscript{144} collected at different temperatures are analyzed in Figure 4.5. The subtle differences among ΔVs and their temperature dependence are revealed in panels B and D by excluding the much larger ΔV of 0.39–0.40 V. This electrochemical gap is attributed to the energy gap between “HOMO−LUMO” frontier states plus charging energy.
Figure 4.5 Temperature dependence of electrochemical properties of Au$_{133}$ and Au$_{144}$. The DPVs of A) Au$_{133}$ and C) Au$_{144}$ at 298, 232 and 195 K. Peak spacing $\Delta V$s are analyzed in B) and D) respectively.

In Au$_{133}$, the peak spacing $\Delta V$ displays a parabolic shape from the energy gap toward higher potentials (positive and negative), corresponding to the gradual and slight decrease in charging energy in more oxidized/reduced states (Figure 4.5.B and Figure 4.6). The trend is consistent with the counterion permeation model in which permeation or intercalation of electrolyte ions would increase the relative permittivity of the ligand monolayer and thus the QDL compact capacitance ($C = 4\pi \varepsilon_0 \varepsilon \frac{r(r+d)}{d}$, where $\varepsilon$ is the dielectric constant or relative permittivity of the dielectric layer of a capacitor, i.e., the ligand monolayer; $\varepsilon_0$ the permittivity of free space; and $r$ the core radius and $d$ the monolayer thickness).$^{141, 161}$ For one-electron transfer
processes, the double layer capacitances ($C_{CLU}$) is correlated to the peak spacing $\Delta V$ and unit charge $e$ by the equation: $\Delta V = e/C_{CLU}$. The smaller $\Delta V$s at lower temperatures correspond to an increase in QDL capacitance which is explained by the increase in the $\varepsilon$ of hydrocarbon monolayer (“oil”). Also shown in the individual temperature plots in Figure 4.6, the parabola shape flattens at lower temperature and can no longer be resolved at 195 K. The phenomenon is explained by the weakened ion/solvent permeation, i.e., decreased molecular motion at lower temperatures.

![Graphs showing peak spacing of Au$_{133}$ at 298, 232 and 195 K. Dash lines are the fitting of $\Delta V$s at 298 and 232 K, which display a parabolic shape. The parabolas show decreased bending radian as the temperature decrease. The data points highlighted in yellow rectangle are attributed to charging energy.](image)

**Figure 4.6** Peak spacing of Au$_{133}$ at 298, 232 and 195 K. Dash lines are the fitting of $\Delta V$s at 298 and 232 K, which display a parabolic shape. The parabolas show decreased bending radian as the temperature decrease. The data points highlighted in yellow rectangle are attributed to charging energy.

Interestingly, the charging energy is much less sensitive to temperature variations in the reduced states versus the oxidized Au$_{133}$ NCs. Given the $\varepsilon$ of hydrocarbon monolayer being a function of ligand numbers, ligand molecular structures determining the conductivity and dielectric layer thickness ($d$), and ion/solvent permeation/intercalation etc., we attribute the asymmetric response (oxidation versus reduction) to the steric hindrance on counterion permeation: the tetrabutylammonium cations are a lot bulkier than perchloride anions to access the core stabilized by a bulky tert-butyl terminal functionality. The relatively larger decrease in charging energy of the oxidized NCs at lower temperatures is explained by the compact
capacitance changes affected by different extent of solvent/counterion permeation accordingly. The leveling behavior of low oxidation states at lower temperatures (compared to higher oxidation states) seems to suggest a threshold for ion/solvent permeation, which is reasonably expected for a given monolayer “free volume”. With less counterion effects in reduced states, the weaker temperature dependence of the charging energy can arise from two factors: the $\varepsilon$ of hydrocarbon monolayer or the diffuse layer capacitance.\textsuperscript{144} The results highlight that an accurate discrimination of different energy states at ca. sub-100 meV resolution should use the appropriate charging energy at the specific charge states and conditions, rather than the average values as widely adopted. Accordingly, the average $\Delta V$ from the points near the energy gap (Figure 4.5.B and Figure 4.6, highlighted by yellow rectangle) is adopted as charging energy for the analysis of energy band gap.

The benzyl mercaptan monolayer on Au\textsubscript{144} is thinner without the bulky tert-butyl portion and more flexible with the CH$_2$ group. With similar core diameter compared to Au\textsubscript{133}, larger capacitance or smaller $\Delta V$s are measured as expected. The peak spacing $\Delta V$s vary almost in an alternative high–low manner, corresponding to consecutive pairs of redox peaks. The oscillatory pattern suggests small splitting of degenerated orbitals/energy states assuming the charging energy remains constant. Besides the large gap at 0.39\textsubscript{1} eV, the next notable gap of 0.28–0.32 eV separates a pair of peaks above the energy gap, i.e., “LUMO”, from higher energy states. The rest splitting between the highs to the next lows is about 0.04 eV or larger, with values listed in Table 4.1. The $\Delta V$s at different temperatures display no clear pattern or difference between oxidation versus reduction, suggesting less discrimination against electrolyte ions of this thinner/leak monolayer.\textsuperscript{161} Ignoring the oscillatory pattern and the two obvious gaps, a parabolic
shape can still be argued centered at the rest potential (0.03 V), instead of between the energy gap in the case of Au$_{133}$ (Figure 4.5.B and Figure 4.6).

Figure 4.7 Peak spacing of Au$_{144}$ at 298, 232 and 195 K. Dash lines are the fitting of $\Delta V$s at 298 and 232 K, which display a parabolic shape. The parabolas show decreased bending radian as the temperature decrease. The data points highlighted in yellow rectangle are attributed to charging energy.

The results suggest two vacancies in the degenerated HOMO frontier orbital set. Following the trend established in Au$_{133}$ and in reference to the $\Delta V$s at higher potentials (O3/O4, O4/O5, and R5/R6), the $\Delta V$ as highlighted is adopted to charging energy (Figure 4.5.D and Figure 4.7, highlighted by yellow rectangle) to calculate the energy gap and to discriminate the splitting of orbitals/states.

Figure 4.8 UV-vis and Electrochemical proprieties of Au$_{144}$(TBBM)$_{60}$ and Au$_{144}$(BM)$_{60}$ nanoclusters. (A) The steady-state UV-visible absorption spectra of Au$_{144}$(TBBM)$_{60}$ and...
To further confirm the more complex patterns of the Au144, the BM ligands were substituted with the bulky TBBM (TBBM is P-tert-butyl benzyl mercaptan) via ligand exchange, which is a nontrivial task. All main redox features are retained, including the two gaps and the oscillatory ΔVs (Figure 4.8 and Figure 4.9). The determined charging energy is about 30 meV higher consistently with the added tert-butyl layer at each temperature, which gives a larger monolayer thickness and thus smaller capacitance or slightly larger ΔVs. However, the discrimination of counterions, cations upon oxidation and anions upon reduction, was not as obvious compared to Au133. Apparently the extra CH₂ group in TBBM made the monolayer more flexible and leakier than TBBT ones.

![Graph showing peak spacing ΔVs and temperature dependence of electrochemical properties of Au144(BM)₆₀ and Au144(TBBM)₆₀ nanoclusters.](image)

**Figure 4.9** Peak spacing ΔVs and temperature dependence of electrochemical properties of Au144(BM)₆₀ and Au144(TBBM)₆₀ nanoclusters.

The highly uniform samples allow us to interpret the irregularities in the ΔVs at resolution. The broadening effects by Boltzmann distribution can be evaluated by the width of half-maximum (W₁/₂) of DPV peaks following the equation for the one-electron process:

W₁/₂ =
3.52RT/F, where $R$ is the gas constant and $F$ the Faraday constant. $W_{1/2}$ is predicted theoretically to be 90, 70, and 62 mV at 298, 232, and 195 K, respectively, though practical parameters such as pulse height (50 mV) can further broaden the redox peaks and lower the resolution. While baseline separation is not necessary in DPV to determine the peak positions, a value larger than one for the parameter of $\Delta V/2W_{1/2}$, that being the resolution between two neighboring peaks with comparable $W_{1/2}$, confirms the accurate determination of the peak spacings. From the experimentally measured $W_{1/2}$ at different temperatures (Table 4.2), any irregularities more than 20–30 mV can be resolved to reflect the properties of the sample.

**Table 4.2** The calculated peak broadening and experimental peak width at half-maximum ($W_{1/2}$) in DPV results. The experimental $W_{1/2}$ is determined from one QDL peak in the middle, i.e. the second oxidation peak (O2/O3), in a sample by using the DPV current at gap as baseline.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Cal. $W_{1/2}$ (mV)</th>
<th>Exp. $W_{1/2}$ of Au$_{133}$ (mV)</th>
<th>Exp. $W_{1/2}$ of Au$_{144}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298 K</td>
<td>90.0</td>
<td>100.0</td>
<td>90.0</td>
</tr>
<tr>
<td>232 K</td>
<td>70.0</td>
<td>89.4</td>
<td>82.4</td>
</tr>
<tr>
<td>195 K</td>
<td>62.0</td>
<td>91.2</td>
<td>73.1</td>
</tr>
</tbody>
</table>

### 4.2.3 Energy Gap between Frontier Orbitals/States.

The values of charging energy used, the calculated capacitance, and energy gap are listed in Table 4.3. Those results at different temperatures from the two samples, though not large, are statistically different. Of note, no electrochemical gap was resolved/claimed for the same Au$_{133}$ in the earlier report by Dass and co-workers, likely from either slight variation in sample quality or the less scrutinization on the inhomogeneous charging energy. The HOMO–LUMO gap of Au$_{144}$ is larger than that of Au$_{133}$, 0.173 eV versus 0.154 eV at room temperature. The difference
increases at lower temperatures, to 0.23\textsubscript{7} eV versus 0.19\textsubscript{2} eV or 45 meV at 195 K. Qualitatively speaking, larger Au cores would correspond to smaller band gaps. In reference to other Au\textsubscript{130/3} NCs and Au\textsubscript{144/6} NCs,\textsuperscript{40, 144, 153, 162} the hollow center in the core atomic structure of Au\textsubscript{144} is believed responsible for the larger HOMO–LUMO gap of Au\textsubscript{144}.\textsuperscript{153, 163, 164}

**Table 4.3** Electrochemical characterizations of Au\textsubscript{133} and Au\textsubscript{144} at 298, 232 and 195 K.

The single mV digit is listed to demonstrate the uncertainty of the calculated capacitance and band gap (one less significant figures). \(\Delta V\) is the appropriate peak spacing determined from \(C_{CLU}\) is the double layer capacitance, and \(Eg\) is the electrochemical energy gap (HOMO-LUMO gap plus \(\Delta V\)).

<table>
<thead>
<tr>
<th></th>
<th>(\Delta V) (V)</th>
<th>(C_{CLU}) (aF)</th>
<th>(\ln C_{CLU})</th>
<th>(Eg) (V)</th>
<th>HOMO-LUMO Gap (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au\textsubscript{133}</td>
<td>298 K</td>
<td>0.24\textsubscript{6}</td>
<td>0.65</td>
<td>-0.43</td>
<td>0.40\textsubscript{0}</td>
</tr>
<tr>
<td></td>
<td>232 K</td>
<td>0.22\textsubscript{8}</td>
<td>0.70</td>
<td>-0.36</td>
<td>0.40\textsubscript{8}</td>
</tr>
<tr>
<td></td>
<td>195 K</td>
<td>0.21\textsubscript{6}</td>
<td>0.74</td>
<td>-0.30</td>
<td>0.40\textsubscript{8}</td>
</tr>
<tr>
<td>Au\textsubscript{144}</td>
<td>298 K</td>
<td>0.21\textsubscript{8}</td>
<td>0.73</td>
<td>-0.31</td>
<td>0.39\textsubscript{1}</td>
</tr>
<tr>
<td></td>
<td>232 K</td>
<td>0.18\textsubscript{5}</td>
<td>0.86</td>
<td>-0.15</td>
<td>0.41\textsubscript{8}</td>
</tr>
<tr>
<td></td>
<td>195 K</td>
<td>0.17\textsubscript{7}</td>
<td>0.90</td>
<td>-0.11</td>
<td>0.41\textsubscript{4}</td>
</tr>
</tbody>
</table>

It is logical to perform similar analysis on Au\textsubscript{246}, another nonmetallic NC and smaller than Au\textsubscript{279}.\textsuperscript{47, 48, 151, 152} DPV results at varied temperatures (Figure 4.10) confirm its nondetectable energy gap, as well as diminished yet distinguishable trends in charging energy such as (1) a parabolic shape with respect to the applied potential and (2) decrease and leveling at lower temperatures and higher potentials. No detectable energy gap or trends in charging energy were resolved from the larger Au\textsubscript{279} (results not shown).
Figure 4.10 DPVs and Analysis of peak spacing of Au$_{246}$ (A) The DPVs and ΔVs of Au$_{246}$ at 232 and 195 K. (B) Analysis of peak spacing of Au$_{246}$. The data of peak spacing at 298 K is cited from the published literature.

The enthalpy and entropy portions in the Gibbs free energy changes associated with the electron transfer reaction of Au$_{133}$ and Au$_{144}$ can be estimated from the intercept and slope of the linear fitting of HOMO–LUMO gap at different temperatures. In Figure 4.11, both the extrapolated intercept and the slope are larger for Au$_{144}$ over Au$_{133}$. The higher enthalpy is attributed to the structure differences in Au$_{144}$. The more sensitive temperature dependence indicates larger entropy which is explained by the more flexible monolayer: short flexible BM ligands without tert-butyl group and thus less steric effect, and slightly larger ligand-to-metal ratio (60/144 for Au$_{144}$ versus 52/133 for Au$_{133}$). It is important to mention that the ion/ solvent permeation should be viewed as a dynamic exchange process between the bulk solution and the monolayer: both the extent/amount and the dynamics will contribute to the measured energy states/parameters and possible shift/splitting after the ET reactions. At lower temperatures, the motion of ligands can be more restricted with a reduced degree of freedom in certain vibration/rotation modes. The counterion/ solvent permeation into the ligand monolayer will be less dynamic and correspondingly more constant relative permittivity of the dielectric layer and
thus the capacitance. At higher charge states, similar reduction in the dynamics of counterions will arise due to the stronger electrostatics. The entropy factor will vary accordingly, which explains the lower linearity of the Au$_{144}$ results.

![Graph](image)

**Figure 4.11** HOMO-LUMO gaps of Au$_{133}$ (pink) and Au$_{144}$ (blue) at 298, 232 and 195 K. Dashed lines are fitting. The error bar represents the standard deviation of the average charging energy highlighted in Figure 4.5.

### 4.2.4 Comparisons of Spectroelectrochemistry and Ultrafast Spectroscopy Features.

In spectroelectrochemistry, the charge state of the sample is altered directly by addition/removal of an electron in a particular energy state via electrode ET reactions. The changes in the steady-state absorption spectrum are then collected. The charge states are unchanged in ultrafast spectroscopy; rather, the populations of electrons/holes in at least two orbitals or energy states are transiently adjusted by a laser and the dynamic changes in spectral features are recorded subsequently. The two techniques can provide complementary insights on energy states and related charge/energy transfer and relaxations, especially when the sample undergoes charge
transfer with other entities, as in many applications. The concept and analysis approach are explained by aligning the spectroelectrochemistry results of the Au$_{133}$ and Au$_{144}$ NCs with previously reported ultrafast data$^{158,163}$ in Figure 4.12.

**Figure 4.12** Absorption changes of Au$_{133}$ (left, A, C and E) and Au$_{144}$ (right, B, D and F) in spectroelectrochemistry and ultrafast spectroscopy. ΔABS calculated by the subtraction with the original spectrum. (A) and (B) are after reductive electrolysis and (C) and (D) after oxidative electrolysis by spectroelectrochemistry. The denoted potentials are the valleys in DPV. (E) and (F) are transit absorption spectra.$^{158,163}$ The eVs listed above the dashed lines are the distinct absorption bands observed in original steady-state spectra. Additional lines are added to highlight the alignment of key features across different panels.

The corresponding original spectra are included in Figures 4.13 for reference. NCs of these types have been extensively studied, so we defer detailed property discussion to earlier literature and only focus on key comparisons between the two techniques.
The first notion is that spectroelectrochemistry offers information over a much larger energy window (Figure 4.12, wavelength at the bottom axis and eV energy on the top) with great flexibility and convenience but lacks dynamics. For example, the distinct 2.95 eV valley in Figure 4.12.A (420 nm peak) and the > 3 eV peak in Figure 4.12.D from spectroelectrochemistry are inaccessible in ultrafast mode, which requires highly complex hardware.

Second, the positive or negative ΔABS in spectroelectrochemistry can be correlated to the excited-state absorption (ESA, positive signals) or ground-state bleaching (GSB, negative signals) in ultrafast results but require deliberation. This is because at least two states (unfilled and occupied) are involved in the laser pumping, whereas the electrolysis changes only one state directly, though indirect changes on other states are inevitable. Of note, a positive ΔABS means an increase in steady-state absorbance after the electrolysis and a one negative for decrease. For
example, the 2.14 and 1.94 eV peaks in Figure 4.12.A in spectroelectrochemistry of Au\textsubscript{133} match the ESA in ultrafast spectra, and 2.95 eV (420 nm) valley to GSB.\textsuperscript{163} For Au\textsubscript{144}, the peak around the 1.64 eV absorption band in Figure 4.12.D matches the onset of interband transition from 5d electrons, which will slightly shift due to the ligand effect.\textsuperscript{27,164,165} The 2.70, 2.41, and 2.27 eV absorption bands (panels B and D) can also find corresponding features in ultrafast spectra (panel F), which mainly originate from the core transitions on the basis of the fast decay. The transition from occupied states across the Fermi energy can contribute to these absorption bands.\textsuperscript{158,166,167}

Third, reduction and oxidation electrolysis modify the unoccupied and occupied orbitals respectively and would generate some symmetric/opposite spectral features.\textsuperscript{44} While the peak/valley positions reveal the corresponding energy states and possible shifts, the intensity of ±ΔABS should reflect the accessible density of states (DOS): a lower DOS or number of degenerated orbitals will display stronger changes per one-electron oxidation/reduction. The reduction-induced changes of Au\textsubscript{133} in Figure 4.12.A display the most changes in ΔABS. Key transition features, such as (1) an isosbestic point (circular dotted line) at about 510 nm and (2) the 580 nm (2.14 eV) and 640 nm (1.94 eV) absorption bands, are consistently observed in both techniques. The oxidation induced ΔABS in Figure 4.12.C is too weak to be reliably interpreted, but the features are under further explorations.\textsuperscript{168-170} For Au\textsubscript{144}, the ΔABS is stronger after oxidation electrolysis (unlike the stronger reduction ones in Au\textsubscript{133}), suggesting stronger perturbation on the frontier occupied orbitals. More consistent with previously reported spectroelectrochemistry features,\textsuperscript{44,46} oxidation/reduction panels (Figure 4.12.D versus Figure 4.12.B) largely mirror each other at respective wavelength ranges qualitatively.

Lastly, irreversible chemical reactions or decomposition could occur in both measurements and affect the analysis. For example, the reduction at high potentials (−1.35 V for
Au_{133} and −1.30 V for Au_{144}) induces significant irreversible decompositions. Additional strong changes in absorbance such as the shift in isosbestic point in panel A of Figure 4.12 should be interpreted in reference to other more consistent features.

4.2.5 **Electrogenerated Chemiluminescence or Electrochemiluminescence.**

In ECL, the excited species are generated by electrode reactions instead of light source. Luminescence occurs when the excess energy, from either oxidation or reduction with optional following reactions, is released via radiative decay. The energy/wavelength and intensity/efficiency of ECL depends on the energy diagram of the ECL reagents and the ECL reaction pathways (self-annihilation or co-reactants).

![Figure 4.14 ECL of Au_{133}, Au_{144} and Au_{279} under potential scanning (A: Au_{133}, B: Au_{144}, C: Au_{279}) and potential step activations (D). The concentration of Au_{133}, Au_{144} and Au_{279} is ~ 15 µM with 0.1 M TBAP electrolyte. The concentration of TPrA coreactant is 10 mM. Potential scan rate is 0.1 V/s in CV-ECL. The electrode potential is held for 5 s in each step over three cycles at the denoted values.](image-url)
Figure 4.14 shows the ECLs from Au\textsubscript{133}, Au\textsubscript{144}, and Au\textsubscript{279} under comparable conditions. Those ECL performances are seven orders of magnitude weaker compared to our recently reported bimetallic Au\textsubscript{12}Ag\textsubscript{13} NCs and not worthy of further explorations. However, ECL is an energy-out measure and much more sensitive over spectroelectrochemistry which analyzes energy-in behaviors. Therefore, combined view of the energy diagram on these three transition-sized nanoclusters-nanoparticles can be established. With high excess tripropylamine (TPrA) as co-reactants for oxidative-reduction ECL generation, the CV/ECL profiles (panels A–C) and potential step ECLs (D) are compared. The prominent current features such as the oxidation current peak of 1.2 V are dominated by the TPrA co-reactant. While all are weak, the ECL from Au\textsubscript{279} is practically non-exist, which further confirms the continuous electronic energy state or innate metallic character. The trends/comparisons of the weak ECL from these samples are qualitatively comparable to their respective near IR photoluminescence (PL; PL results not shown).\textsuperscript{171} In other words, ECL and PL can be potentially used conveniently in a combined or cross-checking fashion to survey or predict high-performing luminophores among the fast growing list of atomic precise nanoclusters or other materials.

4.3 Experimental Section

4.3.1 Chemicals

TetrachloroAuric (III) acid (HAuCl\textsubscript{4} • 3H\textsubscript{2}O, > 99.99% metals basis, Aldrich), Tetraoctylammonium bromide (TOAB, ≥ 98%, Fluka), 2-Phenylethanethiol (PET, C\textsubscript{8}H\textsubscript{9}SH, 98%, Aldrich), L-Glutathione (GSH, reduced, 98+,% Alfa Aesar), Benzyl mercaptan (BM, C\textsubscript{7}H\textsubscript{7}SH, 99%, Aldrich), 4-tert-Butylbenzenethiol (TBBT, C\textsubscript{10}H\textsubscript{13}SH, > 97.0%, TCI), Butylbenzyl mercaptan (BM, C\textsubscript{7}H\textsubscript{7}SH, Aldrich), P-methylthiophenol (pMBT, C\textsubscript{7}H\textsubscript{6}S, 98%,
Aldrich), Sodium borohydride (NaBH₄, Aldrich). Solvents: Methanol (MeOH, HPLC grade, ≥ 99.9%, Aldrich), Ethanol (EtOH, ACS reagent, ≥ 99.5%, Aldrich), Acetone (HPLC grade, ≥ 99.9%, Aldrich), Acetonitrile (ACN, HPLC grade, ≥ 99.9%, Aldrich), Dichloromethane (DCM, ACS reagent, ≥ 99.5%, Aldrich), Toluene (Tol, HPLC grade, ≥ 99.9%, Aldrich). All chemicals were used without further purification. Nanopure water was prepared with a Barnstead NANOpure Diamond system.

4.3.2 Synthesis of Au₁₃₃(TBBT)₅₂, Au₁₄₄(BM)₆₀, Au₁₄₄(TBBM)₆₀, Au₂₄₆(p-MBT)₈₀, Au₂₇₉(TBBT)₈₄ nanoclusters.

Au₁₃₃(TBBT)₅₂ was synthesized by a ligand-exchange reaction from Au₁₄₄(PET)₆₀ according to the literature procedure.

Au₁₄₄(BM)₆₀ was prepared by a ligand-exchange/size-focusing from polydisperse Auₙ(SG)ₓ based on the literature procedure with slight modification. Briefly, HAuCl₄·3H₂O (0.3 mmol, 118 mg) and L-Glutathione (GSH, 1.85 mmol, 570 mg) were dissolved in acetone (30 mL) in a 100 mL round-bottom flask. After vigorously stirring for 15 min, NaBH₄ (3 mmol, 114 mg dissolved freshly in 5 mL of cold Nanopure water) was rapidly added to the solution under vigorous stirring. The solution turned black immediately indicating formation of Au clusters, which then precipitated out of the acetone solution. After stirring for 1 hour, colorless supernatant was discarded and black precipitate was dissolved in 3 mL of Nanopure water. The aqueous solution of polydispersed Auₙ(SG)ₓ was transferred to a 50 mL round-bottom flask with 2 mL of toluene, 0.3 mL of ethanol and 1 ml of benzyl mercaptan. The solution was heated up to 85°C for 28 hours. Then, methanol was added to the reaction mixture to precipitate the product, followed by centrifugation, and the solid product was further washed with methanol to remove excess thiol. This washing step was performed several times and finally pure Au₁₄₄(BM)₆₀
nanocluster was extracted with dichloromethane. The solvent was then evaporated for storage and for further characterization.

\( \text{Au}_{144}(\text{TBBM})_{60} \) was prepared by a ligand-exchange reaction from \( \text{Au}_{144}(\text{PET})_{60} \) nanoclusters that are prepared according to the literature preparation. The prepared \( \text{Au}_{144}(\text{PET})_{60} \) was added into a 50 mL round-bottom flask with 1 mL toluene and 0.5 mL p-tert-butyl benzyl mercaptan. The reaction was allowed for 15 h under 80 °C. Then, 20 mL methanol was added followed by centrifugation, and the precipitate was further washed three times with methanol. Dichloromethane was used to extract the \( \text{Au}_{144}(\text{TBBM})_{60} \) and the solution was removed by rotary evaporator for further experiments.

\( \text{Au}_{246}(\text{p-MBT})_{80} \) was synthesized following the two-step “size-focusing” method.

\( \text{Au}_{279}(\text{TBBT})_{84} \) was synthesized by a ligand-exchange reaction with TBBT using \( \text{Au}_{333}(\text{TBBM})_{79} \) prepared by a stepwise size focusing method according to the literature procedure.

### 4.3.3 Measurements

Cyclic and differential pulse voltammograms (CVs and DPVs) were recorded with a CHI Instrument (Model 750C). In general, the nanoclusters were dissolved in DCM at about 1 mM concentration with 0.1 M TBAP (tetra-n-butylammonium perchlorate) as supporting electrolyte. A Pt disk electrode (d ~ 0.5 mm) as working electrode, and an Ag/AgCl wire and Pt foil as reference and counter electrodes were used. A 20 min purging with argon processing was executed before each measurement. Dry ice in ACN and EtOH was used to maintain the test temperature at 232 and 195 K, respectively.

Spectroelectrochemistry and electrochemiluminescence experiments were performed with a three-electrodes system in a quartz cuvette. A Pt mesh working electrode and cuvette were
aligned at a fixed position with respect to the detector/camera for consistency. An Ag/AgCl wire as reference electrode and a Pt foil as counter electrode were used (away from a light path). A Dropsens μstat200 benchtop potentiostat and Shimazu UV1700 UV–visible spectrometer were used for spectroelectrochemistry. The ECL emission intensity was recorded with an Andor iDUS CCD camera (Model DU401A-BRDD). The camera was externally triggered by the potentiostat (Gamry R600) for synchronized light-current detection. The sample solution was purged for about 20 min with argon prior to the measurements.

4.4 Conclusion

In conclusion, energy differences at < 0.1 eV resolution under charge transfer conditions are determined in three Au nanoclusters spanning the transition from molecular to metallic state. Discrete energy states in Au$_{133}$ and Au$_{144}$ nanoclusters and more continuum redox behaviors of metallic Au$_{279}$ are revealed by scanning and pulse voltammetric analyses at different temperatures. The voltammetric peak spacing of consecutive one-electron transfer reactions, corresponding to the charging energy (eV), is shown inhomogeneous and depends on measurement conditions. Viewed as nanocapacitors described by concentric sphere model, major differences in the charging energy (midtens of milli-eV) and the variations upon charge transfer reactions are explained by the changes in compact capacitance due to counterion permeation. The more rigid and thicker ligand monolayer on Au$_{133}$ imposes stronger steric hindrance toward bulkier counterions, therefore less changes in relative permittivity and thus double layer capacitance or charging energy. The discrimination of subtle energy differences is achieved by using appropriate charging energy under the respective conditions as internal standard. A 0.154 eV HOMO–LUMO gap in Au$_{133}$ is determined after the correction of 0.246 eV charging energy, while a 0.173 eV gap above the highest occupied frontier orbitals is observed in Au$_{144}$. The gaps
and capacitance of $\text{Au}_{133}$ and $\text{Au}_{144}$ increase as the temperature decreases, which reveal the respective enthalpy and entropy contributions to the Gibbs free energy. Optical electronic transitions after electrolysis explored by spectroelectrochemistry are compared to ultrafast spectra. Similarities and differences between the two techniques are discussed to demonstrate a combined approach to analyze steady-state and transient spectral features under charge transfer conditions. ECL of these transition-sized nanoclusters is weak to be of direct practical use alone, but qualitative trends for strong ECL generation such as larger gap or less nonradiative decay pathways can be argued by comparing the ECLs from different nanoclusters. Approaches to resolve the subtle energy differences and density of states, and to gain combined electrospectroscopic understanding of the energy-in and energy-out processes under reaction conditions, are believed important to the explorations of the relative physicochemical properties on other nanoclusters or nanomaterials.

5 SUMMARY

In summary, this dissertation starts with the analysis on the kinetics of the electrochemiluminescence generation from gold nanoclusters. A generalizable multi-parameter ratiometric ECL analysis strategy is then established based on signaling kinetics. The differences in the electronic structures of three Au NCs are determined with spectroelectrochemistry and related methods to elucidate fundamental principles to improve the ECL properties of metal NCs.

An analytical equation to describe ECL kinetics is developed based on the ECE reaction mechanism. This equation is confirmed effective to quantitatively reproduce the representative experimental results with different NCs both on surface and in solution. The assessments of ECL intensity and reaction rates are corroborated with current-time profiles interpreted with classic
electrode kinetics theory. Conditions for approximations and simplifications are discussed for ease of use or generalization. The universal applicability over a wide range of conditions is anticipated to lay the foundation for various ECL based sensing, imaging, and assay type applications.

Several drug compounds contain specific tertiary amine structures that enhance the near IR ECL from Au NCs. A signal-on mechanism for the sensing and drug monitoring type application is further improved by a ratiometric ECL analysis strategy which successfully discriminates similar compounds and against nonspecific interference. Toward this target application, Au NCs are the near-IR ECL luminophores, piperazine drugs and other tertiary amine chemicals are coreactants to enhance the ECL signal as well as analog interferants for one another. Absolute ECL intensity and the ratio/s of ECL at different overpotentials, governed by the differences in signaling kinetics and thus specific to specific compounds, are quantitatively correlated to the concentrations of different compounds. The dual-parameter calibration directly discriminates those similar compounds over several orders magnitudes in concentration with sub-nanomolar detection limit. Validation in the efficacy in synthetic urine suggests the feasibility in therapeutic drug monitoring, especially for those with narrow therapeutic reference ranges.

Effective applications will benefit from improved ECL properties. Electrochemical and spectroscopic methods are used to characterize the electronic structures of several Au NCs at the transition size ranges from molecule-like Au$_{133}$ and Au$_{144}$ nanoclusters to the more continuum metallic Au$_{279}$ nanoclusters. The continuous one-electron transfer reaction, so-called quantized charging energy, is determined to be inhomogeneous, with the variation in the range of tens of milli-electron volts. The differences are explained by different ligand molecular structures and
the impacts on counterion permeation into the ligand monolayer. The corresponding changes in relative permittivity result in a change in charging energy. The understanding of the electronic structures is supported by the comparable features in the steady state spectroelectrochemistry results and transient ultrafast spectra, as well as the comparisons between ECL and photoluminescence behaviors. Larger energy gaps and other factors that suppress nonradiative decays are expected to boost ECL efficiency under otherwise consistent chemical space.

6 REFERENCES


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