Electrochemical Characterizations and Theoretical Simulations of Transport Behaviors at Nanoscale Geometries and Interfaces

Juan Liu

Follow this and additional works at: https://scholarworks.gsu.edu/chemistry_diss

Recommended Citation
Liu, Juan, "Electrochemical Characterizations and Theoretical Simulations of Transport Behaviors at Nanoscale Geometries and Interfaces." Dissertation, Georgia State University, 2012. https://scholarworks.gsu.edu/chemistry_diss/74
ELECTROCHEMICAL CHARACTERIZATIONS AND THEORETICAL SIMULATIONS OF TRANSPORT BEHAVIORS
AT NANOSCALE GEOMETRIES AND INTERFACES

by

JUAN LIU

Under the Direction of Dr. Gangli Wang

ABSTRACT

Since single nanopores were firstly proposed as a potential rapid and low-cost tool for DNA sequencing in 1990s (PNAS, 1996, 93, 13770), extensive studies on both biological and synthetic nanopores and nanochannels have been reported. Nanochannel based stochastic sensing at single molecular level has been widely reported through the detection of transient ionic current changes induced by geometry blockage due to analytes translocation. Novel properties, including ion current rectification (ICR), memristive and memcapacitive behaviors were reported. These fundamental properties of nanochannels arise from the nanoscale dimensions and enables applications not only in single molecule sensing, but also in drug delivery, electrochemical energy conversion, concentration enrichment and separation, nanoprecipitation, nanoelectronics etc. Electrostatic interactions at nanometer-scale between the fixed surface charges and mobile charges in solution play major roles in those applications due to high surface to volume ratio. However, the knowledge of surface charge density (SCD) at na-
nometer scale is inaccessible within nanoconfinement and often extrapolated from bulk planar values. The determination of SCD at nanometer scale is urgently needed for the interpretation of aforementioned phenomena. This dissertation mainly focuses on the determination of SCD confined at a nanoscale device with known geometry via combined electroanalytical measurements and theoretical simulation. The measured currents through charged nanodevices are different for potentials with the same amplitude but opposite polarities, which deviates away from linear Ohm’s behavior, known as ICR. Through theoretical simulation of experiments by solving Poisson and Nernst-Planck equations, the SCD within nanoconfinement is directly quantified for the first time. An exponential gradient SCD is introduced on the interior surface of a conical nanopore based on the gradient distribution of applied electric field. The physical origin is proposed based on the facilitated deprotonation of surface functional groups by the applied electric field. The two parameters that describe the non-uniform SCD distribution: maximum SCD and distribution length are determined by fitting high- and low-conductivity current respectively. The model is validated and applied successfully for quantification and prediction of mass transport behavior in different electrolyte solutions. Furthermore, because the surface charge distribution, the transport behaviors are intrinsically heterogeneous at nanometer scale, the concept is extended to noninvasively determine the surface modification efficacy of individual nanopore devices. Preliminary results of single molecule sensing based on streptavidin-iminobiotin are included. The pH dependent binding affinity of streptavidin-iminobiotin binding is confirmed by different current change signals (“steps” and “spikes”) observed at different pHs. Qualitative concentration and potential dependence have been established. The chemically modified nanopores are demonstrated to be reusable through regenerating binding surface.

INDEX WORDS: Electric-field dependent surface charge density distribution, Single conical nanopores, Surface modification coverage, Stochastic sensing, Finite element simulation
ELECTROCHEMICAL CHARACTERIZATIONS AND THEORETICAL SIMULATIONS OF TRANSPORT BEHAVIORS
AT NANOSCALE GEOMETRIES AND INTERFACES

by

JUAN LIU

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
2012
ELECTROCHEMICAL CHARACTERIZATIONS AND THEORETICAL SIMULATIONS OF TRANSPORT BEHAVIORS
AT NANOSCALE GEOMETRIES AND INTERFACES

by

JUAN LIU

Committee Chair: Gangli Wang

Committee: Jenny J Yang
Stuart Anthony Allison

Electronic Version Approved:

Office of Graduate Studies
College of Arts and Sciences
Georgia State University
December 2012
DEDICATION

This dissertation is dedicated to my family and friends.
ACKNOWLEDGEMENTS

All of my work has been done under the direction of my PhD advisor Dr. Gangli Wang. I owe my deepest gratitude to Dr. Wang. He enlightens my research on nanopores and is extremely patient on mentoring me. He teaches me the way to apply book knowledge into practical application. Most important he also teaches me the way of thinking and doing research. What he has taught me will benefit both research and life. It’s my great honor to be Dr. Wang’s student.

I appreciate my committee members: Prof. Jenny J Yang, Prof. W. David Wilson and Prof. Stuart Anthony Allison for their helpful suggestions.

I thank all the lab members in Dr. Gangli Wang’ group: Maksim Kvetny, Dengchao Wang, Zhenghua Tang, Yan Li, Warren Brown, Tarushee Ahuja, Cecil Conroy and other lab members. I really enjoy the life in the lab together with them.

I also want to thank Dr. Baohua Wu on the help of COMSOL Multiphysics, Dr. Hui Zhao for using AFM, Yidan Liu and Dr. Jie Jiang for providing me samples.

My friends: Xue Qi, Nan Zhao, Qian Wang, Juan Sun and Guchuang Yang have accompanied my PhD life; I appreciate the time with them.

I acknowledge the fellowship from Molecular Basis of Disease Area of Focus (2011-2012), Georgia State University. The financial supports from GWang startup at GSU, Research Initiation Grant at GSU and ORNL FIRST EFRC of DOE (ERKCC61) are acknowledged.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................... v

LIST OF SCHEMES ........................................................................................................................ x

LIST OF TABLES .......................................................................................................................... xi

LIST OF FIGURES ........................................................................................................................ xii

1 INTRODUCTION ..................................................................................................................... 1

1.1 The fabrication of nanochannels and microchannels ..................................................... 3

   1.1.1 Biological ion channels ....................................................................................... 3

   1.1.2 Microchannels ..................................................................................................... 3

   1.1.3 Nanochannels ..................................................................................................... 4

   1.1.4 Arrays and membranes ...................................................................................... 6

1.2 Stochastic sensing based on nanodevices ..................................................................... 7

   1.2.1 The Coulter Counter .......................................................................................... 7

   1.2.2 Polymer sensing ................................................................................................. 7

   1.2.3 Nucleic acid sensing .......................................................................................... 8

   1.2.4 Protein, nanoparticle and other molecule sensing ............................................. 9

   1.2.5 Advantages of channel-type nanodevice based sensing ...................................... 10

       1.2.5.1 The ultimate sensitivity—single molecule .................................................... 11

       1.2.5.2 Heterogeneity analysis ................................................................................. 11

       1.2.5.3 Label-free analytes ...................................................................................... 12

   1.2.6 Challenges in DNA sequencing ........................................................................... 12

1.3 Microchannels and Nanochannels in other applications ........................................... 13

   1.3.1 Nanofluidic electronics ....................................................................................... 13

   1.3.2 Concentration enrichment and separation ........................................................ 14
1.3.3 Drug delivery .................................................................................................................. 15
1.3.4 Electrochemical energy conversion ............................................................................. 15
1.3.5 Spatially confined nanoprecipitation ......................................................................... 16

1.4 Novel properties of nanopore ....................................................................................... 16

1.4.1 Ion current rectification ............................................................................................. 17
1.4.2 Memristive and memcapacitive ion transport .......................................................... 18

1.5 Theoretical studies of ion transport through single nanopores and nanochannels ... 19

1.5.1 Molecular dynamics simulation .................................................................................. 20
1.5.2 Finite element simulation based on continuum theory ............................................. 23

2 GLASS NANOPORES AS SINGLE MOLECULE BINDING SENSORS .................................. 23

2.1 Introduction ..................................................................................................................... 23

2.2 Preliminary results and discussion ............................................................................... 25

2.2.1 Streptavidin binding with iminobiotin modified glass nanopores ......................... 25

2.2.1.1 pH dependence ........................................................................................................ 26
2.2.1.2 Concentration dependence ..................................................................................... 27
2.2.1.3 Potential dependence .............................................................................................. 28
2.2.1.4 Counting the binding sites and binding surface regeneration .......................... 29

2.2.2 The binding of streptavidin-DNA aptamer conjugate ............................................. 30

2.2.3 Challenge in nanodevice based stochastic sensing-data comparison ........................ 34

2.3 Experimental details ...................................................................................................... 35

2.3.1 Materials .................................................................................................................... 35

2.3.2 Nanopore fabrication and surface modification ....................................................... 36

2.3.3 Electrochemical measurements .................................................................................. 40

2.4 Summary ........................................................................................................................ 41
3 SURFACE CHARGE DENSITY DETERMINATION OF SINGLE CONICAL NANOPORES BASED ON NORMALIZED ION CURRENT RECTIFICATION ........................................................................................................ 42

3.1 Introduction ........................................................................................................ 42

3.2 Results and discussion ....................................................................................... 46

3.2.1 Experiments: normalized conductivity in potential scanning measurements ...... 46

3.2.2 Simulation based on continuum theory ............................................................ 48

3.2.2.1 Spatial distribution of ion concentration inside the nanopores ..................... 50

3.2.2.2 Spatial distribution of ion flux inside nanopores ............................................. 53

3.2.2.3 Quantification of the effective SCD of individual nanopores ....................... 56

3.2.2.4 Transference number of K⁺ and Cl⁻ in high- and low-conductivity states ........ 58

3.3 Experimental section .......................................................................................... 59

3.3.1 Materials ........................................................................................................ 59

3.3.2 Conical nanopore fabrication and surface modification ..................................... 60

3.3.3 Electrochemical measurements ....................................................................... 60

3.3.4 Theoretical simulation ................................................................................... 61

3.4 Summary ............................................................................................................ 63

4 ELECTRIC FIELD DEPENDENT SURFACE CHARGE DENSITY DISTRIBUTION IN SINGLE CONICAL NANOPORES ............................................................................................... 65

4.1 Introduction ........................................................................................................ 65

4.2 Results ................................................................................................................ 69

4.2.1 Experiments: i—V responses of conical nanopores in different electrolytes ....... 69

4.2.2 Simulation based on continuum theory ............................................................ 71

4.2.3 Simulation of the measured current at high and low conductivity states ........... 72

4.2.4 The quantitative description and prediction of the experimental i—V results ....... 75
4.2.5 The tolerance of the effective SCD profile determined from $i-V$ measurements 77

4.2.6 The transference number of cations and anions at high- and low- conductivity states .............................................................. 79

4.3 Discussion ........................................................................................................... 81

4.4 Experimental section ......................................................................................... 88

4.4.1 Materials ......................................................................................................... 88

4.4.2 Conical nanopore fabrication and surface modification ................................. 88

4.4.3 Electrochemical measurements ..................................................................... 89

4.4.4 Simulation ....................................................................................................... 89

4.5 Summary ............................................................................................................ 91

5 NONINVASIVE SURFACE COVERAGE DETERMINATION OF CHEMICALLY MODIFIED CONICAL NANOPORES THAT RECTIFY ION TRANSPORT ................................................................. 92

5.1 Introduction ........................................................................................................ 92

5.2 Results and discussion ......................................................................................... 95

5.2.1 Experiments: $i-V$ responses of conical nanopores ...................................... 95

5.2.2 Correlation of the simulated current and SCD ........................................... 97

5.3 Experimental section .......................................................................................... 101

5.3.1 Materials ....................................................................................................... 101

5.3.2 Nanopore fabrication ..................................................................................... 102

5.3.3 Electrochemical measurements .................................................................... 102

5.3.4 Theoretical simulation .................................................................................. 103

5.4 Summary ............................................................................................................ 104

6 CONCLUSIONS AND SIGNIFICANCE .................................................................. 105

REFERENCES ........................................................................................................... 108
LIST OF SCHEMES

Scheme 2.1 Origin of negative charges on inner surface for a glass conical nanopore. Negative charges result from deprotonation of surface silanol groups: $-\text{SiOH} \rightarrow \text{SiO}^- + \text{H}^+$. .......................................................... 35

Scheme 2.2 Scheme of surface modification of a conical glass nanopore for targeting streptavidin. ....40

Scheme 3.1 Ion Flux Confined by Nanopore Geometry and Interaction with the Fixed Charges at the Glass-Solution Interface. The double arrow suggests the electrostatic interaction between mobile ions and those fixed negative charges, which can be divided into two components along and normal to the direction of ion flux. Not drawn to scale. ........................................................................................................44

Scheme 4.1 A gradient SCD distribution inside a conical nanopore. The electric field intensity, represented by the blue and red colors at both ends of the half cross-section of a nanopore, dropped sharply at the first 1~2 µm inside the nanopore, indicated by the color transition........................................68

Scheme 4.2 Surface deprotonation affected by the applied electric field and the formation of an exponential gradient surface charge distribution. ..............................................................84

Scheme 5.1 Surface structures of silica surface (panels A and B) and amine surface (modified with 3-aminopropyltrimethylethoxysilane, panels C and D) at high and low pHs. .................................................94
LIST OF TABLES

Table 1.1 Comparison of biological and synthetic nanopores .......................................................... 3

Table 3.1 Simulated current at different cutline positions under ±0.4 V bias potential (outside vs. inside) in 50 mM KCl solution. The surface charge density is at -170 mC m⁻². The Cl⁻ current is consistent at each cutline: +0.4 V, i_{Cl⁻} = 3.149±0.001 nA; -0.4 V, i_{Cl⁻} = -1.055±0.001 nA. The data were retained after Q test at 90% confidence level ........................................................................................................................................... 55

Table 3.2 Summary of Simulated K⁺ and Cl⁻ Contributions to the Transport Current ........................... 58

Table 3.3 Diffusion Coefficients of K⁺ and Cl⁻ in Different KCl Solutions ............................................ 62

Table 4.1 Cation transference number based on an exponential SCD distribution .............................. 79

Table 4.2 The effective diffusion coefficient of K⁺ and Cl⁻ ions in KCl solution ................................. 90

Table 4.3 The effective diffusion coefficient of Li⁺ and Cl⁻ ions in LiCl solution ................................. 90

Table 5.1 The analysis of surface coverage of several chemically modified nanopores ...................... 100

Table 5.2 The SCD determined for different nanopores in 50 mM KCl at neutral pH (ca.6.2) ............ 101

Table 5.3 The effective diffusion coefficients of K⁺ and Cl⁻ ions in KCl solutions at different concentrations ........................................................................................................................................ 104
LIST OF FIGURES

Figure 2.1 Molecular structures of biotin (left) and 2-iminobiotin (right). .............................................. 24

Figure 2.2 (Left panel) Streptavidin binding with anchored iminobiotin. Not drawn to scale. (Blue area represents glass capillary, green triangles represent anchored iminobiotin, the purple tetramer represent streptavidin). (Middle panel) i—t of streptavidin-iminobiotin binding within a 40 nm (radius) conical nanopore in 0.1 M KCl, plus 20 mM PBS, pH 7.4. (Right panel) i—t of streptavidin-iminobiotin binding within a 20 nm (radius) conical nanopore in 0.1 M KCl, plus 20 mM PBS, pH 6.6.............................. 26

Figure 2.3 Streptavidin-iminobiotin binding within a 40 nm conical nanopore in 0.1 M KCl, plus 20 mM PBS at pH 7.4 with different streptavidin A) 0 nM (background) B) 0.2 nM C) 0.6 nM and D) 3 nM. Panel E: Concentration dependence of streptavidin-iminobiotin binding at pH 7.4... .............................................. 27

Figure 2.4 Streptavidin-iminobiotin binding within a 20 nm conical nanopore in 0.1 M KCl, plus 20 mM PBS at pH 6.6 with different streptavidin A) 0.05 nM B) 0.25 nM and C) 2.5 nM. Panel D: Concentration dependence of streptavidin-iminobiotin binding at pH 6.6... ................................................................. 28

Figure 2.5 Current change over time for streptavidin-iminobiotin binding within a 20 nm conical nanopore in 0.1 M KCl, plus 20 mM PBS at pH 7.4 with 3 nM streptavidin under -0.2 V (left panel) and -0.4 V (right panel) bias................................................................. 29

Figure 2.6 Binding sites quantification by counting “steps” and binding surface regeneration through applying opposite potential to drive bound streptavidin out of single nanopores................................. 29

Figure 2.7 (Panel A) i—t of 1:1 protein-DNA complex binding with anchored iminobiotin of a 50 nm conical nanopore in 0.1 M KCl, plus 20 mM PBS, pH 6.6 under -0.4 V applied bias. (Panel B) Magnified streptavidin-iminobiotin binding events from panel A. (Panel C) A scheme of 1:1 Streptavidin-Biotin Immobilized DNA Aptamer Conjugate. Not drawn to scale. (Green triangle represents biotin group on the DNA aptamer, curved red lines represents the single strand DNA 90mer, and the purple tetramer represents streptavidin)........................................................................................................ 31
Figure 2.8 Potential dependence of current change amplitudes of 1:1 protein-DNA complex binding with iminobiotin functionalized nanopore sensor.................................................................33

Figure 2.9 Potential dependence of current change duration of 1:1 streptavidin-biotin immobilized DNA aptamer conjugate binding with iminobiotin ..................................................................................34

Figure 2.10 Cyclic voltammogram of a nanodisk electrode in 2 mM ferrocene with 0.1 M TBAP acetonitrile solution ..................................................................................................................38

Figure 2.11 Cyclic voltammogram of a conical glass nanopore in 1 M KCl solution ........................................39

Figure 3.1 Normalized conductivity plots of a 26-nm-radius nanopore in different KCl solutions. The current was normalized on the basis of concentration, with the factor listed next to each curve: (red) 0.01 M, X100; (blue) 0.05 M, X20; (green) 0.10 M, X10; (black) 1.00 M, X1. The scan rate was 20 mV/s. 44

Figure 3.2 Normalized conductivity curves of A: 161-nm-radius B: 123-nm-radius nanopore in KCl solutions at different concentration: red 0.01 M, blue 0.05 M, green 0.10 M, black 1.00 M. The current is normalized by the factors listed next to each curve based on the concentration. The nanopore surface was modified with 3-aminopropylmethylethoxysilane, which is found to offer more reproducible measurements as previously described .................................................................47

Figure 3.3 Electric conductivity at +0.4 V (red) and -0.4 V (black) along the centerline of a 26-nm-radius nanopore in 50 mM KCl with surface charge density at -170 mC m^-2 ..........................................................48

Figure 3.4 The effects of nanopore radius and half cone angle on simulated current at +0.4 V (red square) and -0.4 V (blue square). The half-cone angle is 11.2o in Panel A at 1 M KCl solution. The radius is 26 nm in Panel B at 0.1 M KCl solution ........................................................................................................49

Figure 3.5 Concentration profiles of K^+ (red) and Cl^- (green) at (A) + 0.4 and (B) -0.4 V at cut line z = -100 nm. The x axis represents the distance away from the center line along the cut line. The radius of the nanopore is set at 26 nm with the surface charge density defined at -170 mC m^-2 (value based on Figure
The bulk concentration of KCl is 50 mM. The intercept on the concentration axis is enlarged in the inset panel.

**Figure 3.6** Concentration profiles of K⁺ (red) and Cl⁻ (green) at A: +0.4 V; and B: -0.4 V along centerline. Negative values on x axis represents the depth inside the pore, with pore orifice at zero. The radius of the nanopore is set at 26-nm with surface charge density defined at -170 mCm⁻² (value based on Figure 3.9). The bulk concentration of KCl is 50 mM. The maximum and minimum concentration of each ion (overlapped) along Z direction can be seen in the enlarged panel shown on the right.

**Figure 3.7** Spatial distribution of K⁺ and Cl⁻ concentrations inside a nanopore for a 26 nm GNP in 50mMKCl with -170 mC m⁻² SCD. The half-cross section nanopore geometry is shown at the bottom of each panel. The adaptive mesh elements are much denser near the charged interface (boundary 5). The nanopore orifice is at Z = 0 nm, and center line is at R = 0 nm. The concentration profiles inside the nanopore at 3 μm and beyond continuously extend those features shown and thus are not included. The top color scale applies to panels A and B, and the bottom color scale applies to panels C and D. Note that the brushlike features near the interface resulted from the cut-off concentration range, which was set during plotting for a better view. The absolute values at representative positions can be found in Figures 3.5 and 3.6.

**Figure 3.8** Flux distribution of K⁺ (red squares) and Cl⁻ (green triangles) at (A) +0.4 and (B) -0.4 V at cut line z = -10 nm and at (C) +0.4 and (D) -0.4 V at cut line z = -100 nm. The x axis represents the distance away from the center line along the cut line. The radius of the nanopore is set at 26 nm with the surface charge density defined at -170 mCm⁻² (value based on Figure 3.9). The bulk concentration of KCl is 50 mM. The intercept on the flux density axis is enlarged in the inset panel.

**Figure 3.9** Measured conductivity (solid black line) from a ca. 26-nm-radius nanopore in KCl solution at different concentrations. Simulation results for the 26-nm-radius nanopore in (A) 1.00, (B) 0.10, and (C) 0.05 M KCl solutions with different SCDs as indicated.
Figure 3.10 A typical adaptive free triangular mesh used in numerical simulation.

Figure 3.11 Comparison of the computed and measured current from a 26 nm nanopore in 1 M KCl solution. Black symbols represent data calculated with diffusion coefficient at infinite diluted KCl solution. The red symbols represent the data from the effective diffusion coefficient listed in Table 1. Black line is the measured current-voltage curve in 1 M KCl solution.

Figure 4.1 A. Conductivity of a 46-nm-radius nanopore in KCl (blue) and LiCl (green) solutions at different concentration: 10 mM (solid line) and 1 mM (dashed line). Scan rate was at 20 mV/s. B. The ratio of $i_+ / i_-$ (RF) versus absolute potential amplitude.

Figure 4.2 Cyclic voltammograms (solid line) and theoretical simulation (symbols) of a 46 nm nanopore in 1 M electrolyte solution. The simulation parameters for 1 M KCl (blue): -100 mC m$^{-2}$ constant SCD (circle); SCD linearly decreased from -100 mC m$^{-2}$ to -1 mC m$^{-2}$ within 0.94 µm (pentagon); -1 mC m$^{-2}$ constant SCD (triangle). The simulation parameters for 1 M LiCl (green): -120 mC m$^{-2}$ constant SCD (circle); SCD linearly decreased from -120 mC m$^{-2}$ to -1 mC m$^{-2}$ within 1 µm (pentagon); -1 mC m$^{-2}$ constant SCD (triangle).

Figure 4.3 The simulation of the experimental current from a 46-nm-nanopore in 10 mM KCl with different SCD definitions on the interior surface. A. with a constant SCD uniformly distributed. B. an exponentially decreased SCD, with $\sigma_o$=-100 mC m$^{-2}$ and the distribution length varied as indicated in the plot. C. a linearly decreased SCD, with $\sigma_o$=-100 mC m$^{-2}$ and the distribution length varied as indicated in the plot.

Figure 4.4 The simulation of the experimental current from a 26-nm-nanopore in 50 mM KCl with an exponentially decreased SCD, with $\sigma_o$=-170 mC m$^{-2}$ and the distribution length varied as indicated in the plot.

Figure 4.5 The optimized simulation of the measured $i-V$ curves with a 46 nm nanopore based on an exponential SCD distribution in A. KCl and B. LiCl solutions. The solid curves were measured
experimentally and the symbols were from the simulation. Fitting parameters: \( \sigma_o = -100 \text{ mC m}^{-2} \) and \( \tau = 0.4 \mu\text{m} \) for 10 mM KCl; \( \sigma_o = -70 \text{ mC m}^{-2} \) and \( \tau = 1.46 \mu\text{m} \) for 1 mM KCl; \( \sigma_o = -120 \text{ mC m}^{-2} \) and \( \tau = 0.4 \mu\text{m} \) for 10 mM LiCl; \( \sigma_o = -70 \text{ mC m}^{-2} \) and \( \tau = 1.46 \mu\text{m} \) for 1 mM LiCl.

**Figure 4.6** The optimized simulation of the measured \( i-V \) curves with a 46 nm nanopore based on a linear SCD distribution in A. KCl and B. LiCl solutions. The solid curves were measured experimentally and the symbols were from the simulation. Fitting parameters: \( \sigma_o = -100 \text{ mC m}^{-2} \) and \( \tau = 0.94 \mu\text{m} \) for 10 mM KCl; \( \sigma_o = -70 \text{ mC m}^{-2} \) and \( \tau = 3.6 \mu\text{m} \) for 1 mM KCl; \( \sigma_o = -120 \text{ mC m}^{-2} \) and \( \tau = 1 \mu\text{m} \) for 10 mM LiCl; \( \sigma_o = -70 \text{ mC m}^{-2} \) and \( \tau = 3.6 \mu\text{m} \) for 1 mM LiCl.

**Figure 4.7** Cyclic voltammogram (solid line) and optimized theoretical simulation (red squares) of a 26 nm nanopore (left panel) in 50 mM KCl and a 110 nm nanopore (right panel) in 100 mM KCl based on an exponential gradient SCD. Fitting parameters: \( \sigma_o = -170 \text{ mC m}^{-2} \), \( \tau = 0.6 \mu\text{m} \) for the 26 nm nanopore; \( \sigma_o = -480 \text{ mC m}^{-2} \), \( \tau = 2 \mu\text{m} \) for the 110 nm nanopore.

**Figure 4.8** Error analysis of the maximum SCD \( \sigma_o \) at the nanopore orifice and the distribution length \( \tau \) in the simulation of \( i-V \) measurements of a 46 nm GNP in 10 mM KCl. A. high conductivity states at +0.4 V. B. low conductivity state at -0.4 V. Different colors represent different \( \sigma_o \): -90 mC m\(^{-2}\) (blue); -100 mC m\(^{-2}\) (red); -110 mC m\(^{-2}\) (green). Solid symbols represent the simulation based on an exponential SCD distribution, and open ones representing a linear SCD distribution.

**Figure 4.9** The impacts of distribution length on the simulated current at A: high conductivity state at +0.4 V and B: low conductivity state at -0.4 V. Data collected from a 46 nm nanopore in 1 mM KCl with maximum SCD set at -70 mC m\(^{-2}\).

**Figure 4.10** The concentration profiles along radial direction of K\(^+\) (blue) and Cl\(^-\) (red) at A: + 0.4 V; and B: -0.4 V at cutline \( z = 100 \) nm. The x axis represents the distance away from the centerline along the cutline. The radius of the nanopore is set at 46-nm with surface charge density defined at -100 mC m\(^{-2}\) to
-1 mC m$^{-2}$ within 0.4 μm (value based on Figure 4.3). The bulk concentration of KCl is 10 mM. The intercept on the concentration axis is enlarged in the inserted panels. ..........................................................81

**Figure 4.11** Concentration profiles of K$^+$ (red) and Cl$^-$ (green) at A: + 0.4 V; and B: -0.4 V along centerline. The radius of the nanopore is set at 46-nm with surface charge density defined at -100 mC m$^{-2}$ to -1 mC m$^{-2}$ within 0.4 μm (value based on Figure 4.3). The bulk concentration of KCl is 10 mM (indicated by black dash line). The concentration of K$^+$ and Cl$^-$ overlapped. ..........................................................82

**Figure 4.12** Electric field intensity distributions (solid lines) for a 46 nm conical nanopore with neutral surface in 10 mM KCl. (A) centerline and (B) a parallel cutline 3 nm from the interior surface at -0.4 V. The dash lines are exponential fittings of the electric field intensity profiles. Electric field intensity distributions through the whole pore are shown in inserted panels. Pore depth at 0 and 10 μm correspond to the location of orifice and base respectively. ..........................................................83

**Figure 4.13** Electric field intensity distributions (solid lines) for a 46 nm conical nanopore with neutral surface in 10 mM KCl. (A) centerline and (B) a parallel cutline 3 nm from the interior surface at -0.2 V. The dash lines are exponential fittings of the electric field intensity profiles. Electric field intensity distributions through the whole pore are shown in inserted panels. Pore depth at 0 and 10 μm correspond to the location of orifice and base respectively. ..........................................................83

**Figure 4.14** Electric field intensity distributions (solid lines) for a 46 nm conical nanopore with neutral surface in 10 mM KCl. (A) centerline and (B) a parallel cutline 3 nm from the interior surface at +0.4 V (red) and +0.2 V (blue). Pore depth at 0 and 10 μm correspond to the location of orifice and base respectively. ........................................................................................................................................85

**Figure 4.15** The electric field intensity along the cutlines parallel to nanopore interior surface and the comparison with the SCD profile (dash line). The results are from a 46 nm nanopore in 10 mM KCl. The overall electric field $E_{tot}$ is divided by the listed factor for direct comparison of the gradient distribution. ........................................................................................................................................86
Figure 4.16 The electric field intensity along the centerline and the comparison with the SCD profile (dash line). The results are from a 46 nm nanopore in 10 mM KCl. The overall electric field \( E_{tot} \) is divided by the listed factor for direct comparison of the gradient distribution. ................................. 86

Figure 4.17 The electric field intensity along the cutlines parallel to nanopore interior surface and the comparison with linear SCD profile (dash line). The results are from a 46 nm nanopore in 10 mM KCl. The overall electric field \( E_{tot} \) is divided by the listed factor for direct comparison of the gradient distribution. ............................................................................................................. 87

Figure 4.18 The variation of nanopore geometry near the pore orifice. The dash line indicates the geometry before distortion. ........................................................................................................................................... 95

Figure 5.1 The \( i-V \) curves from a 32-nm-radius nanopore in 50 mM KCl pH 3 (red) and pH 9 (blue) solutions. (A) silica surface and (B) surface modified with 3-aminopropyldimethylethoxysilane. The scattered symbols represent the simulated current computed from the optimized surface charge parameters discussed later. The dashed line represents the volume conductivity calculated based on geometric resistance in 50 mM KCl. ........................................................................................................................................... 97

Figure 5.2 The \( i-V \) curves from a 50-nm-radius nanopore before (silica surface) and after (amine surface) modification with 3-aminopropyldimethylethoxysilane in 50 mM KCl solutions in different pH conditions. The solution pH was determined by a pH meter and adjusted by the addition of concentrated HCl or KOH solutions. The dash line represents the volume conductivity of the same nanopore geometry in 50 mM calculated based on pure geometric resistance........................................................................................................... 98

Figure 5.3 Correlation between the simulated current and SCD at +0.4 V applied potential for a 32-nm radius nanopore in 50 mM KCl. A linear fitting \( (R^2 = 0.99) \) is shown as the red dashed line. ................................. 98

Figure 5.4 The correlation between the simulated current and SCD at +0.4 V applied potential of a (A) 26-nm-radius and (B) 110-nm-radius (silica surface, without modification) nanopore in 50 mM (red); 99
mM (green); 200 mM (blue) and 500 mM (magenta) KCl solutions. Linear fittings for each concentration are indicated by dashed lines.

Figure 5.5 The correlation between the simulated current and SCD at +0.2 V applied potential of a 26-nm-radius (silica surface, without modification) nanopore in 50 mM (red); 100 mM (green); 200 mM (blue) and 500 mM (magenta) KCl solutions. Linear fittings for each concentration are indicated by dashed lines.

Figure 5.6 Site density (SCD: the combination of deprotonated silanol groups and protonated amine groups) of the nanopores with different radii with (top panel) amine surface (surface modified with 3-aminopropyl(dimethyl)silane) and (bottom panel) silica surface in 50 mM KCl at different pHs. The site density is calculated based on the SCD determined from the experimental current trajectory from the linear trend for each nanopore at +0.4 V applied potential.

Figure 5.7 A typical adaptive free triangular mesh used in numerical simulation.
1 INTRODUCTION

What is a nanopore or nanochannel? A nanopore or nanochannel is a small pore or channel connecting two solution reservoirs with the radius less than 100 nm in at least one dimension. Nanopores and broadly defined channel-type nanodevices have attracted extensive research interest in the past two decades. The nanopores were proposed as a potential tool for rapid DNA sequencing in the 1990s.\(^1\) Because different DNA bases have different conductivities, the DNA bases might be read out sequentially (one by one base) when single strand DNA passes through the nanodevices in a linear form if sufficient resolution could be reached. The first nanopore based experimental work was reported by Kasianowicz and co-workers in 1996,\(^2\) using a biological nanopore: \(\alpha\)-haemolysin, which is a transmembrane protein embedded in a lipid bilayer membrane separating two solutions. The translocation of nucleotides through \(\alpha\)-haemolysin was indicated by the transient blockage of ionic current compared to a clean background without nucleotides as the analyte. Extensive work has been done based on the nanopore formed in the \(\alpha\)-haemolysin molecule situated in a lipid bilayer membrane.\(^3\)\(^-\)\(^6\)

For bimolecular sensing based on biological nanopores, \(\alpha\)-haemolysin has been widely used based on its stability and excellent performance under physiological conditions. To accommodate for more harsh measurement conditions, synthetic solid-state nanopores have been developed. Extensive work has been done based on solid-state nanopores which allow easier handling for measurement. Unlike \(\alpha\)-haemolysin where the narrowest part only allows the translocation of ss-DNA, ds-DNA transport through a solid-state nanopore has been first reported by Golovchenko’s group.\(^7\) Due to rapid developments on fabrication and characterization of synthetic solid-state nanopores, explosive progresses have been achieved on studying ss/ds-DNA translocations.\(^8\)\(^-\)\(^15\) The difference between polyA and polyC RNA can be recognized using a nanopore.\(^6\)\(^,\)\(^16\) The difference between A and C bases within an RNA molecule
with a heterogeneous sequence of $A_{30}C_{70}$ could also be distinguished. These achievements make nanodevices promising for DNA sequencing.

Synthetic nanopores and $\alpha$-haemolysin based biological nanopores can be used for single molecular sensing and ultimately developed as a rapid and economical tool for DNA sequencing. Meanwhile, nanopores and other channel-type geometries, also have potential application in drug delivery, electrochemical energy conversion, nanofiltration, purification and catalysis, concentration and separation, nanofluidic electronics, nanoprecipitation and so on.

Besides the above discussed sensing applications at single molecular level, novel mass transport properties of nanodevices also attracted tremendous interest. Mass transport inside nanometer scale devices is determined by both geometry and solution-substrate interface factors (i.e. coulomb interactions between solution ions and surface charges). At high surface to volume ratio, a significant contribution from surface leads to several important properties of nanodevices, such as ion current rectification (ICR),\textsuperscript{17,18} memristive and memcapacitive behaviors. These novel properties suggest nanopores and other nanodevices to be fundamentally important for the enhancement of electrochemical energy conversion efficacy in batteries and supercapacitors,\textsuperscript{19-21} and to be used as nanoelectronics.\textsuperscript{17,22}

For mass transport at nanometer scale dimension, due to the high surface to volume ratio, the surface will contribute even dominate the mass transport behavior in comparison to volume effect, which complicates the interpretation of nanodevices based stochastic sensing and ICR, memristive and memcapacitive behaviors. To obtain a better understanding of the mass transport behavior at nanoscale geometries, theoretical simulation has been implemented. The physical origins of ICR have been explained based on the non-uniform ion distribution induced by asymmetric nano-geometries with charged surface, heterogeneous surface charge distribution within a nano-geometry etc. Massive theoretical works with a constant SCD using finite element method based on continuum theory have been done.\textsuperscript{23,24,25}
1.1 The fabrication of nanochannels and microchannels

1.1.1 Biological ion channels

Some protein ion channels are suitable for single molecule sensing due to their natural channel dimensions being comparable to the size of targeted analytes. Among those biological nanochannels, α-haemolysin situated in a lipid bilayer is by far the most widely used for studying DNA translocations.\(^1\),\(^3\),\(^6\),\(^26\)

α-haemolysin is a transmembrane protein, with a diameter of 1.4 nm at the narrowest part. Thus α-haemolysin based nanochannels will only allow translocation of single-strand DNA due to this 1.4 nm dimension limitation at the nanochannel center, double-stranded DNA (~2 nm diameter) has larger dimensions than the pore and is unable to pass through.

Due to limitations of biological nanopores, summarized in table 1.1, synthetic nanochannels and microchannels have been developed which can be superior to biological nanopores in several aspects.

<table>
<thead>
<tr>
<th>Property</th>
<th>Biological nanopores</th>
<th>Synthetic nanopores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Fixed</td>
<td>Flexible</td>
</tr>
<tr>
<td>Surface</td>
<td>Limited</td>
<td>Adjustable</td>
</tr>
<tr>
<td>Analytes</td>
<td>Limited</td>
<td>Versatile</td>
</tr>
<tr>
<td>Stability</td>
<td>Lipid bilayer concerns; Protein stability</td>
<td>High</td>
</tr>
<tr>
<td>Condition</td>
<td>Sensitive to concentration, pH, temperature, external pressure</td>
<td>Flexible</td>
</tr>
<tr>
<td>Application</td>
<td>N/A</td>
<td>Integrated into devices and arrays</td>
</tr>
</tbody>
</table>

1.1.2 Microchannels

In the past thirty years, microchannels with dimension less than 100 µm have attracted extensive interests. Microfluidics, also known as lab-on-a-chip technology is a technique that requires precise control and manipulation of fluids at micro liter scale, has shown broaden applications in biology, chemistry, medicine and engineering.\(^27\)-\(^32\) Believing in the great impacts of microfluidics based high-tech tests
for developing countries, Whitesides at Harvard University has been dedicated to developing microfluidics based low-cost medical diagnostic devices. Microfluidics devices are created using silicon, plastic chips and finally paper is chosen due to its cheap price and is easily disposed by incineration which is convenient for medical waste disposal.\textsuperscript{33-37} Meanwhile, Ramsey at UNC has done a lot of work on microfluidics based MSI-MS,\textsuperscript{38,39} sample filtration, concentration and separation,\textsuperscript{40-42} and single cell analysis.\textsuperscript{43}

Microchannels are mostly fabricated based on ion beam lithography, electron beam lithography and photolithography.\textsuperscript{44-46} Basically a convex three-dimension mold is created by shattering a beam of ions/electrons or photons onto a positive resist film supported by substrate, with the desired part covered by a mask. The obtained mold is then coated with a layer of negative resist. Later the mold and the support substrate are etched away, which leaves the designed geometry with micrometer dimension in the coated film replicating the shape of mold. Polydimethylsiloxane (PDMS) and other polymers are mainly used in this soft lithography, as polymer based fabrication is easier and flexible, also the price is much cheaper than silicon and glass.

Other fabrication techniques include reactive ion etching (RIE), laser ablation and imprinting. RIE is also widely used for microfluidics fabrication, but with quite limited materials (glass, quartz and silicon). Microchannels made of glass by using a mixture of acid solution is used to etch the glass while other parts covered with metal masks have been reported.\textsuperscript{47,48} Laser ablation, similarly to ion beam lithography, involves a shining of laser to polymer substrate which results in polymer decomposition. A cavity is created as decomposition products (CO\textsubscript{2}, CO and C\textsubscript{2}H\textsubscript{2}) are ejected.\textsuperscript{49} Another simple microfluidics fabrication is called imprinting. Generally a micrometer size wire is pressed into a polymer substrate, results in a microchannel in the polymer substrate replicating the wire geometry.\textsuperscript{44}

\subsection{1.1.3 Nanochannels}

As summarized in Table 1.1, synthetic nanochannels/nanopores are more resistive to harsh measurement conditions. They also have controllable size/geometries, high stability, and adjustable sur-
face, rendering them with high versatility for targeting various analytes. Most important, synthetic solid-state nanodevices are capable of being integrated into devices and arrays. All of these advantages have stimulated the development of synthetic nanodevices.

Synthetic nanopores can be made from different kinds of materials such as silicon oxide (SiO$_2$),$^{50,51}$ silicon nitride (SiN),$^7$ aluminum Oxide (Al$_2$O$_3$),$^{52}$ polymers$^{22}$ and graphene.$^{53}$ The solid state nanopores are more robust compared to biological nanopores that are sensitive to pH, pressure and temperature. Recently a lot of success in preparing synthetic nanopores has been achieved. Similar to microchannels, nanochannels are mostly fabricated based on ion beam lithography, electron beam lithography and photolithography as the mold size can be controlled down to nanometer scale resolution. Typically these lithography based fabrication methods included: 1. “Track-etch” method$^{22,54}$, and 2. “Ion-beam sculpting” method$^7$.

The “Track-etch” method uses commercially available polymer membrane with track damages created by shooting heavy ions through a polymer membrane. Then the damaged polymer membrane is etched in alkaline solution on one side, while on the other side a neutralizing electrolyte is used, resulting in a conical nanopore.$^{22}$ The “Ion-beam sculpting” method is based on a solid-state insulating membrane with a cavity on surface. An ion-beam is applied from the other side to remove membrane materials until at the bottom of cavity a through pore is formed.$^7$ The sculpting process is monitored by computer. When a pore is obtained, single ions will transport through the pore and will be detected by the detector. Then the ion beam sculpting process will be stopped immediately.

Besides these lithography based fabrication techniques, nanochannels can also be obtained through the fabrication of carbon nanotubes and other nanotubes (titanium oxide, gold etc.)$^{55-59}$. Carbon nanotubes can be fabricated based on chemical vapor deposition (CVD), where nanotubes will be grown out of crystals under the effects of a furnace and gas flow.
In this dissertation, the “Bench-top” method is used for fabricating single conical glass nanopores using a glass capillary. Basically, a nanopore with pore opening less than 100 nm and a length around ~10 µm is obtained by replicating the geometry of an electrochemically sharpened Pt nanotip.

1.1.4 Arrays and membranes

The techniques mentioned above are used for fabricating single microchannels or nanochannels. To gain a high throughput, arrays of microchannels/nanochannels are created, which normally uses a nanochannel array mold created by electron beam lithography and ion beam milling. Nanochannel arrays are fabricated by pressing a nanochannel array mold into a resist film resulting geometry in the film replicates the shape of the mold. Based on this concept, Chou’s group introduced a nanochannel array fabrication method using “nanoimprinting lithography (NIL)”. The mold is fabricated by NIL techniques with channel size can be manipulated down to 10 nm. Nanochannels fabricated without using NIL techniques has been reported recently. Basically, A layer of nanometer thin amorphous silicon film deposited and etched using RIE, followed by thermal oxidation. Solid channels with the outside to composed of SiO$_2$ of nanometer scale dimension (inside is silicon) are created in the film. Further deposition of amorphous silicon and subsequent chemical-mechanical polishing will expose the gap oxide. Nanochannels are obtained after etching away SiO$_2$. Nanotube based arrays are also achieved using an anodic alumina (AAO) template. Polypyrrole is deposited into the AAO template and further etching of AAO resulted in a gap between the polypyrrole nanowires and AAO. Further deposition of gold and subsequent etching of polypyrrole and AAO results in a gold nanotube array.

Meanwhile, nanoporous membranes have been developed, which are widely used in drug delivery, filtration, and purification. For example, Martin and co-workers reported a carbon nanotube membrane fabricated using a commercial available alumina membrane template by CVD.
1.2 Stochastic sensing based on nanodevices

1.2.1 The Coulter Counter

Nanochannels have been extensively studied due to the nanometer-scale and the resulting novel mass transport behavior. The main motivation is the potential application in stochastic single molecule sensing based on the Coulter Counter concept. The Coulter Counter was patented by W. H. Coulter in 1953. A typical Coulter counter set up includes one or more microchannels or nanochannels separating two chambers of electrolyte solutions. With an external applied potential, particles present in electrolyte solutions are driven through channels. The measured current is non-Faradic current, which results from the transport of ions (charge carriers) through the most resistive region of the channels. Thus when particles with comparable size transport through the channels, they will partially block the geometry, which results in a significant transient ionic current blockage being detected/counted. Based on this concept, nanopores and other nanochannels based stochastic sensing allows a single molecule resolution due to nanometer scale dimension which is comparable to single molecules.

1.2.2 Polymer sensing

Nanopores were first proposed as a potential rapid and economical tool for DNA sequencing. The first experimental work was done by Kasianowicz and co-workers in 1996. The homo-polymers are driven through the biological nanopore in a α-haemolysin molecule under external applied potential. Before adding analytes, mobile ions are electrophoretically driven through the nanopore resulting a constant current (background/baseline), which is limited by the most resistive (the smallest) region. When polymers or other analytes are added into solution, they will be driven into the biological nanopore also. As their sizes are comparable to the nanopore, their translocation will partially block the pore. Thus mobile ions transported through the channel will decrease, causing the current to decrease. After analytes pass through, the current will be restored to the baseline value. Each transient passage of
analytes gives an ionic current blockage event. The current blockage frequency indicates analytes concentration: at high concentration, more analytes are present in solution and transported through the nanochannels, thus more events will be observed within the same time. Meanwhile, the duration of these transient current blockages resulting from molecule binding also provides the binding kinetic information. Different current blockade events induced by translocation of different polymers using α-haemolysin protein nanopore are reported.\textsuperscript{6,16,70} The correlation between induced current blockages and polymer composition, translocation orientation and applied bias has been studied by Gundlach and co-workers.\textsuperscript{70} Recently, they demonstrated DNA sequencing at single nucleotide resolution using a mutated MspA nanopore with phi29 DNA polymerase controlling the translocation velocity.\textsuperscript{71}

Different noises associated with the measured current signals are observed for different PEG polymers using α-haemolysin based nanopore, which suggest an interaction between channel lumen and PEG polymers.\textsuperscript{72,73} The polymer-lumen interaction was showed to be controlled by pH which gives a clue to optimize stochastic sensing.\textsuperscript{73} The translocation duration of different polymers has been studied by Branton and co-worker,\textsuperscript{6} and the translocation duration showed a significant temperature dependence. Biopolymer analysis based on nanochannel arrays has been patented recently.\textsuperscript{74}

\subsection*{1.2.3 Nucleic acid sensing}

As nanopores and nanochannels were mainly proposed as a potential DNA sequencing tool, extensive work based on DNA detection and characterization have been reported.\textsuperscript{6,8,9,11-14,16,75-78}

Since the smallest region of α-haemolysin is only 1.4 nm in diameter, α-haemolysin based nanopores only allows the translocation of single-stranded DNA.\textsuperscript{6,76} Different DNA segments have been driven electrophoretically through a nanopore, giving different translocation durations. The measured translocation duration time is reported to be temperature dependent. Due to the limitations of biological nanopores, synthetic nanopores are developed. Martin and co-workers demonstrate the translocation of single strand phage DNA through a conical nanopore.\textsuperscript{12} The frequency of the ionic current block-
age induced by transient passage of DNA is demonstrated to be concentration dependent. Meanwhile, the duration of transit current change is reported to depend on the external applied transmembrane potential. A much shorter duration of current blockage was observed when detecting a double-stranded DNA; this is explained by the collision of double-stranded DNA with the nanopore orifice as the nanopore used is smaller than double-stranded DNA.

The transport of single strand DNA molecule labeled with nanoparticles through a glass nanopipette has been reported. The nanopipette is fabricated by mechanically pulling a glass capillary upon heating by a laser. The conjugate is often found to transiently enter the pore without a full translocation. Different translocation events are observed when DNA is transported through a ion beam sculpted nanopore in different conformations. The current change amplitude and the corresponding duration of translocation events induced by DNA translocation through a nanopore in different conformations are different. The DNA translocation in unfolded form is found to increase as potential increase and as DNA length increase.

With the development of solid-state nanopore fabrication, translocation of double-stranded DNA through a 5 nm (diameter) nanopore has been achieved. While most analyte translocation through nanopores/nanochannels are under a constant applied potential, DNA transport through a nanopore using a AC signal has also been reported.

1.2.4 Protein, nanoparticle and other molecule sensing

Besides nanopore based polymer and DNA sensing, both biological and synthetic solid-state nanopores have been used for the detection of proteins, nanoparticles, drug molecules, ions and bio-interactions.

Li’s group reported protein characterization based on a single silicon nitride nanopore. BSA and fibrinogen are differentiated based on different ionic current blockages induced by the two different analytes. The relative size and charge of BSA have been estimated based on the mean amplitude,
time duration and integration of current change respect to time. The size of BSA is also confirmed by the current changed induced by BSA translocation through a 55 nm (diameter) nanopore. White’s group reported translocation of nanoparticles with different radius (80 nm and 160 nm) which consequently induce transient current blockages with different amplitude. Nanoparticle detection using carbon nanotubes have also been reported, the size and surface charge of nanoparticles are determined simultaneously.

A similar approach could be used for detecting organic molecules. Organic molecules sensing based on a protein channel with a molecular adapter (cyclodextrins) located inside the nanochannels through non-covalent binding have been reported by Bayley and co-workers. Different ions are differentiated based on a mutated a-haemolysin nanochannel. Characterization and quantification of divalent metal ions are achieved by using a mutated heteromeric α-haemolysin protein which allows metal ion binding.

1.2.5 Advantages of channel-type nanodevice based sensing

Many analytical tool and methods have been developed in the past, for example, fluorescent spectrometry, high performance liquid chromatography (HPLC), and electrochemical analysis etc. By repeated sampling and/or signal amplification, the sensitivity of these techniques can reach as high as picomole/femt mole or even higher. As a chemical or biological molecule is defined by a certain composition, the ultimate sensitivity to detect or analyze a sample would be a single molecule. Compared to these ensemble techniques, the most striking advantage of nanopore based stochastic sensing is the single molecule sensitivity rendered by the nanometer dimension. While for ensemble techniques, this ultimate sensitivity is still missing as the detected signal from a unique molecule is screened by thousands of other molecules in the sample. In addition to this major advantage, other advantages like application in heterogeneity analysis, label-free analytes, reusability and potential application for DNA sequencing etc., all of these have stimulated significant interest in nanopore study.
1.2.5.1 The ultimate sensitivity-single molecule detection

Since the first experimental work done by Kasianowicz using α-haemolysin, stochastic single molecule behaviors have been demonstrated. For nanopore based sensing, the single molecular sensitivity results from their nanometer scale dimensions. The detected current signal results from the ion movement through the nan confinement. If the nanodevice has a signal limiting dimension comparable to the targeted analytes, then the analytes passing through the nanopore orifice will partially block the ion transport, thus resulting in a significant current change to be detected. That is why nanopores and channel-type nanodevices can be used for single molecule sensing. If the size of the analyte is larger than nanopore dimension, the analyte will fully block the pore resulting in no background current. The ability to resolve analyte at the single molecule limit has applications such as clinical diagnosis.

1.2.5.2 Heterogeneity analysis

Heterogeneity analysis is significantly important for human life. For example, cancer therapy, which requires the early detection and characterization of mutated biomolecules. Resolution at the single molecule limit by nanopore based sensing is the prerequisite for applications in heterogeneity analysis. As the presence of analyte in solution is indicated by the transient ionic current blockage, the heterogeneity of sample can be analyzed. Typically, the ionic current blockage can be described by two parameters: current change amplitude $\Delta i$ and time duration $\tau$ for each transient translocation event. The current change amplitude indicates size and charge state of analytes. The time duration $\tau$ is the time it takes analyte to traverse the most sensitive (current limited) region, which probably indicates the analytes structure/conformation. If the current change is induced by single molecule binding confined in nanopores, then the time duration $\tau$ indicates the binding ability. If different analytes go through nanopores or bind to surface immobilized receptors, the two parameters (amplitude $\Delta i$ and time duration $\tau$) will be different, which allows the sample heterogeneity analysis.
1.2.5.3 Label-free analytes

Nanodevice based sensing is widely studied not only for single molecule sensitivity and potential application in analyzing sample heterogeneity, but also for label-free analyte detection. Unlike other single molecule detection techniques such as fluorescence imaging or radioisotope labeling, the analyte is detected in the native state without any modification. Analytes present in solution can be driven by the externally applied field due to electrophoresis if they are charged under the measurement conditions. Since no analytes labeling/modification is involved, the nanodevice based sensing often requires less sample and less time consuming sample preparation, thus it is more efficient and economical compared to techniques which require labeling. More important, as the analytes are detected in their native state, this excludes signal distortion introduced by molecule labeling or mutation.

Besides the sensitivity to detect single molecules, the sample heterogeneity analysis, and the practical advantages in label-free analyte handling, nanodevices possess other advantages that make them a powerful sensing tool. For example, fabricated solid-state nanodevices can be durable and reusable. Furthermore, as solid-state nanopores and nanodevices can be integrated into devices and arrays, sensors based on solid-state nanodevices can be made as portable devices, as the whole set up simply involves an external applied electric field through the sensing part. This is overwhelmingly competitive than other related tools.

1.2.6 Challenges in DNA sequencing

Resolution at the single molecule level has been achieved in stochastic sensing applications using various nanodevices. The feasibility and efficacy of nanopore based rapid and low-cost DNA sequencing have been demonstrated. Significant progresses have been achieved. However, there are still challenges to be resolved.

Typical biological nanopore systems involve a protein situated in a lipid bilayer membrane. The main challenges for biological nanopores arise from the stability issues of protein and lipid bilayers. Lipid
bilayers are fragile. Meanwhile, lipid bilayers are sensitive to solution pH, electrolyte concentration, pressure and temperature, thus do not last long. All of these concerns need to be resolved.

On the one hand, the detected intrinsic low current signal through nanodevices allows resolution of a single molecule. On the other hand, as the current is so small any noise from surrounding environment arise as a major concern. Nanodevice performance can be improved through data averaging which minimizes the noise. Further efforts in eliminating surrounding noise are still required.

To resolve a single base, the translocation duration of DNA through a nanopore should be equal or larger than 1 millisecond based on Deamer and Branton’s report. Solutions for these concerns are urgently needed. Recent work done by Gundlach and co-workers has demonstrated the ability to control the DNA translocation at millisecond scale mediated by phi29 DNA polymerase inside a mutated MspA nanopore.

1.3 Microchannels/Nanochannels in other applications

Besides single molecule detection, nanochannel devices have potential applications in many areas such as nanofluidics, nanoelectronics, concentration and separation, drug delivery, electrochemical energy conversion, and controlled nanoprecipitation.

1.3.1 Nanofluidic electronics

Siwy, Martin and coworkers have pioneered studies of mass transport behaviors through asymmetric nanochannel/nanopore devices. Controlled mass transport is achieved by changing the geometry and surface charge distribution inside those nanodevices. For example, a bi-conical nanopore with the narrowest part positioned at the center has been created. Bipolar surface charge has been introduced in a conical nanopore, with positive surface charges on one end and negative charges on the other end. A typical diode-like current-potential behavior has been observed in nanopores with different geometries. In the electronics industry, a solid-state diode carries a higher current under one potential
polarity but allows a very low current to pass through at the opposite bias polarity. For a conical nanopore with a bipolar surface charge distribution, the “on” and “off” state can be switched by reverting the distribution of opposite surface charges. The diode behavior can also be generated using a cylindrical nanochannel with a unipolar surface charge distribution, where the channel is chemically modified so that one side is positively charged while the other side is neutral. The control of mass transport through solid nanodevices by designing the geometry and inner surface charge distributions enables their application in nanofluidics and nano electronics.

1.3.2 Concentration and separation

Recently, Crook’s group reported the application in separation and electrolyte concentration enhancement by bipolar electrodes (BPEs) created inside microchannels or nanochannels. A bipolar electrode is composed of an electric conductor, normally a long nanowire in a conductive ionic environment inside a nanochannel/microchannel. Electron transfer processes, reduction or oxidation will occur on either end of the conductor when a high electric field is created between the two ends of the conductor. The conductor is normally supported on the insulating channel interior surface. Therefore there is no direct connection between the conductor and external power supply. High throughput analysis can be achieved with such simplified design. The ability of BPEs to change the local electric field allows species to concentrate locally, which is the prerequisite for the separation of analytes. Since the mobility of different electrolytes is different, the position of molecule concentration zone for different electrolytes will be different in the channel, thus separation of analytes can be achieved. In the paper, 1, 3, 6, 8-pyrene tetrasulfonic acid (PTS\(^4\)), 8-methoxypyrene-1, 3, 6-trisulfonic acid (MPTS\(^3\)) and BODIPY\(^2\) were separated based on the respective electrophoretic mobility. Other nanodevice based separations are reported.
### 1.3.3 Drug delivery

Another important application of nanodevices is the application in drug delivery. The type of nanopore membrane materials include silicon, poly (ethylene terephthalate) (PET) and polycarbonate (PC) polymers, and etc. The nanopores are combined with a drug storing reservoir first. This combined device is then implanted through direct injection using a specially designed trochar. A pump with a titanium chamber that can store drugs at a volume about 75-300 ml is introduced. The rigid titanium shell allows easy implantation and removal, and titanium is resistive to body fluids for protecting the stored drugs. At one end of the titanium chamber a silicon nanopore membrane is attached. The nanopore membrane is used for controlled drug delivery. Note if the nanopore size approaches the dimensions of the drugs molecules, the release is not simply concentration-dependent, as the diffusion based on Fick’s law of diffusion is not the sole transport mechanism. The size and density of nanopores in the membrane could be designed for accommodating release of different drugs based on their sizes, molecular structures, and other related properties. The nanopore pump is superior compared to the commercially accessible Viadur/Duros devices, in which the drugs are released by an osmotic engine, thus limiting the volume for drug storage.

### 1.3.4 Electrochemical energy conversion

Most electrochemical energy conversion happens at the electrode surfaces. To increase the energy density for better device performance, high surface area electrodes are desired. Due to the high surface to volume ratio at nanometer scale, the transport of cations and anions as charge carriers, carrying the total current and quantified by the respective ion transference numbers, can be significantly affected by the nanostructured geometries and interfaces. The selective transport of either cation or anion could cause internal energy loss of energy devices. Fundamental understanding of ion transport through nanostructures could lead to rational design of energy devices with enhanced performances.
Kamat has reported a new generation of solar energy conversion device by using nanostructures. Three major ways are proposed: 1. Donor-acceptor assemblies for mimicking photosynthesis. 2. Fuel production through semiconductor assisted photocatalysis. 3. Solar cells based on semiconductors made of nanostructures. In each of those routes, the transport of charges will be significantly affected by the nanostructures thus affecting the energy harvesting efficiency.

Recently, Jiang and co-workers reported an energy harvesting device by using a charged nanochannel. The maximum power for a single nanochannel is reported to be ~26 pW. A concentration gradient at the two ends of a nanochannel with negatively charged surface is firstly established. This device then converts Gibbs free energy in the form of diffusion current induced by concentration gradient into electric power. The obtained electric power shows a pH dependence, which allows for further optimization for.

### 1.3.5 Spatially confined nanoprecipitation

If the electrolyte concentration is near saturation, the current measured using a nanopore will oscillate due to the precipitation of electrolytes at the current limiting nanopore region. The electrolyte system being tested included as CaHPO₄ and CoHPO₄. Theoretical analysis suggests that the precipitation is induced by the ions enrichment and subsequent nanoprecipitation. Further systematic investigation is needed.

### 1.4 Novel properties of nanodevices

Two types of novel mass transport behaviors through nanopores will be introduced next: 1. Steady-state ion current rectification and 2. Transient memristive and memcapacitive ion transport. Both result from a combined asymmetric nanogeometry and electrostatic interactions between surface charges and mobile ions (surface effects) due to the high surface to volume ratio at nanometer scale dimensions.
1.4.1 Ion current rectification

The substrate used to fabricate the nanopore/nanochannel devices impact the mass transport behavior due to the surface properties of the material. In the case of glass, the deprotonation of silanol groups results in negatively charged surface at the solution/substrate interface. At nanometer dimensions, the mass transport behaviors within nanoscale pores and channel devices will be affected by the electrostatic interaction between the fixed interfacial charges on the surface and the mobile ions in solutions. The ionic transport through these nano-geometries are not only affected by geometry/volume but also by the fixed interfacial charges from surface, the measured current-voltage curves deviate away from the well-known linear ohm’s behavior (V=I*R) and show non-linear curvatures. In other words, current measured at one potential doesn’t equal to those measured at the same potential amplitude with opposite polarity, which is well known as ion current rectification (ICR). This convoluted overall current-potential behavior, and the attempts to explain the physical origins of this ICR phenomenon, have led to several qualitative models that have been summarized in recent reviews. Briefly, the rectification behavior has been explained by potential ratchet, inhomogeneous conductivity, or ion mobility differences in the nanopore region as a result of the combined volume and surface effects.

ICR is characterized using ICR ratio, which is the ratio of current measured at the same bias amplitude but opposite polarities. The ICR is concentration dependent. The linear Ohm’s law determines the current-potential responses at high concentration because the surface effects are mostly screened (ICR ratio=1). If concentration is lowered, the surface effects are less screened, thus ICR shows up (ICR>1). However, as concentration further decrease, ICR ratio will reach a maximum then decrease due to the limitation of available counter ions for interfacial surface charge neutralization. ICR can be engineered through geometry and surface design. A diode-like behavior based on a polymer nanopore has been reported through artificially designing the charge distribution through inner nanopore surface.
1.4.2 Memristive and memcapacitive ion transport

Another important property of charged nanopore geometries is the memristive and memcapacitive ion transport, which has been reported by Dengchao Wang in our group based on single conical nanopore in SiO$_2$ substrate.\(^9\)\(^9\) Memristance and memcapacitance, as the name implies, refer to resistive and capacitive behaviors with memory effects. In other words, their values are time or previous history dependent. In comparison, their traditional solid-state counterparts are known to have constant values. This memory effects is due to the finite mobility of ions, which limits the redistribution of ions in response to the changing applied bias, thus the ion behavior is affected by previous state. Due to this memory effect, pinched hysteresis loops are observed in the \(i-V\) behaviors of a single conical nanopore at different scan rates.\(^9\)\(^9\) Note such frequency-domain measurements offer additional phase shift information unavailable from those steady-state studies that reveal ICR behaviors. The current-potential responses suggest a dynamic concentration polarization process under the cyclic sweeping potential. More interestingly, the \(i-V\) curves at different scan rates cross at a constant non-zero point independent of the scan rates and the bias potential window. The potential at this cross point represents the effect of the surface electric field on the mobile ions. Meanwhile, different trends are observed for high- and low-conductivity states as the scan rate of the applied bias changes. At high-conductivity state, as scan rate increases the measured current decreases. While at low-conductivity state, the current increases slightly instead of decrease when scan rate increases. The relation between the cross point potential and concentration has been quantitatively established. An exponential relationship is found between the cross potential and square root of concentration, which qualitatively agrees with classic double layer theory.

1.5 Theoretical studies of ion transport through nanoscale devices

As discussed above, nanopores and channel-type nanodevices have drawn great attention due to their promising potentials in broad applications. Those interesting properties and corresponding
Important applications result from the nanometer scale dimension where surface effect begins to contribute even dominates mass transport behaviors. The measured signals, either the intrinsic current or the change of the overall current, are a convolution of geometric/volume and surface effects. For a better understanding of the detected sensing signal and the fundamental mass transport mechanism through a nano-geometry, theoretical simulations of this physical process are carried out as a complement to experiments. Two types of theoretical simulation are mainly used: 1. Molecular dynamics simulation based on interactions at molecular level. 2. Simulation based on finite element method by solving Poisson, Nernst-Planck equation, and Navier-Stokes equations.

1.5.1 Molecular dynamics simulation

Molecular dynamics (MD) simulation is theoretical simulation of a system based on interactions at molecular level, which allows the accessibility of dynamic properties of investigated system. Basically, MD simulations is achieved by solving a series of equations of all the species present in an atomic system as shown in Equations 1.1 and 1.2

\[ m_i \ddot{r}_i = f_i \quad \text{(Eq 1.1)} \]
\[ f_i = -\frac{\partial}{\partial r_i} u \quad \text{(Eq 1.2)} \]

Equation 1.1 describes the force \( f_i \) applied on atom \( i \), which equals mass \( m_i \) multiplied by acceleration \( \ddot{r}_i \). \( r_i \) represents the position of atom \( i \) in a three-dimensional coordinate. Equation 1.2 correlates the atomic force \( f_i \) and electric potential \( u \) results from all the other atoms present in the system.

MD simulations are a principle tool for studying the biomolecules and their complex, such as conformation fluctuations and changes, dynamics, and thermodynamics. Study molecule dynamics using MD simulations in designed environment that is used to mimic the experimental conditions has been achieved.\textsuperscript{100-102} And MD simulations has been successfully used for studying the translocation dynamics
of single stranded DNA through α-haemolysin based biological nanopores and other systems.\textsuperscript{26,101,103-108}

Some simulation packages, for example, GROMOS, AMBER and CHARMM, are available for MD simulations.

However, as shown by Eqs 1.1 and 1.2, MD simulations require a solution for solving the equations of all the atoms involved in the systems, which require extensive computation efforts. Even for the smallest systems, there are still thousands of atoms. MD simulations are more suitable for processes lasting up to a few nanoseconds, and thus are not appropriate for processes that last for longer periods. These drawbacks lead to the application of finite element simulation in this study.

1.5.2 Simulation based on continuum theory

Theoretical studies of mass transport through nanodevices by solving PNP equation have been reported.\textsuperscript{24,109} The simulation of mass transport behaviors through a charged nanometer scale geometry using finite element simulation based on continuum theory is established by White and Bund.\textsuperscript{23} The simulation is carried out based on a conical glass nanopore using the commercially available software COMSOL Multiphysics. The method is validated through the agreement of simulated results with analytical solutions for two systems (flat surface and a cylindrical nanochannel) involving double layer structures. COMSOL Multiphysics allows solving of coupled governing equations for an arbitrary geometry. An accurate flux/current can be simulated based on the weak constraint feature of COMSOL Multiphysics. The constraints are implemented by using finite element on constraint domain for the Lagrange multiplier and solving the Lagrange multiplier based on original problems.

In White and Bund’s simulation, a two dimensional geometry representing half of the cross-section of a conical nanopore is used to simplify computation. The flux/current through the conical nanopore is obtained by surface integration of flux density. The geometry extends out with two reservoirs at micrometer dimension to stabilize the mass transport behavior. Boundaries representing glass surfaces that will affect the mass transport behavior are set to be charged. The modules of Electrostatics and
Transport of Diluted Species were employed to solve Poisson equation, Nernst-Planck equation and Navier-Stokes equation. More detail information could be found in White and Bund’s paper.\textsuperscript{23}

For a species of ion i, the ion transport behavior through certain geometry with net charges can be physically described by Nernst-Planck equation (eq 1.3).

\[ J_i = -D_i \nabla c_i - \frac{z_i F}{RT} D_i c_i \nabla \Phi + c_i u \quad \text{ (Eq 1.3)} \]

\( J_i \) is flux. \( D_i \) is diffusion coefficient. \( c_i \) and \( z_i \) are ion concentration and charge of ion species i. \( F \) is Faraday constant. \( R \) is gas constant. \( T \) is absolute temperature. \( \Phi \) is local electric potential. \( u \) is solution velocity due to electroosmotic flow under the effects of surface charge. The electric potential and concentration is correlated by Poisson equation

\[ \nabla^2 \Phi = - \frac{F}{\varepsilon} \sum_i z_i c_i \quad \text{ (Eq 1.4)} \]

\( \varepsilon \) is dielectric constant of the medium.

The electroosmotic effect is negligible thus is not included in the theoretical simulation for simplification based on Siwy’s report,\textsuperscript{109} which is described by the Navier-Stokes equation (Eq 1.5), where pressure, local surface electric potential and the fluid velocity are correlated.

\[ u \nabla u = -\frac{1}{\rho} (-\nabla p + \eta \nabla^2 u - F \sum_i z_i c_i \nabla \Phi) \quad \text{(Eq 1.5)} \]

\( u \), \( \rho \) and \( \eta \), respectively, are velocity, density and viscosity of the medium fluid. \( p \) is pressure.

The following parameters were used at room temperature (298.13 K): density, \( \rho = 1000 \text{ kg/m}^3 \); viscosity, \( \eta = 1 \times 10^{-3} \text{ Pa} \text{ s} \); relative dielectric constant, \( \varepsilon = 80 \); Faraday constant, \( F = 96485 \text{ C/mol} \); \( D_{K^+} = 1.957 \times 10^{-9} \text{ m}^2/\text{s} \); \( D_{Cl^-} = 2.032 \times 10^{-9} \text{ m}^2/\text{s} \).

Siwy et al. compared the analytical solution with numerical solutions based on a bipolar ionic diode nanopore platform.\textsuperscript{109} They also found that the contribution from electroosmosis is negligible. The effect of electroosmotic flow on ion current rectification of a conical nanopore has also been studied by Qian et al.\textsuperscript{110} The geometry and surface effect on the ion current rectification have also been
reported. Simulated current, current rectification ratio, concentration distribution of both cations and anions based on different linear surface charge distribution was reported, but the physical origin of such a gradient SCD is missing. The mass transport behavior ($i-V$ response) and concentration distribution of a single conical nanopore has been calculated based Poisson and Nernst-Planck model by Ramirez et al.24

As overall current or current change signals obtained in nanodevice based sensing is complicated by contributions from both volume and surface factors, the nanogeoemetry (determining volume conductance) and SCD inside the transport-limiting nanopore (determining surface effects) need to be characterized. The quantification of SCDs at nanoconfinement is previously unavailable and always extrapolated from bulk planar surface. Based on the earlier theoretical work, our group successfully quantified the surface charge density (SCD) within a conical nanopore through the fitting of experimental data. The SCD within nanoconfinement has been determined for the first time. In comparison to the earlier theoretical modes that successfully simulated the trend of ICR quantitatively, our model actually quantitatively fitted the experimental responses. In our simulation, an effective diffusion coefficient is used, calculated based on practical solution conductivity instead of infinite dilute solution and corresponding transference number. Based on this, the efficiency of surface chemical modification of a single nanopore has been reported. This is significant in that the available functional sites of individual nanodevices could be non-invasively determined. Also, an exponential gradient surface charge distribution has been proposed. The physical origin is attributed to the non-uniform electric field distribution inside the conical nanopore. This model has been validated by perfect fitting of experiments in KCl and predicting the mass transport behaviors of the same nanodevice in another type of electrolyte LiCl (manuscript in revision, chapter 4).
2 GLASS NANOPORE MEMBRANE AS SINGLE MOLECULE BINDING SENSOR

This chapter presents preliminary results (unpublished) of streptavidin-iminobiotin binding at the single molecular level based on a chemically modified conical glass nanopore. Instead of using well known non-covalent avidin and biotin binding (Kd =10^{-15} \text{ M}), a much weaker streptavidin-iminobiotin binding (10^{-7} \text{~}10^{-8} \text{ M}) pair was selected. The ultimate goal of this project is to develop a universal analytical tool for single molecule analysis based on chemically functionalized individual nanoparticles. The concept is established via a systematic investigation of the streptavidin-iminobiotin binding. Each observed current oscillation corresponds to an individual binding event of protein streptavidin or protein-DNA complex. The binding was found to be pH dependent and concentration dependent. To demonstrate the reproducibility of these single binding signals, the binding surface, located inside the nanopore sensor, was recovered through application of an externally controlled potential waveform. Those results also demonstrate the reusability of the nanopore sensors.

2.1 Introduction

Since nanopores in protein ion channels were proposed as a potential tool for rapid and low-cost DNA sequencing, various nanopores and nanochannels have been explored. The advantages of synthetic nanodevices over biological nanopores are summarized in Table 1.1. With the rapid development of fabrication and characterization, \cite{7,50,53,60,64,65,92,115} the detection of protein and DNA molecules, at the single molecule level, based on nanochannels have been extensively studied. \cite{1,3,9-11,14,15,81,116-118} The non-covalent binding and dissociation between the analytes in solution and recognition elements anchored on the interior nanopore surface induce conductivity changes within the transport-limiting region of the nanopore. Corresponding to the size blockage and reopening of nanopore upon analyte binding and dissociation, the detected signals are often the transient decrease and recovery of the steady-state ionic current during continuous measurements over time. Note only those binding and dissociation events...
occurring at the signal-limiting nanopore orifice region can be detected. The challenges associated with such single molecule detection reside in the extremely low current and heterogeneity of the detected signals.

This chapter presents some preliminary stochastic sensing results based on the streptavidin binding inside individual iminobiotin modified nanopores. Instead of using the well known avidin-biotin binding (Kd =10^{-15} M), streptavidin-iminobiotin binding was chosen (Kd =10^{-7} \sim 10^{-8} M), because the low binding affinity of streptavidin-iminobiotin binding will allow the dissociation therefore regeneration of the binding sites. Streptavidin (60 kDa) is a protein consists of 4 identical subunits, each with 159 amino acids. That means streptavidin has 4 binding sites that can bind to iminobiotin. Biotin is a 240 dalton vitamin. Iminobiotin has similar structure; the only difference is the carbonyl group in biotin is substituted by guanidine groups.

\[
\begin{align*}
\text{HO} & \quad \text{HN} \\
\text{HO} & \quad \text{HN} \\
\text{HO} & \quad \text{HN} \\
\text{HO} & \quad \text{HN}
\end{align*}
\]

**Figure 2.1** Molecular structures of biotin (left) and 2-iminobiotin (right).

The non-covalent binding of streptavidin and iminobiotin is through hydrogen bond. The pH-dependent binding of streptavidin and iminobiotin has been reported by Hendrickson using crystallographic analysis.\textsuperscript{119} Binding with a protonated biotin will results in an unstable structure, thus at low pH streptavidin and iminobiotin won’t bind.

To target streptavidin, the interior nanopore surface is modified with 3-aminopropylmethyloxy silane, this process is detailed in Chapter 2.3.2. After the modification of the glass surface through silane chemistry, part of the surface functional groups become amine terminated, which allows for the attachment of iminobiotin through formation of an amide bond using commercial available N-Hydroxysuccinimidoimino biotin Trifluoroacetamide. Streptavidin has a mild acidic pl, thus in most of our measurements (pH 6.6 and pH 7.4), it is always negatively charged. The negatively
charged streptavidin migrates into nanopore orifice driven by external applied potential. The working electrode was set outside in the bulk solution while the reference electrode was inside nanopore.

2.2 Preliminary results and discussion

This section will present binding studies of iminobiotin functionalized conical nanopores. Two types of analytes are tested: 1. streptavidin and 2. The complex of streptavidin conjugated with biotinylated DNA aptamer at 1:1 mole ratio.

2.2.1 Streptavidin binding with iminobiotin

For nanodevice based stochastic sensing, the signal to monitor the analyte binding is the ionic (non-faradic) current through the nano-geometry. This current is determined by the most resistive orifice region. To detect the analytes that have the size at ~5nm, the sensing region of the nanopore device needs to have comparable dimension. Due to the nanometer scale sensing region, the detected current is very low, normally ca. nano amps or pico amps. When an anlyte molecule is driven through the nanopore sensing region by external applied potential, it will transiently block the transport of electrolyte ions through the nanopore resulting in a drop in current, thereby allowing for its detection. The amplitude and duration of the current changes could be used to identify the analytes and to elucidate the binding and dissociation kinetics. Meanwhile, the frequency of binding/dissociation events can be used for determining the concentration of analytes.

As the binding affinity of streptavidin-iminobiotin biotin is pH dependent, two typical binding events (steps and spikes) are observed which support the reported pH dependent binding affinity. Besides pH dependence, preliminary concentration dependence and potential dependence have been established for streptavidin-iminobiotin binding.
2.2.1.1 pH dependence

**Figure 2.2** (Left panel) Streptavidin binding with anchored iminobiotin. Not drawn to scale. (Blue area represents glass capillary, green triangles represent anchored iminobiotin, the purple tetramer represent streptavidin). (Middle panel) $i$ vs $t$ of streptavidin-iminobiotin binding within a 40 nm (radius) conical nanopore in 0.1 M KCl, plus 20 mM PBS, pH 7.4. (Right panel) $i$ vs $t$ of streptavidin-iminobiotin binding within a 20 nm (radius) conical nanopore in 0.1 M KCl, plus 20 mM PBS, pH 6.6.

Two types of typical ionic current blockages: “steps” (at pH 7.4) and “spikes” (at pH 6.6) are shown in Figure 2.2 (unpublished results). The observed current decreases were induced by streptavidin-iminobiotin binding within a single iminobiotin modified conical glass nanopore. The left panel illustrate half of the cross section of a modified nanopore. Streptavidin molecules (~5 nm, negatively charged) are driven into nanopore by an external applied potential and then bind to surface anchored iminobiotin through non-covalent binding. The binding of streptavidin with immobilized iminobiotin will partially block pore orifice, thus induce a significant and detectable drop in current.

At higher pH, the binding affinity is high. The bounded streptavidin won’t dissociate, thus the decreased current will remain constant until another binding happens. Therefore “steps” are observed at pH 7.4. At lower pH, the binding affinity is low. The weakly bounded streptavidin will quickly dissociate from surface immobilized iminobiotin thus the current will increase and restore to the original level, results in a “spike”. The two types of binding events are in agreement with the reported pH dependent binding affinity of streptavidin and iminobiotin. The relative high noise during binding probably indicates the ion redistribution inside the conical nanopore originated from the perturbation from binding and dissociation that require further investigation.
2.2.1.2 Concentration dependence

Figure 2.3 Streptavidin-iminobiotin binding within a 40 nm conical nanopore in 0.1 M KCl, plus 20 mM PBS at pH 7.4 with different streptavidin A) 0 nM (background) B) 0.2 nM C) 0.6 nM and D) 3 nM. Panel E: Concentration dependence of streptavidin-iminobiotin binding at pH 7.4.

A stable background current is observed without streptavidin in solution as shown in Figure 2.3 panel A. Panels B-D shows that step-wise current decrease events are observed after the addition of streptavidin into outside solution. Each current decrease step corresponds to partial pore blockages induced by streptavidin-iminobiotin binding. As streptavidin concentration increases, the frequency of detected “steps” increases. To better illustrate the concentration dependence, the event frequency (number of events per 300 s) is plotted over streptavidin concentration as shown in panel E. Though the data is limited, the frequency clearly increases with respect to the increase in analyte concentration qualitatively. Dissociate was not observed due to the strong binding during the measurement period. This complicate further quantitative analysis, as the sensing region is irreversibly occupied during the measurements. To overcome such complications, the solution pH was lowered in the next series of measurements.
The binding results at pH 6.6 are shown in Figure 2.4. As expected, the current restores to original values after the dissociation of bounded streptavidin. This allows the release of those occupied iminobiotin sites. Therefore, more binding activities can be recorded at comparable analyte concentration during comparable period. As concentration increases, the event frequency increases. Because the sensing region is limited to the nanopore orifice region, simultaneous binding to multiple iminobiotin sites are readily observed in panel B and C. Ultimately, a plateau is reached corresponding to the saturation of the binding sites. The total effective sensing region is believed to be ca. several hundred nanometers inside the conical nanopore based on the simulation studies discussed in Chapters 3 and 4. Due to the taper geometry inside the nanopore orifice, those binding sites closer to the orifice will generate larger current changes in comparison to those further inside. This explains the heterogeneous amplitudes of the presented current change events.

2.2.1.3 Potential dependence

The ionic current corresponds to the number of charges (carried by mobile ions in solution) being transported through the nanopore orifice per time. Therefore, the detected current should depend on applied potential since the velocity of ions is proportional to applied bias. The same principle holds
for the detected current change sensing signal. This has been confirmed in the results shown in Figure 2.5. The amplitude of the current changes induced by streptavidin-iminobiotin binding at two different biases: -0.2 V and -0.4 V, are 0.2 nA and 0.4 nA respectively based on an average. “Steps” with larger amplitude are observed at higher potential (-0.4 V).

![Figure 2.5](image)

**Figure 2.5** Current change over time for streptavidin-iminobiotin binding within a 20 nm conical nanopore in 0.1 M KCl, plus 20 mM PBS at pH 7.4 with 3 nM streptavidin under -0.2 V (left panel) and -0.4 V (right panel) bias.

2.2.1.4 Counting the binding sites and binding surface regeneration

![Figure 2.6](image)

**Figure 2.6** Binding sites quantification by counting “steps” and binding surface regeneration through applying opposite potential to drive bound streptavidin out of single nanopores.

To establish more quantitative correlation between the detected current signals with the analyte binding, reproducible and systematic measurements are needed. Ideally, those measurements should be collected with a certain nanopore sensor and then compared with the responses from different nanopore sensors. A key prerequisite would be the reusability of individual nanopore sensors, which is also significant for practical applications. Interestingly, the binding surface of chemically modified
glass nanopores can be regenerated through bias control as shown in Figure 2.6. In the left and right $i-t$ panels, comparable number of binging events can be seen. The blank panel in the middle indicates the application of a potential with opposite polarity between the two $i-t$ measurements. In our experimental setup, a working electrode was placed outside in the bulk solution while reference electrode was set inside the nanopore. With negative applied potential (-0.4 V), negatively charged streptavidin in the exterior solution will be driven into nanopore. The process enables the binding with surface immobilized iminobiotin and induces current changes to be detected, indicated by the “steps” shown in the two $i-t$ curves. After all available binding in the sensing region are occupied by streptavidin molecules; the current reaches a minimum and remains constant. Any additional binding that occurred beyond the sensing region would not induce detectable current decreases. If a positive potential is applied, the bounded negatively charged streptavidin will experience a driving force toward exiting the nanopore imposed by the applied electric field. This facilitates the dissociation and thereby leads to the regeneration of the binding sites. By comparing the baseline current (no streptavidin is added) of the two $i-t$ curves, a +0.4 V bias is sufficient to fully regenerate the specific nanopore under the measurement conditions. Furthermore, the current reached the same minimum roughly after 20-30 minutes in both measurements, indicating comparable transport and binding/dissociation kinetics. Because the two $i-t$ curves are measured using the same nanopore continuously, the comparable baseline current (lower dashed line) and saturated current (minimum current, upper dashed line) indicate the availability of comparable binding sites. This can also be inferred by the same number of “steps” observed.

2.2.2 The binding of streptavidin- DNA aptamer conjugate

The studies of streptavidin-iminobiotin binding are in agreements with the volume blockage mechanism proposed in stochastic sensing literature using various channel-type nanodevices. The main thrust of my research is the surface charge effects that are less explored. In the following section, a biotinylated DNA aptamer with 90 nucleotides was conjugated with the same protein streptavidin at 1:1
mole ratio as the analytes, referred to as protein-DNA complexes herein. The charges on the long DNA backbone will amplify the electrostatic interactions with fixed surface charges on the nanopore sensor, thus reveal the surface effects of interest. The characteristic binding behaviors of the protein-DNA complexes inside iminobiotin modified conical glass nanopore sensors are shown in Figure 2.7. Compared to the binding behaviors of protein streptavidin alone, the introduction of DNA aptamer which has more negative charges obviously induced different binding behaviors. The most striking notion is that the current increase instead of decrease in the case of protein-DNA complex binding. This suggests a fundamentally different sensing mechanism other than the volume blockage. The observation can be qualitatively explained by the dense charges on each protein-DNA complex. The binding of such highly charged species would drastically change the conductance, resulting in an increase in the measured current signal. Apparently, the measured binding signals result from the enhancement by the charges on the analytes minus the decrease by the volume blockage. The highly charged analytes also display a much higher frequency of “current spikes”, which probably results from the larger driving force imposed under comparable applied electric field therefore highly dissociation rates.

**Figure 2.7** (Panel A) $i-t$ of 1:1 protein-DNA complex binding with anchored iminobiotin of a 50 nm conical nanopore in 0.1 M KCl, plus 20 mM PBS, pH 6.6 under -0.4 V applied bias. (Panel B) Magnified streptavidin-iminobiotin binding events from panel A. (Panel C) A scheme of 1:1 Streptavidin-Biotin Immobilized DNA Aptamer Conjugate. Not drawn to scale. (Green triangle represents biotin group on the DNA aptamer, curved red lines represents the single strand DNA 90mer, and the purple tetramer represents streptavidin).
Meanwhile, some transient spikes with larger amplitude are shown in Figure 2.6 panel B. There are two possibilities that could cause such observations. Firstly, an overlap of two simultaneous binding events could induce a current spike with larger amplitudes (twice in an ideal case). The translocation and binding of one protein-DNA complex prior to the dissociation of a previous binding would also generate current spikes with larger amplitudes. The quantification of the amplitude and integrated area of those peaks would offer further insights as discussed later. The other type of possible cause results from the preparation of the protein-DNA complexes. It is possible that a trace amount of dimer complexes exist, corresponding to a streptavidin molecule conjugated with two biotinylated DNA aptamer chains instead of one. The dimer could be formed during preparation of 1:1 streptavidin-biotin immobilized DNA aptamer conjugate monomer, which is prepared through the addition of biotinylated DNA into a streptavidin solution under rapid stirring.

Qualitative concentration dependence is found for the 1:1 protein-DNA complex binding with iminobiotin on the nanopore interior surface. To offer quantitative insights of this system, the potential dependence from a nanopore sensor under the same solution conditions is shown in Figure 2.8. Two $i-t$ curves under -0.4 V and -0.25 V bias are shown. The histograms of current change amplitudes at the two potentials were plotted. At -0.25 V, two types of transient “spikes” are observed, reflected by two Gaussian type distributions shown in the histogram. As discussed previously, the one with larger amplitude could indicate simultaneous binding or the presence of a minor species, 1:2 protein-DNA dimer complexes in solution. At higher potential (-0.4 V), only one type of binding events is observed. The amplitudes in the histogram displays a broader distribution compared to those at -0.25 V. Overall, it is unlikely based on our current understanding that the amplitude differences of the monomers and dimers could be screened by the enhanced velocity at higher bias, thus we propose those spikes with larger amplitudes indicate overlapping binding events. The preliminary analysis demonstrates the efficacy of nanodevice based sensing for sample heterogeneity analysis through controlling the measurement con-
ditions. The peak amplitude of “spikes” with the most population, are plotted over applied bias (indicated by the arrow), as shown in the dashed frame. A qualitative potential dependence is clearly established. The two points out of the dashed frame results from the overlapping events during measurements and not further interpreted.

**Figure 2.8** Potential dependence of current change amplitudes of 1:1 protein-DNA complex binding with iminobiotin functionalized nanopore sensor.

The same approach has been applied for analyzing the duration of those transient binding and dissociation “spikes” measured at -0.25 V bias. Similar types of plots are shown in Figure 2.9. The binding events induced by single 1:1 streptavidin- DNA aptamer conjugates are labeled with red frames, while those corresponding to simultaneous binding events are labeled with black frames. The duration (half-width) is also potential dependent. As applied bias increases, the duration decreases. This is due to the velocity enhancement at higher bias, thus the time spent on approaching and leaving the anchored iminobiotin decreased.
2.2.3 **Challenges in nanodevice based stochastic sensing-data comparison**

As discussed above, stochastic single activity binding of both protein streptavidin and protein-DNA aptamer complexes with iminobiotin has been demonstrated. However, direct data comparison for the two systems is impossible because the measured current is a convolution of volume exclusion and surface enhancement. For most nanodevices used in these sensing applications, the surfaces are normally charged due to substrate property and/or fabrication procedure. At such high surface-to-volume ratio environments, the surface effects will contribute to and even dominate the mass transport behaviors, thus complicating the detected sensing signal (current or current changes). As the surface charge densities (SCD) within nano-geometries of individual nanodevices are not available, which is known to be heterogeneous but routinely extrapolated from bulk value or planar surfaces, the data comparison for the same nanodevice at different measurement conditions is impossible, let alone different nanodevices. This is a long standing barrier for nanodevice based stochastic sensing in broad applications.

To overcome this challenge, a theoretical simulation of experimental data by solving Poisson and Nernst-Planck equations using COMSOL Multiphysics has been established. The SCD of individual single conical glass nanopores is noninvasively quantified in the following chapters (Chapter 3&4). This is for the first time, the SCD within nanoconfinement is determined, which also provides a better under-
standing of the fundamental mass transport mechanism through a charged nano-geometry. The negative charges of our nanopores result from the deprotonation of surface silanol groups, which has been demonstrated in Scheme 2.1. Furthermore, the SCD quantification method has been successfully applied to quantify the surface modification efficacy of single nanopores. The surface coverage is calculated ranging from 8%~27%, in excellent agreement with literature report around 20% over a large range (Chapter 5).

![Scheme 2.1](image.png)

**Scheme 2.1** Origin of negative charges at interior surface of a glass conical nanopore. Negative charges result from deprotonation of surface silanol groups: $-\text{SiOH} \rightarrow \text{SiO}^- + \text{H}^+$.  

2.3 **Experiment**

2.3.1 **Materials**

Water ($\sim 18.2$ MΩ-cm) was purified with a Barnstead E-pure water purification system. All other chemicals and materials were used as received. Corning 8161 glass capillaries (o.d. 1.50 mm, i.d.1.10 mm) were from Warner Instruments, and tungsten rods were from A-M System, Inc. Platinum wire (99.95%, diameter 25 μm), silver conductive paste, silver wire (99.9985%, diameter 0.5 mm), and ferrocene were from Alfa Aesar. KCl was from J. T. Baker. CaCl$_2$, FeCl$_3$ (97%), HNO$_3$, H$_2$SO$_4$, H$_2$O$_2$ (30%), acetone, acetonitrile, and tetrabutylammonium perchlorate (TBAP) were from Sigma-Aldrich. 3-Aminopropyl(dimethyl-ethoxysilane was from Gelest Inc. Immunopure streptavidin and EZ-link NHS Iminobiotin (N-Hydroxysuccinimido-9minotin Trifluoroacetamide) are from Thermo Scientific.
2.3.2 Nanopore fabrication and surface modification

The “Bench-top” method was used for fabricating single conical nanopores using a glass capillary. This method is simple and quite straightforward; it can be carried out in the lab and doesn’t involve heavy ion irradiation. The fabrication procedure basically involves four steps: 1. Preparation of a sharpened Pt nanotip through electrochemical etching. 2. Sealing of sharpened Pt tip into a glass capillary. 3. Fabrication of nanodisk electrode by removal of excess glass to expose the sealed Pt nanotip. 4. Fabrication of a conical glass nanopore by removal of sealed Pt nanotip. The success of fabricating a small nanopore requires success for each step. Step 1 is the prerequisite for a small nanopore, while the sealing (step 2) determine whether a through nanopore can be obtained since only a short tip of Pt is allowed to be sealed so that at step 4, the sealed Pt in glass can be fully removed to create a through pore. If a long Pt tip was sealed, a full removal is impossible. Step 3 also plays an important role in obtaining a small pore as it requires a prompt stop to avoid over polishing which will resulting a larger pore due to the conical shape of the sharpened Pt tip.

Step 1. Fabrication of Pt nanotip. A 25 µm (diameter) Pt wire with 3-4 cm length was attached to a tungsten (W) rod using silver conductive paste. The Pt/W assembly was then dried in air overnight or baked in oven at 120°C for 1 hour to secure the attached Pt wire. After the silver conductive paste was dried, the Pt wire was straightened and Pt/W assembly was inserted into a glass capillary (o.d. 1.50 mm, i.d. 1.10 mm). The Pt wire was straightened so that when inserted into glass capillary, it was centered in capillary without touching glass surface which is necessary for later sealing. The tungsten rod was bent to secure Pt/W assembly to protect Pt wire during the whole fabrication procedure. Then the whole Pt/W/capillary assembly was suspended vertically in air using an iron stand, and Pt wire was pushed out of capillary about 1-2 cm long with the end (~1 cm) dipped into 15% CaCl₂ solution prepared using a mixture of 1:1 H₂O and acetone (v/v). With a 5 V AC voltage applied, the Pt was electrochemically etched to form a sharp nanotip, during which substantial bubbles were formed. The addition of acetone in CaCl₂
was used to disperse these bubbles formed. As bubble formation ceased indicated electrochemical etching was completed, the AC bias was removed. The obtained sharp nanotip was dipped into piranha solution to clean tip surface, followed by dipping into H₂O to remove piranha solution residue. Then the etched Pt tip was withdrawn back into capillary for the next step.

Step 2. Sealing of Pt nanotip. The electrochemically etched Pt nanotip was sealed with the aid of an optical microscope with X20 magnification. As described in step 1, the obtained Pt nanotip was withdrawn back into glass capillary leaving a distance of 4-5 mm between the end of capillary and Pt tip. Before sealing, the Pt tip was positioned at the center of capillary without touching the surface. The 4-5 mm space left is used to achieve a complete sealing of Pt tip into a glass ball. The glass was then slowly melted using a Bunsen burner. Remove the Pt/W/capillary assembly away from the Bunsen burner as the sharpened Pt nanotip penetrate the air/glass interface into the formed glass ball with a depth ≤ 1 mm, this will allow a full removal of sealed Pt nanotip in step 4. In this sealing process, the shape of electrochemically etched Pt nanotip remain unchanged as the melting point of Pt is 1769 °C higher than the softening temperature (1100 °C). The other end of glass capillary is sealed used a non-conductive epoxy to fix the Pt/W assembly, and the next step is to polish glass to expose the sealed Pt nanotip.

Step 3. Fabrication of nanodisk electrodes. The Pt/W/glass assembly is first manually polished using coarse to fine sand papers (400, 800, 1200 grit) sequentially to remove excess glass. Sand papers are wetted with DI water. The polishing process was monitored under an optical microscope; a mirror image will be seen if a certain amount of glass (glass at the end where Pt is sealed) has been removed. As the distance between mirror image and real Pt tip is getting relative close, switch to 800 grit sand papers then switch to 1200 grit sand papers when the two images are quite close. The Pt/W/glass assembly is then connected into an electrical circuit based on a metal oxide semiconductor field effect transistor (MOSFET). When the Pt nanotip is almost exposed, only a thin layer of glass is left indicated by a discontinuous beeping, which results from the capacitive current. Then switch to a Buehler Microcloth pol-
ishing pad with a slurry of Al$_2$O$_3$ nanoparticles to finish the final polishing indicated by a clear and continuous beeping since a close circuit is formed as the Pt nanotip is exposed. A nanodisk electrode is fabricated.

Step 4. Fabrication of a conical glass nanopore. The obtained nanodisk electrode was etched in 15% KCl solution prepared using a mixture of 1:1 H$_2$O and acetone (v/v) overnight, the same as described in step 1. CaCl$_2$ is replaced with KCl to avoid Ca$^{2+}$ absorbance to the exposed negatively charged surface due to electrostatic interaction during etching. After etching, remove epoxy and mechanically pulling the W rod to fully remove the seal Pt wire. To ensure a pore was obtained, the glass capillary was filled with 1 M KCl (insert W rod into capillary as working electrode) and etching in the same solution again to dissolve Pt residue. Finally, clean the glass capillary with nanopore H$_2$O. A through conical glass nanopore was fabricated replicating the shape of sharpened Pt nanotip.

![Cyclic voltammogram of a nanodisk electrode in 2 mM ferrocene with 0.1 M TBAP acetonitrile solution.](image)

**Figure 2.10** Cyclic voltammogram of a nanodisk electrode in 2 mM ferrocene with 0.1 M TBAP acetonitrile solution.

The size of nanodisk electrodes and nanopores are characterized based on cyclic voltammetry. For nanodisk electrodes, the radius is determined by measuring the diffusion-limited steady state current (id) from oxidation of 2mMferrocene (FC) with 0.1 M tetrabutylammonium perchlorate (TBAP) acetonitrile (CH$_3$CN). A typical cyclic voltammogram is shown in Figure 2.10. The radii is calculated based on Equation 2.1

$$id = 4nFDC * r \quad (Eq \ 2.1)$$
n is the number of electrons transferred during ferrocene oxidation. F is Faraday constant. D is diffusion coefficient \((2.4 \times 10^{-5} \text{ cm}^2/\text{s})\). C is the bulk concentration of redox molecule, here FC. r is nanodisk electrode radius. The scan rate is set 20 mV/s.

The nanopore radius characterization is based on conductivity measurement \((i-V)\) in 1 M potassium chloride (KCl). A typical cyclic voltammogram is shown in Figure 2.11. The scan rate is set 20 mV/s. The radius is calculated based on the resistance from +0.05 V to -0.05 V where the surface effect is mostly screened by low potential and high electrolyte concentration. Using equation 2.2, the radius can be determined.

![Cyclic voltammogram of a conical glass nanopore in 1 M KCl solution.](image)

**Figure 2.11** Cyclic voltammogram of a conical glass nanopore in 1 M KCl solution.

\[
R_p = \frac{1}{kT} \left( \frac{1}{\pi \tan \theta} + \frac{1}{4} \right)
\]

\(R_p\) is nanopore resistance in 1 M KCl solution. K is conductivity of 1 M KCl solution (11.19 S/m). r is nanopore radius. \(\theta\) is half-cone angle of conical nanopore \((8.5 \pm 1^\circ)\), which has been well characterized.

For nanodisk size characterization, the measured current is Faradaic current, which results from the oxidation of ferrocene involve electron transfer. But for nanopore characterization the measured current is non-Faradaic ion current, the current is from the movement of ions transport through a confined nanopore orifice.
For sensing application, the fabricated nanopore surface needs to be modified to target different analytes. Single conical nanopore based single molecule sensing with streptavidin-iminobiotin binding pair requires a surface modification using silane chemistry. The nanodisk surface and nearby cylindrical glass surface is modified with 3-cyanopropyltrimethylchlorosilane (Cl(Me)2Si(CH3)3CN) to prevent surface adsorption. The surface is cleaned sequentially with H2O, ethanol (EtOH), CH3CN and H2O. Then the nanodisk is soaked in 1 M nitric acid HNO3 solution for 15 minutes to activate the surface (to form free surface silanol groups). After cleaning with H2O, EtOH and CH3CN, the activated nanodisk electrode is soaked in 2% (V/V) 3-cyanopropyltrimethylchlorosilane CH3CN solution overnight. The modified nanodisk electrode is cleaned with CH3CN, EtOH and H2O. The pore is loaded and soaked in 2% (V/V) 3-aminopropyltrimethoxy silane CH3CN solution following the same procedure for creating binding sites. The 3-aminopropyltrimethoxy silane CH3CN solution is loaded to the nanopore tip to restrict the binding at the pore orifice. The remaining interior surface is modified with 3-cyanopropyltrimethylchlorosilane to avoid analyte absorbance to increase sensitivity. The surface modification procedure of conical glass nanopores for sensing application is demonstrated in Scheme 2.2.

![Surface Modification with Silanes](image)

**Scheme 2.2** Scheme of surface modification of a conical glass nanopore for targeting streptavidin

Measurements based on modified nanopores were more reproducible compared to those in bare glass. The surface coverage of 3-aminopropyltrimethoxy silane CH3CN is reported to be 20%.

### 2.3.3 Electrical measurements

A CHI potentiostat (750C) was used in the conductivity studies (cyclic voltammetry software). Two Ag/AgCl wires were used to control the bias potential. Both reference and counter electrode leads
were connected to the same Ag/AgCl electrode immersed inside the nanopore, and the working electrode was outside, in the bulk solution. The measured current is non-Faradaic current. Nanopore radius is determined on the basis of the absolute current values at +0.050 and -0.050 V in 1M KCl, where the surface effect is more effectively screened by the high concentration of electrolytes. The current change over time is recorded using both CHI potentiostat (750C) and Axopatch 200B.

2.4 Summary

As shown in this chapter, single streptavidin, 1:1 streptavidin-biotin immobilized DNA aptamer conjugate binding with iminobiotin were detected. Qualitative pH, concentration and potential dependence of streptavidin-iminobiotin binding have been established. Charge effects on the mass transport have been confirmed by the introduction of 1:1 streptavidin-biotin immobilized DNA aptamer conjugate as the analytes. The synthetic conical glass nanopores have been demonstrated to be reusable. Also, binding sites on nanopore surface can be determined through counting the number of events measured. However, the detected current is also a function of SCD, which is not available at nanoconfinement. This hampers the interpretation of detected current signals and the establishment of a universal method, as the results for different solution conditions and/or different analytes could not be compared even for the same nanodevice. The data comparison from one nanodevice to another is even more challenging to surmount. Meanwhile, the binding efficacy is observed only from a small portion of those fabricated nanopores, which could result from heterogeneous surface modification and/or the cancelation of volume and surface effects (i.e. analytes binding would decrease the conductive volume causing current to decrease and an increase in SCD would will increase local conductance causing current to increase. All of these intrigued the work shown in Chapters 3-5, where surface charge distribution within individual nanoconfinement is determined for the first time and surface modification efficacy is determined correspondingly.
3 SURFACE CHARGE DENSITY DETERMINATION OF SINGLE CONICAL NANOPORES BASED ON NORMALIZED ION CURRENT RECTIFICATION

Current rectification is well known in ion transport through nanoscale pores and channel devices. The measured current is affected by both the geometry and fixed interfacial charges of the nanodevices. In this article, an interesting trend is observed in steady-state current-potential measurements using single conical nanopores. A threshold low-conductivity state is observed upon the dilution of electrolyte concentration. Correspondingly, the normalized current at positive bias potentials drastically increases and contributes to different degrees of rectification. This novel trend at opposite bias polarities is employed to differentiate the ion flux affected by the fixed charges at the substrate—solution interface (surface effect), with respect to the constant asymmetric geometry (volume effect). The surface charge density (SCD) of individual nanopores, an important physical parameter that is challenging to measure experimentally and is known to vary from one nanopore to another, is directly quantified by solving Poisson and Nernst—Planck equations in the simulation of the experimental results. The flux distribution inside the nanopore and the SCD of individual nanopores are reported. The respective diffusion and migration translocations are found to vary at different positions inside the nanopore. This knowledge is believed to be important for resistive pulse sensing applications because the detection signal is determined by the perturbation of the ion current by the analytes.

3.1 Introduction

Fundamental transport properties in individual synthetic nanopores and nanochannel devices have attracted extensive research interest.\textsuperscript{19,51,87,120-124} Unique mass transport (MT) behaviors at high surface-volume ratios render the synthetic nanopores and nanochannels as promising platforms for rapid, lower-cost analytical sensors.\textsuperscript{3,4,81,85,125} With a nanopore or nanochannel connecting two solution reservoirs through which a bias potential is applied, the nanopore region limits the ionic transport process
and thus the overall conductivity. Because both the nanoscale geometry (radius, length, etc.) and inter-
face (Coulombic interaction between fixed surface charges and mobile ions in solution) affect the ion
transport signal, the overall current or conductance measured experimentally does not reveal the re-
spective impacts of surface and volume factors. This knowledge is important for quantitative sensing
applications because the analytes perturb those factors differently in different regions of the
nanodevices and generate signals differently.

The MT resistance of a nanopore is not a constant value at different applied potentials, which
causes the measured current voltage ($i-V$) curve to deviate from linearity (Ohm’s law). The current at
one potential polarity is often different from that at the same amplitude but the opposite potential po-
ularity, which is well known as ion current rectification (ICR). The deviation from linear Ohmic beha-
vior is generally believed to be a consequence of the asymmetrical geometry and charge distribution at
the nanoscale interface. The asymmetrical device geometry and asymmetry between the radial influx
and pseudoplanar efflux at the opening region are referred to as volume effects in this paper. At a high
surface to volume ratio, the transport of cations and anions is significantly affected by the Coulombic
interaction with the fixed charges at the substrate-solution interface, referred to as surface effects. The
concept is illustrated in Scheme 3.1.

A quantitative correlation of the measured ICR with the physical parameters of the nanodevices,
specifically, the surface charge density (SCD) that establishes the surface electrical field inside the na-
opore, has not been established experimentally. The fixed charges at the substrate-solution interface
inside the nanopore have opposite impacts on the transport of cations and anions via Coulombic interac-
tion. The density of fixed charges at the solid solution interface is fundamentally important to sensing
applications and directly affects the electrochemical energy conversion in high-energy-density devices. The
understanding of SCD within nanodevices is generally extrapolated from the values obtained from
bulk measurements, which varies widely in the literature and is known to depend on the measurement
The direct experimental determination of SCD on the nanometer scale of individual nanodevices remains a challenge. Average SCD values from a flat surface are frequently used in theoretical approaches. However, it is well known that the efficacy and responses of individual nanodevices used in experiments vary from one to another, depending on the measurement conditions.

Scheme 3.1 Ion Flux Confined by Nanopore Geometry and Interaction with the Fixed Charges at the Glass-Solution Interface. The double arrow suggests the electrostatic interaction between mobile ions and those fixed negative charges, which can be divided into two components along and normal to the direction of ion flux. Not drawn to scale. (Adapted with permission from ref. 113. Copyright (2012) American Chemical Society.)

At present, the transport behavior is mostly studied experimentally by time domain conductivity measurements at constant or scanning potentials. The measured ionic current, carried by cation and anion transport, reflects both volume and surface impacts. This convoluted overall current-potential behavior has led to several qualitative models that have been summarized in recent reviews. Briefly, the rectification behavior has been explained by potential ratchet, inhomogeneous conductivity, or ion mobility differences in the nanopore region as a result of the combined volume and surface effects. Analytical and numerical simulations based on Poisson, Nernst-Planck, and Navier-Stokes equations have been reported to describe the overall conductivity through nanopores and nanochannels with various geometries. Although those models describe the experimental trend correctly, they often fail to explain the different degrees of ICR observed experimentally from the same types of substrate materials with comparable geometry.
In stochastic nanopore sensing based on the Coulter counter concept,\textsuperscript{78,117,118,128-130} the binding and transport of analytes perturb the ion flux and generate the current signal. Volume effects (size of analytes over nanopore volume) are routinely considered in the analysis, with the prerequisites of uniform flux distribution inside the nanopore and negligible surface effects. Experimentally, the efficacy of the nanosensors varies from one to another, which is generally postulated to be the heterogeneity of the fabrication and functionalization process. A noninvasive characterization of individual nanosensors, both geometry and surface features, is critical for broad applications but is a significant challenge to address.

Our recent ac impedance analysis has revealed ion-transport processes with different time constants, with the physical origin attributed to volume and surface impacts.\textsuperscript{131} The experimental evidence that reveals (1) the spatial distribution of ion flux in addition to ion concentration in the nanopore region, (2) the respective volume and surface impacts on the ionic transport process, and (3) the respective contributions of cations and anions to the overall measured ionic current will greatly advance the fundamental understanding of the transport phenomenon. In this report, a combined experimental and theoretical approach is employed to differentiate the volume and surface effects in ion transport through single conical nanopores. By normalizing the current-voltage responses measured from high to low ion concentrations, surface effects via columbic interaction are found to enhance the conductivity at positive bias potentials (outside vs inside). A threshold in normalized conductivity was observed at low-conductivity states upon dilution. This opposite trend differentiates surface effects from geometric parameters that normally have the same impacts at both potential polarities. The direct characterization of the SCD of individual nanopores is achieved by fitting the experimental results based on the continuum theory.
3.2 Results and Discussion

3.2.1 Experiments: Normalized Conductivity in Potential Scanning Measurements

Conductivity measurements have been widely used to noninvasively characterize the dimension of nanodevices based on Ohm’s law (resistance = solution factor × geometric factor).\textsuperscript{132,133} The prerequisite is the negligible contribution from surface conductivity, which often leads to an increase in the measured conductance and therefore overestimates the size. Contrary to the symmetric nanochannels in which the surface charges enhances the measured conductance at both potential polarities,\textsuperscript{134} a novel trend was discovered in the normalized $i-V$ curves in conical nanopore measurements, which allows the differentiation of surface and geometric effects. The $i-V$ curves of a 26-nm-radius nanopore measured at different KCl concentrations were analyzed in Figure 3.1. The current amplitude in each curve was normalized by the corresponding concentration value to display the normalized conductivity. Almost linear ohmic behavior is observed in 1 M KCl (black) solution. When the electrolyte concentration decreases from 1 M, the normalized current at positive potentials increases whereas that at negative potentials remains largely unchanged. Glass is negatively charged upon the deprotonation of surface silanol groups. The electrostatic interaction of mobile charge carriers (cation and anion) with the fixed surface charges is less screened at lower ionic strength. Because the volume effect is determined by Ohm’s law, the deviation from linearity will reflect the surface effects.

It is important to observe different trends at positive and negative potentials in the normalized conductivity plots. At positive potentials (outside vs inside), the normalized current continues to increase as the electrolyte concentration decreases. At negative potentials, the almost overlapping current curves reveal a threshold-normalized conductivity. Because the increase in nanopore radius always results in the increase in current at both potential polarities, the opposite trend, especially the threshold low-conductivity states, can be employed to differentiate size and surface effects.
The current was normalized on the basis of concentration, with the factor listed next to each curve: (red) 0.01 M, X100; (blue) 0.05 M, X20; (green) 0.10 M, X10; (black) 1.00 M, X1. The scan rate was 20 mV/s. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)

The deviation from linearity has been routinely quantified by the rectification factor (RF), which is the current ratio at an arbitrary potential value but the opposite polarity (e.g., current at +0.4 V over current at -0.4 V). The increase in the RF at low ionic strength can be explained by less screening of surface charge effects at low ionic strength because the nanopore structure remain unchanged. The analysis suggests that at lower electrolyte concentrations ICR mainly originates from the enhanced conductivity at the positive bias end. It also reveals a threshold low conductivity state limited by the surface charge impacts on ion flux that is further discussed by simulation.

A similar trend was observed from other nanopores of different sizes shown in Figure 3.2. The specific bulk concentration reaching the apparent low-current threshold varies from one GNP to another. Note that the measured current reflects the non-Faradic transport of cations and anions through the nanopore. Because of the conical geometry, the smallest orifice region of the nanopore limits the transport process whereas the impacts at the larger opening at the other end and the length of the nanopore are less significant and generally ignored in the literature and in this study.
Figure 3.2 Normalized conductivity curves of A: 161-nm-radius B: 123-nm-radius nanopore in KCl solutions at different concentration: red 0.01 M, blue 0.05 M, green 0.10 M, black 1.00 M. The current is normalized by the factors listed next to each curve based on the concentration. The nanopore surface was modified with 3-aminopropyl-dimethylethoxysilane, which is found to offer more reproducible measurements as previously described.\textsuperscript{109} (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)

3.2.2 Simulation Based on the Continuum Theory

The interesting trend in the normalized $i-V$ curves reveals opposite impacts by surface effects on the total conductivity at opposite bias potentials: enhanced and reduced conductivity at positive and negative bias potentials (outside vs inside), respectively. The finding is in accordance with the ac impedance analysis previously reported by our group and by molecular dynamic simulation.\textsuperscript{131,135} To explain the observed phenomena and surface impacts quantitatively, the experimental results are fitted by classic theory simulation. The total ion flux is calculated through the surface integration of flux at the exterior boundary electrode. The flux distribution inside the nanopore is validated by the conservation of integrated flux at a specified cut line position inside the nanopore. Although the ion concentration inside different types of nanopores and nanochannels has been simulated (often under conditions different from experimental measurements),\textsuperscript{23,24,109-112} the spatial distribution of ion flux in the nanopore region reveals an interesting spatial variation of migration and the diffusion contribution to the total flux. Information about the ion flux distribution in the signal-limiting nanopore region is more relevant to resistive pulse sensing applications because the current signals being detected originate from the perturba-
tion of the ion flux by the analytes. The model geometry is described in the Experimental Section and illustrated in Figure 3.10.

To gain an understanding of the ion distribution and flux distribution in the signal-limiting region, a cut line along the radial direction at a designated depth (Z) inside the nanopore was introduced. The distribution of the z-component flux and the ionic concentration along the cut line were computed. The distance (R direction) is zero at the center line and ends at the nanopore interior surface (boundary 5). Because the applied bias potential is limited to a relatively low range, the contribution by electroosmotic flow is known to be small and is thus ignored.\textsuperscript{23,24,109-112} The model design was first validated by reproducing reported results\textsuperscript{23,25} in which the conductivity and ionic concentration along the center line of a 50-nm-radius nanopore have been computed. The electrical conductivity at +0.4 and -0.4 V along the center line of the 26-nm-radius nanopore is included in Figure 3.3. The variation of pore geometry such as the pore diameter and cone angle displays similar impacts as reported in the literature. Representative results are included in Figure 3.4.

\textbf{Figure 3.3} Electric conductivity at +0.4 V (red) and -0.4 V (black) along the centerline of a 26-nm-radius nanopore in 50 mM KCl with surface charge density at -170 mC m\textsuperscript{-2}. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)
Figure 3.4 The effects of nanopore radius and half cone angle on simulated current at +0.4 V (red square) and -0.4 V (blue square). The half-cone angle is 11.2° in Panel A at 1 M KCl solution. The radius is 26 nm in Panel B at 0.1 M KCl solution. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)

3.2.2.1 Spatial Distribution of Ion Concentration Inside the Nanopores

Figure 3.5 Concentration profiles of K⁺ (red) and Cl⁻ (green) at (A) + 0.4 and (B) -0.4 V at cut line z = -100 nm. The x axis represents the distance away from the center line along the cut line. The radius of the nanopore is set at 26 nm with the surface charge density defined at -170 mC m⁻² (value based on Figure 3.9). The bulk concentration of KCl is 50 mM. The intercept on the concentration axis is enlarged in the inset panel. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)

The concentration profiles of K⁺ and Cl⁻ along a 100 nm cut line (radial direction) are shown in Figure 3.5. Within a few nanometers from the negatively charged glass surface at ±0.4 V potentials, K⁺ is highly concentrated whereas the concentration of Cl⁻ is much lower because of electrostatic interactions. The concentration at the center line can be seen in the inset panel, which can be compared to
those along the center line, as included in Figure 3.6. Unlike the general perception that cations are enriched and anions are repelled inside the nanopore by the negatively charged surface, interesting

**Figure 3.6** Concentration profiles of K⁺ (red) and Cl⁻ (green) at A: +0.4 V; and B: -0.4 V along centerline. Negative values on x axis represents the depth inside the pore, with pore orifice at zero. The radius of the nanopore is set at 26-nm with surface charge density defined at -170 mCm⁻² (value based on Figure 3.9). The bulk concentration of KCl is 50 mM. The maximum and minimum concentration of each ion (overlapped) along Z direction can be seen in the enlarged panel shown on the right. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)

collection profiles are observed at different potential polarities. At positive potentials where high-conductivity states are observed experimentally, both the K⁺ and Cl⁻ concentrations away from the charged surface are found to be higher than the defined bulk concentration of 50 mM (panel A). The high concentration of K⁺ inside the pore is attributed to the radial influx of K⁺ driven by the applied potential, which is also favored by the negatively charge surface via Coulombic interaction. The high influx of K⁺ in turn requires excess Cl⁻ to maintain the charge neutrality inside the nanopore. The intercepts on the concentration axis at different cut lines are in agreement with the concentration profile in Figure 3.6 and in accordance with the trend along the center line as previously reported.²³,²⁴ The results suggest that anions also contributed to the higher conductivity in ICR, though to a less significant extent than did cations. At a -0.4 V bias potential (low-conductivity states), the concentration away from the charged
interface is lower than the bulk concentration. In this case, the geometric factor, radial influx, and pseudoplanar efflux (note that the half cone angle is ca. 11°, Scheme 3.1) tend to cancel the Coulombic effects.

Figure 3.7 Spatial distributions of K⁺ and Cl⁻ concentrations inside a nanopore for a 26 nm GNP in 50mMKCl with -170 mC m⁻² SCD. The half-cross section nanopore geometry is shown at the bottom of each panel. The adaptive mesh elements are much denser near the charged interface (boundary 5). The nanopore orifice is at Z = 0 nm, and center line is at R = 0 nm. The concentration profiles inside the nanopore at 3 μm and beyond continuously extend those features shown and thus are not included. The top color scale applies to panels A and B, and the bottom color scale applies to panels C and D. Note that the brushlike features near the interface resulted from the cut-off concentration range, which was set during plotting for a better view. The absolute values at representative positions can be found in Figures 3.5 and 3.6. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)

To provide a comprehensive view, the 2D concentration profiles inside the nanopore are shown in Figure 3.7. In each panel, the cross section of the half nanopore is projected at the bottom, with the pore orifice and center line at zero for the Z and R directions, respectively. The concentration range differs in each panel for a better observation of the spatial distribution. Accordingly, the high concentration of K⁺ and the low concentration of Cl⁻ near the charged interface were cut off and displayed brushlike features. The results emphasize that anions are enriched to a different extent under high-conductivity states in different regions of the nanopore. The absolute concentration value along the center line can be found in Figure 3.6.
3.2.2.2 Spatial Distribution of Ion Flux Inside Nanopores

Figure 3.8 Flux distribution of K⁺ (red squares) and Cl⁻ (green triangles) at (A) +0.4 and (B) -0.4 V at cut line z = -10 nm and at (C) +0.4 and (D) -0.4 V at cut line z = -100 nm. The x axis represents the distance away from the center line along the cut line. The radius of the nanopore is set at 26 nm with the surface charge density defined at -170 mCm⁻² (value based on Figure 3.9). The bulk concentration of KCl is 50 mM. The intercept on the flux density axis is enlarged in the inset panel. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)

Representative flux distributions of K⁺ and Cl⁻ at 10 and 100 nm cut lines under the applied potentials of ±0.4 V are presented in Figure 3.8. In the vicinity of the nanopore interior surface, the z-direction flux of K⁺ is much higher than that of Cl⁻. This mainly results from the high concentration of K⁺ due to Coulombic interaction with the negatively charged nanopore surface. A few nanometers away from the boundary toward the center line, the K⁺ and Cl⁻ fluxes become comparable. The intercepts of the flux distribution at the center line (R = 0) can be seen in the inset panel. It is interesting that the differences between the K⁺ and Cl⁻ fluxes vary at different positions and bias potentials (inset panels), though the concentration profiles overlap away from the charged interface. The spatial variation of K⁺ and Cl⁻ flux differences can be explained by diffusion, driven by the concentration gradient. At positive bias potentials (outside vs inside), the ion concentration reaches its maximum at ca. 100 nm inside the nanopore as shown in Figures 3.6 and 3.7. At the 100 nm cut line, the concentration gradient is ca. zero.
Therefore, no diffusion is expected and migration is the sole mechanism of ion flux. As the cut line is positioned near the orifice, the concentration gradient increases. Importantly, the directions of diffusion and migration are the same for the Cl⁻ flux, which increases the total Cl⁻ flux. In the case of K⁺ flux, the direction of diffusion is opposite to the direction of migration, causing the total K⁺ flux to decrease. Consequently, the total flux of K⁺ is less than that of Cl⁻ because of the cancellation effect at a certain spatial position that has the same concentration profile for both ions. The same rationale applies to any charged analytes in sensing applications. Correspondingly, the nature of the signal (i.e., current amplitude and duration) would depend on the binding location or transport trajectory.

To validate the flux distribution and to correlate with experimental i—V responses, the total flux through the cross-section of the nanopore at the boundary and different Z positions was computed by the surface integration of the flux distribution (R from zero to boundary 5). The total flux and current at the cut line ranging from 1 to 200 nm inside the nanopore have been computed, and the results are included in Table 3.1. Because the conductivity was measured under steady state, the total flux across the cross section at any Z position should be conserved. This is confirmed by comparing the average flux values to the flux at the boundary electrode. It is worth pointing out that in continuum theory the sizes of hydrated ions and ion-solvent interactions were not accounted for. Molecular-level modeling would provide better fitting near the interface. Unfortunately, its applicability is generally limited to the dimension of a few nanometers or less and therefore could not describe the overall experimental behaviors of the nanopores used in this study.
Table 3.1 Simulated current at different cutline positions under ±0.4 V bias potential (outside vs. inside) in 50 mM KCl solution. The surface charge density is at -170 mC m⁻². The Cl⁻ current is consistent at each cutline: +0.4 V, $i_{\text{Cl}^-} = 3.149±0.001$ nA; -0.4 V, $i_{\text{Cl}^-} = -1.055±0.001$ nA. The data were retained after Q test at 90% confidence level.

<table>
<thead>
<tr>
<th>Cutline (nm)</th>
<th>Current by K⁺(nA)</th>
<th>Total Current (nA)</th>
<th>Current by K⁺(nA)</th>
<th>Total Current (nA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>5.07</td>
<td>8.22</td>
<td>-2.91</td>
<td>-3.96</td>
</tr>
<tr>
<td>-2</td>
<td>5.04</td>
<td>8.19</td>
<td>-2.94</td>
<td>-3.99</td>
</tr>
<tr>
<td>-5</td>
<td>5.10</td>
<td>8.25</td>
<td>-2.87</td>
<td>-3.93</td>
</tr>
<tr>
<td>-8</td>
<td>5.07</td>
<td>8.22</td>
<td>-2.90</td>
<td>-3.96</td>
</tr>
<tr>
<td>-10</td>
<td>5.07</td>
<td>8.22</td>
<td>-2.91</td>
<td>-3.97</td>
</tr>
<tr>
<td>-20</td>
<td>5.06</td>
<td>8.21</td>
<td>-2.92</td>
<td>-3.97</td>
</tr>
<tr>
<td>-30</td>
<td>5.09</td>
<td>8.24</td>
<td>-2.89</td>
<td>-3.94</td>
</tr>
<tr>
<td>-40</td>
<td>5.06</td>
<td>8.21</td>
<td>-2.92</td>
<td>-3.97</td>
</tr>
<tr>
<td>-50</td>
<td>5.08</td>
<td>8.23</td>
<td>-2.90</td>
<td>-3.95</td>
</tr>
<tr>
<td>-60</td>
<td>5.11</td>
<td>8.26</td>
<td>-2.87</td>
<td>-3.92</td>
</tr>
<tr>
<td>-70</td>
<td>5.04</td>
<td>8.19</td>
<td>-2.94</td>
<td>-3.99</td>
</tr>
<tr>
<td>-80</td>
<td>5.12</td>
<td>8.27</td>
<td>-2.86</td>
<td>-3.92</td>
</tr>
<tr>
<td>-90</td>
<td>5.05</td>
<td>8.19</td>
<td>-2.93</td>
<td>-3.99</td>
</tr>
<tr>
<td>-100</td>
<td>5.05</td>
<td>8.20</td>
<td>-2.93</td>
<td>-3.98</td>
</tr>
<tr>
<td>-120</td>
<td>5.13</td>
<td>8.28</td>
<td>-2.85</td>
<td>-3.90</td>
</tr>
<tr>
<td>-150</td>
<td>5.11</td>
<td>8.26</td>
<td>-2.87</td>
<td>-3.92</td>
</tr>
<tr>
<td>-180</td>
<td>5.04</td>
<td>8.19</td>
<td>-2.94</td>
<td>-3.99</td>
</tr>
<tr>
<td>-200</td>
<td>5.07</td>
<td>8.21</td>
<td>-2.91</td>
<td>-3.97</td>
</tr>
<tr>
<td>Average</td>
<td>5.1±0.1</td>
<td>8.2±0.1</td>
<td>-2.9±0.1</td>
<td>-4.0±0.1</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>RE</td>
<td>5.1</td>
<td>8.2</td>
<td>-2.9</td>
<td>-4.0</td>
</tr>
</tbody>
</table>
3.2.2.3 Quantification of the Effective SCD of Individual Nanopores

Although nanodevice geometry can be determined by various imaging techniques, a noninvasive characterization of the interior surface is a challenge. With various nanostructures being developed for sensing and other applications, it is significant if the conductivity measurements could be employed to quantify the SCD of individual nanodevices noninvasively.

In aqueous solution, the SCD of glass substrates is mainly determined by the protonation/deprotonation of silanol groups (Si–OH). Because the deprotonation process is associated with charge separation at the functional group, the pKa of those groups is known to be affected by the local surface electric field (surface morphology or geometry) and vary under different conditions. Furthermore, glass is known to form a gel layer and respond to pH, which is the foundation of pH electrodes. Accordingly, SCD in this article quantifies the accumulative fixed charges at the substrate-solution interface from multilayers instead of a monolayer of charges at the substrate surface.

![Figure 3.9](image)

**Figure 3.9** Measured conductivity (solid black line) from a ca. 26-nm-radius nanopore in KCl solution at different concentrations. Simulation results for the 26-nm-radius nanopore in (A) 1.00, (B) 0.10, and (C) 0.05 M KCl solutions with different SCDs as indicated. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)
The experimental $i-V$ responses were fitted by a systematic variation of the SCD of the constructed nanopore. As shown in Figure 3.9, the simulated current (scattered points, total flux times the Faraday’s constant) matches the experimental $i-V$ curve at ca. -170—240 mCm$^{-2}$ in different electrolyte concentrations at different potentials. In 1 M KCl solution, the surface charge impacts become less obvious. All results at different SCDs converge to the linear ohmic behavior and match the measured results well. As the electrostatic interaction with the charged interface becomes less screened at lower ion concentrations, ICR effects intensify. The simulated current in high-conductivity states increases with the increase in SCD in a well-defined fashion. The simulated current in low-conductivity states displays a rather weak dependence on the SCD variation. Therefore, the determination of SCD in the following discussion is focused on high-conductivity responses. Experimentally, the trend in low-conductivity state current also varies from nanopore to nanopore in different electrolyte concentrations, as attested to by the results provided in Figure 3.2. The offset between the simulated and measured low-conductivity currents is addressed in a separate study and does not affect the SCD determination. Again, the variation of geometric parameters could not describe the increase in the normalized conductivity at positive bias potentials and the threshold low conductivity.\textsuperscript{139} In fact, the pore geometry is not expected to change in different electrolyte solutions.

To the best of our knowledge, no direct measurement of the charge density has been reported at the nanometer-scale interface inside single nanodevices. The determined SCD is validated by the literature based on ensemble studies that actually have a wide range: from ca. -0.002enm$^{-2}$ at low electrolyte concentration (μM range) up to ca. 1enm$^{-2}$ (160 mC m$^{-2}$) at higher electrolyte concentrations.\textsuperscript{126,136} The determined SCD at ca. -170—240mC m$^{-2}$ is marginally higher but is considered to be reasonable under the measurement conditions with a high electric field. Glass is well known to form a gel layer in an aqueous environment. The charge density in the gel layer is determined by the pKa of the functional groups in the SiO$_2$ network and the solution pH. Note that a high electric field could signifi-
cantly facilitate Si—O—H bond dissociation, which causes the variation of effective pKa values under different surface curvatures. Furthermore, recent reports suggest that the deep hydration of the glass surface could be as much as hundreds of nanometers,\textsuperscript{140,141} which also supports the quantified effective SCD.

3.2.2.4 Transference Number of K$^+$ and Cl$^-$ in High- and Low- Conductivity States

The respective cationic (K$^+$) and anionic (Cl$^-$) contributions to the total current are summarized in Table 3.2. At $+0.4$ V (outside vs inside), the normalized current of both K$^+$ and Cl$^-$ increases as the concentration decreases. The high conductivity states are established with K$^+$ being the main charge carrier.\textsuperscript{142} The low-conductivity changes are much smaller and limited by both the measurement uncertainty and the simulation offset and thus are not discussed. At higher concentration (1 M), the electrostatic interaction with the negatively charged interface is less obvious. The transference number of K$^+$ and Cl$^-$ are comparable and primarily determined by their respective ion mobilities (with respect to the bulk values listed in Table 3.1). The finite differences suggest that the ion strength at 1 M is not sufficient to eliminate the surface effects at $\pm 0.4$ V fully for this nanopore. The normalized currents of both ions significantly increase in more dilute solutions. With the normalized current in 1 M representing the volume conductivity, the differences at lower concentrations quantify the surface conductivity that leads to the high-conductivity states.

<table>
<thead>
<tr>
<th>conc (M)</th>
<th>E (V)</th>
<th>measured i (nA)</th>
<th>K$^+$ current (nA)</th>
<th>Cl$^-$ current (nA)</th>
<th>total simulated i (nA)</th>
<th>$t_{K^+}$</th>
<th>$t_{Cl^-}$</th>
<th>normalized K$^+$ current (by conc)</th>
<th>normalized Cl$^-$ current (by conc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0500</td>
<td>0.40</td>
<td>8.84</td>
<td>5.1</td>
<td>3.1</td>
<td>8.23</td>
<td>0.62</td>
<td>0.38</td>
<td>102</td>
<td>62.0</td>
</tr>
<tr>
<td>0.100</td>
<td>0.40</td>
<td>13.8</td>
<td>8.3</td>
<td>5.6</td>
<td>13.9</td>
<td>0.60</td>
<td>0.40</td>
<td>83.0</td>
<td>56.0</td>
</tr>
<tr>
<td>1.00</td>
<td>0.40</td>
<td>66.8</td>
<td>34.3</td>
<td>33.6</td>
<td>67.9</td>
<td>0.51</td>
<td>0.49</td>
<td>34.3</td>
<td>33.6</td>
</tr>
</tbody>
</table>

The simulated current and corresponding transference number listed are based on the SCD that best fit the experiments at each concentration.
The asymmetrical nanopore geometry defines a radial influx of ions and a pseudoplanar efflux. Note that the ion species available within a certain mass transport distance is determined by the ion concentration on either side of the nanopore orifice. As divided by the cone angle (ca. 11°), the component of Coulombic interaction along the Z direction directly affects the ion current being detected, and the component along the R direction changes the ion concentration and flux distribution. At positive potentials (outside vs. inside), the radial K⁺ influx is facilitated by the negatively charged nanopore interior surface. The migration of Cl⁻ under the applied potential is also enhanced by the surface effects, but the total Cl⁻ flux is less significant compared to that of K⁺ because the efflux is planar and the Cl⁻ concentration is small near the interface. Therefore, the overall normalized current and K⁺ transference number increase upon the dilution of the electrolyte concentration. At negative potentials, the impacts of volume and surface potential attenuate each other. The radial influx of Cl⁻ is repelled by the Coulombic interaction with the negatively charged interface. The planar efflux of K⁺ is also suppressed by surface effects. Because the volume/geometric parameters are constant for individual nanopores, surface effects intensify upon the dilution of ion concentration. A threshold low conductivity state is established if surface effects reach their maxima. If conventional double-layer theory applies, then this effect is expected if the double layer overlaps inside the nanopore. The specific concentration reaching this threshold current obviously depends on the size and surface charge density of individual nanopores.

3.3 Experimental Section

3.3.1 Materials

Water (~18.2 MΩ-cm) was purified with a Barnstead E-pure water purification system. All other chemicals and materials were used as received. Corning 8161 glass capillaries (o.d. 1.50 mm, i.d.1.10 mm) were from Warner Instruments, and tungsten rods were from A-M System, Inc. Platinum wire (99.95%, diameter 25 μm), silver conductive paste, silver wire (99.9985%, diameter 0.5 mm), and ferro-
cenewere from Alfa Aesar. KCl was from J. T. Baker. CaCl$_2$, FeCl$_3$(97%), HNO$_3$, H$_2$SO$_4$, H$_2$O$_2$ (30%), acetone, acetonitrile, and tetrabutylammoniumperchlorate (TBAP) were from Sigma-Aldrich. 3-Aminopropyl(dimethyl-ethoxysilane was from Gelest Inc.

### 3.3.2 Conical Nanopore Fabrication and Surface Modification

The fabrication of glass nanopores followed previous reports.$^{50, 51, 131}$ Briefly, one end of a 25-μm Pt wire was electrochemically etched to create a sharpened nanotip. The sharpened Pt tip was then sealed inside a glass capillary. Excess glass was manually polished using sand paper and 50 nm Al$_2$O$_3$ nanoparticles (from Alfa Aesar) sequentially until the nanotip was exposed. After that, the exposed Pt-nanotip inside the glass shroud was electrochemically etched again, followed by mechanical pulling from the other end. Full removal of the Pt wire inside the glass capillary results in a conical nanopore, with the length of ca. 10-20 μm and the shape replicating that of a Pt nanotip. The nanopore structure has been extensively characterized by imaging and electrochemical methods in previous reports and thus is not reported in this article.$^{50, 51, 131}$ After a nanopore was fabricated, it was rinsed with H$_2$O, EtOH, and H$_2$O sequentially. Prior to silane modification, the glass surface was activated in 1 M HNO$_3$ for 15 min. After a thorough rinsing with H$_2$O, EtOH, and CH$_3$CN, the nanopore was loaded and soaked in 2% (v/v) 3-aminopropyl(dimethyl-ethoxy silane acetonitrile solution overnight. The modification by monoethoxy- silane was found to stabilize the electrochemical responses in the measurements based on previous studies.$^{131}$ The surface coverage is ca. 20% on the basis of those estimated from bulk measurements (Gelest).

### 3.3.3 Electrochemical Measurements

A Gamry Reference 600 potentiostat was used in the conductivity studies (cyclic voltammetry software). The scan rate was generally at 20 mV/s. Two Ag/AgCl wires were used to control the bias po-
tential. Because the nanopore region limits the detected current signals, the Faradic processes at the macroscopic Ag/AgCl wires are not discussed in the article. Both reference and counter electrode leads were connected to the same Ag/AgCl electrode immersed inside the nanopore, and the working electrode was outside, in the bulk solution. The radius of the nanopore is determined on the basis of the absolute current values at +0.050 and -0.050 V in 1M KCl, where the surface effect is more effectively screened by the high concentration of electrolytes.

### 3.3.4 Theoretical Simulation

COMSOL Multiphysics Package (Version 4.0a) was used. The modules of Electrostatics and Transport of Diluted Species were employed to solve Poisson equation and Nernst-Planck equation. The nanopore geometry and mesh elements are shown in Figure 3.10 with 1.3 maximum element growth rate, 0.3 curvature resolution and 1993912 degree freedom. In consideration of the symmetry along the centerline Z direction, half of the cross-section is used in the computation. Boundary 1 represents the center line. The nanopore orifice is at z = 0. To provide sufficient materials for the transport studies with reasonable computation expenses, and to maintain bulk concentration near electrodes for steady state response, the Z dimension extends to 10 microns inside the pore and 2 microns outside. The bias potential is applied between Boundary 3 (working electrode, out of pore) and Boundary 2 (reference electrode, inside pore). Boundary 5 represents negatively charged interior glass surface that are expected to affect the ion transport. To minimize the computation expenses without affecting the charge distribution near the mass transfer limiting region, negative charges are placed on the exterior surface represented by boundary 4, which is 20 times of the pore radius. Boundary 6 and 7 define the bulk dimension. As Boundary 6 and 7 are away from the mass transport limiting region, they are not charged to save the computation expenses. Note that the 0.3—nm—mesh element is approaching the size of solvated ions, which is a fundamental limit of continuum theory. The following parameters were used at
room temperature (298.13 K): density, $F = 1000 \text{ kgm}^{-3}$; viscosity, $\eta = 1 \times 10^{-3} \text{ Pa s}$; relative dielectric constant, $\varepsilon = 80$; Faraday constant, $F = 96485 \text{ Cmol}^{-1}$. The diffusion coefficients of $K^+$ and $Cl^-$ at each concentration are listed in Table 3.3. It is known that the diffusion coefficient varies in different concentration. Instead of using the value at infinite dilution, effective diffusion coefficient of $K^+$ and $Cl^-$ in each concentration is calculated shown below, with the details discussed in “Electrochemical Systems”, Third Ed. (Ch 11 & 12).

$$\frac{1}{\kappa} = -\frac{RT}{c_0 Z^+ Z^- F^2} \left( \frac{c_0 t^-}{c_+ D_-} \right)$$
in which $D_\pm$ is related to drag or friction coefficient and addresses ion-ion interaction. Based on the solution conductivity and ion transference number from literature, the conductivity and diffusion coefficient of cation and anion are calculated respectively.

$$\frac{1}{\kappa} = \frac{-RT}{c_T z_i z_j F^2} \left( \frac{1}{D_\pm} + \frac{c_0 t^0}{c_+ D_0^-} \right)$$

$$\kappa_i = \kappa \times t_i$$

$$D_i = \frac{\kappa_i RT}{|z_i| F^2 c_i}$$

The conductivity of species (ion) $i$ equals to its transference number multiply solution conductivity. Since the conductivity already includes the correction of ion-ion interaction, the calculated ion diffusion coefficient in each concentration offers better fitting of experimental results by simulation. Note the diffusion coefficient at infinite dilution could not be correlated to the solution conductivity following the above definition (reference data in Table 3.1). The comparison of 1M KCl results can be found in Fig. 3.11.

### 3.4 Summary

Ion transport at single conical nanopores is investigated experimentally by conductivity measurements under scanning potentials and theoretically through the simulation by solving the Poisson and Nernst-Planck equations. The volume and surface charge effects are differentiated by normalizing the current voltage responses measured at different ion concentrations. Surface effects via columbic interaction cancels the volume effects at negative potentials but enhances the volume effects at positive bias potentials, leading to low- or high-conductivity states in the measurements, respectively. The SCDS of individual nanopores are directly determined by fitting the experimental results based on continuum
theory. The spatial distribution of the ion concentration and, more importantly, the ion flux distribution inside the nanopore are reported. Correspondingly, the migration and diffusion contributions to the total flux are demonstrated to vary at different locations inside the nanopore. The flux distribution and its physical origin in the signal-limiting nanopore region are believed to be significant for sensing applications because the magnitude and duration of the sensing signal depend on the location and trajectory of analytes (along the center line vs near the interface).

**Figure 3.11** Comparison of the computed and measured current from a 26 nm nanopore in 1 M KCl solution. Black symbols represent data calculated with diffusion coefficient at infinite diluted KCl solution. The red symbols represent the data from the effective diffusion coefficient listed in Table 1. Black line is the measured current-voltage curve in 1 M KCl solution. (Reprinted with permission from ref. 113. Copyright (2012) American Chemical Society.)
4 ELECTRIC FIELD DEPENDENT SURFACE CHARGE DISTRIBUTION IN SINGLE CONICAL NANOPORES

Electrostatic interaction of mobile charges in solution with the fixed charges at nanometer-scale interface is known to strongly affect stochastic sensing and electrochemical energy conversion. The key parameter to describe this interaction, surface charge density (SCD), is not directly accessible at nanometer scale and often extrapolated from bulk values. In this report, an exponential distribution of SCD is introduced inside a single nanopore instead of a uniform distribution throughout the nanopore surface in theoretical simulation. The steady-state current-voltage ($i-V$) curves measured in different electrolyte concentrations are fitted with simulated current by solving Poisson and Nernst-Planck equations based on the predefined exponential gradient SCD. A maximum SCD value at the pore orifice is determined from the fitting of the high conductivity state current, while the distribution length of the exponential SCD gradient is determined by fitting the low conductivity state current. Quantitative fitting of the rectified $i-V$ curves is achieved and validated by the responses in different electrolytes. The SCD distribution, originating from the density of deprotonated surface functional groups, is proposed to dependent on local electric field and ionic strength. The exponential SCD gradient is correlated with the local electric field distribution.

4.1 Introduction

Rectified ionic conductance, resistance-capacitance with memory effects, and other interesting properties have been observed in the ion transport (IT) through individual synthetic nanopores and nanochannels connecting two solution reservoirs. The overall steady-state (SS) and non-SS ionic current signals are limited by the most constrained, and thereby most resistive nanopore region. This feature makes these nanodevices promising platforms as stochastic analytical sensors and other functional devices. At the signal limiting nanopore region, both nanoscale geometry (radius, length, etc.) and interface (i.e. coulombic interaction between the charges on substrate surface and mobile ions
in solution) affect the transport process. The resulting signal, obtained experimentally often in the format of overall current or conductance, reveals the combined impacts from the nanoscale geometry and interface. While significant advances have been achieved in the fabrication and characterization of nanodevices with known geometry, it remains a significant challenge to characterize nanoscale interfacial features such as the density and distribution of the fixed surface charges. It is even more complicated if the interface is confined inside a nanochannel that limits the direct accessibility for analysis.

The physical origin of the fixed charges on SiO$_2$ substrates, as in the case of glass and quartz nanopores and nanochannels, are a consequence of the deprotonation of surface silanol groups. Since the reaction involves the separation of charges (H$^+$ and SiO$^-$), the equilibrium is obviously a function of solution ionic strength. $^{126}$ Furthermore, the deprotonation of surface functional groups is facilitated by applied electric field. This effect may become more obvious for conical nanopore geometry, as the resulted electric field is always very high (~1 MV/m). Thus the resulted high driving force on protons is proposed to have a higher chance to drive dissociated protons away, resulting in more negative charges (-SiO$^-$). As the electric field inside a conical nanopore exponentially decreased from pore orifice to base, the ability to drive dissociated protons is different, thus results in an exponential surface charge gradient correspondingly with more negative charges at pore orifice (high electric field) and less at base (low electric field). For nanodevices with high surface-to-volume ratio, the surface effects greatly impact the ion transport processes. Consequently, the characterized nanogeometry is often found inadequate to quantitatively describe the observed transport behaviors in different electrolyte concentrations or at different solution pHs. This dampens the significance of the nanogeometry characterization, as it no longer predicts the transport responses or the detection of signals in sensing and other analytical applications that frequently employ various solution conditions. To address the heterogeneity of the nanodevice functionality/efficacy, which has been a well-known barrier for broad applications, quantitative description of the interface at nanometer scale and its impacts on IT are required.
The heterogeneity of the surfaces from different nanodevices further complicates the analysis under same solution or measurement conditions. This leads to the well-known heterogeneity in stochastic sensing and other related studies and limits broader applications.

Due to the surface effect, the SS conductivity of asymmetric nanopore devices with charged interface deviates from the linear Ohm’s behavior, known as ionic current rectification (ICR).\textsuperscript{17,18} This is confirmed in impedance analysis where different resistances are obtained at different potentials. Meanwhile, with a small perturbation by applying a sine potential waveform, the impedance analysis reveals a complex multi-time-constant transport process.\textsuperscript{131} The facilitation and inhibition by the charged interface to the overall ion transport induce intriguing memory effects in potential scanning measurements.\textsuperscript{99,135,143,144} The ICR is generally described by rectification factor (RF), which is calculated from the current ratio at arbitrarily selected potential amplitude at both positive and negative polarities. Both asymmetric nanogeometry and interfacial charges contribute to this ICR behavior. Due to the non-linear $i-V$ features, the RF values vary at different potential amplitudes in the same electrolyte solution and different ionic strength would result in different RF values as well. By the variation of the surface charge density (SCD) and the introduction of a linear gradient, the general trend of ICR has been successfully demonstrated in theoretical analysis for various nanogeometries.\textsuperscript{24,98,111,112,145,146} However, the physical origin of the proposed SCD gradient has been lacking. Furthermore, the current responses from experimental data have not been quantitatively correlated to the interfacial quantities in the simulation, especially at low conductivity states (constant SCD). One possible disconnection is that the diffusion coefficient frequently used in computation studies is obtained from infinite dilute solution, which will lead to systematically larger bulk conductivity than the experimentally measured solution conductivity.\textsuperscript{113} Note this deviation is partially cancelled in the RF related discussions.

In this report, the impact of the electric field, localized near the nanopore orifice, on the surface charge inside the conical nanopore is studied. With the proposed electrical field driving protons in the
protonation/deprotonation equilibrium of surface functional groups, a gradient SCD on the nanopore interior surface is introduced. The distribution length defining the SCD gradient is correlated with the distribution of electrical field intensity inside the nanopore. Illustrated in scheme 4.1, the SCD will decrease from a maximum value at the pore orifice to the bulk value of -1 mC m⁻² at a certain depth, at which position the electric field becomes negligible. The simulation model offers an excellent fit for the experimental results at both high and low conductivity states. We further demonstrate that the maximum SCD at pore orifice can be determined from the high conductivity state, while the low conductivity state reveals the distribution of the SCD gradient inside the pore. Though various inhomogeneous SCD distributions inside nanopore have been proposed in previous simulations, the physical origin is offered for the first time to address the relatively high maximum and the exponential SCD gradient to the best of our knowledge. Both exponential and linear distribution models are tested and further validated by predicting the ion transport behavior of other electrolytes (LiCl).

Scheme 4.1 A gradient SCD distribution inside a conical nanopore. The electric field intensity, represented by the blue and red colors at both ends of the half cross-section of a nanopore, dropped sharply at the first 1~2 µm inside the nanopore, indicated by the color transition.
4.2 RESULTS

4.2.1 Experiments: $i-V$ responses of conical nanopores in different electrolytes

![Graph showing conductivity of a 46-nm-radius nanopore in KCl (blue) and LiCl (green) solutions at different concentrations: 10 mM (solid line) and 1 mM (dashed line). Scan rate was at 20 mV/s. B. The ratio of $i_+/i_-$ (RF) versus absolute potential amplitude.](image)

Figure 4.1 A. Conductivity of a 46-nm-radius nanopore in KCl (blue) and LiCl (green) solutions at different concentration: 10 mM (solid line) and 1 mM (dashed line). Scan rate was at 20 mV/s. B. The ratio of $i_+/i_-$ (RF) versus absolute potential amplitude.

Representative experimental $i-V$ curves in Figure 4.1 are from a 46-nm-radius nanopore in different electrolyte solutions. A linear $i-V$ curve in 1 M KCl, shown in Figure 4.2, reflects the volume conductance defined by the nanopore geometry. At lower electrolyte concentration, the surface effects intensify. Correspondingly, the $i-V$ curves in Figure 4.1 deviate from the linear Ohm’s behavior. The non-linear correlation of the measured ion current with different potential amplitudes and polarities is in agreement with the literature. The $i-V$ responses from different electrolyte concentrations and different type of cations from the same nanopore (geometry, or volume remains constant) allow quantitative fitting in simulation and therefore the elucidation of surface parameters discussed next. The high conductivity states appear at positive applied potential because the bias is applied on the outside electrode with the inside electrode as reference. At the same concentration, the current from KCl solution is always larger than that of LiCl due to the larger ion mobility of $K^+$ over $Li^+$. 
Figure 4.2 Cyclic voltammograms (solid line) and theoretical simulation (symbols) of a 46 nm nanopore in 1 M electrolyte solution. The simulation parameters for 1 M KCl (blue): -100 mC m\(^{-2}\) constant SCD (circle); SCD linearly decreased from -100 mC m\(^{-2}\) to -1 mC m\(^{-2}\) within 0.94 µm (pentagon); -1 mC m\(^{-2}\) constant SCD (triangle). The simulation parameters for 1 M LiCl (green): -120 mC m\(^{-2}\) constant SCD (circle); SCD linearly decreased from -120 mC m\(^{-2}\) to -1 mC m\(^{-2}\) within 1 µm (pentagon); -1 mC m\(^{-2}\) constant SCD (triangle).

The nonlinear correlation with ionic concentration of either RF (i+/i-) or absolute current amplitude makes quantitative comparison of the measurements under different conditions challenging, which is the prerequisite for the detection of unknown analytes in sensing applications. From Figure 4.1B, it is obvious that the RF values differ at different potential amplitudes as well as different ions or ion strength. Meanwhile, in recent theoretical study, the RF values are predicted to decrease at both extremely high and low electrolyte concentrations.\(^{127,143}\) This is confirmed in our experiments. For example, RF first increase from 1 (1 M) to 4.3 (10 mM), and then decrease to 3.8 (1 mM) for KCl solution. The same trend was observed in LiCl, 1/4.3/5.5 (1 M/10 mM/1 mM).

In an earlier report, the simulated current at high and low conductivity states has been shown to display different responses upon electrolyte concentration variation.\(^{149}\) Those observations suggest that the high and low conductivity states might depend on more than one physical constraint and require separate parameters to describe in the modeling. This has been confirmed in the following discussion, where the experimental results will be subject to theoretical simulation for quantitative fitting. A predictive model is developed for the quantitative comparison of the \(i-V\) responses collected in either differ-
ent electrolytes or different concentrations from a certain nanodevice, and to determine the surface parameters of individual nanodevices.

### 4.2.2 Simulation based on Continuum Theory

The measured current signal originates from the combined volume and surface conductivity. For the same nanopore in different electrolyte solutions, the volume conductance is solely determined by the solution conductivity since the geometry remains constant. Therefore, the deconvolution of the volume contribution from the overall current will reveal the surface impacts and vice versa. Aiming at quantitative fitting of the characteristic experimental data shown in Figure 4.1 and not just to mimic the trend of current rectification qualitatively, three types of SCD definition are employed. After the effectiveness of the proposed model is validated by the quantitative fitting, the physical meaning of the parameters is discussed.

In the first type SCD definition, a constant SCD is applied to the nanopore surfaces that affect the ion transport processes. This enables the comparison with most simulation reports, in which the value of SCD is varied to demonstrate ICR effects. A linear SCD gradient is also proposed in the literature to better mimic ICR responses.\(^{112}\) To evaluate the impacts of SCD definition on the fitting and find out the physical origin to induce those non-uniform distributions, in the next two scenarios, the SCD is defined to have an exponential distribution inside the nanopore, as defined by the following equations:

- **Exponential SCD:**
  \[
  \sigma(z) = (\sigma_a - \sigma_b) \cdot \left( e^{-\frac{z}{\tau}} \right) + \sigma_b \; (\tau < 3 \mu m) \tag{Eq. 4.1}
  \]

- **Linear SCD:**
  \[
  \sigma(z) =\begin{cases} 
  \frac{\sigma_a - \sigma_b}{\tau} \cdot z & (z \leq \tau) \\
  \sigma_b = -1 \text{ mC m}^{-2} & (z > \tau)
  \end{cases} \tag{Eq. 4.2}
  \]

\(\sigma(z)\) is the SCD at depth \(z\) inside the nanopore. At the pore orifice (\(z = 0\)), \(\sigma\) reaches a maximum value \(\sigma_a\). At the pore base (\(z = 10 \mu m\)), \(\sigma = \sigma_b = -1 \text{ mC m}^{-2}\), a commonly used value from a planar silica surface. \(\tau\) represents a characteristic distribution length established by the experimental conditions. For
τ > 3 μm, in the exponential SCD definition, to be able to define the SCD at the pore base at -1 mC m$^{-2}$, a practical expression is provided in Equation 4.3.

$$\sigma(z) = a \cdot \left(e^{-\frac{z}{\tau}}\right) + b \quad (\tau \geq 3\mu m)$$ (Eq. 4.3)

At a given distribution length τ, a and b are calculated by: at $z = 0$, $\sigma_o = a+b$; and at $z = 10 \mu m$, $\sigma_b = a \cdot \left(e^{-\frac{10}{\tau}}\right) + b = -0.001 \text{ mC m}^{-2}$. These definitions approach the linear SCD gradient and serve as the boundary conditions for the analysis. At τ < 3 μm, this equation is mathematically identical with Eq 4.1.

4.2.3 Simulation of the measured current at high and low conductivity states

The impacts of the nanopore SCD on the ion transport current are demonstrated and compared with the experimental results. Panel A in Figure 4.3 shows the results by the variation of a constant SCD value that is defined uniformly throughout the nanopore interior surface, a common approach employed in simulation literature. The nanopore geometry used in the simulation is validated by the perfect fitting of 1 M KCl and LiCl experiments shown in Figure 4.2. As the SCD value increases, the simulated current increases drastically at high conductivity states as previously reported. Unlike the simulation literature that mostly focus on the RF (the ratio of current at opposite potentials), the current at low conductivity states is found to remain basically unchanged if the SCD is below ca. 50 mC m$^{-2}$, and then slightly increases at higher SCD values as indicated by arrows. It is obvious that in the fitting of the experimental current, a discrepancy arises from the overestimation of the low conductivity current. Even though the simulated RF ($i_+ / i_-$) could match that of the experiments if the SCD value is further increased, both high and low conductivity state currents would be larger than real experimental data. The results suggest that a simple constant SCD is not enough to fully describe the responded ionic current at all bias potentials.
Figure 4.3 The simulation of the experimental current from a 46-nm-nanopore in 10 mM KCl with different SCD definitions on the interior surface. A. with a constant SCD uniformly distributed. B. an exponentially decreased SCD, with $\sigma_0 = -100 \text{ mC m}^{-2}$ and the distribution length varied as indicated in the plot. C. a linearly decreased SCD, with $\sigma_0 = -100 \text{ mC m}^{-2}$ and the distribution length varied as indicated in the plot.

The simulations with an exponential and a linear SCD gradient are presented in panels B and C respectively. The $\sigma_0$ (-100 mC m$^{-2}$) is determined from the best fitting for high conductivity current in panel A while the distribution length is systematically varied. The change in the simulated high conductivity current appears to be negligible upon the variation of the SCD distribution length (panels B and C). Excitingly, the simulated current at low conductivity states decreases as distribution length is lowered for both exponential and linear SCD gradient. Ultimately, the experimental value is approached at a certain distribution length. This trend is also confirmed using other nanopores, with one example shown in Figure 4.4.
Figure 4.4 The simulation of the experimental current from a 26-nm-nanopore in 50 mM KCl with an exponentially decreased SCD, with $\sigma_o=-170 \text{ mC m}^{-2}$ and the distribution length varied as indicated in the plot.

Qualitatively, the observation can be explained with the two factors $\sigma_o$ (SCD at pore orifice) and $\tau$ (distribution length) separately. Since the cation is known to be the main charge carriers, the following discussion will mainly focus on cation transport. At high conductivity states (positive bias, outside vs. inside), the cations will migrate from outside solution into the pore and then move from the tip to base. This process is limited by the most resistive region along the transport trajectory, which is at the tip orifice. Since the nanopore orifice remain constant in different electrolyte concentrations, the rectified high conductivity current is therefore mainly correlated with the $\sigma_o$ at the pore orifice. This explains why the simulated high conductivity current remains unaffected with a fixed $\sigma_o$ upon the variation of the distribution length inside the nanopore. At low conductivity states, the cations will migrate from the base toward the tip. Therefore, the fixed charges along the nanopore interior surface, described by both $\tau$ and $\sigma_o$, will affect the cation flux, and thus the detected current. An overestimation of SCD, either by defining a uniform distribution of $\sigma_o$ or a gradient with longer $\tau$, will introduce more negative charges on the nanopore interior surface in the simulation. Consequently, the cation concentration and cation flux will be arbitrarily increased inside the nanopore due to the electrostatic interactions, leading to an overestimation of low conductivity state current in the simulation. This has been confirmed in both panels B
and C, where $\sigma_0$ is fixed, the ionic flux/current solely depends on $\tau$. Only as $\tau$ reaches the real value determined by measured condition, the experimental current is fitted. The minor contribution from anion is discussed in the transference number analysis.

### 4.2.4 The quantitative description and prediction of the experimental $i-V$ results

![Graph showing $i-V$ curves for KCl and LiCl](image)

**Figure 4.5** The optimized simulation of the measured $i-V$ curves with a 46 nm nanopore based on an exponential SCD distribution in A. KCl and B. LiCl solutions. The solid curves were measured experimentally and the symbols were from the simulation. Fitting parameters: $\sigma_0 = -100$ mC m$^{-2}$ and $\tau = 0.4$ $\mu$m for 10 mM KCl; $\sigma_0 = -70$ mC m$^{-2}$ and $\tau = 1.46$ $\mu$m for 1 mM KCl; $\sigma_0 = -120$ mC m$^{-2}$ and $\tau = 0.4$ $\mu$m for 10 mM LiCl; $\sigma_0 = -70$ mC m$^{-2}$ and $\tau = 1.46$ $\mu$m for 1 mM LiCl.

Next we demonstrate that the proposed model can be employed to quantitative describe and even predict the experimental $i-V$ responses in a range of concentrations, and predict the response of electrolytes with different monovalent cations (main charge carriers) at comparable concentration ranges.

Using the $\sigma_0$ and $\tau$ determined at $\pm 0.4$ V in each concentration (as demonstrated in Figure 4.3), the simulated $i-V$ results in both 1 mM and 10 mM KCl solutions are presented in Figure 4.5A. The simulated current based on an exponential SCD distribution fits the experimental data (solid curves) perfectly at all potential amplitudes. The fitting with a linear SCD distribution is shown in Figure 4.6. The results from other sized nanopores (26-nm and 110-nm radius) are included in Figure 4.7, which display similar fittings.
Figure 4.6 The optimized simulation of the measured $i-V$ curves with a 46 nm nanopore based on a linear SCD distribution in A. KCl and B. LiCl solutions. The solid curves were measured experimentally and the symbols were from the simulation. Fitting parameters: $\sigma_o = -100$ mC m$^{-2}$ and $\tau = 0.94$ µm for 10 mM KCl; $\sigma_o = -70$ mC m$^{-2}$ and $\tau = 3.6$ µm for 1 mM KCl; $\sigma_o = -120$ mC m$^{-2}$ and $\tau = 1$ µm for 10 mM LiCl; $\sigma_o = -70$ mC m$^{-2}$ and $\tau = 3.6$ µm for 1 mM LiCl.

Figure 4.7 Cyclic voltammogram (solid line) and optimized theoretical simulation (red squares) of a 26 nm nanopore (left panel) in 50 mM KCl and a 110 nm nanopore (right panel) in 100 mM KCl based on an exponential gradient SCD. Fitting parameters: $\sigma_o = -170$ mC m$^{-2}$, $\tau = 0.6$ µm for the 26 nm nanopore; $\sigma_o = -480$ mC m$^{-2}$, $\tau = 2$ µm for the 110 nm nanopore.

To further demonstrate the effectiveness of this approach, the experiments using the same nanopore but in LiCl solutions and the corresponding optimized simulations are shown in Figure 4.5B. By replacing the K$^+$ with Li$^+$ in the simulation (change diffusion coefficient accordingly), the simulated current matches experiments as well. The fitting parameters $\sigma_o$ and $\tau$ of the optimized simulation are slightly differently. Considering the variation of SCD at ±10 mC m$^{-2}$ corresponds to 0.6 e/10 nm$^2$, or the uncertainty of ±1 deprotonated site per 17 nm$^2$, the fitting parameters are consistent under experimental
conditions. The differences in $\sigma_o$ and $\tau$ between KCl and LiCl could also indicate the difference in ion size and/or surface binding/adsorption of cations with Si-O’ groups.\textsuperscript{150}

The fitting results suggest that as electrolyte concentration decreases, $\sigma_o$ decreases while $\tau$ increases. This can be qualitatively explained by the deprotonation of surface silanol groups that involves the separation of charges (H\textsuperscript{+} and Si-O\textsuperscript{−}). Lower ionic strength will inhibit charge separation, thus less surface charge is expected in this case. The argument is also supported by the experiments using fluorescence dyes confined inside nanodevices: the fluorescence intensity varies when the SCD is changed from 100 mC m\textsuperscript{−2} to 2 mC m\textsuperscript{−2} (Figure 3d in ref. 103).\textsuperscript{134} The SCD variation is well correlated with the electrolyte concentration dilution from 1 M to 1 mM in this study. With less charge carriers available at lower concentration inside the nanopore, the applied electric field will drop along a longer distribution length, therefore longer $\tau$.

The comparison of the simulated and experimental currents at relative low concentration reveals surface contribution to the overall measurements. At high electrolyte concentration, the surface effects are effectively screened by high ionic strength. The simulated current should no longer be affected by $\sigma_o$ and $\tau$. This is confirmed by the comparison of the simulation with different SCD definitions in 1 M KCl and 1 M LiCl solutions, which are included in Figure 4.2.

\textbf{4.2.5 The tolerance of the effective SCD profile determined from $i$—$V$ measurements}

Retrospectively, the quantitative fitting of the experimental current by simulation reveals surface charge parameters at nanoscale interfaces that are very challenging to determine. The $\sigma_o$ and $\tau$ in the proposed model could be non-invasively determined by fitting the high and low conductivity current respectively. From Figure 4.3 panels B and C, the simulated high conductivity current appears to be sensitive to $\sigma_o$ and less dependent on $\tau$. As $\sigma_o$ increases, the simulated current increases, shown in Figure 4.3, panel A. To better demonstrate how $\sigma_o$ and $\tau$ affects the simulated current, $\sigma_o$ and $\tau$ are systematically varied as shown in Figure 4.8. The solid and open symbols represent the exponential and linear SCD
distribution defined on the nanopore interior surface. To demonstrate the resolution of the simulation (tolerance of the determined $\sigma_o$ and $\tau$), a ±5% variation from experimental current is added to represent the possible measurement uncertainty.

![Figure 4.8](image)

**Figure 4.8** Error analysis of the maximum SCD $\sigma_o$ at the nanopore orifice and the distribution length $\tau$ in the simulation of $i-V$ measurements of a 46 nm GNP in 10 mM KCl. A. high conductivity states at +0.4 V. B. low conductivity state at -0.4 V. Different colors represent different $\sigma_o$: -90 mC m$^{-2}$ (blue); -100 mC m$^{-2}$ (red); -110 mC m$^{-2}$ (green). Solid symbols represent the simulation based on an exponential SCD distribution, and open ones representing a linear SCD distribution.

At +0.4 V bias shown in panel A, a ±10 mC m$^{-2}$ SCD (0.6 e per 10 nm$^2$) from the optimized SCD of 100 mC m$^{-2}$ gives ca. 5% variation of the simulated current, regardless of the variation of $\tau$. This suggests that $\sigma_o$ can be directly determined by the fitting of high conductivity state current. At -0.4 V bias shown in panel B, a ±10 mC m$^{-2}$ SCD (blue and green symbols) results in negligible difference of simulated current at each distribution length. However, the distribution length $\tau$ affects the simulated current significantly. At the same $\sigma_o$ ± $d\sigma$ (the three types of symbols), shorter $\tau$ defines less surface negative charges, which will decrease the local cation concentration thus its conductivity. Accordingly, the simulated current decreases and matches the measured current at a certain $\tau$ value. The analysis of the results in 1 mM KCl follows similar trend as presented in Figure 4.9. A unique combination of $\sigma_o$ and $\tau$ can therefore be determined for a certain nanopore under the measurement condition following the above approach.
Figure 4.9 The impacts of distribution length on the simulated current at A: high conductivity state at +0.4 V and B: low conductivity state at -0.4 V. Data collected from a 46 nm nanopore in 1 mM KCl with maximum SCD set at -70 mC m⁻².

4.2.6 The transference number of cations and anions at high and low conductivity states

Table 4.1 Cation transference number based on an exponential SCD distribution.*

<table>
<thead>
<tr>
<th>Conc. (M)</th>
<th>KCl</th>
<th>LiCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>H (+0.4 V)</td>
<td>0.72</td>
<td>0.57</td>
</tr>
<tr>
<td>L (-0.4 V)</td>
<td>0.95</td>
<td>0.92</td>
</tr>
<tr>
<td>0.001</td>
<td>0.60</td>
<td>0.43</td>
</tr>
<tr>
<td>0.01</td>
<td>0.76</td>
<td>0.64</td>
</tr>
<tr>
<td>1</td>
<td>0.49</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* The transference number listed in this table is calculated from the simulations that best fit the experiments in each concentration. H and L represent high and low conductivity states respectively. $t_{Cl}^- = 1 - t_{cation}$. The respective contribution of cations and anions to the overall measured current is quantified in Table 4.1. The cation transference number is determined from the optimized simulation with an exponential gradient SCD that best fits the corresponding experimental current in each KCl or LiCl solution. Anion transference number can be obtained by $t_{Cl}^- = 1 - t_{cation}$ thus not listed. As expected, at high electrolyte concentration (1 M), surface effect is negligible. The $K^+$ transference number (0.49) approximately equals to that of $Cl^-$(0.51) due to the similar ion mobility. Because the mobility of $Li^+$ is ca. half of that from $Cl^-$, the $Li^+$ transference number is lower accordingly. As bulk electrolyte concentration decreases, the surface effects become more prominent. Being the counter ions compensating the negative surface
charges, the cations always have larger transference number than the anions Cl\(^-\) at both high and low conductivity states. This validates the earlier argument that K\(^+\) is the major current carrier at each condition. Therefore, as concentration decreases, the surface contributes more and more, correspondingly the cations transference number increases, for both polarities.

Interestingly, at low conductivity states (\(-0.4\) V), the K\(^+\) transference number increases from 0.49 in 1 M KCl (and 0.30 for Li\(^+\) in 1 M LiCl) to almost unity in 1 mM. The observation supports previous theoretical prediction\(^{342}\). Furthermore, at lower electrolyte concentrations, the transference number of K\(^+\) is higher than that at high conductivity states though the current amplitude is lower. Those behaviors can be explained by the electrostatic interactions between mobile solution ions with the fixed negative surface charges. Due to the surface effects, cations will concentrate near the negatively charged surface, while anions will be repelled. The high conductivity states are established by the enrichment of both cations and anions inside the nanopore as previously reported. Cations are obviously more abundant, giving larger transference number. However, at low conductivity states, the concentration of both ions are lower than bulk at the nanopore region. The depletion effect is more significant for anions, leading to a much lower current primarily carried by the accessible cations, or approaching unity for cation transference number. The concentration profiles of K\(^+\) and Cl\(^-\) are provided in Figure 4.10 and 4.11.
Figure 4.10 The concentration profiles along radial direction of K⁺ (blue) and Cl⁻ (red) at A: +0.4 V; and B: -0.4 V at cutline z = 100 nm. The x axis represents the distance away from the centerline along the cutline. The radius of the nanopore is set at 46-nm with surface charge density defined at -100 mC m⁻² to -1 mC m⁻² within 0.4 µm (value based on Figure 4.3). The bulk concentration of KCl is 10 mM. The intercept on the concentration axis is enlarged in the inserted panels.

Figure 4.11 Concentration profiles of K⁺ (red) and Cl⁻ (green) at A: +0.4 V; and B: -0.4 V along centerline. The radius of the nanopore is set at 46-nm with surface charge density defined at -100 mC m⁻² to -1 mC m⁻² within 0.4 µm (value based on Figure 4.3). The bulk concentration of KCl is 10 mM (indicated by black dash line). The concentration of K⁺ and Cl⁻ overlapped.

4.3 DISCUSSION

The physical origin of the gradient SCD distribution and the rationale of its variation are proposed in the following discussion. The SCD of silica surface, even planar at macroscopic scale, could vary in a wide range depending on measurement conditions as reported in literature. Unfortunately, direct characterization of the SCD and SCD distribution (τ and σ₀) inside a nanodevice at sub-micron resolution is unavailable to the best of our knowledge. Since the surface charges are from the deprotonation of
surface functional groups and the surface equilibrium is a function of local electrical field, the gradient SCD distribution inside a nanopore is attributed to the applied electric field and the available charge carriers (electrolytes in solution) inside a conical nanopore.

![Electric field intensity distributions](image)

**Figure 4.12** Electric field intensity distributions (solid lines) for a 46 nm conical nanopore with neutral surface in 10 mM KCl. (A) centerline and (B) a parallel cutline 3 nm from the interior surface at -0.4 V. The dash lines are exponential fittings of the electric field intensity profiles. Electric field intensity distributions through the whole pore are shown in inserted panels. Pore depth at 0 and 10 μm correspond to the location of orifice and base respectively.

The distribution of an applied electrical field inside a nanopore with neutral surface (no surface charge) is firstly presented in Figure 4.12. This corresponds to a simplified scenario that the surface electric field established by fixed surface charges is negligible. Figure 4.12 shows the overall computed electric field intensity profiles at low conductivity states (-0.4 V). Similar electric field intensity profiles at -0.2 V and at high conductivity states (+0.4 V and +0.2 V) are included in Figure 4.13 and 4.14 respectively. Both along the nanopore centerline in panel A and near the interior surface in panel B (Figure 4.12), a high electric field (1-2 MV/m) at the orifice is observed due to the taper geometry. Because no surface electric field is defined, the computed field intensity corresponds solely to the applied electric field, which drops primarily within the first 1~2 μm inside the nanopore. The electric field intensity curves display an exponential gradient by fitting (dash lines).
Figure 4.13 Electric field intensity distributions (solid lines) for a 46 nm conical nanopore with neutral surface in 10 mM KCl. (A) centerline and (B) a parallel cutline 3 nm from the interior surface at -0.2 V. The dash lines are exponential fittings of the electric field intensity profiles. Electric field intensity distributions through the whole pore are shown in inserted panels. Pore depth at 0 and 10 μm correspond to the location of orifice and base respectively.

Figure 4.14 Electric field intensity distributions (solid lines) for a 46 nm conical nanopore with neutral surface in 10 mM KCl. (A) centerline and (B) a parallel cutline 3 nm from the interior surface at +0.4 V (red) and +0.2 V (blue). Pore depth at 0 and 10 μm correspond to the location of orifice and base respectively.

The rationale of an exponential SCD gradient inside the nanopore established in accordance with the applied electric field is illustrated in Scheme 4.2. Within ca. 1 μm inside the nanopore orifice, the higher electric field will impose stronger force to facilitate the proton dissociation compared to the lower field region toward the base. If the electric field becomes negligible, the surface reaction equilibrium is less affected. The SCD should therefore maintain the value comparable to literature. Consequently, the SCD at the nanopore tip should be higher than the SCD at the base which adopts the bulk value at ca. 1-10 mC m⁻² (1 mC m⁻² used in the simulation).
**Scheme 4.2** Surface deprotonation affected by the applied electric field and the formation of an exponential gradient surface charge distribution.

It is worth pointing out that a slight redistribution of the surface deprotonation sites will be sufficient to induce such SCD gradient. As $1 \text{e} = -1.6 \times 10^{-19} \text{C}$, a SCD of $-100 \text{ mC m}^{-2}$ corresponds to ca. 6 deprotonated silanol groups per 10 nm$^2$. Taking the total site density of surface silanol groups at 4.9 per nm$^2$, or 49 per 10 nm$^2$, only ca. 10% variation is expected. This variation corresponds to ca. 0.1 shift in surface pKa at sub-micron scale, making it very challenging to characterize at such spatial resolution. Note the surface pKa and the site density at 4.9 per nm$^2$ are based on statistics. For the dynamic surface chemical process, the probability of the deprotonation at different locations at nanoscale is not accurately reflected in those ensemble descriptions. Furthermore, individual nanopore devices could have different site density of different types of silanol groups (isolated and neighboring ones linked by hydrogen bonding) and unknown distributions of each type at nanoscale. Therefore, the SCD profile of individual nanodevices could vary, which accounts for the heterogeneity in current responses and device functionality. It is therefore significant to be able to non-invasively address the surface parameters of individual nanodevices for transport related applications.

The electric field near a charged surface is known to be strong (more than MV/m in most cases). Next we discuss whether the applied electric field is sufficient to alter the surface charge distribution that establishes a stronger surface electric field. The surface electric field inside the nanopore, with the vector normal to the interior surface, can be divided into two components normal and parallel to the
applied electric field direction separated by the half-cone angle $\theta$. The parallel component directly affects the ion flux driven by the applied electric field, or the detected current signal, and corresponds to ca. 10-20% of the surface electric field intensity (by $\sin \theta$). Considering the applied electric field only needs to redistribute ca. 10% of the deprotonation sites, or change the probability by 10% inside the nanopore, an applied electric field that is one or two orders magnitude lower than a surface field could establish the SCD gradient as proposed at a first degree approximation.

**Figure 4.15** The electric field intensity along the cutlines parallel to nanopore interior surface and the comparison with the SCD profile (dash line). The results are from a 46 nm nanopore in 10 mM KCl. The overall electric field $E_{\text{tot}}$ is divided by the listed factor for direct comparison of the gradient distribution.

Quantitative comparison is shown in Figure 4.15. The overall electric field intensity $E_{\text{tot}}$ is the sum of the applied and surface electric fields. The $E_{\text{app}}$ is computed with a neutral surface, corresponding to the pure externally applied field that is proposed to establish the SCD gradient. Because the SCD needs to be predefined during the simulation, an exponential SCD profile replicating the electric field distribution is firstly introduced. The $\tau$ and $\sigma_0$ are then systematically varied to fit the experimental results as shown in Figure 4.3. The SCD profile is computed using the $\tau$ and $\sigma_0$ that best fit the experimental current responses. Two $E_{\text{tot}}$ curves near the surface are included, each divided by the listed factor to better illustrate the curve gradient. The factors affirm that the applied electric field is comparable with the surface electric field component in parallel. Note that the applied electric field intensity at dif-
Different bias (i.e. -0.2 V vs. -0.4 V) is within the same order of magnitude. Similar comparison with electric field along the centerline is provided in Figure 4.16.

**Figure 4.16** The electric field intensity along the centerline and the comparison with the SCD profile (dash line). The results are from a 46 nm nanopore in 10 mM KCl. The overall electric field $E_{tot}$ is divided by the listed factor for direct comparison of the gradient distribution.

**Figure 4.17** The electric field intensity along the cutlines parallel to nanopore interior surface and the comparison with linear SCD profile (dash line). The results are from a 46 nm nanopore in 10 mM KCl. The overall electric field $E_{tot}$ is divided by the listed factor for direct comparison of the gradient distribution.

A quantitative correlation between the electric field strength and the shift in the deprotonation probability is not available at this scale. Qualitatively, $E_{app}$, $E_{tot}$ and the defined SCD primarily dropped within ca. 1 µm at similar gradient. It is also interesting to notice that $E_{tot}$ curve approaches SCD toward the surface. The results within 1 nm from the surface are not discussed due to the unknown interior surface roughness from experiments and the continuum theory being used in the simulation. Shown in Figure 4.17, a linear SCD gradient generates discontinuous sharp transitions at the defined transition point.
Since the dimension discussed here is still in the continuum regime (10s-100s nm), and based on the physical picture discussed, we believe the exponential gradient is a better description of the real experiments.

It is worth evaluating the impacts of finite geometric variation near the nanopore orifice on the aforementioned analysis. In a computational study, the nanopore geometry has been systematically varied by a mathematical description. Higher rectification could be achieved following the ICR trend of: \( RF_{\text{bullet}} > RF_{\text{conical}} > RF_{\text{trumpet}} \). The geometry near the pore orifice is arbitrarily adjusted to test whether the systematical larger negative current is due to the finite uncertainty of nanopore geometry limited by the characterization resolution.

![Diagram of nanopore geometry](image)

**Figure 4.18** The variation of nanopore geometry near the pore orifice. The dash line indicates the geometry before distortion.

Because the overall nanopore geometry has been well characterized in previous reports and validated by the volume conductivity measurements in 1 M electrolyte solutions, the half cone angle was fixed at 11° to maintain the volume conductivity. Note the systematic geometric variation in ref. 91 leads to the change of volume conductivity (much smaller overall half-cone angle toward the pore base) thus not applicable to this study. With a small variation of the orifice geometry to create bullet-like geometry shown in Figure 4.18, the impacts on the simulation results appear to be negligible.
Meanwhile, the effect of SCD distribution of the external surface is also studied. The high electric field at the nanopore orifice will have similar effects following the same rationale discussed earlier. The SCD distribution is determined following the same approach as that inside the nanopore. Accordingly, an exponentially decreased SCD on the exterior surface near the orifice is found to cause ca. 10% variation in the simulated current compared to that from a constant (uniform) SCD distribution ($\sigma_o$). The difference is comparable with the 5% tolerance thus not further discussed. The absolute values of the fitting parameters could change slightly but the trend of the analysis will not be affected.

4.4 Experimental Section

4.4.1 Materials

Water (~18.2 MΩ•cm) is purified by a Barnstead E-pure water purification system. Silver wire (99.9985%, diameter 0.5 mm), silver conductive paste, platinum wire (99.95%, diameter 25 µm), and ferrocene were used as received from Alfa Aesar. Tungsten rods (A-M System, Inc.), Corning 8161 glass capillaries (OD 1.50 mm, ID 1.10 mm, Warner Instruments) were used as received. All other chemicals and materials were from Sigma-Aldrich, with 3-aminopropyltrimethoxy silane from Gelest Inc.

4.4.2 Conical Nanopore Fabrication and Surface Modification

The fabrication procedure of the glass nanopores followed previous reports. Briefly, a Pt nanotip is created by electrochemically etching, followed by sealing the Pt nanotip inside a glass capillary. Manual polishing of excess glass from the end leads to the exposure of the Pt nanotip or nanodisk. A through conical glass nanopore is obtained by full removal of the Pt wire via electrochemical etching and mechanical pulling. The nanopore shape replicates that of sharpened Pt nanotip. The interior surface of the nanopores is modified with 3-aminopropyltrimethoxy silane to improve the stability of the current responses. Note the surface coverage is ca. 20%, thus the surface remains to be negatively
charged at ambient pH. The fabrication, surface modification, and characterization of the nanopores have been well documented in previous reports thus not elaborated.\textsuperscript{50,51,131}

4.4.3 Electrochemical Measurements

The current-potential ($i-V$) responses (cyclic voltammetry) are measured using a Gamry Reference 600 potentiostat. The potential scan rate is 20 mV/s. Two Ag/AgCl wires are used to control the applied bias. The bias is defined by working versus reference, with the working electrode in the outside solution while reference inside the nanopore. The radius of the nanopore is determined based on the conductivity at $+0.050$ V to $-0.050$ V in 1 M KCl solution, at which condition the surface effect is effectively screened by the high concentration of electrolytes and small potential amplitudes (Figure 4.2).

4.4.4 Simulation

COMSOL Multiphysics Package (Version 4.0a) was used. Two models: Electrostatics and Transport of Diluted Species were employed to solve Poisson equation and Nernst-Planck equation. Adaptive triangle mesh was used. The geometry and SCD distribution are represented in Scheme 4.1. Briefly, half of the nanopore cross-section is used in the computation to save computation time since geometry is symmetric along the centerline (Z direction). Adaptive mesh with free triangular element was used. The mesh size within the first 300 nm inside the nanopore orifice was limited to 0.01 nm in an attempt to minimize the simulation noise. Other mesh elements at the charged boundaries (interior and exterior next to the nanopore orifice) were set to range from 0.3 nm to 0.6 nm. The maximum element growth rate is 1.3, 0.3for curvature resolution and 1993912 degree freedom. The nanopore orifice is at $z=0$. The pore length (Z) is set to be 10 µm (Pore length will affect the ion transport behavior if it is not long enough). One bulk reservoir with dimension of 2 µm X 2 µm connects the tip of nanopore for the ion transport. The bias potential is applied between this reservoir (working electrode, out of pore) and the bottom of nanopore (Z=10 µm, reference electrode inside pore).
One key notion is the introduction of a gradient SCD distribution instead of a uniform (constant) SCD on interior surface. The following parameters at room temperature were used \( T = 298.13 \text{ K} \): density \( \rho = 1000 \text{ kg/m}^3 \), relative permittivity \( \varepsilon = 80 \), Faraday constant \( F = 96485 \text{ C/mol} \). Instead of using the values at infinite dilution, an effective diffusion coefficient \( D_i \) is used to compute the volume conductivity (reflecting geometry effects) in simulation as previously reported.\(^{113} \) The effective diffusion coefficients of \( K^+ \) and \( Cl^- \) in KCl solutions, and \( Li^+ \) and \( Cl^- \) in LiCl solutions at different concentrations were listed in Tables 4.2 and 4.3 respectively.

**Table 4.2** The effective diffusion coefficient of \( K^+ \) and \( Cl^- \) ions in KCl solution.*

<table>
<thead>
<tr>
<th>conc (M)</th>
<th>conductivity (S/m)</th>
<th>( t_{K^+} )</th>
<th>( t_{Cl^-} )</th>
<th>( K^+ ) conductivity (S/m)</th>
<th>( Cl^- ) conductivity (S/m)</th>
<th>effective ( D_{K^+} ) (m(^2)/s)</th>
<th>effective ( D_{Cl^-} ) (m(^2)/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0010</td>
<td>0.01470</td>
<td>0.4905</td>
<td>0.5095</td>
<td>0.007</td>
<td>0.007</td>
<td>( 1.92\times10^{-9} )</td>
<td>( 1.994\times10^{-9} )</td>
</tr>
<tr>
<td>0.010</td>
<td>0.1413</td>
<td>0.4902</td>
<td>0.5008</td>
<td>0.069</td>
<td>0.072</td>
<td>( 1.840\times10^{-9} )</td>
<td>( 1.918\times10^{-9} )</td>
</tr>
<tr>
<td>1.0</td>
<td>11.19</td>
<td>0.4882</td>
<td>0.5118</td>
<td>5.463</td>
<td>5.727</td>
<td>( 1.455\times10^{-9} )</td>
<td>( 1.525\times10^{-9} )</td>
</tr>
</tbody>
</table>

*The solution conductivity and ion transference number are from “Electrolyte Solutions”, Second edition, by Robinson and Stokes (table 7.7 on page 158; and Appendix 6.3 on page 466).

**Table 4.3** The effective diffusion coefficient of \( Li^+ \) and \( Cl^- \) ions in LiCl solution.*

<table>
<thead>
<tr>
<th>conc (M)</th>
<th>conductivity (S/m)</th>
<th>( t_{Li^+} )</th>
<th>( t_{Cl^-} )</th>
<th>( Li^+ ) conductivity (S/m)</th>
<th>( Cl^- ) conductivity (S/m)</th>
<th>effective ( D_{Li^+} ) (m(^2)/s)</th>
<th>effective ( D_{Cl^-} ) (m(^2)/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0010</td>
<td>0.01112</td>
<td>0.334</td>
<td>0.666</td>
<td>0.0038</td>
<td>0.0075</td>
<td>( 9.996\times10^{-10} )</td>
<td>( 1.993\times10^{-9} )</td>
</tr>
<tr>
<td>0.010</td>
<td>0.107</td>
<td>0.3289</td>
<td>0.6711</td>
<td>0.0353</td>
<td>0.0720</td>
<td>( 9.399\times10^{-10} )</td>
<td>( 1.918\times10^{-9} )</td>
</tr>
<tr>
<td>1.0</td>
<td>7.32</td>
<td>0.297</td>
<td>0.703</td>
<td>2.174</td>
<td>5.146</td>
<td>( 5.789\times10^{-10} )</td>
<td>( 1.370\times10^{-9} )</td>
</tr>
</tbody>
</table>

*0.001 M and 0.01 M LiCl conductivity: “The Physical Chemistry of Electrolytic Solutions”, Third edition, by Harned Owen (table 6-2-1A on page 697).

1M LiCl conductivity: “Electrolyte Solutions”, Second edition, by Robinson and Stokes (Figure 11.5 on page 320).

0.01 M and 1M LiCl transference number: “Electrolyte Solutions”, Second edition, by Robinson and Stokes (table 7.7 on page 158).

0.001 M LiCl transference number: Longsworth, L. G. J. Am. Chem. Soc.1932, 54, 2741, figure 4.

The correction is based on Equations 4.4 and 4.5 using the values obtained from ensemble bulk measurements. \( \kappa_i \) is the conductivity of species (ion) i which equals to its transference number (\( t_i \)) multi-
plies solution conductivity ($\kappa$). $R$, $F$, $T$ are the gas constant, Faraday constant, and the absolute temperature, respectively. $z_i$ and $c_i$ represent charge and concentration of species $i$.

\[ \kappa_i = \kappa * \ell_i \quad \text{(Eq. 4.4)} \]

\[ D_i = \frac{\kappa_i RT}{|z_i|^2 F c_i} \quad \text{(Eq. 4.5)} \]

4.5 Conclusion

The surface charge distribution through a conical glass nanopore is determined through fitting the experiments with theoretical simulation by solving Poisson and Nernst-Planck equations. An exponentially decayed distribution of surface charge density is introduced based on the electric field dependent deprotonation of surface silanol groups. The two parameters that define the SCD profile are noninvasively determined independently: a SCD maximum at the pore orifice is determined by optimized fitting of the experimental current at high conductivity states; the distribution length of SCD is determined by fitting the low conductivity current. A quantitative match between the experiments and simulation of ion transport through single nanopores is achieved for different electrolytes at different concentration.
5 NONINVASIVE SURFACE COVERAGE DETERMINATION OF CHEMICALLY MODIFIED CONICAL NANOPORES THAT RECTIFY ION TRANSPORT

Surface modification will change the surface charge density (SCD) at the signal-limiting region of nanochannel devices. By fitting the measured $i-V$ curves in simulation via solving the Poisson and Nernst–Planck equations, the SCD and therefore the surface coverage can be noninvasively quantified. Amine terminated organosilanes are employed to chemically modify single conical nanopores. Determined by the protonation–deprotonation of the functional groups, the density and polarity of surface charges are adjusted by solution pH. The rectified current at high conductivity states is found to be proportional to the SCD near the nanopore orifice. This correlation allows the noninvasive determination of SCD and surface coverage of individual conical nanopores.

5.1 Introduction

Synthetic nanopores, nanopipettes, and various nanochannel devices have attracted extensive research interest due to their novel transport properties resulting from the high surface-to-volume ratio.\textsuperscript{17-19,51,87,99,120-122} They have found broad applications in sensing,\textsuperscript{3,4,81,85,125} bioseparation,\textsuperscript{116,156} and implications in high efficacy electrochemical energy conversion.\textsuperscript{20,96,157} The functions of those nanodevices are highly dependent on their surface features. However, noninvasive or in situ characterization of the surface properties at the signal-limiting region, normally at the nanometer scale, of individual nanodevices is not yet accessible.\textsuperscript{123} The information from the planar surface orensemble systems is often adopted, which could not address the heterogeneous responses from individual nanodevices, a well-known barrier for broad applications.

Conductivity based stochastic single-molecule or single-event sensing is a representative application of the channel-type nanodevices.\textsuperscript{115,118,158} The detection signal is generally the analyte-induced disturbances or changes in steady-state (SS) ionic transport (IT) current. This SS ion flux current, often
rectified and previous-state-dependent (memory effects),\textsuperscript{99,131,135} is determined by both the geometric volume of the most resistive region as well as the surface charges at that effective region. Consequently, both SS current and the analyte-induced disturbance signals vary nonlinearly with respect to the detection conditions including electrolyte concentrations, solution pHs, and measurement parameters such as the applied bias potential. This frequently leads to heterogeneous responses from measurement to measurement or from one nanodevice to another.

One significant advantage of synthetic nanopores is the versatility to functionalize the device surface to target specific analytes.\textsuperscript{123,155} Surface chemical modification will alter the surface functional groups and could change the SCD accordingly. By analyzing the change in SCD, direct characterization of the efficacy of surface functionalization can be achieved. In previous reports,\textsuperscript{23-25,109,113} the SCD in individual nanopores can be determined by fitting the measured $i-V$ curves with theoretical simulation. In this letter, we report the noninvasive analysis of the surface coverage near the signal-limiting nanopore orifice region of individual nanopores upon modification. This is achieved by the analysis of the rectified ion transport current at high conductivity states via combined experiments and simulation. Note the interior surface of the nanochannel devices is not accessible for most direct characterizations such as imaging or below the resolution of those tools.

The proof-of-concept analysis is based on the results from single conical glass nanopores, which are chemically modified with 3-aminopropyltrimethoxysilane. The surface functional groups before and after modification and their charge states at different pHs are indicated in the four scenarios in Scheme 5.1. Before chemical modification, the silanol groups on silica surface deprotonate at high pH and establish a negatively charged surface (case A) and get protonated at low pH, thus resulting in a neutral surface (case B). After modification, amine groups were introduced. Depending on the efficiency of the reaction, the terminal functional groups on the surface will be a mixture of silanol and amine groups. At high pH, the surface will still be negatively charged with the neutral amine groups and nega-
tively charged silanol groups upon deprotonation (case C). At low pH, a positively charged surface (case D) will be created upon the protonation of silanol groups (neutral) and amine groups (NH$_3^+$).

Scheme 5.1 Surface Structures of Silica Surface (Panels A and B) and Amine Surface (Modified with 3-Aminopropyltrimethoxysilane, Panels C and D) at High and Low pHs. (Reprinted with permission from ref. 114. Copyright (2012) American Chemical Society.)

The efficacy of the surface modification is directly related to the change of charge states of the surface functional groups. As the positive charges on the surface solely come from the attached amine groups through modification, the positive surface charges determined directly reflect the site density of amine groups, thus the surface coverage. Because independent characterization of SCD at the nanoscale is inaccessible, the results are compared with those from the planar surface based on ensemble measurements. The SCD is determined by fitting the high conductivity state responses ($i-V$ curves) with theoretical simulation via solving Poisson and Nernst-Planck equations. The details of the measurements and simulation are provided in Experimental Section.
5.2 Results and Discussion

5.2.1 Experiments: $i-V$ Responses of Conical Nanopores

Figure 5.1 The $i-V$ curves from a 32-nm-radius nanopore in 50 mM KCl pH 3 (red) and pH 9 (blue) solutions. (A) silica surface and (B) surface modified with 3-aminopropyldimethylethoxysilane. The scattered symbols represent the simulated current computed from the optimized surface charge parameters discussed later. The dashed line represents the volume conductivity calculated based on geometric resistance in 50 mM KCl. (Reprinted with permission from ref. 114. Copyright (2012) American Chemical Society.)

Representative experimental $i-V$ curves from single conical nanopores corresponding to cases A–D are shown in Figure 5.1. A nonlinear $i-V$ curve, well-known as ion current rectification (ICR), originates from the asymmetric ion flux defined by a charged surface inside an asymmetric nanochannel device. Before surface modification, the silica surface at pH 9 is negatively charged (case A). The current at a positive applied potential (the bias is applied between an Ag/AgCl working electrode outside versus another Ag/AgCl electrode inside the nanopore) is larger than that at a negative applied potential (Figure 5.1 A, blue line) referred to as high and low conductivity states, respectively. For the same nanopore in a pH 3 solution, the silica surface is mostly neutralized due to the protonation of surface silanol groups (case B). Thus the surface effect diminished and the $i-V$ curve approached linearity (Figure 5.1A, red line). A linear $i-V$ curve (dash line) calculated based solely on the volume/geometric conductivity (no surface factors) was provided for comparison.

To avoid cross-linking of trifunctional (alkoxy or halide) silanes that could clog the nanopores, 3-aminopropyl(dimethylethoxy)silane was used. The surface coverage is known to be ∼20% on planar sur-
After modification, some surface silanol groups are converted into amine terminal groups. Therefore, the surface will be less negatively charged (case C) compared to that from unmodified nanopore (case A). This is supported by the less rectified $i-V$ curves at pH 9 in Figure 5.1B versus that in Figure 5.1A. At pH 3, the surface became positively charged as $-\text{NH}_3^+$ carry positive charges and silanol groups are neutralized (case D). A reversed ICR is observed accordingly, attesting the surface modification. The change in nanopore geometry is considered negligible in all four scenarios.

In an earlier report\textsuperscript{113} and shown next, the SCD near the nanopore orifice can be quantitatively determined from the current at a high conductivity state by fitting the experimental results via solving the Poisson and Nernst−Planck equations in simulation. The positive charges from the amine modified surface in a pH 3 solution can be determined similarly. The fitting of the results shown in Figure 5.1 from the amine surface give a SCD of $+70 \text{mC m}^{-2}$ corresponding to $\sim 0.4$ amine groups per nm$^2$ ($1 \text{ e nm}^{-2} = -160 \text{ mC m}^{-2}$) at pH 3. Unfortunately, neither the SCD nor the coverage of surface functional groups could be directly characterized at nanometer scale spatial resolution. Considering the total silanol density at 4.9 per nm$^2$ and type I (isolated) silanols at $\sim 20\%$ are most reactive for monoethoxysilanes,\textsuperscript{154} the results find reasonable agreements with the bulk values determined from the planar surface. The analysis of bare glass (Figure 5.1A) gives a SCD of $-50 \text{ mC m}^{-2}$ (0.3 silanol groups per nm$^2$) and $-32 \text{ mC m}^{-2}$ (0.2 silanol groups per nm$^2$) after silane modification (Figure 5.1B) in pH 9 solution. The decrease in negative SCD after modification is due to the replacement of silanol groups with amino groups. The variation of total surface sites (no. at pH 3 + no. at pH 9) before and after modification might indicate the conversion of different types of silanol groups during the surface chemical reaction.\textsuperscript{153,154} Similar results have been observed from other nanopores with different sizes. The data from a 50 nm radius nanopore before and after modification is provided in Figure 5.2, which shows much more significant ICR at both positive and
negative potential polarities. The higher surface effects from a slightly larger nanopore (50 nm vs 32 nm) demonstrate the heterogeneity of the nanodevices, demanding a surface charge description. Note one charge (or one functional group) per nm$^2$ corresponds to $-160$ mC m$^{-2}$, thus the distribution or charge variation of surface functional groups within the mass transport limiting nanopore region could easily cause these observed heterogeneous responses elaborated on next. It is therefore significant to have the ability to noninvasively characterize the surface features of individual nanopores to describe and ultimately to predict the transport responses under different measurement conditions.

### 5.2.2 Correlation of the Simulated Current and SCD

The characterization of surface coverage is based on the linear correlation between the SCD and the simulated current at high conductivity states. For a known nanogeometry, it is a common approach to systematically vary the local SCD at the nanoscale in the simulation to describe the experimental trend. The simulated current at high conductivity states has been shown to mainly depend on the SCD at the nanopore orifice.$^{113}$ Accordingly, this report focuses on the analysis of high conductivity state responses. Shown in Figure 5.3, the simulated correlation with the defined SCD for the nanopore con-
structured based on experiments. From this trend, the specific SCD that describes the experiments can be read. The surface coverage at the nanopore orifice, indicated by the SCD, can therefore be determined. The experiment currents at +0.4 V in parts A and B of Figure 5.1 at pH 9 correspond to a SCD of -50 and -32 mC m⁻² as labeled in Figure 5.3, respectively.

![Graph](image)

**Figure 5.3** Correlation between the simulated current and SCD at +0.4 V applied potential for a 32-nm radius nanopore in 50 mM KCl. A linear fitting (R² = 0.99) is shown as the red dashed line. (Reprinted with permission from ref. 114. Copyright (2012) American Chemical Society.)

This linear correlation is further confirmed by other nanopores at concentrations equal or higher than 50 mM KCl and under different applied potential amplitudes. Those results are shown in Figures 5.4 and 5.5 respectively.

![Graphs](image)

**Figure 5.4** The correlation between the simulated current and SCD at +0.4 V applied potential of a (A) 26-nm-radius and (B) 110-nm-radius (silica surface, without modification) nanopore in 50 mM (red); 100 mM (green); 200 mM (blue) and 500 mM (magenta) KCl solutions. Linear fittings for each concentration are indicated by dashed lines. (Reprinted with permission from ref. 114. Copyright (2012) American Chemical Society.)
Figure 5.5 The correlation between the simulated current and SCD at +0.2 V applied potential of a 26-nm-radius (silica surface, without modification) nanopore in 50 mM (red); 100 mM (green); 200 mM (blue) and 500 mM (magenta) KCl solutions. Linear fittings for each concentration are indicated by dashed lines. (Reprinted with permission from ref. 114. Copyright (2012) American Chemical Society.)

For each specific nanopore in different pH solutions, its SCD will vary due to the shift of protonation-deprotonation equilibrium. From the linear trend illustrated in Figure 5.3, the SCD near the orifice of the nanopore under different pHs can be determined. The results from different nanopores with different surfaces (silica and amine surfaces) are plotted in Figure 5.6. The obtained SCDs were converted to site density to reflect the functional groups on the nanopore interior surface.

Figure 5.6 Site density (SCD: the combination of deprotonated silanol groups and protonated amine groups) of the nanopores with different radii with (top panel) amine surface (surface modified with 3-aminopropyldimethylethoxysilane) and (bottom panel) silica surface in 50 mM KCl at different pHs. The site density is calculated based on the SCD determined from the experimental current trajected from the linear trend for each nanopore at +0.4 V applied potential. (Reprinted with permission from ref. 114. Copyright (2012) American Chemical Society.)
The surface pKa of silanol groups is known to have a broad pH range centered at \( \sim \text{pH 5.} \)\textsuperscript{153,154} Therefore, \( \sim 10\% \) would still be deprotonated and contribute to negative surface charges at pH 4, confirmed in the bottom panel in Figure 5.6. At pH 3, the SCD of bare silica became negligible. The positive SCD of amine modified nanopores will therefore directly represent the site density of the amine groups (top panel). The pH variation is limited between 3 and 9 to avoid protonation of silanol groups at extreme acidic pHs and glass dissolution at extremely basic pHs. The change of the SCD upon pH variation qualitatively agrees with the titrations curves from various SiO\textsubscript{2} surfaces.

The SCD calculated based on the \( i-V \) curve in pH 3 solution of Figure 5.1B is +70 mC m\textsuperscript{-2}, corresponding to 0.4 amine groups per nm\textsuperscript{2}, or a surface coverage of 8\% based on a site density of 4.9 per nm\textsuperscript{2} for a fully hydrated silica surface.\textsuperscript{136,151,152} To further validate the proposed analysis, the surface coverages of several amine modified nanopores of different sizes are presented in Table 5.1. The calculated site density (surface coverage) of amine groups ranges from 0.4 to 1.3 nm\textsuperscript{2}, corresponding to 8\% to \( \sim 27\% \) surface coverage. Considering the variation of the surface coverage reported even for planar ensemble surfaces, the results are in reasonable agreement with the \( \sim 20\% \) coverage known for monooethoxysilane on planar silica surfaces.\textsuperscript{153,154}

### Table 5.1 The analysis of surface coverage of several chemically modified nanopores

<table>
<thead>
<tr>
<th>Nanopore radius (nm)</th>
<th>Conc. (mM)</th>
<th>SCD (mC m\textsuperscript{-2})</th>
<th>Site density (#nm\textsuperscript{-2})</th>
<th>Surface coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>50</td>
<td>+70</td>
<td>0.4</td>
<td>8%</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>+213</td>
<td>1.3</td>
<td>27%</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>+109</td>
<td>0.7</td>
<td>15%</td>
</tr>
<tr>
<td>69</td>
<td>50</td>
<td>+202</td>
<td>1.3</td>
<td>27%</td>
</tr>
<tr>
<td>171</td>
<td>50</td>
<td>+106</td>
<td>0.7</td>
<td>14%</td>
</tr>
</tbody>
</table>

Note: The SCD is directly determined through theoretical fitting of experiments thus is no error bar. Nanopore radius is calculated from the conductivity equation.
At the end, we would like to comment on the distribution of the SCD or the surface coverage determined from different sized nanopores and the nanopores of comparable dimension. The variation of one charge or functional group per 10 nm$^2$ corresponds to 16 mC m$^{-2}$. This SCD variation will lead to significant current variation demonstrated in Figure 5.3. For a certain nanopore, the distribution of silanol groups on the interior surface near the signal-limiting-orifice region is already established. Therefore, a consistent trend can be observed upon the variation of solution pH. Additional data are presented in Table 5.2, which demonstrate that the SCD and the size of the nanopore are not correlated with each other.

**Table 5.2** The SCD determined for different nanopores in 50 mM KCl at neutral pH (ca.6.2)

<table>
<thead>
<tr>
<th>Radius (nm)</th>
<th>5</th>
<th>32</th>
<th>32</th>
<th>42</th>
<th>55</th>
<th>82</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD (mC m$^{-2}$)</td>
<td>-40</td>
<td>-45</td>
<td>-38</td>
<td>-24</td>
<td>-20</td>
<td>-80</td>
<td>-52</td>
</tr>
</tbody>
</table>

Note: The SCD is directly determined through theoretical fitting of experiments thus is no error bar. Nanopore radius is calculated from the conductivity equation.

Rather, the SCD depends on the statistic distribution of the terminal silanol groups. The distribution of different types of silanols of individual nanopores will lead to their SCD variation at the signal limiting region. Therefore, it is critical to noninvasively characterize the SCD of individual nanopore devices because the characterized geometric factors are insufficient to describe the ion transport through nanodevices in fundamental studies and sensing applications.

### 5.3 Experimental Section

#### 5.3.1 Materials

Water (~18.2 MΩ·cm) was purified by a Barnstead E-pure water purification system. Silver wire (99.9985%, diameter 0.5 mm), silver conductive paste, platinum wire (99.95%, diameter 25 μm), and ferrocene were from Alfa Aesar. Tungsten rods (A-M System, Inc.), Corning 8161 glass capillaries (OD
1.50 mm, ID 1.10 mm, Warner Instruments) were used as received. 3-aminopropyldimethylethoxysilane was from Gelest Inc. All other chemicals and materials were used as received from Sigma-Aldrich.

### 5.3.2 Nanopore Fabrication

The fabrication and characterization procedures have been fully documented in previous reports. Briefly, a 25 μm platinum wire was electrochemically etched in 15% CaCl₂, H₂O/acetone solution under 5 V peak-to-peak AC potential at 300 Hz frequency to obtain a sharp nanotip. After cleaning with piranha solution and nanopure water sequentially, the sharpened platinum nanotip was sealed into one end of a glass capillary through thermal-melting. The excess glass at the sealed end was removed manually using polishing discs (from rough to fine). This process was monitored using a conductivity tester built with metal oxide semiconductor field effect transistor (MOSFET)-based circuit. The exposure of the sealed platinum nanotip closes the circuit and signals when to stop the polishing. The sealed nanotip was electrochemically etched again and fully removed by mechanically pulling to create a nanopore with internal geometry replicating the removed nanotip.

### 5.3.3 Electrochemical Measurements

The conductivity response (i—V curves) was studied using a Gamry Reference 600 potentiostat (using the provided cyclic voltammetry software) with 20 mV/s scan rate. Two Ag/AgCl electrodes were used to control the bias potential. Electrodes were prepared by immersion of silver wire into a saturated solution of Fe(III)Cl₃ The working electrode was placed into bulk solution, outside of the nanopore; while both reference and counterelectrode leads were connected to another Ag/AgCl electrode immersed inside the nanopore. The detected current signal in this report is non-Faradic ion transport current, determined by the most resistive nanopore region (near the nanopore orifice). As the nanopore region limits the current, Faradic processes at the macroscopic Ag/AgCl electrodes are not discussed. The nanopore radius is calculated based on the resistance determined with potential range from +0.050 to -
0.050 V in 1M KCl, as the high electrolyte concentration and low potential make the surface effects less significant and negligible.

5.3.4 Theoretical Simulation

COMSOL Multiphysics 4.0a software package was used for the theoretical simulation to solve Poisson and Nernst-Planck equations. The 2D conical nanopore geometry in the simulation is shown in Figure 5.7, in which half of the cross section is used based on the centerline (red) symmetry. The model design and rationale are detailed in previous reports. The interior glass surface of conical nanopores is negatively charged, represented by Boundary 5 (highlighted in blue). As the exterior surface near the mass transport limiting region affects the charge distribution, charges are placed on exterior surface but limited to 20 times of the pore radius (indicated by boundary 4, highlighted in blue) to minimize the computation expenses without affecting results. A uniform SCD along boundary 4 and 5 was defined during each simulation. This uniform SCD definition fits the high conductivity state responses as previously reported. At low conductivity states, the simulated current is consistently larger than the experiments. This discrepancy does not affect the determination of the SCD near the nanopore orifice and is addressed separately. Adaptive free triangular mesh element was used in the simulation. The mesh size around the sharp corner of nanopore was limited to 0.1 nm to minimize the simulation noise. A 0.3
nm (min.) to 0.6 nm (max.) mesh was used at the charged boundaries (4 and 5) to obtain the best resolution within a reasonable time. Note that the 0.3 nm mesh element is approaching the size of solvated ions, which is a fundamental limit of continuum theory. The following parameters were used: room temperature, \( T = 298.13 \) K; viscosity, \( \eta = 1 \times 10^{-3} \) Pa·s; density, \( F = 1000 \) kg m\(^{-3}\); relative dielectric constant, \( \varepsilon = 80 \); Faraday constant, \( F = 96485 \) C mol\(^{-1}\). Instead of using values for infinitely dilute solutions, the diffusion coefficients of \( K^+ \) and \( Cl^- \) of each concentration are calculated based on the conductivity and corresponding transference number explained in previous report (Table 5.3).\(^{113}\)

**Table 5.3** The effective diffusion coefficients of \( K^+ \) and \( Cl^- \) ions in KCl solutions at different concentrations*

<table>
<thead>
<tr>
<th>conc (M)</th>
<th>conductivity (S/m)</th>
<th>( t_K^+ )</th>
<th>( t_{Cl^-} )</th>
<th>( K^+ ) conductivity (S/m)</th>
<th>( Cl^- ) conductivity (S/m)</th>
<th>( D_K^+ ) (m(^2)s(^{-1}))</th>
<th>( D_{Cl^-} ) (m(^2)s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.050</td>
<td>0.6670</td>
<td>0.4899</td>
<td>0.5101</td>
<td>0.327</td>
<td>0.340</td>
<td>1.740\times10^{-9}</td>
<td>1.812\times10^{-9}</td>
</tr>
<tr>
<td>0.100</td>
<td>1.288</td>
<td>0.4898</td>
<td>0.5102</td>
<td>0.631</td>
<td>0.657</td>
<td>1.680\times10^{-9}</td>
<td>1.750\times10^{-9}</td>
</tr>
<tr>
<td>0.200</td>
<td>2.482</td>
<td>0.4894</td>
<td>0.5106</td>
<td>1.214</td>
<td>1.267</td>
<td>1.617\times10^{-9}</td>
<td>1.687\times10^{-9}</td>
</tr>
<tr>
<td>0.500</td>
<td>5.864</td>
<td>0.4888</td>
<td>0.5112</td>
<td>2.866</td>
<td>2.998</td>
<td>1.526\times10^{-9}</td>
<td>1.596\times10^{-9}</td>
</tr>
</tbody>
</table>

*The solution conductivities and ion transference numbers are from “Electrolyte Solutions”, Second edition, by Robinson and Stokes (table 7.7 on page 158; and Appendix 6.3 on page 466).

### 5.4 Summary

Fixed surface charges are well-known to strongly affect the transport through channel-type nanodevices but a quantitative description has been missing. A noninvasive characterization of surface coverage and surface charge density at the nanometer scale is presented based on a combined experimental \( i-V \) measurements and theoretical simulation. The surface parameters reported in this paper has significant implications in addressing the heterogeneity in individual nanodevice efficacy and single molecule sensing analysis.
6 CONCLUSIONS AND SIGNIFICANCE

Since nanopores and broadly defined channel-type devices were first proposed as a rapid and low-cost DNA sequencing tool in the past decades, tremendous research interests have been stimulated in the creation and functionalization of various nanodevices. A major breakthrough is the transition from biological nanopores to synthetic nanopores. The fast development in the fabrication and characterization of synthetic solid-state nanodevices has broadened the application of nanodevices to a great extent. Based on the Coulter Counter concept, the nanodevices have been applied in stochastic sensing at single molecule resolution which cannot be achieved by current ensemble techniques. The translocation of molecules through nanodevices will partially block the pore which induces a significant change in the ionic current through the nanopore to be detected. A conventional analysis of unknown sample includes a series of procedures including separation, concentration and characterization etc. The single molecule resolution offers the nanodevice based sensors tremendous advantages in simplified sample handling and more importantly, directly analyzing sample heterogeneity that could enable trace detection/early diagnosis. The analytes are detected in the native states so that additional labeling is not required. The molecular binding kinetics (binding affinity) is also revealed by the detected current change signals. Since the background current is extremely small, the conformation change induced by molecular binding can also be read from the current change. All of these advantages make nanodevices superior over other sensing techniques.

Besides sensing application, nanodevices have applications in various fields such as electrochemical energy conversion, nanofluidic electronics, drug delivery, concentration enrichment and separation etc. At nanometer scale, due to the high surface to volume ratio, both surface and volume effects will contribute to the detected ionic current. The i—V responses deviate from linear ohm’s behavior, well known as ion current rectification. All of these applications result from a convolution of volume and surface contributions, therefore further developments require the quantification of surface charge den-
sity (surface factor) and geometric contributions at nanometer scale. Due to nanometer spatial limitation, access to the interior surface of nanoconfinement is limited.

The aim of this dissertation is to resolve these surface and geometric effects by determining the surface change density at nanometer scale, through theoretical simulation of experimental data by solving Poisson and Nernst-Planck equations. A linear correlation between the simulated current and surface charge density is found. This allows the prediction of the intrinsic ionic current through the nanopore. The recognition sites of the nanodevices targeting different analytes for sensing applications are normally introduced via chemical modification. However, the surface modification efficacy is inaccessible for individual nanodevices. Since the surface charge density can be quantified by the simulation of experiments, this problem is easily solved. As surface modification will change surface properties, the surface charge density before and after modification allows the quantification of surface modification efficacy as demonstrated in Chapter 5.

Due to the asymmetric geometry, the electric field inside a conical glass nanopore displays pseudo exponential distribution according to the simulation. At high applied electric field, deprotonation of surface functional groups increases thus inducing a higher surface charge density than the bulk value. Correspondingly, an exponential gradient surface charge distribution is proposed and validated by perfect fitting of the experimental current at high- and low-conductivity states simultaneously. The model enables the prediction of mass transport behavior of other electrolytes, which is demonstrated in Chapter 4. The information revealed from theoretical simulation of experiments provides a better understanding of the fundamental mass transport mechanism through a charged nanodevice. Furthermore, the determined transference numbers showed that at low conductivity cation transference number almost approach unity, in agreement with previous prediction. The results provide insights in designing high efficacy supercapacitors and batteries and other energy devices with nanostructures with high surface to volume ratio.
In conclusion, nanodevices and nanostructured electrodes are widely used in single molecule sensing, electrochemical energy conversion, drug delivery, concentration enrichment and separation, nanofluidic electronics, and so on. Further applications have been hampered by the lack of understanding of surface charge effects on the transport behaviors, which is the main thrust of this dissertation. Therefore this dissertation focused on two projects of my PhD work: single molecule sensing based on chemically modified conical nanopores and theoretical and experimental study of mass transport through a charged nano-geometry. For nanopore based stochastic sensing, qualitative pH, concentration and potential dependence of streptavidin-iminobiotin have been established. To better understand the detected current/current change signal, SCD quantification is carried out. The SCD quantification within nanoconfinement has been determined for the first time by theoretical simulation of experiments through solving Poisson and Nernst-Planck equations using finite element simulation. Two significant improvement are introduced in simulated the ion transport behavior through nanoconfinement. 1. Introduction of an effective diffusion coefficient, which allows an accurate prediction of experiments. 2. An exponential gradient SCD distribution is proposed, which results from electric field facilitated deprotonation of surface functional groups. The maximum SCD at pore orifice is determined by fitting high conductivity current, while low conductivity is used for determining distribution length. The introduction of an effective diffusion coefficient and exponential gradient SCD distribution allows a perfect fitting of experiments. The obtained information provides a quantitative understanding of fundamental mass transport mechanism at a charged nanoconfinement and eliminates practical barriers in stochastic sensing, which allows a better interpretation of detected signal. Since the quantification of surface charge density is realized, the detected sensing signal and baseline current can be predicted and compared for different nanodevices. Also, quantification of surface modification efficiency is achieved by comparing SCD change before and after modification. Furthermore, the obtained information helps optimization of high efficacy electrochemical battery design.
REFERENCES

(18) Daiguji, H. *Chemical Society Reviews* 2010, 39, 901.


(68) Thombre, A. G.; Cardinal, J. R.; DeNoto, A. R.; Herbig, S. M.; Smith, K. L. *Journal of Controlled
Release 1999, 57, 55.

(70) Butler, T. Z.; Gundlach, J. H.; Troll, M. Biophysical Journal 2007, 93, 3229.
(74) Austin, M.; Deshpande, P. A.; Sharonov, A.; Bogdanov, V.; BIONANO GENOMICS, INC.: USA, 2012; Vol. 2012024635
(87) Schoch, R. B.; Han, J. Y.; Renaud, P. Rev. Mod. Phys. 2008, 80, 839.


(139) Ramirez, P.; Apel, P. Y.; Cervera, J.; Mafe, S. *Nanotechnology* **2008**, *19*.


(158) Lan, W. J.; White, H. S. Acs Nano 2012, 6, 1757.