Determination of Protein Titration Curves Using Si Nanograting FETs*

Silu Zhang, Student Member, Pengyuan Zang, Student Member, Yuchen Liang, Student Member, and Walter Hu, Senior Member, IEEE

Abstract—This study describes a methodology of measuring protein titration curve by Si Nanograting FETs (NGFETs). Two proteins were measured with each of the titration curves showing specific characters for identification. The measurements at high ionic concentration were performed to eliminate the effect of SiO$_2$ surface. The intrinsic protein titration curves extracted are consistent with prior researches.

I. INTRODUCTION

The net charges of protein is an important feature that has been drawn great attention in biomolecular researches.[1-5] The titration curve, which is known as the protein surface net charge behavior as a function of pH values, has been theoretically computed based on the Henderson–Hasselbalch equation [3, 4]. As the shape of the curve is determined by the acid dissociation constant (pKa) of each amino-acid residue, the titration curve can be presented as a representative of certain type of protein. Therefore, determination of titration curve is of great value in terms of the potential in identifying proteins. Surface charge is typically measured by zeta potential [5, 6]. R. Kun et al. studied the net charge of bovine serum albumin (BSA) by applying titration with polyelectrolytes in the Mu¨ tek Particle Charge Detector[7]. However, through these methods it can barely get enough data points to form an accurate titration curve.

Silicon-based field effect transistors (Si FETs) have been proved to be effective biosensors in detecting surface charges (or potential).[8] Among various types of FETs, Si Nanograting FETs (Si NGFETs) shows better stability.[9] P. Zang et al. have shown the outstanding performances of NGFETs as a pH sensor.[10]

A new methodology was applied in this study to determine the titration curve of proteins via NGFETs. It was demonstrated to be a convenient method providing more accurate outcomes. Firstly, by showing the respective titration curves of BSA and immunoglobulin G from mouse serum (mouse IgG), the feasibility of distinguishing two types of proteins was proved. Secondly, the effect cause by SiO$_2$ surface of the device was measured at high ionic strength and could, therefore, be eliminated to extract intrinsic protein characteristic. Finally, the outcome matches with the simulating results and also can be verified by the zeta potential data.[5]

II. MATERIALS AND METHODS

A. NGFETs Fabrication

The schematic of the n-type NGFET used in this study is shown in Fig. 1. The nanowires with 3-nm silicon dioxide grown on the surfaces are designed as 20 µm in length, 50 nm in width and 30 nm in height. This dimension of nanowires was chosen to achieve optimal device sensitivity.[11] The channel consists of 100 nanowires and was proved to have better device performance in stability.[9] The detailed fabrication process of NGFETs is described in previous work [9, 10].

B. Surface Modification

The surfaces of NGFETs have been treated by piranha ($H_2O_2:H_2SO_4=1:3$) for 30 min and dried with N$_2$. To coat the surface with self-assembled monolayer (SAM) for protein immobilization, the device was immersed in 0.1% 11-(triethoxysilyl) undecanal (TESU, Gelest, Inc) in toluene for 5 hours and dried with N$_2$. 50 µg/mL mouse IgG or BSA (both from Sigma-Aldrich) was dissolved in 1 mM NaCNBH$_3$ and reacted with TESU modified surface for 3 hs, followed with rinsing by 2 mM PBS. The device was stored in a sealed holder with its surface covered by 2 mM PBS before test.

<table>
<thead>
<tr>
<th>TABLE I. REPRESENTATIVES OF FUNCTION GROUP CHARGE STATUS</th>
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<tbody>
<tr>
<td>Function Group</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Hydroxyl</td>
</tr>
<tr>
<td>Adedhe</td>
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<td>Amino</td>
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<td>Thiol</td>
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C. Electrical Measurement

PBS buffers with various pH values were selected by a selector valve and were delivered to the gate of the sensor through PDMS channel at a flow rate of 100 μL/min. The fluidic pressure at 5 psi was supplied by a nitrogen gas cylinder, so as to eliminate the effect of CO₂ dissolution to ensure a constant pH value for each buffer during test. The gate voltage was applied to solutions through an Ag/AgCl electrode at a proper potential where the sensor worked in its sub-threshold region. The conductance of device at this time can respond to changes in surface potential. For n-type NGFETs as used in this study, increased surface (or gate) potential accumulates carriers in the channel and therefore cause increase in drain current, while decreased surface potential depletes carriers and reduce drain current. The drain current was measured and recorded by Keithley 4200-SCS. Buffer of each pH value was injected to the sensor for ~100 s to obtain a stable signal. Each titration curve was obtained by averaging two sweeps.

III. RESULTS AND DISCUSSION

Three different NGFETs surfaces shown in Fig. 2 were studied. The SiO₂ surface was hydroxyl-terminated after the piranha treatment. In Fig. 2a, TESU molecules were assembled to the surface through forming Si-O bonds and having –CHO terminals to anchor the protein of study. The amino groups of each protein reacted with –CHO bonds and then reduced by NaCNBH₃, as depicted in Fig. 2b and c. Part of the –CHO groups which have not reacted with the proteins were possibly oxidized to –COOH by the O₂ in the air. Therefore, the net charge of the surface was contributed by the combination of protonated or deprotonated –OH, –CHO, –COOH and residues of protein molecules. The charge status of main functional groups are presented in TABLE I. In low pH environment, protonation tended to dominate to have functional groups positively charged; whereas in high pH environment, deprotonation was the dominant factor for functional groups being negatively charged. At certain pH value, which is called isoelectric point (pI), the extent of protonation equals to deprotonation, results in the net charge at the surface equal to zero.

However, not all charges of the multi-component surface can be sensed by the device. Only those that are close enough to the device surface can be detected, others are screened. The detectable distance is set by Debye length, which is determined by ionic concentrations of solutions. So by measuring at a specific ionic concentration, the region above the surface within a given distance can be selectively detected. The detectable region given by Debye length and its corresponding ionic concentration is schematically marked in Fig. 2 (Dimensions are not scaled). The orientation of each protein is arbitrary instead of necessarily in the status shown in Fig. 2. Therefore, even though a protein molecule may be larger than 5 nm in height, it is still sensible as being partially lying in the detectable region.

A. Titration Curve Measured at Low Ionic Concentration

Titration curves for each of the three surfaces described in Fig. 2 were consistently measured at 4 mM ion concentration and are shown in Fig. 3a. The current was converted to surface potential according to Id-Vg curve of NGFET. Since theoretical Debye length at this concentration is 4.873 nm [11], charges of SiO₂, TESU and proteins can all contribute to the signal detected by device. The plots in Fig. 3a are the titration curves of the three surfaces, respectively. Despite the effect of SiO₂ surface, these curves exhibited different shapes that were distinguishable from each other. The behavior at the ends of each titration curve reflects a screening effect that matches the simulation result in Yang’s work. At low/high pH values, the hydroxyl groups of SiO₂ surface are largely charged due to extensive protonation/deprotonation. The charges above the TESU molecular layer are, hence, screened by the highly

<table>
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<th>Curve Legend</th>
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<tr>
<td></td>
<td>pKₐ,₁</td>
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<tr>
<td>TESU_100 mM</td>
<td>4.0</td>
</tr>
<tr>
<td>TESU_4 mM</td>
<td>5.0</td>
</tr>
<tr>
<td>TESU+BSA_4 mM</td>
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charged SiO₂ surface. In the range of low pH values (< 3.5), increasing amount of positive charges are screened with the declining pH values. As a result, the surface potential drops as the screening effect dominates. Similar behavior appears in the range of high pH values (>10.5).

**B. Titration Curve Measured at High Ionic Concentration**

To further observe the intrinsic pH dependent behavior of BSA and mouse IgG, the impacts of SiO₂ surface and uncovered TESU terminal groups were eliminated from the titration curves. To achieve this, the titration curve of TESU modified surface was measured at 100 mM ionic concentration with the Debye length reported as 0.975 nm[11], so the potential change was only from the functional groups existing within the height of 1 nm above the surface. The result is shown as the black curve in Fig. 3b. No screening effect was observed at the two ends because charges at the higher position of the layer had been screened by high concentration of salt in the entire pH region. As expected, the curve reflects the intrinsic behavior of hydroxyl group at the SiO₂ surface and is therefore different from the black curve in Fig. 3a. The result was verified by taking same measurement on mouse IgG modified surface. The outcome is shown as the red curve in Fig. 3b. The curve has identical shape as that of TESU modified surface, due to the fact that the higher portion of the surface molecule layer failed to give a signal.

<table>
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<td>pKa₁</td>
<td>pKa₂</td>
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<tr>
<td>TESU+IgG_4 mM</td>
<td>4.3</td>
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Figure 3. Titration curve measured at ionic concentration of a) 4 mM and b) 100mM.

Figure 4. a) Titration curves converted to surface charge. b) Titration curves after elimination of –OH effect. c) Protein intrinsic titration curve extracted.

### Conversion of Surface Potential to Surface Charge

In order to get the information of surface charges, the zero-charge potential (the surface potential at pH equal to pI) need to be determined first. Although a titration curve does not directly provide its pI, it does give the information of the pKₐ₁ and pKₐ₂ values where the curve suddenly declines [12]. Notice that the pKₐ₁ and pKₐ₂ values discussed here, represent the apparent behavior of the multi-molecule functionalized surface, rather than being the intrinsic pKₐ₁ or pKₐ₂ value of a specific functional group. The pKₐ₁ and
pKa values of the three curves in Fig. 3a and the black curve in Fig. 3b were extracted and listed in TABLE II. The red curve in Fig. 3b has same pH-dependent behavior of SiO2 surface as the black curve does; therefore further analysis is not necessary. The pI of each curve can then be calculated according to the following equation:

\[ pI = \frac{pK_{a1} + pK_{a2}}{2} \]  

(1)

The calculated pIs are also listed in TABLE II. The reference potential of each curve is set at zero-charge potential where the pH value equals pI. Then the relative surface potential to the zero-charge potential is now representing the surface charge, annotated as 'Charge/Cg (gate capacitance)'. The surface potential of the black curve in Fig. 3b and the three curves in Fig. 3a are converted to charge/Cg, and are entitled the legends of -OH, TESU, TESU+BSA and TESU+IgG, respectively, as shown in Fig. 4a.

D. Extraction of Protein Intrinsic Titration Curve

The net charges on protein are normally more concerned, so a method to extract intrinsic titration curves of proteins is proposed here. Considering that the -OH groups influence the charges of the curve in Fig. 4a, it is possible to eliminate the effect of -OH by subtracting the black curve from the others. The charges of the -OH group are weighted by a factor of 0.4 before subtraction and the result is shown in Fig. 4b.

In Fig. 4b, the black curve represents the charging behavior of the -CHO group only. Since TESU surface is not fully covered by BSA or IgG, the -CHO charges indicated in the black curve are partially contributing to the titration curves of the BSA (red) and IgG (blue) modified surfaces. The coverage of BSA and IgG are estimated to be around 0.7, thus, the intrinsic titration curve of each protein is similarly extracted. The black curve is scaled by 0.3 and subtracted from the red and the blue curves, respectively, to obtain the titration curves of pure BSA and IgG. The outcomes are shown in Fig. 4c.

The reliability of the result was verified by the consistency with prior works. The pIs of BSA and mouse IgG obtained from Fig. 4c are 5.7 and 6.6, respectively, which are close to the values appeared in literatures[13]. In addition, both the titration curves of BSA and IgG show a relatively flat region from pH 7.5 - 9.5, which is commonly observed in typical protein simulation results[4, 12]. Similar experimental results are also obtained by E. Feller[5].

Just for the purpose of protein identification through NGFETs, it is not necessary to obtain the intrinsic protein titration curve with high degree of accuracy. The most significant contribution of this work is the ability to distinguish different proteins by only measuring their apparent titration curves. A standard titration curve can be measured by NGFETs in practical use.

IV. Conclusion

This paper describes a new methodology of measuring protein titration curves by NGFETs. This is a more convenient and reliable method to experimentally obtain titration curves than the conventional ones. In the study two proteins -BSA and mouse IgG - were tested with the apparent titration curves graphed to show the ability of identification. The intrinsic titration curves of the two proteins were also extracted based on proper assumptions and estimations. The resulted curves provide information consistent to both the simulation and experimental results presented in previous researches. In practical use, this method obtains an electronic symbol of a specific protein and shows great potential in the application of protein identification.

Acknowledgment

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References
