Determination of Protein Titration Curves Using Si Nanograting FETs*

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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 SiO₂ 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 Muˆtek 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 caused by SiO₂ 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]

Figure 1. Schematics of NGFETs: a) Optical image; b) Scanning electron microscope (SEM) image of nanowire channels.

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 (H₂O₂:H₂SO₄=1:3) for 30 min and dried with N₂. 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 N₂. 50 μg/mL mouse IgG or BSA (both from Sigma-Aldrich) was dissolved in 2 mM PBS containing 1 mM NaCNBH₃ 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 I. REPRESENTATIVES OF FUNCTION GROUP CHARGE STATUS

Function Group	Charge Status		
	Positive	Neutral	Negative
Hydroxyl	–OH ₂ ⁺	–OH	–OH [–]
Aldehyde	–CH ₂ O ⁺	–CHO	–COO [–]
Amino	–NH ₃ ⁺	–NH ₂	
Thiol		–SH	–S [–]

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Curve Legend	Key Values		
	$pK_{a,1}$	$pK_{a,2}$	PI
TESU+IgG_4 mM	4.3	9.3	6.8

Figure 3. Titration curve measured at ionic concentration of a) 4 mM and b) 100mM.

charged SiO_2 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 SiO_2 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 SiO_2 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.

Figure 4. a) Titration curves converted to surface charge. b) Titration curves after elimination of $-\text{OH}$ effect. c) Protein intrinsic titration curve extracted.

C. 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 $pK_{a,1}$ and $pK_{a,2}$ values where the curve suddenly declines [12]. Notice that the $pK_{a,1}$ and $pK_{a,2}$ values discussed here, represent the apparent behavior of the multi-molecule functionalized surface, rather than being the intrinsic $pK_{a,1}$ or $pK_{a,2}$ value of a specific functional group. The $pK_{a,1}$ and

$pK_{a,2}$ 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 SiO_2 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:

$$pI = \frac{pK_{a,1} + pK_{a,2}}{2} \quad (1)$$

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 B. 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.

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