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RAPID COMMUNICATION

A reversible surface functionalized nanowire transistor to study protein—protein interactions

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Summary Taking advantage of the reversible association—dissociation between glutathione (GSH) and glutathione S-transferase (GST), we have developed a novel method by surface-functionalizing GSH on silicon nanowire field-effect transistors (SiNW-FET) and then anchoring a particular GST-fused protein for the studies of protein—protein interactions. The reversible surface functionalization method has been proven by analytical techniques, fluorescence imaging, atomic force microscopic imaging, and electrical measurements. The reversible GSH—GST functionality on the SiNW-FET has made this sensorial device consecutively reusable and calibratable, allowing for quantitative analysis. In this work, we have demonstrated that the biologically modified SiNW-FET can be used as a screening sensor for the future studies of biomolecular associations, such as protein—protein interactions, protein—DNA interactions, protein—carbohydrate interactions, etc.

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Miniaturized biological sensors made of nanotube/nanowire field-effect transistors (NT/NW-FET) recently have attracted wide attention because of their high selectivity, extreme sensitivity, rapid response, and potential for integration into full electronic on-chip systems for high-throughput analysis [1—3]. A particular advantage of employing NT/NW-FET biosensors is their capability of in situ and real-time recording the signal changes caused by single cells and individual neurons [4,5]. NT/NW-FET can be massively produced and are relatively cost-effective in requiring less amount of sample than other large-scale biosensors [6,7]. Several applications of surface-functionalized NT/NW-FET have been reported for the detections of ions [8], DNA [9—11], proteins [12—19], glucose and hydrogen peroxide [8,20], and viruses [4].

In the current applications of NT/NW-FET for biomedical diagnosis of a particular antigen (target molecule), the corresponding antibody (probe molecule) is usually modified on the surfaces of NT/NW-FET prior to the detection. By virtue of the strong and specific binding affinity between antigen and antibody under a normal physiological condition, the surface-modified NT/NW-FET can serve as an extremely sensitive sensor with high selectivity. By the same token, because of this strong binding between antigen and antibody, it is difficult to remove the antigen-antibody complex from the surface of NT/NW-FET in the end of detection, yielding that a NT/NW-FET could be used just for a single measurement. With this limitation, consecutively quantitative analysis by a calibratable NT/NW-FET has been problematic to achieve.

Here, we report a novel strategy to design a reusable sensor by judiciously selecting a reversible association—dissociation molecular system to be functionalized on the surface of silicon nanowire field-effect transistor (SiNW-FET). It is well studied that protein fused with glutathione S-transferase (GST) can associate with glutathione (GSH)-conjugated resin for purification or studying protein—protein interaction [7,21,22]. GST binds GSH specifically with high affinity and the associated GST-fused protein can be eluted easily by a high concentration of GSH [23]. However, the traditional approach requires a large amount of proteins and tedious process. In this study, we chose GSH to be immobilized on a SiNW-FET (termed GSH/SiNW-FET), which can then anchor a specific protein fused with GST (termed protein—GST/GSH/SiNW-FET) to screen possible interacting proteins. Particular emphasis will be given to the reversible surface functionalization on the sensorial SiNW-FET. A schematic diagram for this retrieval surface-modified SiNW-FET is illustrated in Fig. 1 including the association of a GST-fused protein on a GSH/SiNW-FET to screen possible interacting proteins, the dissociation of the used GST-fused protein after detection, and the iterative retrievable cycles. In the pursuit of a fast high-throughput screening method, a myriad of protein—protein interactions can be examined by this protein—GST/GSH/SiNW-FET, where various GST-fused proteins can be easily prepared in a large scale via bacterial or mammalian expression systems [7,24,25].

In the immobilization of GSH on a SiNW-FET, a self-assembled monolayer (SAM) of 3-aminopropyltrimethoxysilane (APTMS) was first bonded to the silicon-oxide sheath on the SiNW. This SAM technique has been proven to be one of the most popular methods for the surface functionalization of devices [1,2,26,27]. A linker of 3-maleimidobenzoic acid *N*-hydroxysuccinimide ester (MBS) was then applied to bond APTMS on one end and to anchor GSH on the other end (Fig. 1). The GSH/SiNW-FET could subsequently be used for protein sensing experiments by anchoring a specific protein. To examine each-step modification on a Si substrate, we identified the elemental compositions and chemical-structure characteristics by electron spectroscopy for chemical analysis (ESCA), including APTMS anchoring, MBS linking, GSH immobilization, and GST association. The ESCA spectra in Fig. 2 show the spe-

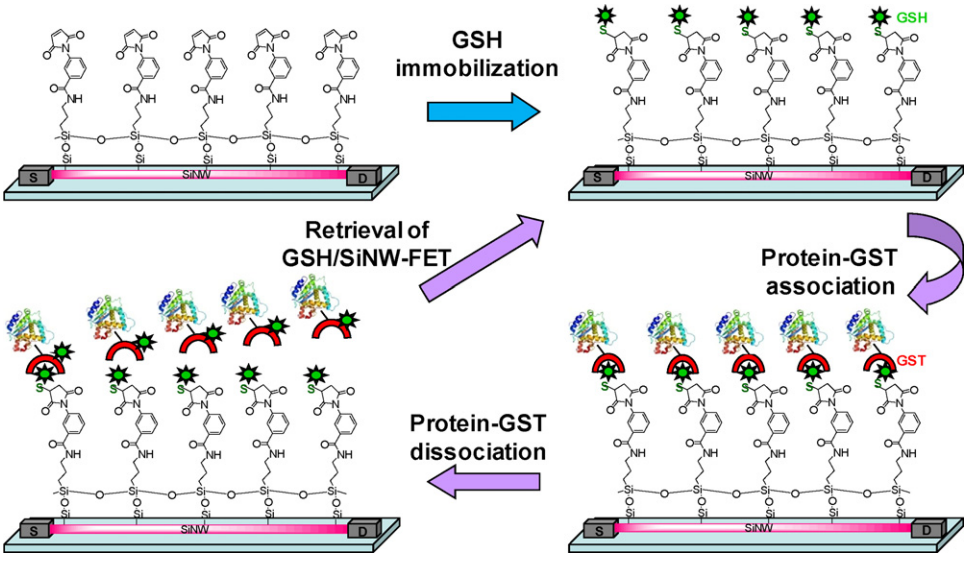

Figure 1 Schematic illustration for the reversible functionalization of protein-GST on a GSH/SiNW-FET for rapid high-throughput screening protein interactions. A SiNW-FET is first modified with APTMS and MBS linkers prior to the immobilization of GSH. A particular protein-GST is then associated with the GSH/SiNW-FET for screening possible interacting proteins. In the end of each measurement, the used protein-GSTs are removed with 10 mM GSH washing solution, making the GSH/SiNW-FET a reusable biosensor.

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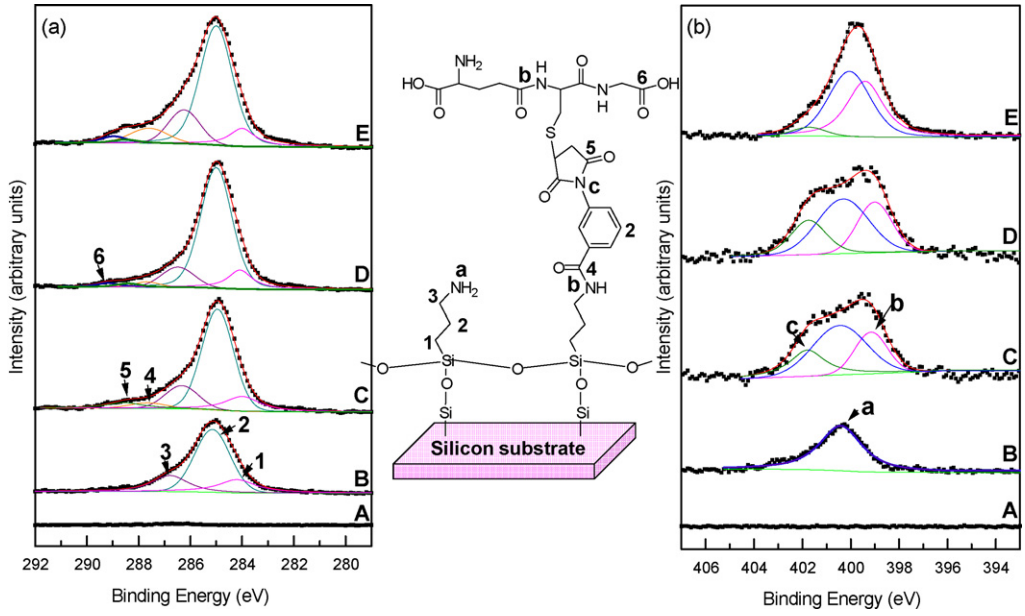

Figure 2 The observed (a) C 1s and (b) N 1s ESCA spectra to examine the serial surface modification steps of (A) a clean Si substrate, (B) the modification of APTMS, (C) the insertion of an MBS linker, (D) the immobilization of GSH, and (E) the association with GST. The characteristic functional groups responsible for particular ESCA signals are identified. In the C 1s spectra, 1: Si—C, 2: C—C, 3: C—NH_2, 4: CONH, 5: C=O in $C_{11}H_5NO_3$, and 6: COOH. In the N 1s spectra, a: C—NH_2, b: CONH, and c: $C_{11}H_5NO_3$.

cific functional groups of the added molecule in each-step modification. For example, after an APTMS-SAM is anchored on the Si surface via silanol group, the spectral features in the C 1s ESCA spectrum (the B panel in Fig. 2a) are observed at 283.3, 285.0, and 286.6 eV corresponding, respectively, to the silicon carbide (Si—C), carbon—carbon (C—C), and amine (C—NH_2) groups of APTMS. Because of the coupling of MBS with APTMS, the specific peaks appear at 287.8 eV (amide) and 288.7 eV (C=O in maleimidobenzoyl) in the C 1s spectrum (the C panel in Fig. 2a). Meanwhile, a significant increase of two new peaks at 399.3 eV (amide) and 402.0 eV (maleimidobenzoyl) in the N 1s spectrum (the C panel in Fig. 2b) has also evidenced the existence of MBS. Owing to the very weak ESCA feature for the S element, we have chosen the carboxylic acid and amide groups as spectral indicators to demonstrate the presences of GSH and GST. In the D and E panels of Fig. 2a, the carboxyl groups of both GSH and GST are responsible for the peak at 289.0 eV. Furthermore, apparent increases of the amide group in the C 1s (287.8 eV) and N 1s (399.3 eV) spectra were observed after the association of GST with the GSH-modified Si substrate. Except the S element, the relative atomic percentages among Si, O, C, and N analyzed from the ESCA spectra for each-step modification are listed in Table 1. Before any surface functionalization, the main compositions of a Si substrate have the Si/O ratio of 32.3/67.7. After the APTMS modification, while the relative contents for both Si and O decrease, two additional elements of C and N appear in the ESCA spectra. Consistent with a previous report [28], the changes in the atomic compositions of our ESCA data correctly reflect the immobilization of APTMS on the Si surface. Table 2 lists the compositions of amine (C—NH_2), amide (CONH), and maleimidobenzoyl ($C_{11}H_5NO_3$) groups for each-step modification calculated from the N 1s ESCA spectra (Fig. 2b). It is noteworthy that the percentage of amide reaches 44.4% after associating GST with GSH and decreases to 28.6% after removing GST by 10 mM GSH. In comparison with the original 29.7% of amide in a GSH-modified Si substrate, this ESCA examination has indicated a thorough removal of GST by 10 mM GSH, demonstrating that the reversible GSH—GST association—dissociation is a suitable molecular system to be adapted for a reusable SiNW-FET sensor. In the

Table 1 ESCA analysis for the atomic compositions in each-step surface modification on a Si substrate. Results represent the mean ± SEM of readings obtained from four samples.

	Atomic composition (%)			
	Si	O	C	N
Si substrate	32.3 ± 0.3	67.7 ± 0.7	–	–
APTMS—Si substrate	16.9 ± 0.4	25.9 ± 0.3	45.7 ± 0.8	11.5 ± 0.1
MBS—APTMS—Si substrate	13.0 ± 0.2	26.9 ± 0.4	47.7 ± 0.3	12.4 ± 0.5
GSH—MBS—APTMS—Si substrate	10.0 ± 0.4	27.2 ± 0.5	49.8 ± 0.2	13.0 ± 0.6
GST—GSH—MBS—APTMS—Si substrate	5.3 ± 0.4	29.6 ± 0.2	50.7 ± 0.5	14.4 ± 0.3

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Table 2 The compositions of amine, amide, and maleimidobenzoyl groups in each-step surface modification on a Si substrate analyzed from the observed ESCA N 1s spectra.

	N-containing group composition (%)		
	Amine (C—NH_2)	Amide (CONH)	Maleimidobenzoyl ($C_{11}H_5NO_3$)
Si substrate	–	–	–
APTMS—Si substrate	100	–	–
MBS—APTMS—Si substrate	49.5	25.3	25.2
GSH—MBS—APTMS—Si substrate	47.2	29.7	23.1
GST—GSH—MBS—APTMS—Si substrate	50.8	44.4	4.8
After 10 mM GSH washing the GST—GSH—MBS—APTMS—Si substrate	49.2	28.6	22.2

experiments, we have been able to conduct repeatedly the association—dissociation cycles of GST on a GSH-modified Si substrate for more than 30 times. Shown in Fig. 3a is the N 1s ESCA spectrum of a GSH-modified Si substrate after 30 cycles of the GSH—GST association—dissociation, which is noted to have the same pattern as the original GSH-modified Si substrate (the panel D in Fig. 2b).

Since fluorescent images can provide visual evidence of efficient surface modification [11,14,17,26], we have applied a microscopic fluorescence imaging technique to further demonstrate the antibody against GST and fluorescein isothiocyanate-labeled secondary antibody against primary antibody (termed FITC-aGST/GST) on a GSH-modified Si substrate and the thorough removal of FITC-aGST/GST by 10 mM GSH washing solution. In the fluorescence imaging examination of the surface modification, a micropattern was fabricated on a Si substrate with a standard photolithographic procedure using an SU-8 photoresist. The modification procedures of GSH and GST on the Si surface are the same as those described in the above ESCA spectroscopic examination (details in Supplementary Information (SI)). Fig. 3b shows the green fluorescence after the association of FITC-aGST/GST on a GSH-modified substrate. In comparison, Fig. 3c—e shows three negative controls with no fluorescence, because the FITC-aGST/GST could not be effectively associated on the Si substrate without an advanced immobilization of GSH. To demonstrate that the associated FITC-aGST/GST could be removed thoroughly, the sample substrate was washed with 10 mM GSH for at least 5 min. As a result, the green fluorescence from FITC-aGST/GST (Fig. 3b) vanishes as displayed in Fig. 3f.

These successful surface functionalization procedures have been adapted to modify n-type SiNW-FETs in this study for real-time and label-free sensing experiments. The real-time conductance changes (ΔG) inside a GSH/SiNW-FET in response to the association and dissociation of GST are presented in Fig. 4a and b. In the electrical measurements, a polydimethylsiloxane (PDMS) microfluidic channel (6.25 mm × 0.5 mm × 0.05 mm) [29], aligned to couple with the SiNW-FET arrays, was designed to deliver a sample solution to the location of the SiNW-FET arrays by a syringe pump at a flow rate of 0.3 mL h^{-1}. It is noted in FET sensing experiments that an electric-field screening caused by the electrolytic environment is crucial for the detection sensitivity of an FET sensor. In this study, we dissolved samples, such as GST and GSH, in either phosphate buffered saline (1× PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4 with NaOH) or phosphate solution (1× PS, 2.4 mM NaH_2PO_4, 7.6 mM Na_2HPO_4, pH 7.4 with NaOH) of different concentrations, thus with different Debye—Hückel screening lengths (λ_D). It is well known that biomolecules keep their characteristics from denaturing in a normal physiological condition (1× PBS). However, the short $\lambda_D \sim 0.8$ nm for the ~0.14 M biological electrolyte renders a severe screening effect in the sensing measurement, therefore deteriorating the detection sensitivity of the FET sensor [6,30,31]. In Fig. 4a, we observed that the electrical measurement of 15 nM GST by a GSH/SiNW-FET in either 0.1× PBS (black curve) or 1× PS (red curve) are about the same, where $\lambda_D = 2$ nm has been calculated for both of environmental electrolytes. Shown in Fig. 4b are the electrical measurements of 15 nM GST in further diluted 0.01× PBS (black curve) or 0.1× PS (red curve), where the measured results are also of no significant difference in either solution except a fourfold enhancement in the ΔG, compared with those in Fig. 4a, due to the longer $\lambda_D = 6.6$ nm in these diluted electrolytes. In view of the similar electric responses, both PBS and PS buffer solutions with the same λ_D should possess equal buffer ability for GSH and GST without altering their expressions during the measurements. The decreases of the conductance in the n-type SiNW-FET device after adding GST is caused by a gating effect because of the negatively charged GST (pI ~ 6.72) at pH 7.4. In line of the same reasoning, the conductance returned to the initial level after the removal of GST with 10 mM GSH washing solution, also indicating a complete retrieval of the GSH/SiNW-FET as demonstrated in the two repeated cycles in Fig. 4a and b. These repeated cycles for the association and dissociation of GST on the GSH/SiNW-FET have been tested comprehensively in our experiments with one example of six consecutive retrieval cycles being provided in Fig. S1 of SI.

In a parallel examination to evidence the association and dissociation of GST on a GSH/SiNW-FET, we scanned the surface topographs of the SiNW-FET before and after the electrical measurements by atomic force microscopy (AFM). Prior to the surface modification, the top-view and profile AFM images of an unmodified SiNW-FET are displayed in Fig. 4c and d, respectively. After an electrical measurement, the association of GST on the GSH/SiNW-FET is clearly seen from the lumpy AFM image of Fig. 4e. It is noted that before the AFM imaging, the substrate of GST/GSH/SiNW-FET was washed for 15 min in vigorously stirred deionized water assisted by a mechanical rotator. The illustrative AFM image of Fig. 4e for the association of GST on GSH/SiNW-

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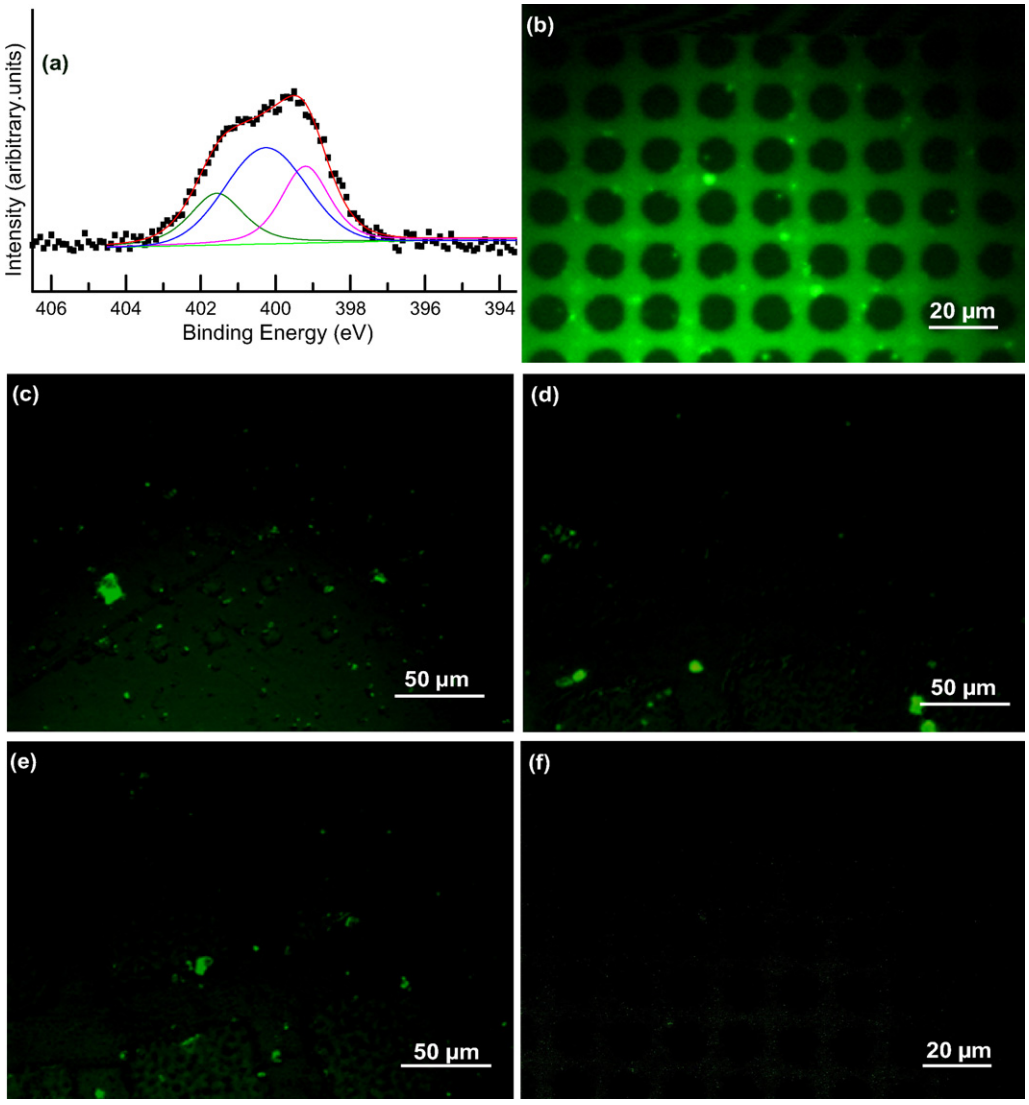

Figure 3 Demonstrations of the reversible association—dissociation of GST on a GSH modified Si substrate. (a) An N 1s ESCA spectrum obtained for a GSH modified Si substrate after 30 cycles of the GSH—GST association—dissociation, which has the same pattern as the original GSH-modified Si substrate (the panel D in Fig. 2(b)). (b) A microscopic fluorescence image of FITC-aGST/GST in association with a GSH immobilized Si substrate via the linkers of MBS and APTMS (termed FITC-aGST/GST/GSH/MBS/APTMS). The micropattern was fabricated with a standard photolithographic procedure. (c—e) Negative control tests show that FITC-aGST/GST could not be effectively associated on the Si substrate without an advanced immobilization of GSH. The surface modifications were (c) FITC-aGST/GST, (d) FITC-aGST/GST/APTMS, and (e) FITC-aGST/GST/MBS/APTMS. (f) The green fluorescence of FITC-aGST/GST in (b) vanishes after the sample substrate was washed with 10 mM GSH.

FET also illuminates the strong GSH—GST interaction. The effective removal of GST by 10 mM GSH washing is confirmed by the clean surface topograph of the SiNW-FET device in Fig. 4f.

In the electrical measurements with the SiNW-FET arrays immersed in solution, we found that the devices were easier contaminated by salts due to the more complex compositions in PBS than those in PS. We therefore dissolved biological samples in 0.1× PS (λ_D = 6.6 nm) in the following protein sensing experiments. The protein-binding selectivity of a GSH/SiNW-FET was tested by choosing avidin (pI ~ 10.5) as a negative control. In Fig. 5(a), after balanced with 0.1× PS, the GSH/SiNW-FET showed no significant ΔG to 15 nM avidin (positively charged at pH 7.4), thus manifesting the binding specificity of the GSH/SiNW-FET. On the contrary, a substantial ΔG was monitored when 15 nM GST reached the GSH/SiNW-FET sensor. In total, we have measured the ΔG of GSH/SiNW-FET in response to various concentrations (2, 3, 6, 15, 50, and 100 nM) of GST as plotted in Fig. 5(b) with a working concentration range before saturation spanning around 2—15 nM comparable to other biosensors for GST sensing [7].

We also applied the SiNW-FET sensor to quantitatively analyze protein—protein interactions by taking the well known biotin—streptavidin interaction as a demonstrative example. To this end, we first added biotin-tagged antibody against GST (termed biotin-aGST) to bind a GST/GSH/SiNW-FET (termed biotin-aGST/GST/GSH/SiNW-

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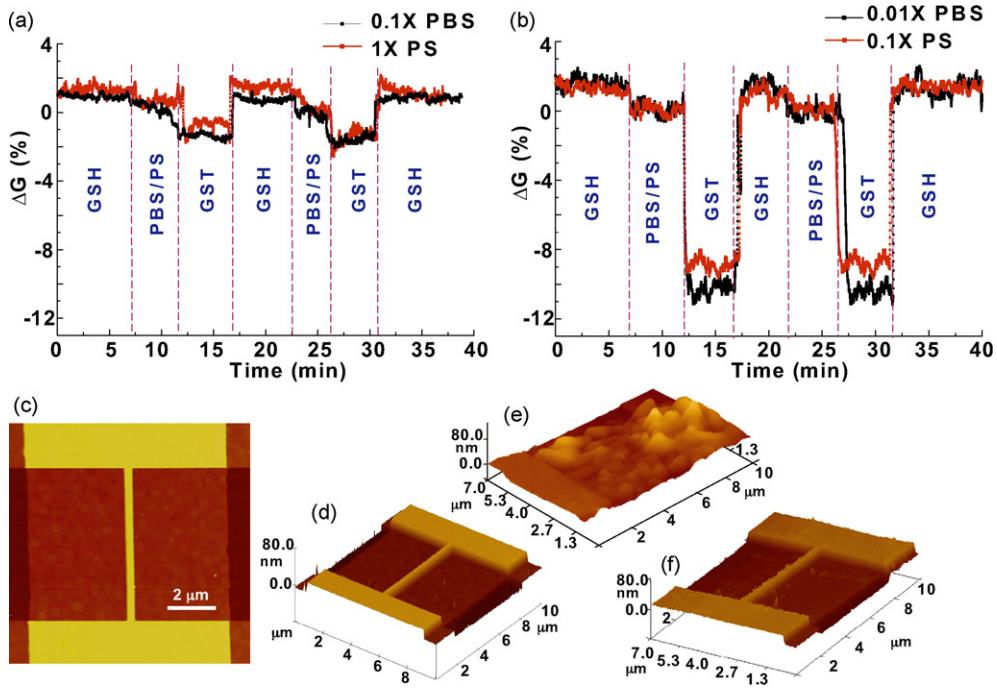

Figure 4 Environmental electrolytes with different Debye—Hückel screening lengths and the AFM surface topographs of a SiNW-FET. Real-time electrical measurements of the association and dissociation of GST on a GSH/SiNW-FET in (a) 0.1× PBS (black curve) and 1× PS (red curve) of $\lambda_D = 2$ nm and (b) 0.01× PBS (black curve) and 0.1× PS (red curve) of $\lambda_D = 6.6$ nm. The (c) top-view and (d) profile images of an unmodified SiNW-FET scanned by AFM. The wire has width, length, and thickness of 200 nm, 6 μm, and 50 nm, respectively. (e) The lumpy AFM image evidences the association of GST with the GSH/SiNW-FET. (f) The effective removal of GST by 10 mM GSH washing is confirmed by the clean surface topograph of the SiNW-FET device.

FET) in 0.1× PS ($\lambda_D = 6.6$ nm). In Fig. 5(c), a sizable variation of ΔG in the GST/GSH/SiNW-FET indicates the specific recognition between biotin-aGST and GST. The electric responses of biotin-aGST/GST/GSH/SiNW-FET upon adding 10 and 20 nM streptavidin, respectively, and the returns of the conductance level after 10 mM GSH washings are also displayed in Fig. 5(c). The variation of ΔG in the biotin-aGST/GST/GSH/SiNW-FET to streptavidin at 10, 20, 40, 100, and 200 nM are depicted in Fig. 5(b) with a working concentration range before saturation around 10—40 nM. The decrease of conductance in the n-type SiNW-FET after adding streptavidin (pI ~ 5.5—6.5) [32] is due to the gating effect caused by the negatively charged streptavidin at pH 7.4. From our measurements, the sensitivity of the biotin-aGST/GST/GSH/SiNW-FET to detect streptavidin in 0.1× PS ($\lambda_D = 6.6$ nm) could reach a value better than 10 nM, despite the quite long distance of ~15 nm from the biotin—streptavidin complex to the surface of SiNW-FET, encompassing five linkers of biotin-aGST, GST, GSH, MBS, and APTMS. Further improvement of the detection limit by diluting the buffer solution of 0.1× PS was not tested to prevent the proteins from denaturing.

The construction and expression of a GST-tagged protein are routine procedures in a molecular biology laboratory. Although the target protein expressed is amid many interfering molecules in the analyte, the tagged protein can be concentrated by several approaches, like fractionation and fast protein liquid chromatography, to increase its concentration so as to minimize the background. Furthermore, this expression can be conducted in a cell-free in vitro system with high protein yield and low background interference [33].

Without the reversible surface functionalization, a tedious surface modification process has to be carried out for each individual chip, and device-to-device variations in the number of probe molecules modified on an FET sensor would cause uncertainty in quantitative analysis. Since the reversible functionalization process does not change the number of GST molecules on the GSH/SiNW-FET, it provides an economic and convenient approach for quantitative analyses. Although the probe—nanowire distance was increased ~6 nm by the GSH—GST molecule chain, thus decreasing the sensitivity of the device, our current design could still response to the protein—protein interaction with high sensitivity. This, together with the reusability, makes the proposed technology well suited for biosensor researches.

In conclusion, we have demonstrated a novel method by imparting the reversible functionality of GSH—GST on the surface of SiNW-FETs to make the sensor reusable. The surface functionalizations on Si substrates were confirmed by ESCA and fluorescent imaging in all modification steps, including APTMS anchoring, MBS linking, GSH immobilization, and GST association. Removal of the associated GST from GSH/SiNW-FET with 10 mM GSH was verified by AFM imaging. The reversible functionalization of GST on the GSH/SiNW-FET has made this sensorial device consecutively reusable and calibratable, allowing for quantitative analysis. The detection of streptavidin by the biotin-aGST/GST/GSH/SiNW-FET in the concentration range of 10—200 nM has demonstrated this biologically modi-

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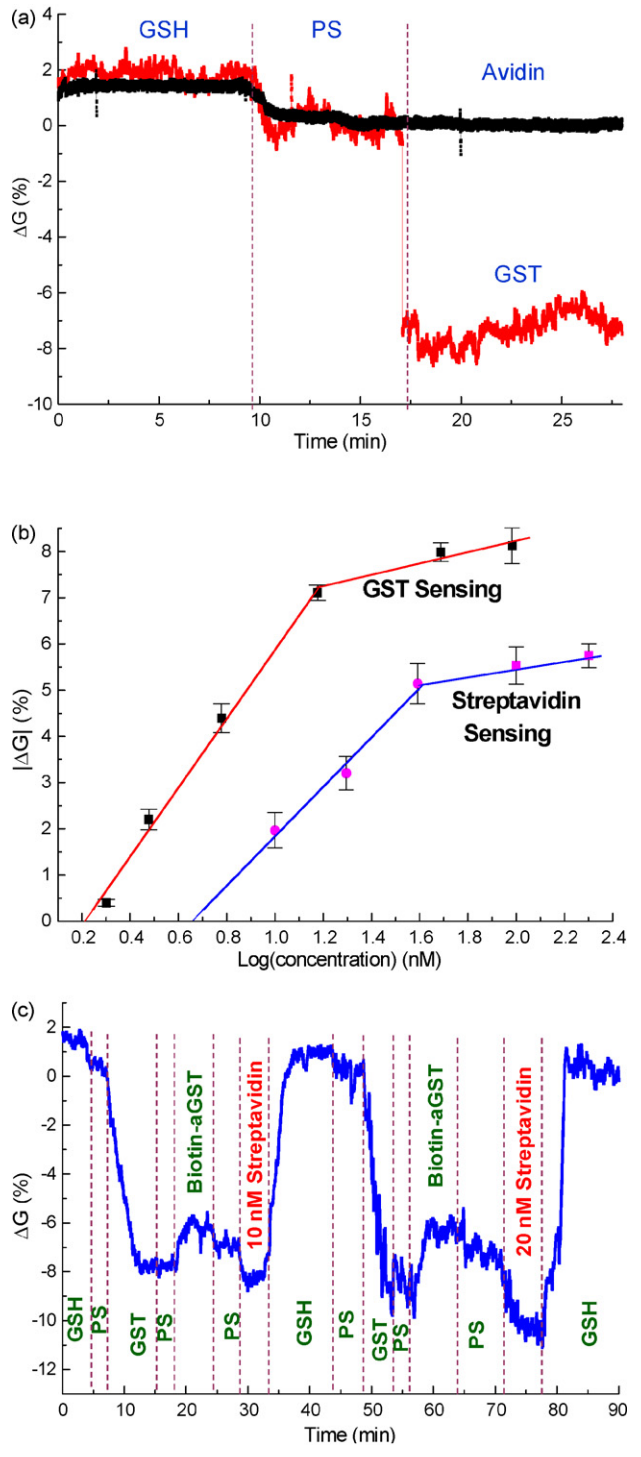

Figure 5 Protein sensing and detection limit. (a) In the specific recognition of GST, a prominent ΔG in a GSH/SiNW-FET is monitored, which is in contrast to no clear electric response in the detection of avidin as a negative control test. (b) The ΔG of a GSH/SiNW-FET in the detections of GST for various analyte concentrations (red curve) and the ΔG of biotin-aGST/GST/GSH/SiNW-FET to streptavidin (blue curve). Each data point represents the mean ± SEM from four measurements. (c) Real-time detections of the binding of biotin-aGST to GST/GSH/SiNW-FET and the subsequent sensing of streptavidin. All measurements were carried out in 0.1× PS of $\lambda_D = 6.6$ nm.

fied SiNW-FET capable of rapid high-throughput screening biomolecular associations, such as protein–protein interactions, protein–DNA interactions, protein–carbohydrate interactions, etc.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nantod.2009.04.005.

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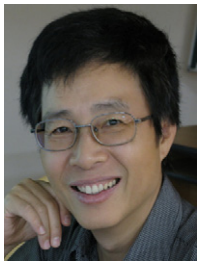

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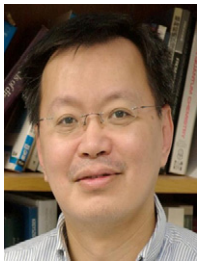

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