LOW COST THIN-FILM TRANSISTOR NANORIBBON SENSORS FOR DETECTION OF PROTEINS USING A MINIATURE BEAD-BASED ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

C. Hu, I. Zeimpekis, K. Sun, P. Ashburn and H. Morgan*

Department of Electronics and Computer Science, and Institute for Life Sciences, University of Southampton, United Kingdom

ABSTRACT

We demonstrate a low cost thin-film transistor (TFT) nanoribbon sensor for measuring enzyme-substrate reactions. The sensor is used to measure the inflammatory biomarker C-reactive protein (CRP) in human serum using a miniature bead-based Enzyme-Linked Immunosorbent Assay (ELISA) with the sensor measuring pH. Rather than binding antibodies directly to the sensor surface, a magnetic bead-based ELISA was used. This keeps the functionalization steps and capture moieties away from the sensor surface, increasing the binding sites and improving speed and sensitivity. The ability to sense proteins in physiological buffer via enzyme activity overcomes the Debye length limitation associated with nano-biosensors.

KEYWORDS: nanoribbon, bead-based ELISA, protein sensing, thin-film transistor

INTRODUCTION

Quantification and analysis of molecules at low concentrations is important in healthcare and has many biomedical applications. Recently, Field Effect Transistor (FET) devices such as silicon nanowire and nanoribbon biosensors have been developed for direct, high sensitivity, label-free sensing of biomolecules. These devices are small and could be integrated into point of care (PoC) systems. However, there are technological challenges in using these devices. One is the limitation on suspending medium composition imposed by the screening effect of the electrical double layer. Coupling of molecules and non-specific binding to the sensor surface can also be problematic. To overcome these limitations, we describe a method for detecting CRP in human serum using a miniature bead-based ELISA. The approach is similar to conventional bead-based ELISA, but instead of measuring the signal optically, an enzyme-induced pH change is measured using the nanoribbon as pH sensor. The proteins are measured indirectly through an enzyme-substrate reaction thereby circumventing the Debye screening problem. Furthermore immobilizing the capture molecules onto the beads rather than the sensor surface increases the capture cross section and also the reaction rate. This also decouples the semiconductor manufacturing processes from the chemical functionalization – beads can be pre-prepared and measured on the device as part of the assay.

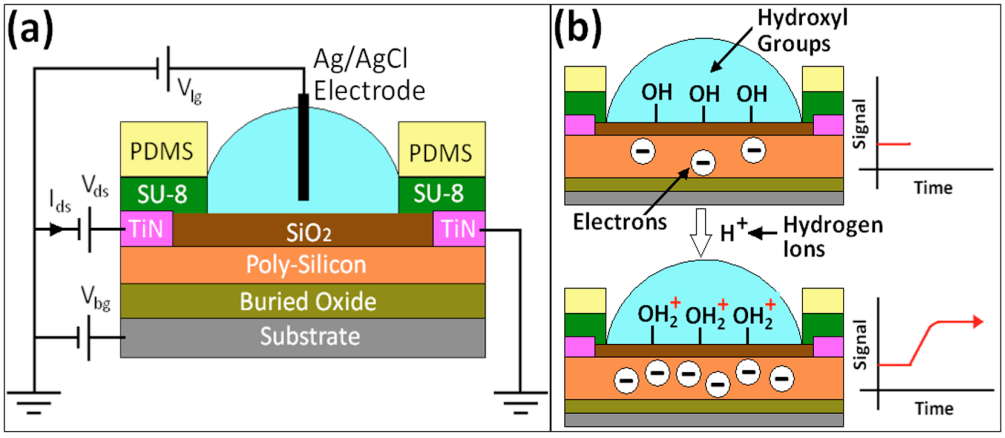

Figure 1: (a) Schematic diagram of the NR sensor together with the measurement configuration. (b) Schematic diagram of the working principle of pH sensing with n-type NR sensors.

EXPERIMENTAL

A three-mask fabrication method [1] was used to make the TFT silicon nanoribbon sensors (Fig. 1). The fabrication process involves polysilicon definition, metallization, and patterning of SU8 to create a sensing window. The nanoribbon transistor has two gates. The top gate is formed by a liquid gate with the voltage controlled by an Ag/AgCl electrode (Fig. 1a). The bottom gate is accessed from the back of the substrate and is grounded in all the measurements described in this paper. The sensor gate is made from silicon dioxide. The principle of pH sensing is shown in Fig. 1b. When immersed in solution, OH^- groups are formed on the SiO_2 surface. A baseline source-drain current I_{ds} is obtained for a given source-drain voltage V_{ds} and liquid gate bias V_{lg}. The local pH change changes the oxide surface potential, which leads to accumulation or depletion of electrons in the n-doped polysilicon channel modulating the source-drain current.

RESULTS AND DISCUSSION

The system was tested using the urea-urease enzyme-substrate reaction. Urea serves an important role in metabolic processes and is the main nitrogen-containing substance in the urine of mammals. Its concentration is an important indicator of some diseases like heart and renal failure [2]. For our measurements, urease (50 µL, 0.45 mg/mL) was pipetted onto the device and different concentrations of urea (100 µL) were reacted and the pH monitored (Fig. 2a). The source-drain current decreases with increasing pH as the enzyme reaction proceeds.

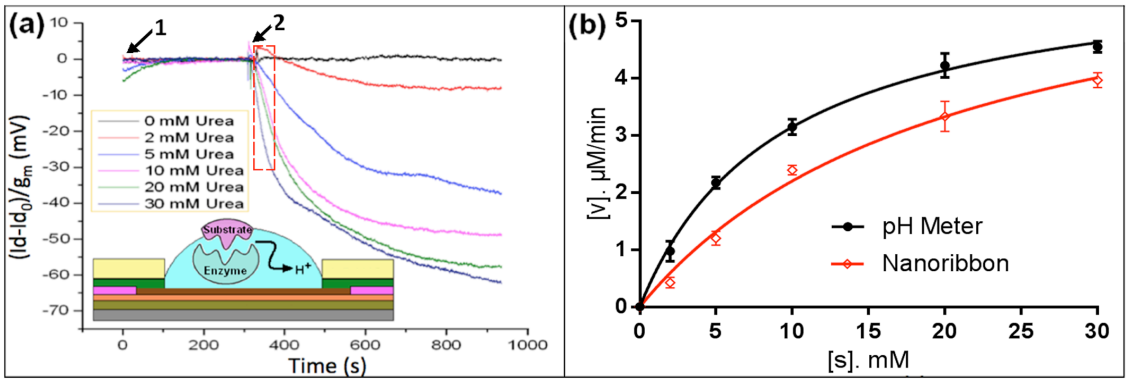

Figure 2: Detection of the urea-urease reaction using an n-type nanoribbon field effect transistor. (a) Normalised source drain current at different urea concentrations with device operating in sub-threshold mode. Urea is pipetted onto the sensor at time point 1 and urease is added at point 2. (b) Michaelis–Menten curves obtained from urea-urease reaction (data in dashed square in Figure 2a) for two detection methods: Direct measurement of pH in a test tube with pH meter and electrical detection with Nanoribbon. Data are the mean+SEM (n=3).

The reaction rate was determined from the initial slope of the reaction (dashed square in Fig. 2a) and also fitted to the Michaelis–Menten equation (Fig. 2b). The enzyme activity was also measured using a lab pH meter to verify the result obtained from NR sensors. The Michaelis constant K_M values for these two methods are 15.9 ± 2.1 mM (nanoribbon) and 9.2 ± 0.7 mM (pH meter) respectively, indicating that the TFT nanoribbon sensor is capable of measuring the enzyme-substrate reaction.

A bead based electronic ELISA was implement using magnetic beads that were modified sequentially as shown in Fig. 3a: Commercial magnetic beads with CRP capture antibody were purchased from R&D systems. These beads were re-suspended in PBS to a concentration of 500 beads/µL. A 10 µL aliquot of these beads was mixed with a 10 µL solution of CRP spiked into human serum. This mixture was incubated at room temperature for 30 minutes on a horizontal orbital microplate shaker set at 800±50rpm. The beads were then washed three in PBS by concentrating the beads with magnet and aspirating the supernatant. 10 µL of biotinylated CRP detection antibody solution (0.1 mg/ml in PBS) was added to the well and incubated at room temperature for 10 minutes on a horizontal orbital microplate shaker set at 800±50rpm, followed by three washes. 10 µL of streptavidin (0.1 mg/ml in PBS) was then added to the

well and incubated at room temperature for another 10 minutes on a horizontal orbital microplate shaker set at 800±50rpm, followed by three washes. Finally, 10 µL of biotinylated urease (0.2 mg/mL) was added and incubated at room temperature for 10 minutes followed by washing. The bead suspension was then re-suspended in the working buffer (0.01×PBS+150 mM NaCl) to a volume of 3 µL and transferred to the sensor. Five different concentrations of CRP were measured, ranging from 0 to 100 ng/ml. The amount of urease immobilized on the bead surface is proportional to the concentration of CRP, which is determined by that addition of urea (3µL, 12 mg/ml) and the pH change recorded by the nanoribbon sensor. The measured signal from a device for the five concentrations of CRP is shown in Fig. 3b.

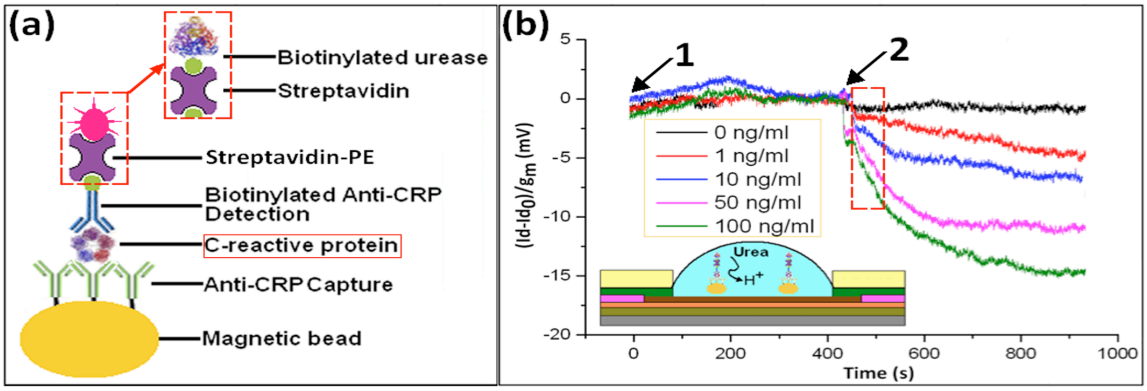

Figure 3: Detection of CRP using a bead-based ELISA with nanoribbon transistor. (a) Schematic diagram of the bead surface chemistry and functionalization. (b) Electrical response for different concentrations of CRP. The signal is the change in source-drain current divided by the sensor transconductance. Beads were pipetted on the sensor at time point 1 and urea added at point 2.

The data shows that these sensors can be used to measure enzyme-substrate reaction and to detect the inflammatory biomarker C-reactive protein in human serum using a simple bead-based ELISA. The method could be used for electrical detection of a wide range of proteins in human samples and brings the nano-FET a step closer to a point of care device.

ACKNOWLEDGEMENTS

The authors acknowledge the Technology Strategy Board (TSB) and the Engineering and Physical Sciences Research Council (EPSRC: EP/K502327/1) for funding. We also thank Sally Anderson, Gregory Gay, Ben Hadwen and Chris J. Brown of Sharp Laboratories Europe for useful discussions.

REFERENCES

[1] K. Sun, I. Zeimpekis, M. Lombardini, N. M. J. Ditshego, S. J. Pearce, K. S. Kiang, O. Thomas, M. R. R. de Planque, H. M. H. Chong, H. Morgan and P. Ashburn, "Three Mask Polysilicon Thin Film Transistor Biosensor," *IEEE Trans. Electron Dev*, 2014, 61, 2170-2176.

[2] L. Y. Mu, I. A. Droujinine, N. K. Rajan, S. D. Sawtelle and M. A. Reed, "Direct, Rapid, and Label-Free Detection of Enzyme–Substrate Interactions in Physiological Buffers Using CMOS-Compatible Nanoribbon Sensors," *Nano Lett*, 2014, 14, 5315-5322.

[3] E. Stern, A. Vacic, C. Li, F. N. Ishikawa, C. W. Zhou, M. A. Reed and T. M. Fahmy, "A nanoelectronic-enzyme linked immunosorbent assay (ne-ELISA) for detection of proteins in physiological solutions," *Small*, 2010, 6, 232-238.

CONTACT

* Hywel Morgan; phone: +44 (0) 2380593330; hm@ecs.soton.ac.uk