Detection of Small Molecule-Protein Binding with Amorphous Silicon Nanostructures

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ABSTRACT

We present the fabrication and characterization of a nano-scale sensor made of amorphous silicon (a-Si) for electronic detection of small molecule–protein binding. The main component of the detector is a nano-scale a-Si wire with a surface functionalized with receptor molecules. Upon exposure, target molecules bind to these receptors and effectively change the surface dipole moment of the wire. This change of dipole moment in turn bends the semiconductor bands near the surface resulting in a change in charge carrier density. Due to the extremely small cross section of the conductive path (50 nm x 50 nm), changes close to the surface significantly affect the apparent conduction in the wire and can be monitored electronically to determine the occurrence of molecular binding events.

Keywords: protein detection, biotin, streptavidin, sensor, nanowire

1 BACKGROUND

The field of Biological Microelectromechanical Systems (BioMEMS) has seen a rapid increase in the number of micro-scale and nano-scale tools to detect objects of biological interest over the past decade. These "Biochips"[1] offer many advantages over their traditional counterparts, including the abilities to handle small sample volumes, to detect rapidly, and to sense very low molar concentrations. Of particular interest to the field of BioMEMS is the detection of proteins. Proteins are involved in almost every intracellular and extracellular process.[2] Not only are proteins used as tools to perform various tasks in cells, they are also responsible for regulating these tasks. The state of a cell is defined in part by protein concentrations. By knowing the concentration of proteins within a cell or in cell's vicinity, one can begin to determine the details of the cell's causal relationships. The ultimate goal of researchers is to develop biochips that can detect different proteins quickly and inexpensively in the environment occupied by a single cell. One feature of protein sensors that could make both goals attainable is the utilization of a transduction mechanism that is nano-scale and can be easily integrated with the Complementary Metal Oxide Semiconductor (CMOS) integrated circuit fabrication technology. The small dimensions of such devices would produce dense sensor arrays with each sensor capable of detecting very low concentrations of a target protein. CMOS compatibility would allow these devices to be incorporated with modern high-speed analog and digital circuits capable of rapid transcription and analysis of binding events. To allow for such integration and arraying, we present the fabrication and characterization of a nano-scale sensor made of amorphous silicon (a-Si) for direct electronic detection of small molecule –protein binding.

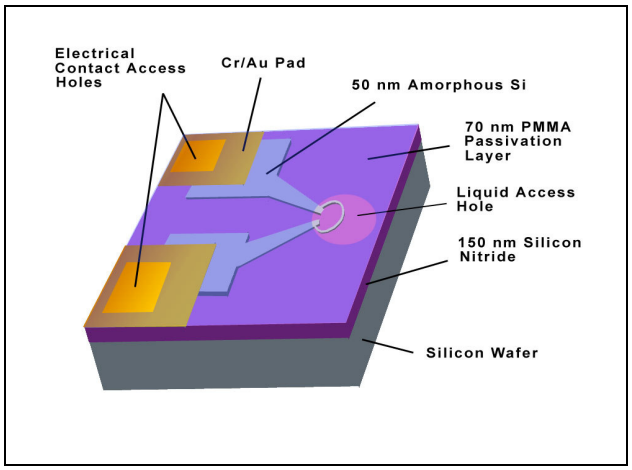

Figure 1: 3D view of sensor structure (not to scale).

The key feature of the sensor is a nano-scale semicircle loop of a-Si. The surface of this sensor is functionalized with receptors that are selected to preferentially bind to specific target molecules. Depending on the residual charges of the target molecules, this binding event can alter the surface dipole moment of the semicircle structure. The alteration of the surface dipole moment results in a shifting of the energy bands within the a-Si material, leading to an observable change in conductivity. Due to the nano-scale dimensions of the sensor's cross-sectional conductive path (50 nm x 50 nm), a change in the conductivity limited to a region near the surface results in a noticeable change in the overall sensor conduction. This allows us to monitor electronically molecular binding events by observing changes in the sensor's conductivity.

2 MATERIALS AND METHODS

2.1 Device Fabrication

The sensor architecture is illustrated in Figure 1. The sensor is comprised of a 50 nm wide amorphous silicon semi-circle fabricated on a 150 nm thick silicon nitride insulating layer. The entire structure is protected with a polymeric insulating layer so that only the semi-circle and

small electrical contact access holes are exposed. There is sufficient separation between the semi-circle and electrical access holes to ensure that only the semi-circle is exposed to the test solution.

We began the device fabrication with a clean silicon wafer (Figure 2). We grew a 150 nm thick silicon-nitride layer on the wafer surface using low-pressure chemical vapor deposition (LPCVD) followed by a 50 nm layer of amorphous silicon using plasma-enhanced chemical vapor deposition (PECVD). We spin-coated a 70 nm layer of PMMA on the wafer and used electron-beam lithography to

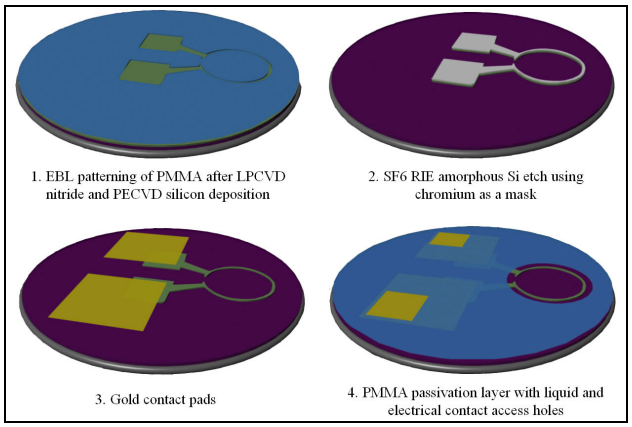

Figure 2: Fabrication process for the a-Si semicircle.

pattern nano-scale features in the PMMA. The pattern consisted of a 270º semicircle loop with a width of 50 nm and a radius of 1 µm. Each end of the semicircle was connected to a large (40 µm x 40 µm) pad by an arm that tapered from 5 µm at the pad to 150 nm at the ends of the semicircle. Following electron-beam lithography, we thermally evaporated a 20 nm layer of chromium on the wafer and performed a liftoff in acetone. We used the chromium patterns that remained as a reactive ion etch (RIE) mask. All amorphous silicon not covered by chromium was removed using an SF6 RIE etch. We used wet etching (Transene Chromium Mask Etchant) to remove the remaining chromium mask. An SEM image of the resulting a-Si nanostructure is shown in Figure 3. We

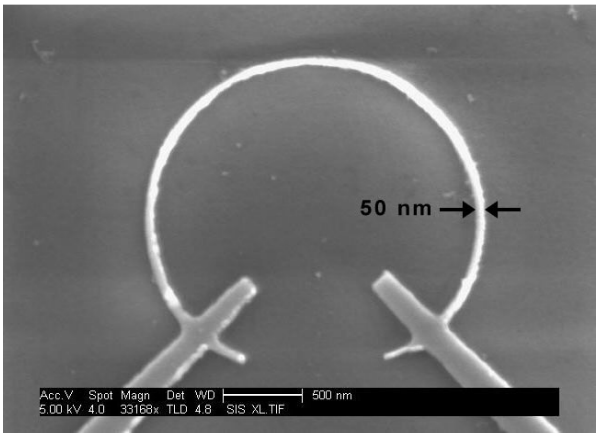

Figure 3: SEM image of amorphous silicon semicircle on a nitride substrate (top view).

performed a second electron-beam lithography step, metal evaporation, and lift-off to pattern 200 µm x 100 µm metal pads; the pads were made of 5 nm of chromium used as an adhesion layer and 35 nm of gold. These larger pads overlapped the silicon pads and allowed for more reliable electrical measurements. We used a final electron-beam

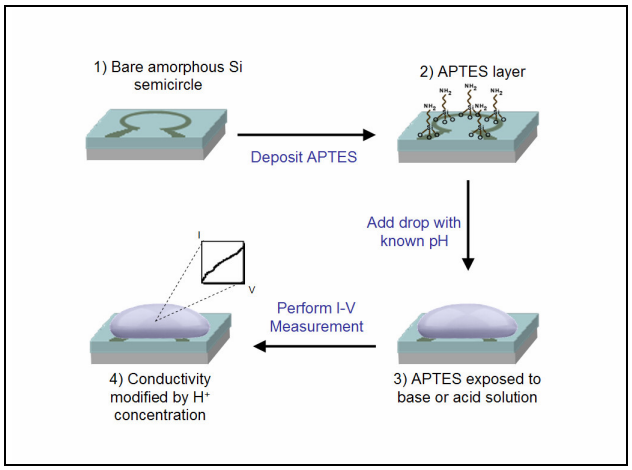

Figure 4: Semicircle molecular functionalization.

lithography step to create a 70 nm passivation layer of PMMA with a small opening for liquid access to the semicircle and two openings for electrical contact to the Cr/Au pads. This passivation layer ensured that the test solution only contacted the semicircle and prevented the ionic solution from contacting the probe tips or gold pads.

2.2 Sensor Functionalization

We modified the a-Si semicircle with two different self-assembled molecular monolayers to detect two different target molecules. We formed a monolayer of aminopropyltriethoxysilane (APTES) on the native silicon dioxide on the a-Si surface for the purpose of detecting H+ concentration (pH)[3], and a layer of biotinamidocaproyl-labeled bovine serum albumin (BAC-BSA) on the semicircles for the detection of streptavidin.

We formed the APTES monolayer by soaking the wafer in a solution of 0.5% v/v APTES in ethanol for 30 minutes. Afterward, we placed the wafer in a vacuum chamber for 8 hours to remove unbound molecules.

We created a layer of biotin-modified BSA on the semi-circle surface by incubating the amorphous silicon with a solution containing BAC-BSA.

BSA is a common protein with a well-studied molecular structure. It is also known to readily attach to silica surfaces with an adsorption that is modulated by pH.[4-6] The BSA we used was tagged with Biotinamidocaproate N-hydroxysuccinimidyl ester (Sigma). Biotinamidocaproate N-hydroxysuccinimidyl ester is a biotinylation reagent incorporating an aminocaproyl spacer. This spacer can reduce the steric hindrance in binding streptavidin to biotinylated compounds. We incubated the sensors in a 10

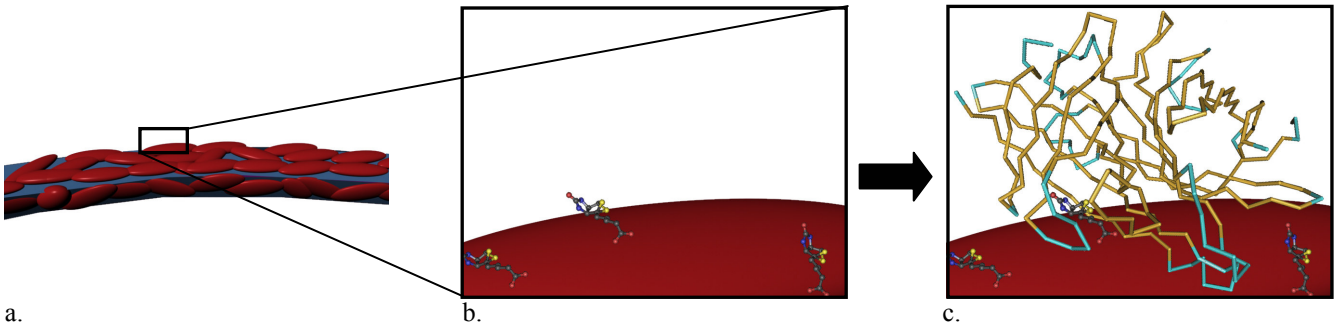

Figure 5: graphical rendition of BSA denatured on the amorphous silicon semicircle (a), a close-up showing biotin molecules attached to the BSA (aminocaproyl spacer not shown) (b), and the attachment of a streptavidin molecule to one of the biotins (c).

µL drop of 3.6 µM biotinamidocaproyl labeled BSA (prepared in 1 mM phosphate buffer with 5 mM NaCl, pH 5.6) for 2 hours, followed by five times rinse with the buffer solution.

3 EXPERIMENT PROCEDURE

Detection of pH using APTES is possible because, depending on the pH of the solution it is in, the primary amine of APTES molecules can protonate or deprotonate. We tested the functionality of our APTES-modified a-Si semicircles by exposing them to solutions with different pH values.

Solutions with pH 2, 4, 5, 6, 7, and 9 were prepared by combining double distilled H_2O with various concentrations of HCl and ammonia. As indicated in Figure 4, we placed a droplet of solution with known pH on the silicon semicircle and an I-V measurement was performed. We then rinsed the droplet with double distilled H2O and the dried the wafer before the next solution was tested. We repeated this process for all six pHs.

To verify the ability of the BAC-BSA to detect streptavidin, we first conducted a control experiment by placing a 1 uL drop of d-biotin-saturated streptavidin (prepared by adding four equivalents of d-biotin to one equivalent streptavidin in 1 mM phosphate buffer) on the functionalized sensor and measuring the conductance. Following the control, we rinsed the sensor with buffer and placed a 1 uL drop of 18.9 uM streptavidin (New England BioLabs) on the sensor and repeated the conductance measurement. We also found the transient response of the sensor to the addition of streptavidin by monitoring the conductance of the sensor while adding a drop of the streptavidin solution.

4 RESULTS

Electrical measurements performed on the APTES-modified semicircle showed an almost linear relationship between measured current at a constant voltage and the pH

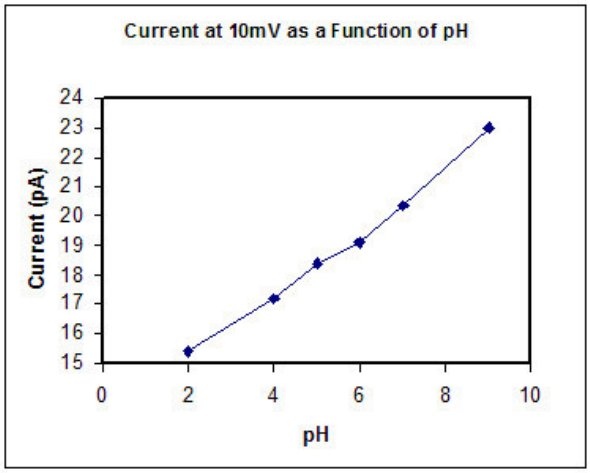

Figure 6: Measured dependency of sensor current under constant bias on pH

of the solution the semicircle was exposed to. As shown in Figure 6, there is a substantial dependence of sensor current at a constant bias on the pH of the solution. Current increases by 40% from pH 2 to pH 9.

As can be seen from Figure 7, for the streptavidin detection experiment there was almost no change in conductance with the addition of d-biotin-modified

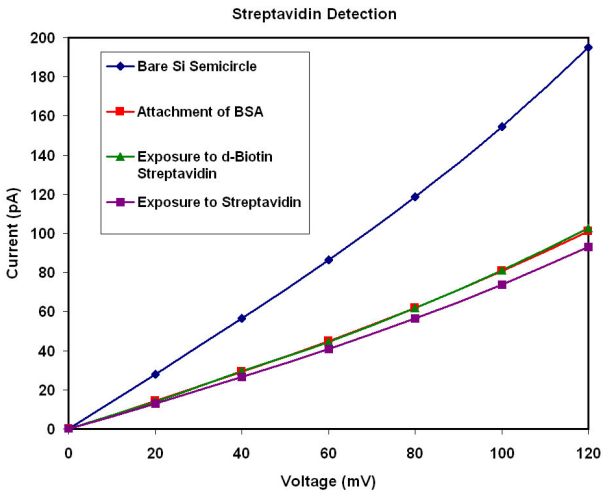

Figure 7: Streptavidin detection

streptavidin (control), while there was a noticeable change upon exposure to the streptavidin. This indicates that the sensor is detecting the actual binding of streptavidin to the biotin-tagged BSA. Figure 8 shows the transient response of the sensor to the addition of streptavidin.

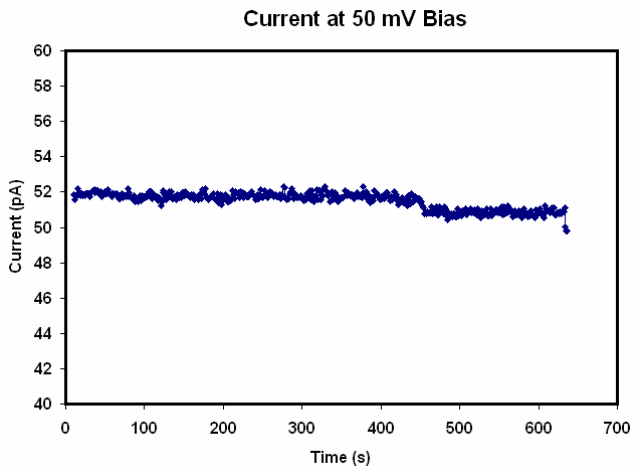

Figure 8: Transient graph showing the current change in a typical sensor upon addition of streptavidin at time = 460 s.

5 CONCLUSION

The need to further understand the operation of molecular biological processes has greatly increased the desirability of biological molecule detection tools which offer compact design and simple, efficient signal transduction. We have presented a simple sensor structure which we have demonstrated is capable of detecting pH and streptavidin. Moreover, the sensor structure has a footprint of approximately 1 um^2. The sensor directly converts a protein binding event into an electronic signal and can be integrated over CMOS-based read-out and data manipulation circuitry.

6 ACKNOWLEDGEMENTS

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