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Detection of the biotin–streptavidin interaction by exploiting surface stress changes on ultrathin Si membranes

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ABSTRACT

The detection of the biotin–streptavidin interaction is presented based on surface stress changes of functionalized Si membranes. During the biomolecular interaction, the surface stress of the functionalized membranes alters resulting in their deflection and finally in capacitance variations. The biological interaction on the sensors surface was verified after each experiment using fluorescent microscopy. Successful detection of 21 nM of streptavidin using 3.55 mM biotin probes is presented.

1. Introduction

In recent years the detection of biological species based on surface stress changes of functionalized cantilevers has received a lot of attention as they are sensitive and relieve sample preparation treatment from labeling. For example, using microcantilever sensors, Fritz et al. [1] have studied the DNA hybridization of 12-mer oligonucleotides and report detection of single nucleotide mismatches, whereas Zhang et al. [2] and Arntz et al. [3] have detected biomarker transcripts in human RNA and cardiac biomarker proteins, respectively. In proteins, the induced surface stress change may either be compressive or tensile [4,5].

During the biotin–streptavidin interaction, the surface stress change depends on the nature of the biotin modified surfaces. In particular, biotin–SS–NHS coated microcantilevers has been reported to exhibit tensile stress increase of 17.8 mN/m, biotin–HPDP coated microcantilevers exhibit compressive stress of 88.7 mN/m and biotin–PEG coated microcantilevers do not bend upon the injection of streptavidin [4].

One of the main drawbacks of the cantilever technique for biosensing is the limitations it presents when used in practical systems with the optical setups needed for the cantilever deflection readout. Optical setups are usually costly and bulky systems that are difficult to use as they need continuous alignment and calibration. In addition, they are sensitive to changes in the optical density of the sample and they cannot be used in opaque solutions, such as blood [6–9].

On the other hand, capacitive readout although highly sensitive is also not efficient for microcantilever arrays in biological solutions because of the faradaic currents that appear between the cantilever and the fixed electrode. In contrast, when a membrane is used instead of a cantilever, the membrane seals the area underneath it and the biological solutions cannot slip into the area between the electrodes, thus enabling capacitive readout. In this work, we present a capacitive type sensor built around a thin Si membrane on which receptor molecules are immobilized (Fig. 1).

The device performance is tested in the detection of a standard biological interaction using biotin–NHS probes and 21 nM concentration of streptavidin target molecules. A typical reported sensitivity of the optical cantilever technique, in the case of biotin–streptavidin system, is in the range of a few nM of streptavidin concentration [4,6]. The presented sensor sensitivity, although not tested yet, can be maximized by optimizing the geometry of the sensor (e.g. membrane thickness, cavity depth etc.).

2. Sensing element description

The presented capacitive biosensor element comprises a silicon membrane of 700 nm thickness and 250 μm diameter. The

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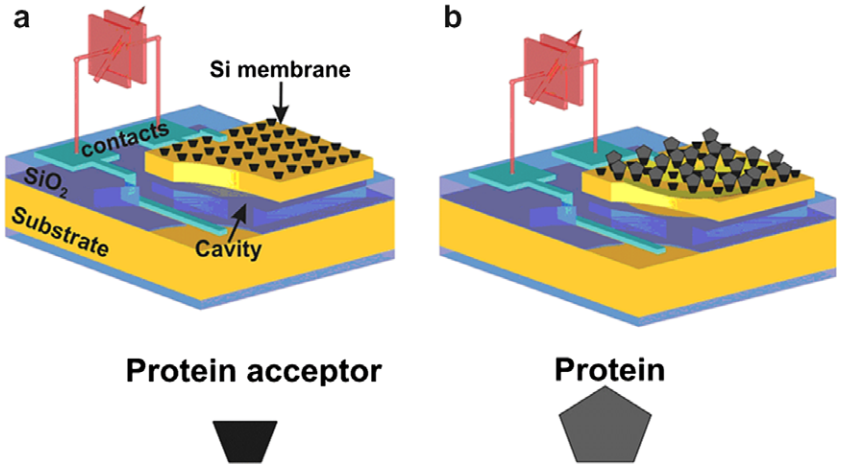

Fig. 1. Schematic of the capacitive sensing element consisting of the flexible Si membrane over the cavity formed on a thermal SiO_2 layer. Upon protein binding, a capacitance change between the membrane and the fixed electrode on the substrate is observed.

membrane is formed by the epitaxial SiGeB layer of a Si wafer which has been silicon fusion bonded to a substrate wafer. The Young's modulus of the membrane is expected to be the same with that of undoped Si (169 GPa) as the effect of the boron doping is counterbalanced by the simultaneous incorporation of germanium atoms in the silicon lattice. The membrane is passivated by a 500 nm thick low temperature oxide (LTO) layer, on which the biotin probes are immobilized. A schematic of the sensing element is depicted in Fig. 1 and is composed of a rigid fixed electrode implemented on a thick silicon substrate, a cavity etched in the SiO_2 layer, and the ultrathin flexible silicon membrane electrode overhanging the cavity. The membrane deflects due to surface stress variations when the probe molecules bind to their respective target counterparts. The deflection is then translated into a change in capacitance between the flexible electrode and the substrate, thus allowing for unlabeled sensing and ease of electrical detection. The fabrication of an array of such sensing elements has been presented in detail in [10].

The functionalization of the sensing material took place directly on the passivation LTO layer. In particular, the surface of the sensor was first coated with 3-[2-(2-aminoethylamino)ethyl amino] propyltrimethoxysilane (AEEPTMS) by shaking the sample for 19 h into the coating solution (1% aminopropyltriethoxysilane in solution of 95% ethanol), washing the sample two times with 95% of ethanol solution and one time with distilled water. Afterwards, the functionalized surface was dried at the specific temperature of 80 °C for 2 h. Then biotin–NHS probes where immobilized on the membrane surface. Biotin–NHS is able to covalently attach to the amino-modified LTO substrate. Biotin probe drops where dispensed, using a pipette, on the sensor surface in 3.55 mM concentration and drying the samples at 80 °C for 20 min. In order to remove the unbound biotin, the sensors were then washed two times with 50 mM Tris–HCl (pH 7.4) and four times with phosphate buffered saline (PBS). Finally, for the interaction between biotin and streptavidin the prepared samples were exposed to a streptavidin–R–phycoerythrin solution as described next.

3. Experimental setup

A Polydimethylsiloxane (PDMS) gasket covers the sensing membranes and limits the bioreaction over the active area. The gasket is part of a larger enclosure which accommodates two fluidic inputs and two outputs thus allowing for the controlled insertion of reaction fluids and necessary buffers within the limited space above the membranes. The sensor itself is mounted onto a printed circuit board (PCB) which is hosted within the hybridization cassette and provides the necessary electrical connections to a Labview program running on a PC and an LCR bridge enabling capacitance measuring (Fig. 2).

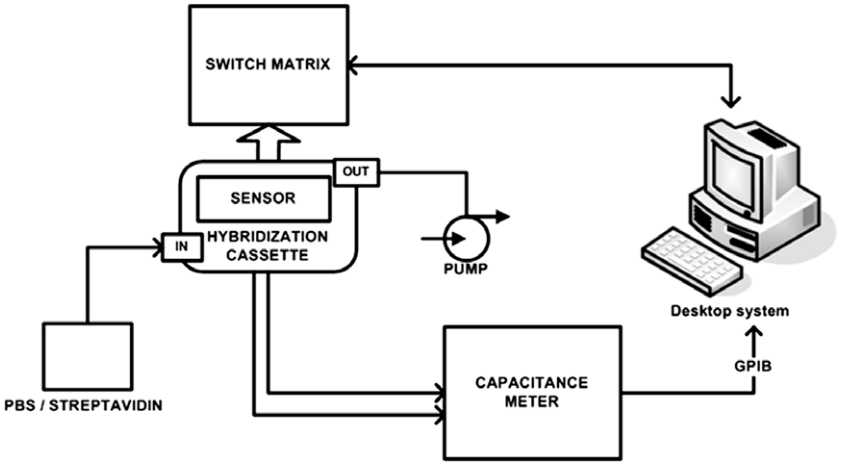

Fig. 2. Schematic of the experimental setup. A switch matrix is controlled by a Labview program for switching the capacitance measurement between several sensing elements, while the biological solutions pass over the sensors through silicone tubing.

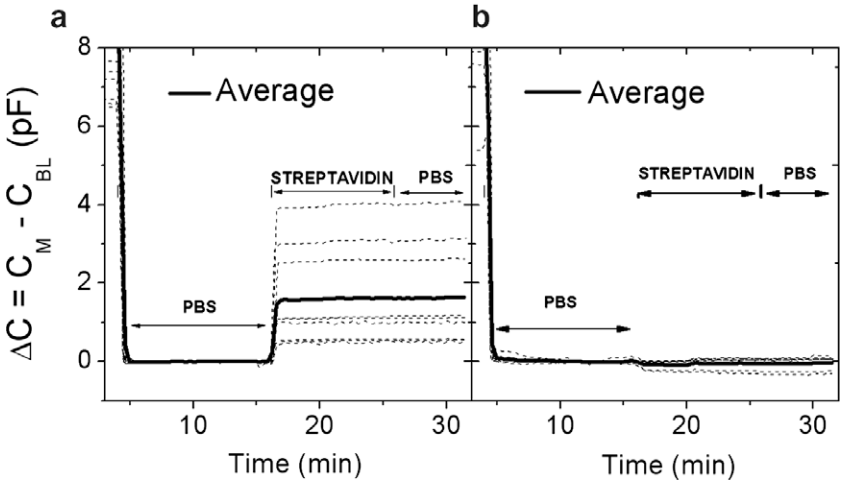

Fig. 3. Isolated and average responses of (a) biotin functionalized sensors and (b) biotin unfunctionalized reference sensors. The signal corresponds to the measured capacitance (C_M) minus the capacitance baseline (C_{BL}). The baseline is the mean capacitance value of each sensor in the PBS solution prior to streptavidin insertion.

To operate the system, 0.5 μm ID silicone tubing is used to implement the fluidic ways and a peristaltic pump is introduced at the exit of the microfluidic system. The pump draws the solution out of the tank and into the hybridization cassette and chamber and then out to an external waste tank.

4. Experimental results

The biotin–streptavidin reaction took place at room temperature. During the experiments PBS buffer is first inserted into a PDMS chamber that covers the sensing area and wets the sensor. Then 21 nM of streptavidin is introduced into the chamber for several minutes and after its removal the chamber and sensor are washed with PBS buffer, so unspecifically bound streptavidin is PBS washed away.

The streptavidin molecules are labeled with fluorescent molecules in order to confirm the interaction through fluorescent microscopy after the end of the experiment. At first, the fluorescent microscopy confirmed that 5 min after the introduction of the streptavidin solution are enough for the interaction to occur and obtain a strong fluorescent signal. In fact, the biotin–streptavidin binding needs only a few seconds to occur since the proteins are mixed together and the interaction is efficient at room temperature.

The capacitance signals of several functionalized sensors as well as of membranes that are not biotin functionalized and used as reference are shown in Fig. 3. Initially, all sensors acquire a stable value (C_{BL}) in the PBS solution prior to streptavidin insertion. It is observed that upon streptavidin insertion the capacitance of the sensors that have been functionalized with biotin (BFS) is increased (Fig. 3a), as the protein binds to the immobilized biotin on the sensor surface. On the contrary, the corresponding capacitance of the biotin unfunctionalized sensors (BUS) remains relatively stable (Fig. 3b). The change in capacitance during the interaction amounts up to 4 pF (~1.5 pF on average) for the BFS while the reference ones remain relatively stable with unspecific changes of at least one order of magnitude less (~0.06 pF on average). These unspecific changes may be attributed to possible artifact from the solution mass change upon insertion of streptavidin or unspecific binding. However, these effects are eliminated using the differential capacitive readout. Even after washing with PBS buffer almost no drop is observed in the capacitance of biotin functionalized sensors confirming specifically bound streptavidin molecules on the sensor surface.

The capacitance increase indicates that the gap between the fixed and the flexible electrode decreases, thus the membrane bends towards the substrate upon the reaction. The downward deflection of the membrane indicates that the biotin–streptavidin binding induces tensile stress, coming into agreement with previously reported results [4]. Nevertheless, the value of surface stress cannot easily be extracted for further comparison, as the membrane displacement cannot be estimated by the capacitance change. This is due to the fact that the membranes are not flat, but they exhibit slightly different deflections because of stress induced during the fabrication process. For the same reason, each

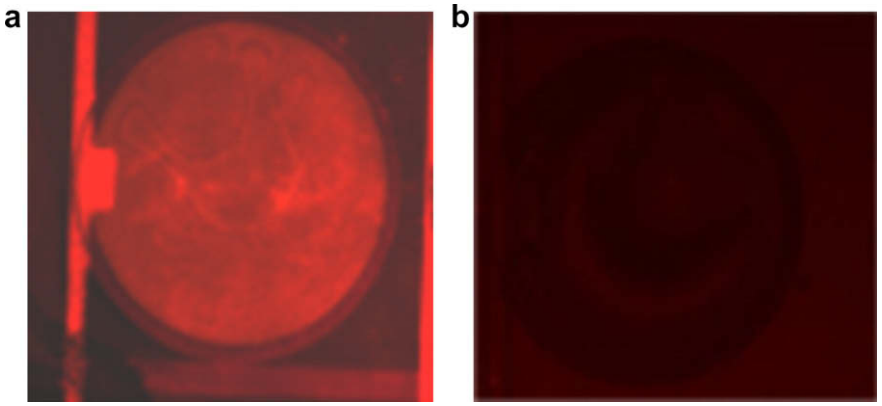

Fig. 4. Fluorescence images of a typical (a) biotin functionalized and (b) biotin unfunctionalized sensor after the reaction with fluorescent streptavidin.

membrane has a somewhat different initial capacitance value, depending on its initial deflection, and finally, the capacitance difference between similar sensors for the same experimental conditions may diverge. As a result, dispersion is observed in the sensor response shown in Fig. 3.

In Fig. 4a the fluorescent signal of a biotin functionalized membrane after the reaction experiment is depicted. On the contrary, membranes that have not been functionalized with biotin exhibit almost no fluorescence signal after the experiments (Fig. 4b). It is observed that the fluorescence signal of the biotin functionalized sensors (BFSs) is about six times larger than that of the biotin unfunctionalized sensors (BUSs) that are used as reference.

5. Conclusion

The capacitive detection of the biotin–streptavidin binding utilizing the surface stress changes during the reaction is demonstrated. The capacitive readout for the biological applications has been enabled using an ultrathin Si membrane as the flexible electrode. Contrary to the common method wherein cantilevers are covered by a thin gold layer for the immobilization of probe molecules, the immobilization of biotin molecules took place directly on the amino-modified membrane surface which is composed of a thin LTO passivation layer. Fluorescence microscopy confirmed the biotin–streptavidin binding above the surface of the membranes after the end of the experiments. A significant capacitance change is observed when the interaction takes place on the surface of biotin functionalized membranes as opposed to minor capacitance change of unfuctionalized sensors. These results encourage the efforts on using capacitive readout for miniaturizing in situ, fast and label free analysis of biomolecular interactions.

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