Microdroplet Protein Sensors on a Gold Surface with a Self-assembled Monolayer Treatment

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Abstract: A new kind of microdroplet-based biological protein sensor is presented. The sensor was made by placing silicon oil on gold film with a self-assembled monolayer (SAM). The surface tension dominates the sensitivity of the sensor. Using mercaptoundecanoic acid (11-MUA) as the sensor’s SAM layer, the sensor can detect 0.5 mg/ml, 20 μg/ml, and 0.4 μg/ml bovine serum albumin (BSA) protein solutions in a control volume of 0.5ml. The sensor’s reaction time for concentrations of 0.5 mg/ml, 20 μg/ml, and 0.4μg/ml protein solutions was 15, 60 and 120 minutes, respectively. As the size of microdroplet decreased, the change of contact angle increased.

Keywords: microdroplet; protein sensor; self-assembled monolayer (SAM)

Introduction

A biosensor is a bio-analytical device that can detect biological material and microorganisms. It can also monitor exogenous or endogenous in-vivo chemical reactions, and display the variations in sensors via a built-in transducer. These signals can then be provided to researchers for analysis [1, 2]. In recent years, advances in biotechnology have found broad applications, including food and drug safety tests, clinical trials of new drugs and disease detection. Proteins are usually key factors in these processes, and protein sensors are a type of biosensor used in a broad range of examinations. Analysis of the protein assays is an important field of study. Applications of protein-ligand recognition reveals harmful substances in foods and can detect the presence of infection by testing potential combinations of antigens and antibodies.

The physical properties of the sensor’s surface will change due to the formation of an antibody-antigen complex. These changes can easily be detected and then converted into an electrical signal via acoustical (e.g. quartz crystal microbalances (QCM) device [3-5]), optical (e.g. surface plasmon resonance (SPR) detector [6-9]), or mechanical (e.g. microcantilever beams [10, 11]) transducers. Biosensors are categorized by their transducer type (e.g., electrochemical, optical, acoustical, thermometric, mechanical, etc.). Despite their relatively high expense, optical biosensors have become more popular due to their high efficiency and sensitivity. Optical biosensor formats involve direct detection of analytes, or indirect detection through optically-labeled probes. Optical transducers can detect changes in absorbance, luminescence, polarization or refractive index.

Biomedical science has developed quickly in recent years, spawning the development of myriad related technologies. Previous methods have tended to be subject to certain disadvantages in protein-ligand recognition: a broader sample base is required, the use of immunofluorescent assays can be harmful to test subjects, and reaction times take anywhere from a few days to several weeks.
In this study, silicon oil droplets with diameters measured in tens of micrometers are used to detect proteins, and gold film is used as a substrate for detecting changes in the protein’s contact angle. The system with oil droplet in liquid was reported to adjust the droplet profile by voltages for optical focus tuning. [12-15] This recent-developed technique is simple to use, does not require highly-precise measurement equipment, and only requires 0.5 ml of protein liquid for testing.

Materials and Methods

Materials

A gold thin film was used as the substrate to ensure biocompatibility. A 4-inch square glass substrate was coated with a 100 nm-thick gold thin film by the E-gun system (ULVAC) to ensure biocompatibility. The glass was then cut into squares measuring 1 mm on a side.

The Self-Assembled-Monolayer (SAM) is a key component of a protein sensor, and the choice of SAM depends on the material used as the substrate. This study adopted mercaptooundecanoic acid (11-MUA) as a SAM. The molecule contains a twelve-carbon alkyl chain, and its functional group is carboxyl (-COOH) which attaches with the Amino (-NH2+) in the protein. Its head group for linking with the gold film is thiol (-SH), and the molecular formula of the SAM is HS(CH2)10COOH.

Bovine serum albumin (BSA) was used in three concentrations for testing. The microdroplet liquid was silicon oil No. 704 (Dow Corning). To maintain a constant pH value, we used a protein liquid buffer of phosphate buffered saline (PBS), which contains sodium chloride, sodium phosphate, and (in some formulations) potassium chloride and potassium phosphate.

The monolayer deposition process had been successfully used for a number of long chain molecules on metal surfaces, and offers a simple method for surface modification at the single monolayer level [16-18].

One of the most successful techniques employed for adsorption on metal surfaces is the attachment of thiol molecules (-SH) on gold surfaces. Many self-assembly systems have been investigated, but a monolayer of alkylthiols on gold film is the most widely studied. Most of the thiol compounds investigated to date contain a long alkyl chain, which enables the compounds to form a compact monolayer on Au surfaces. However, for protein binding, the –COOH group can react with the –NH2+ in most protein structures. The combination of protein and SAM molecules is a covalent bond, and is thus good for signal capture.

Surface treatment

We used 250 ml 99.5% ethanol solution as the solvent and added 50 mg 11-MUA powder to make 1 mM 11-MUA solution. The gold film was washed following standard procedures: washing separately in Acetone, IPA, and DI water, followed by cleaning with 90°C vitriol solution (H2O2 : H2SO4 = 3 : 7) for ten minutes. The gold film was immersed in the 1 mM 11-MUA solution for twenty-four hours. We then used 95% ethanol to wash away the SAM molecule physical adhesion on surface of the gold film, followed by 5 min washing in an ultrasonic cleaner before pouring off the solution. We repeated this procedure three times to ensure the removal of the physical adhesive 11-MUA from the gold film. Following this procedure, the SAM film was coated on the gold film.

Droplet forming

A precisely-controlled dispenser (MUSASHI, SUPER 2x V2) was used to generate microdroplets of 20 μm to 80 μm in diameter by controlling the air pressure to form the volume of silicon oil required on the gold film. The test fragments were placed in a 2 cm x 1 cm x 0.5 cm acrylic receptacle boxes, submerged in 0.5 ml PBS solution for testing. At the start of the experiment, the protein solution was added to the box and the contact angle was measured (Figure 1).
Theory

Young formulated the relationship between the interfacial tensions at a point in a 3-phase contact angle line (Figure 2) as follows [19]:

$$\cos(\theta) = \frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}},$$  \hspace{1cm} (1)

When liquid 1 immersed in liquid 2, the equation can be rewritten as (Figure 3):

$$\cos(\theta) = \frac{\gamma_{SV} - \gamma_{SI}}{\gamma_{LV}},$$  \hspace{1cm} (2)

where

$$\gamma_{12} \approx \gamma_1 + \gamma_2 - 2\sqrt{\gamma_1\gamma_2},$$  \hspace{1cm} (3)

where $\gamma$ is surface tension which depends on the properties of materials.

$$\gamma = \left(\frac{\text{# of molecules}}{\text{area}}\right) \left(\frac{\text{binding energy}}{\text{molecules}}\right)$$  \hspace{1cm} (4)

Since $\gamma$ is related to the surface tension, this property is investigated here. More rigorously, Young’s equation (Equation (1)) can be rewritten as [20, 21]

$$\cos(\theta) = \frac{\gamma_{SV} - \gamma_{SI}}{\gamma_{LV}} - \frac{\sigma}{\gamma_{12} R},$$  \hspace{1cm} (5)

where $\sigma$ is line tension, and $R$ is the radius of the three-phase contact circle on the plane of the solid surface. The line tension is defined as the free energy per unit length of the three-phase contact line.

When liquid 1 immersed in liquid 2, the equation can be modified as

$$\cos(\theta) = \frac{\gamma_{SV} - \gamma_{SI}}{\gamma_{12}} - \frac{\sigma}{\gamma_{12} R}.$$  \hspace{1cm} (6)

The contact angle changes as the protein is bound on the gold surface, and measuring the change in the contact angle enables protein sensing in the solution.

Observation system

A microscope with a 60x objective was used to observe images captured by a CCD at a resolution of 1280 to 1024.

Concentrations of bovine serum albumin (BSA) for sensing, 0.5 mg/ml, 10 μg/ml and 0.4 μg/ml, were tested on each of the 20 μm, 40 μm, and 80 μm diameter droplets.

Once the sample had been prepared in the acrylic receptacle, the protein solution was added to the PBS solution to result in respective concentrations of 0.5 mg/ml, 20 μg/ml and 0.4 μg/ml per 0.5 ml control volume. We then measured each change of contact angle several times.
Results

The droplet images were captured by CCD camera. As shown in Figure 4, the contact angle of the 40 μm droplet in the 20 μg/ml protein solution changed after ten minutes. Figures 5 to 7 show the contact angle change results for the different sized microdroplets in three concentrations of protein solution. The sensitivity of the sensor is seen to vary with the size of the microdroplet and the concentration of the protein. In a high concentration protein (0.5 mg/ml), all three microdroplets sizes achieved the final contact angle in fifteen minutes. The largest change of contact angle occurred in the smallest microdroplet, while the smallest change occurred in the largest microdroplet. In addition, for all microdroplet sizes, a reduction in the protein solution concentration slows the reaction time. For the 20 μg/ml and 0.4 μg/ml protein solutions, equilibrium was reached after sixty and one hundred and twenty minutes, respectively. Alternatively, Figure 8 shows the contact change transformed into a curvature change with different concentrations in the same microdroplet size. For the same droplet size, the radius of the curvature increases with the protein concentration. For the same concentration, the curvature change increases with the size of the microdroplet.
Discussion and Conclusion

Error estimation

Suppose the droplet is one part of a circle and the estimated error is +/- one pixel. For the calculated result, the error is 0.64°. Alternatively, MATLAB is used to fit the droplet profile to the CCD image, as shown Figures 9 and 10. The black line is the profile of the microdroplet captured by MATLAB, and the red line is the fitting curve with the polynomial.

Contact angle change

The contact angle change is shown in Figures 5 to 7. The droplet size is seen to be negatively correlated to the degree of contact angle change. For example, in Figure 5, the respective contact angle changes from 20 μm, 40 μm, and 80 μm are 15°, 11°, 6°. In addition, the protein concentration is positively correlated with the degree of contact angle change. For the 40 μm droplets, the respective change of contact angle in 0.5 mg/ml and 0.4 μg/ml was 11° and 5°. Reaction time is also found to depend on protein concentration, with the 0.4 μg/ml solution taking 120 minutes to reach equilibrium, as opposed to only 15 minutes for the 0.5 mg/ml solution.

In conclusion, the proposed protein sensor can detect a 0.4 μg/ml protein solution in 120 minutes, with the change in contact angle varying with microdroplet size, protein concentration, and reaction time.

References


