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Biomolecular kinetics analysis using long-range surface plasmon waveguides

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ABSTRACT

A novel, cost effective, label free and real time biosensor based on straight gold waveguides supporting long range surface plasmon polaritons (LRSPPs) is used to measure the kinetics constants of protein–protein interactions. Bovine serum albumin (BSA) and anti-bovine albumin antibody (anti-BSA) produced in rabbit are used as the bio-pair for the purposes of demonstrating and investigating the extraction of binding kinetics. BSA protein is immobilized on the surface using thiol coupling. The output power of the system is recorded continuously over time, and then converted into surface mass concentration. The kinetics constants are extracted from binding curves using the integrated rate equation. Linear least squares and non-linear least squares analysis are employed to obtain the constants and the results are compared.

1. Introduction

The characterization of biomolecular affinity and interaction kinetics is important to understand antibody-antigen or generally protein–protein interaction for the selection of appropriate biomaterials for immunoassays, or for drug discovery within the pharmaceutical fields [1]. Methods that detect changes in refractive index based on changes in surface mass concentration (*e.g.*, optical biosensors) [2,3] or changes in fluorescence [4–6] can be employed for kinetics analysis. Optical biosensors are capable of providing rapid, direct and real-time detection and characterization of biomolecular interactions. Among all of the technologies, surface plasmon resonance (SPR) has demonstrated strong potential as an optical biosensor technology for label-free and real-time detection of reagents. SPR biosensors utilize the Kretschmann-Raether configuration which is based on total internal reflection from a metal-coated prism [7–9].

Long-range surface plasmon polaritons (LRSPPs) are transverse magnetic (TM) polarized optical surface waves propagating along a thin metal film or stripe. LRSPPs on stripes can be excited by butt-coupling to a polarisation-maintaining single-mode optical fiber (PM-SMF) [10,11]. LRSPPs can propagate over appreciable lengths, allowing a long optical interaction with the sensing medium, and thus high-sensitivity, real-time, label-free biosensors. LRSPPs have been excited in prism-coupled sensors in order to increase the sensitivity of the device, and used in the detection of *E. coli* bacteria [12] and for studying the effects of toxins on HEK-293 cells [13]. LRSPP biosensors based on metal stripe waveguides offer advantages over prism-coupled sensors, in the form of increased sensitivity, compactness, and ease of manufacturing. Dengue virus detection [14,15], bacteria detection in urine [16] and the detection of leukemia markers [17] has been performed using LRSPP biosensors. In the case of Dengue detection, the surface was activated using EDC/NHS, patient blood samples were immobilized on the surface and the results show similar or better detection than ELISA [14]. In the case of Leukemia detection, the surface was activated using protein G and antibodies from patient samples were immobilized selectively on the surface [17].

In this paper, we demonstrate LRSPPs biosensors based on metal stripe waveguides for the analysis of the affinity and kinetics rate constants of biomolecular interactions. We work with bovine serum albumin (BSA) and antibodies against BSA (anti-BSA) produced in rabbit. BSA is a protein of molecular weight ~66 KDa used extensively in routine biosensor work and it is inexpensive. The analysis technique employed here is based on the immobilisation

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of BSA on the gold stripe surface, while anti-BSA is injected over the surface and its reaction with BSA is observed. Changes in the output power are recorded continuously over time, which are then translated to changes in surface mass density [18]. On this basis, the kinetics rate constants can be extracted, *e.g.*, by fitting responses to the rate or integrated rate equations. Compared to conventional SPR systems, LRSPP biosensors based on metal stripes are more compact and cost-effective [19,20].

This paper is organised as follows: First we summarise our theoretical model for the system and interaction kinetics, then the materials and methods. We then present our results along with a discussion and close the paper with concluding remarks.

2. Theoretical model

Kinetics analysis for the interaction of immobilized receptor and soluble analyte, BSA and Anti-BSA in our case, can be done through real-time monitoring of the output signal. In our LRSPP biosensors, changes in the output power indicate binding events occurring along the surface of the metal stripe. The output power is monitored continuously over time and plotted as the sensorgram.

In general, the interaction between two biomolecules A and B can be modelled as:

$$A + B \leftrightarrow AB \tag{1}$$

The rate of this reaction can be expressed as:

$$d[AB]/dt = k_a[A][B] - k_d[AB] \tag{2}$$

where k_a is the association rate constant and k_d is the disassociation rate constant (concentrations are implied by square brackets). The concentration of B can be expressed in terms of its initial concentration and the concentration of complex AB, after an interaction time t, as $[B] = [B]_0 - [AB]$. Substituting this into Eq. (2) yields:

$$d[AB]/dt = k_a[A]([B]_0 - [AB]) - k_d[AB] \tag{3}$$

Based on the above, the net rate of formation of complex AB depends on the concentrations of A and B as well as the interaction kinetics. In our biosensor application, B represents the receptor immobilized on the surface of the device, and A soluble analyte, so complex AB forms on the surface of the device as A in solution binds to B immobilized thereon. The output signal is dependent on the concentration of complex AB forming on the surface which can be expressed, generally, in terms of surface mass density Γ. By analogy with Eq. (3), we write the time rate of change of the surface mass density accumulating on the surface as:

$$d\Gamma/dt = k_a C(\Gamma_{max} - \Gamma) - k_d\Gamma \tag{4}$$

where C is the concentration of the injected analyte. At equilibrium $d\Gamma/dt = 0$ and rearranging Eq. (4) yields:

$$\Gamma/C = k_a/k_d(\Gamma_{max} - \Gamma) \tag{5}$$

where k_a/k_d is defined as the affinity constant K. The affinity constant can thus be obtained as the slope of a plot of Γ/C vs. Γ. Rearranging Eq. (4) yields:

$$d\Gamma/dt = k_a C\Gamma_{max} - (k_a C + k_d)\Gamma \tag{6}$$

where the concentration C and the maximum surface mass density Γ_{max} are known. The rate constants k_a and k_d can be extracted from this equation by plotting $d\Gamma/dt$ *vs.* Γ. Different slope values k_s for such plots are obtained for different analyte concentrations C:

$$k_s = k_a C + k_d \tag{7}$$

Using this equation, the association and disassociation rate constants can be obtained from the slope and the intercept of the k_s *vs.* C plot, respectively. The extraction of the disassociation rate constant is more error prone, because k_d values are usually small.

The integrated rate equation is obtained by solving Eq. (6) directly, yielding:

$$\Gamma = \frac{k_a C\Gamma_{max}\left[1 - e^{-(k_a C + k_d)t}\right]}{(k_a C + k_d)} \tag{8}$$

Alternatively, the integrated rate equation can be used directly to extract the binding kinetics through fitting as will be discussed in the results and discussion section of the paper.

In our LRSPP biosensors, changes in the output power indicate binding events occurring along the surface of the metal stripe, and thus changes in surface mass density thereon. The surface mass density can be related to the output power via [18,21]:

$$\Gamma(t) = \frac{1}{k}\left(\frac{n_a - n_c}{\partial n/\partial c}\left(\frac{P_{out}(t)}{P_{out}(t_b)} - 1\right)\right) \tag{9}$$

where Γ is the surface mass density (pg/mm^2), k is 0.0318/nm [18] (a device constant), n_a and n_c are the refractive index of the biomaterial accumulating on the surface and of the carrier fluid, 1.5 and 1.338, respectively [18], $\partial n/\partial c$ is 0.185 mm^3/mg [22], and $P_{out}(t_b)$ and $P_{out}(t)$ are the output powers measured before and during analyte binding.

3. Materials and methods

3.1. Chemicals and reagents

16-Mercaptohexadecanioc acid (16-MHA), phosphate buffered saline (PBS) 0.01 M, pH 7.4, bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), 2- isopropanol alcohol (IPA), acetone HPLC grade ≥99.9, octane, glycerol (electrophoresis grade), N-Hydroxysuccinimide sodium salt (NHS), 1 -ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, ≥99% (EDC) and anti-BSA antibody produced in rabbit were obtained from Sigma-Aldrich. Distilled water was deionized using Millipore filtering membranes (Millipore, Milli-Q water system at 16 MΩ cm).

The buffer used in the experiments was prepared by mixing PBS and glycerol (PBS/Gly) to achieve a refractive index of n = 1.338 at the wavelength of operation (~1310 nm), in order to achieve an approximate refractive index match to Cytop and high sensitivity [18]. The pH of the solution remains constant throughout the experiments. The lab temperature also remains constant at 25 °C and all solutions are allowed to stabilise to lab temperature before use.

100 μg/mL and 20 μg/mL BSA solutions were prepared by mixing lyophilized BSA with PBS/Gly buffer. Different antibody concentrations, (1 μg/mL, 5 μg/mL, 30 μg/mL and 100 μg/mL) were prepared by mixing powdered antibody with PBS/Gly buffer.

1% SDS was prepared in PBS/Gly for the purposes of disrupting Anti-BSA to BSA binding in order to regenerate the receptor surface.

3.2. Sensing device and instrumentation

LRSPP straight waveguides composed of gold stripes embedded in CYTOP with a fluidic channel etched into the top cladding were used as the sensing structure in the experiments. The device is fabricated using optical lithography to define Au features on a bottom cladding of CYTOP. A fluidic channel of length L = 1.65 mm (sensing length) was formed by etching the top CYTOP cladding down to the gold surface. Fabrication details can be found in [23]. The sensor die incorporated straight waveguides with a Au stripe 5 μm wide, 35 nm thick and 3.8 mm in length.

An optical setup comprised of a DFB laser, a cleaved PM-SMF, a collimating objective, an aperture, a beam splitter and a power sensor, as sketched in Fig. 1, was used to capture the output of a straight