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A comparison of binding surfaces for SPR biosensing using an antibody–antigen system and affinity distribution analysis

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ABSTRACT

The application of optical biosensors in the study of macromolecular interactions requires immobilization of one binding partner to the surface. It is often highly desirable that the immobilization is uniform and does not affect the thermodynamic and kinetic binding parameters to soluble ligands. To achieve this goal, a variety of sensor surfaces, coupling strategies and surface chemistries are available. Previously, we have introduced a technique for determining the distribution of affinities and kinetic rate constants from families of binding and dissociation traces acquired at different concentrations of soluble ligand. In the present work, we explore how this affinity distribution analysis can be useful in the assessment and optimization of surface immobilization. With this goal, using an antibody–antigen interaction as a model system, we study the activity, thermodynamic and kinetic binding parameters, and heterogeneity of surface sites produced with different commonly used sensor surfaces, at different total surface densities and with direct immobilization or affinity capture.

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1. Introduction

Biosensors have become an important tool in the study of macromolecular interactions, and many different design principles have been described that measure surface binding of molecules label-free and with exquisite detection limits. These include optical biosensors based on surface plasmon resonance [1–3], resonant mirrors [4], interference reflectometry [5,6], and other optical evanescent wave principles [5,7,8], as well as quartz crystal microbalance biosensors [9]. In order to fully take advantage of the detection limits for analytes, all biosensors have in common the need for a sensing surface with high sensitivity and specificity. The latter is usually achieved through the creation of a surface layer of binding sites, usually through the surface immobilization of macromolecules that can capture with high affinity soluble binding partners (the analyte) flowing across the sensor surface, but is otherwise inert. Furthermore, for the purpose of biosensing in the study of macromolecular interactions, it is highly desirable, and often essential, that the surface attachment of the stationary binding partner does not diminish its binding energy or kinetics for the soluble analyte [1].

It is widely appreciated that creating such a specific surface with uniform ensemble of sites is a non-trivial task. For example, the proximity of the surface can add steric constraints and surface potentials contributing to the free energy of binding. Similar to the attachment of fluorophores or other extrinsic moieties in other biophysical techniques, the surface attachment of the macromolecule – covalent or through high-affinity capture – has the potential of altering the macromolecular conformation and/or access to the binding site. Due to the rugosity and microheterogeneity of the surface environment, heterogeneity of the surface sites may result [10]. Considering that in the overwhelming majority of published SPR biosensor studies random immobilization chemistries are used, and that often a significant fraction of the surface bound molecules has become inactive after immobilization (or after exposure to chemical 'regeneration' conditions that are designed to reversibly reduce the life-time of the bound state), it is easily conceivable that this could render a subset of molecules partially active. For these reasons, an ensemble of molecules that is well-described by a single set of thermodynamic parameters in solution may be expected to experience some dispersion of binding energies once immobilized to a surface. Many examples for heterogeneity of surface binding sites caused by immobilization have been reported [11–16].

In SPR biosensing, the most commonly employed surfaces have flexible polymeric linker layers, such as a carboxymethyl dextran brush. This has the virtue of separating the macromolecule from

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the surface to provide better access to the binding partner, suppress non-specific surface binding, and facilitate surface attachment [6,10,17]. On the other hand, diffusion through this layer has the potential to pose a limiting step for the binding kinetics [6,18], and the non-uniform density distribution of the macromolecules in this layer could create microenvironments with different charge, pH, and surface crowding [10]. Interactions between immobilized protein and matrix are evident from the altered dextran structure after immobilization or ligand binding [6,19], for the fundamental reason that these forces must also act, vice versa, on the proteins. In some published cases, these matrix effects appear to be absent [20], but in others they dominate the surface binding signals [21,22]. Clearly, tools to control for surface heterogeneity and other differences in the analyte binding energetics between soluble and immobilized forms of macromolecules are of critical importance for the use of optical biosensors to characterize macromolecular interactions.

SPR biosensing can provide data with excellent reproducibility and signal/noise characteristics, and therefore offers the possibility of a detailed computational analysis. This contrasts with misfits of experimental data by overly simplistic, one or few site models found frequently in the literature. However, even though it is possible to embark on the development of *ad hoc* models with more complex reaction schemes that will invariably fit the data better, in the absence of independent confirmation they do not inspire much confidence [23,24], especially in view of the experimental difficulties outlined above [25]. Recently, we have taken the opposite approach and introduced a data analysis model that we believe is closer to the experimental reality by not requiring the assumption of discrete classes of surface binding sites. Instead, it is based on modeling the data with an integral equation that describes the surface sites as a (quasi-)continuous two-dimensional distribution of affinity and kinetic rate constants [15]. Remarkably, this model routinely provides fits of the measured data with root-mean-square deviations (rmsd) on the order of the noise of data acquisition. We have previously used this model to demonstrate, with different antibody–antigen systems, the presence of heterogeneity and microheterogeneity in immobilized Fab fragments, as well as various classes of non-specific sites ascribed to the sensor surface [13–15]. For the study of protein interactions, resolving these sites allows us in a second stage to focus on the peak in the affinity/kinetic rate distribution that is presumed to reflect best native binding of molecules in solution [25–30].

The purpose of the present work was to demonstrate how the continuous affinity/kinetic rate distribution approach can be used to compare the performance of different sensor surfaces. To this end, we have collected SPR biosensor data of antigen binding to a monoclonal antibody immobilized with different chemistries, and different total immobilization density, to three different commercial sensor surfaces with different dextran coatings. We found significant differences in the surface site distributions, including the average values in the main peak for the affinity and kinetic rate constants, but also for the degree of transport limitation as well as the degree of non-specific binding. Although this pilot study is very limited in the repertoire of surface chemistries applied, it highlights the impact of the surface properties on the observed surface site affinity distributions, and indicates the utility of this analysis approach for more systematic experimental optimization.

2. Materials and methods

2.1. Reagents

β_2-Microglublin (B2MG) from human urine (catalog number: M4890) was purchased from Sigma–Aldrich (St. Louis, MO). A randomly biotinylated form of mouse monoclonal antibody to β_2-microglublin (anti-B2MG-biotin) was acquired from Abcam (Cambridge, MA, catalog number: ab21899). The capture molecule, streptavidin (SA) and D-biotin were purchased from Thermo Scientific Pierce Protein Biology Products (Rockford, IL, catalog numbers: 21125 and 29129). HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005% surfactant P20), reagents for amine coupling (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, *N*-hydroxysuccinimide, and acetate buffers) as well as sensor chips C1, CM3 and CM5 were acquired from Biacore, GE Healthcare (Piscataway, NJ).

2.2. Sensor surfaces and binding experiments

The antibody was coupled to the sensor surfaces C1, CM3, and CM5 by amine coupling using the standard protocol [31,32]. Immobilization was performed with the antibody at 30 μg/mL at pH 5.5 using a flow rate of 5 μL/min. Alternatively, affinity capture by SA was used. For this purpose, SA at a concentration of 100 μg/mL in sodium acetate buffer, pH 5.5, was immobilized to the sensor surfaces C1, CM3, and CM5 using standard amine coupling, followed by injection of anti-B2MG-biotin at 25 μg/mL in the working buffer (HBS-EP) to capture the antibody, and application of biotin to block unoccupied sites. Consistent with reported binding constants of SA for biotin [33], dissociation of anti-B2MG-biotin from the SA functionalized surface was negligible during the course of the binding study. In both direct immobilization and capture approaches, the surface density of immobilized antibody on CM3 and CM5 sensor chips was varied; the immobilization level of SA (1000–2000 RU) and anti-B2MG-biotin (800–6000 RU), respectively, was controlled by varying the exposure time during immobilization. For each sensor chip, a reference surface was generated by mock derivatization without anti-B2MG-biotin antibody, and for affinity capture the same amount of SA was immobilized on the reference surface as the working surfaces on the same sensor chip.

SPR binding experiments were conducted in Biacore X and Biacore 3000 instruments (GE Healthcare, Piscataway, NJ), at a temperature of 25 °C, using HBS-EP as working buffer for all the experiments. A concentrated stock of B2MG (8.6 μM) was diluted in HBS-EP, and a series of 5 or 8 concentrations (0.1–100 nM) of B2MG were injected across the sensor surface at a flow rate of 5 or 10 μL/min. The time-course of binding was observed between 500 and 2000 s, dependent on B2MG concentration. This was followed by the observation of the dissociation process for 2000–6000 s, during which the surface was rinsed with HBS-EP buffer. Because the signal decreased to baseline level at the end of dissociation, a regeneration step was not necessary.

2.3. Data analysis

Data analysis was conducted with the software EVILFIT [15]. First, sensorgrams were preprocessed using BIAevaluation (version 4.0.1, Biacore GE Healthcare, Piscataway, NJ), to subtract the signal response from the designated reference cell (including bulk refractive index changes), and to subtract remaining background signals measured in blank buffer injections. The net binding traces were aligned to assign 0 s to the start of the injections, and exported into an .xls file. This file was loaded into EVILFIT, where the kinetic traces in each data set were globally fit at all concentrations with a model for continuous distributions of affinity constants and dissociation rate constant (see below). For a few data sets from surfaces with high surface density of immobilized molecules, the binding traces at all concentrations were globally analyzed using the distribution model combined with a two-compartment approximation of mass transport [14,25]. The peaks of the