# Binding Kinetics Analysis with SPR: Interaction between Bovine Serum Albumin (BSA) and Anti-BSA

In this application note, the use of flow injection SPR for real-time monitoring of the association and dissociation of anti-bovine serum albumin (anti-BSA) antibody with surface-confined BSA molecules is demonstrated. In restriction digestion, BSA has been used to stabilize enzymes during DNA digestion. It is also widely used as a biomolecule to block active sites on surfaces. Formation of the anti-BSA/BSA immune complex is relevant to studies of the receptor site of the red blood cells [1]. By injecting anti-BSA samples of different concentrations onto BSA-modified gold sensor chips and fitting the resultant sensorgrams with the Biosensing Instrument (BI) Kinetic Analysis Program, the anti-BSA/BSA association and dissociation rate constants can be deduced.

BSA was immobilized onto a carboxylated PEG/PEG[2] mixed monolayer (Figure 1). The activation of the carboxyl groups on the PEG film occurs with an injection of 0.1 M N-hydrosuccinimide (NHS) and 0.4 M N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (EDC) solution mixture. The high binding affinity of the BSA/Anti-BSA complex and the high sensitivity of the BI-SPR instrument permits that only a small amount of BSA be immobilized for evaluation. For a close up view, the inset plot shows the rise in baseline due to the immobilization of BSA by injecting 10 nM BSA solution. The remaining active NHS ester groups on the surface are deactivated or blocked with the injection of 1 M ethanolamine (EA) solution. A regeneration solution of 20 nM NaOH was injected to remove any loosely bound BSA protein.

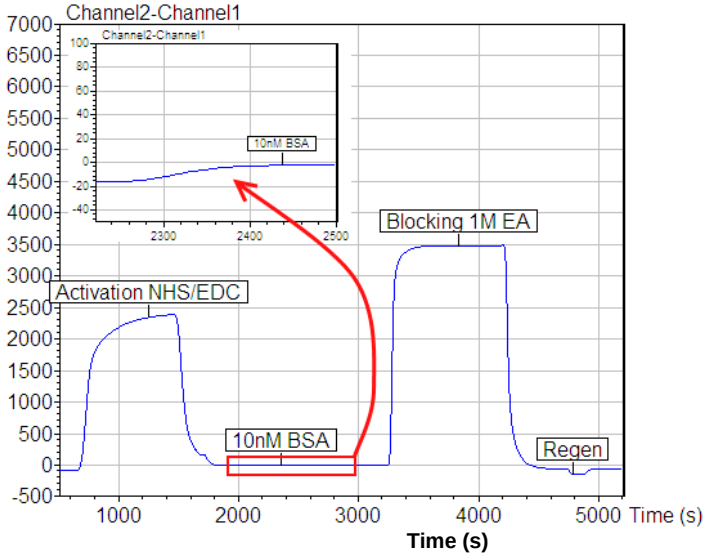


FIG.1 A SPR sensorgram showing the activation of the PEG film with NHS/EDC, the attachment of BSA via amine coupling (inset plot), and the deactivation/blocking of the unreacted surface sites with ethanolamine. The flow rate is 20 μL/min and the running buffer is a phosphate buffered saline solution.

Next, a series of anti-BSA solutions were injected serially into both the sample channel (channel 2 in this example) and the reference channel (channel 1), serially. In serial flow mode, the presence of any secondary effects due to non-specific absorption or bulk refractive index shifts may be removed by subtracting the sample channel from the reference channel. Figure 2 depicts the reference-subtracted sensorgram corresponding to the injections of the anti-BSA solutions and the regeneration of the sensor chip with 20 mM NaOH injections. Notice that the injection of an anti-BSA solution results in a rapid rise in the baseline, indicating that the association reaction between BSA and anti-BSA is rather facile. This is in contrast with the dissociation reaction, which shows a slow decay. The combination of rapid binding and slow decay suggests that the binding of anti-BSA to BSA is quite strong.

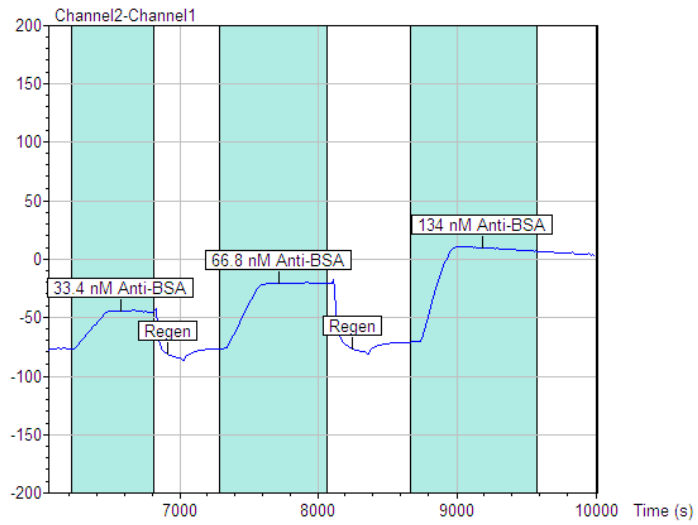


FIG.2 A SPR sensorgram showing the injections of 33.4, 66.8, and 134 nM anti-BSA solutions (*cf.* the labels on the injection peaks). The negative-going peaks after then sample injection are due to regeneration injections of 20 mM NaOH which result in the desorption of the anti-BSA from the complex. A flow rate of 80 μL/min was used.

Using the BI software region selection tool, data regions may be conveniently selected from any section or for any duration of the sensorgram. The selected data regions can then be reorganized by the BI software for a detailed kinetics evaluation. Using this feature, multiple binding regions and blank injections (not shown) were selected from the SPR plot in Figure 2. Figure 3 displays the selected binding regions overlaid (black curves) with theoretical kinetics fits (red curves). As can be seen, the fit is excellent and yields an associated rate constant $k_a$ of $7.1 \times 10^4$ $M^{-1}s^{-1}$ and a dissociate rate constant $k_d$ of $2.55 \times 10^{-4}$ $s^{-1}$. Since the affinity binding constant $K_D$ is the ratio of $k_d$ over $k_a$, the calculated binding constant $K_D$ was determined to be 3.6 nM. All these values are in reasonable agreements with other studies [3]. After these experiments, we injected anti-IgG into the reference channel and did not observe any binding between anti-IgG and BSA (data not shown). The lack of binding here indicates that the interaction between BSA and anti-BSA is highly specific.

Finally, we carried out the same assay on gold sensor chips that were modified with carboxymethylated dextran using a protocol similar to a literature procedure [4]. The kinetic constants determined compare well with the above values, suggesting that the PEG film used in this study is as effective as the dextran film in immobilizing proteins via amine coupling and in preventing nonspecific protein adsorption. This is consistent with previous studies [2-3].

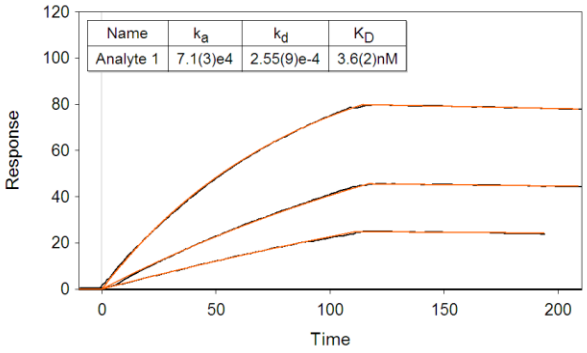


FIG.3 Experimental (black curves) and fitted (red curves) SPR binding profiles at different anti-BSA concentrations. The BI software easily calculates the kinetic coefficients and affinity value from the overlaid fits.

**References**

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[3] www.colby.edu/chemistry/PChem/Lab/SI
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