bioanalytics xantec

Application Note 01

Biotinylated Anti-HSA-HSA Binding

Human Serum Albumin (HSA) Binding to Biotinylated Anti-HSA IgG Captured on Planar Neutravidin Surface

Human serum albumin (HSA) is a 67 kDa protein and is the most abundant protein in human blood serum. HSA is essential to the blood transport system as it is responsible for the transport of several physiologically important compounds such as fatty acids, bilirubin, calcium, steroid hormones, and various drugs. This Application note presents the binding kinetics of a model antibody-antigen system, HSA binding to anti-HSA IgG. Anti-HSA is biotinylated with approximately 6 biotin groups (Solulink, part no. B-1001-105) and captured on a planar neutrAvidin sensor slide.

Experimental

The experimental conditions for this assay are summarized below:

- Ligand: Anti-HSA
- Analyte: HSA
- Analyte Concentrations: 80,40,20,10,5,2.5,1.25 nM
- Association Time: 3 min
- Dissociation Time: 5 min
- Regeneration Solution: Cocktail

Results

The SR7000DC SPR Dual Channel System monitors this antibody-antigen interaction in real-time with simultaneous monitoring of sample and reference channels.

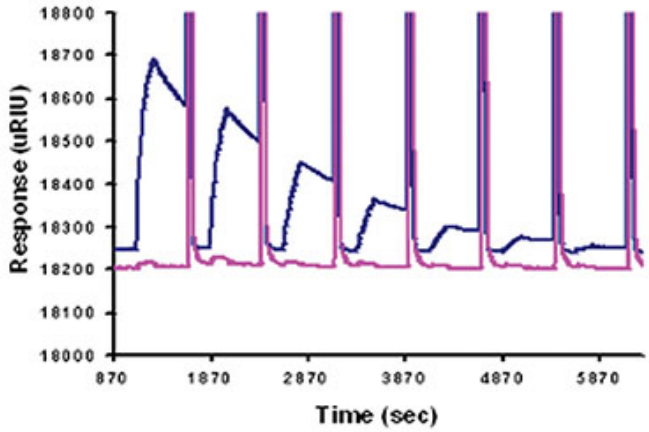

Figure 1 presents raw data from a cycle of HSA injections across the two channels; one being the sample side (with captured anti-HSA) and the other serving as a reference (surface without anti-HSA). The results show that the binding is highly specific with very minimal non-specific binding on the reference channel.

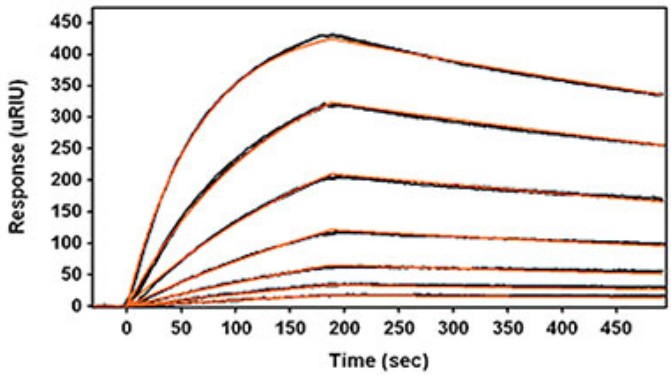

Figure 2 presents the normalized HSA binding curves along with the fit (red lines) to a simple bimolecular model using Scrubber (Biologic Software). Each concentration is injected at least twice to verify reproducibility. The association rate constant (k_a) is found to be 1.66e5 M-1s-1 and the dissociation rate constant (k_d) is determined to be 7.70e-4 s-1. Thus, the equilibrium dissociation constant (K_D) (i.e., the ratio of k_d to k_a) is 4.65 nM for this interaction.

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