

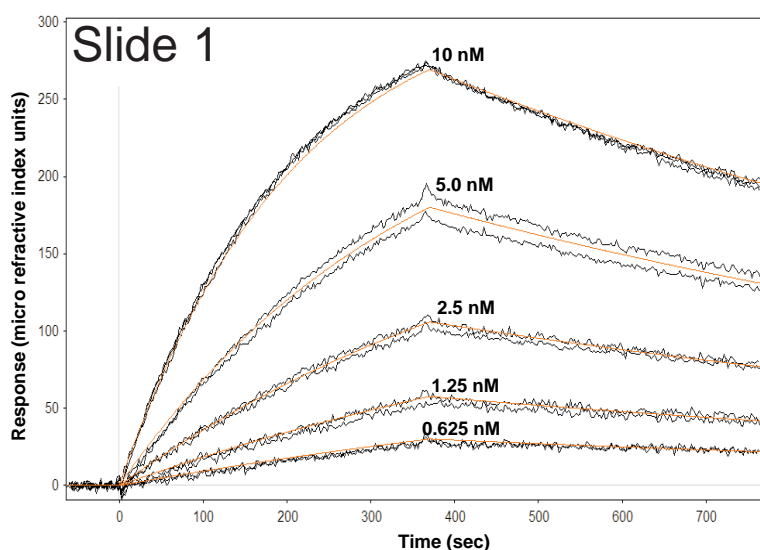
**Surface Plasmon Resonance Dual-Channel Refractometer  
SR7000DC  
Application Note I**

Human Serum Albumin Binding to  
Anti-Human Serum Albumin IgG Immobilized on a Planar Surface

## Introduction

The Reichert SR7000DC surface plasmon resonance instrument is a powerful tool to monitor biomolecular binding events. This application describes the binding of a well-known antibody-antigen pair; human serum albumin (HSA) binding to the monoclonal antibody anti-HSA IgG. The SR7000DC monitors this binding event in real-time without the need for labels. The dual channel capabilities allow the instrument to simultaneously monitor the sample and reference channels against non-specific binding. The anti-HSA is immobilized on a planar surface and solutions of HSA are then flowed over the sensor surface. As binding occurs, the change in mass on the surface is detected by the SR7000DC. The data in this study was fit to a simple bimolecular model using Scrubber (BioLogic Software). Normalized data is imported directly into Scrubber. The associations and dissociations were fit globally, providing a stringent test of the reaction model while improving the reliability of the results. The following Figures present the normalized HSA binding curves along with the fits to the bimolecular model from Scrubber shown by the red lines. The HSA concentrations that were injected are 10, 5, 2.5, 1.25 and 0.625 nM. Each concentration was injected at least twice to illustrate reproducibility. Three experiments were performed on different days with varying anti-HSA immobilization levels to show the system reproducibility.

## Results

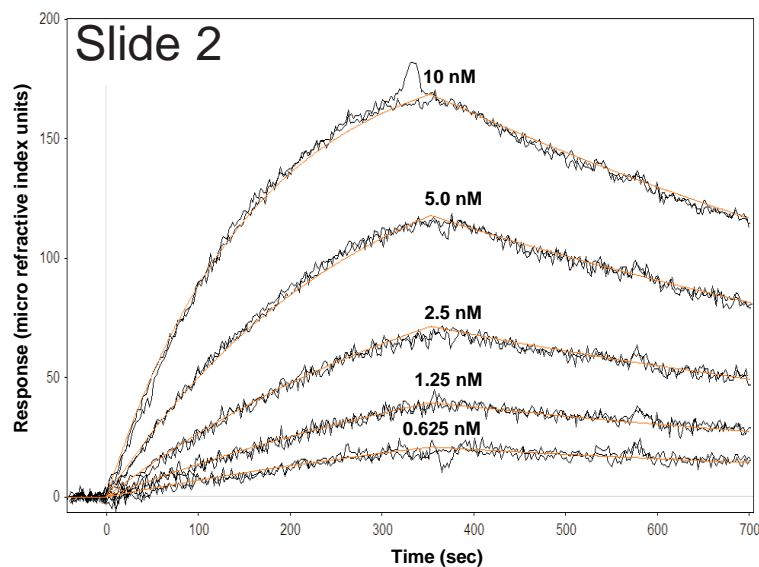


High anti-HSA surface immobilization level  
(3000  $\mu$ RIU immobilized)

Reaction Model:  
 $A + B \leftrightarrow AB$

$k_a$ ( $M^{-1} s^{-1}$ )	$k_d$ ( $s^{-1}$ )	$R_{max}$ ( $\mu$ RIU)	$K_D$ (nM)
$4.091 \times 10^5$	$8.087 \times 10^{-4}$	385.6	1.980

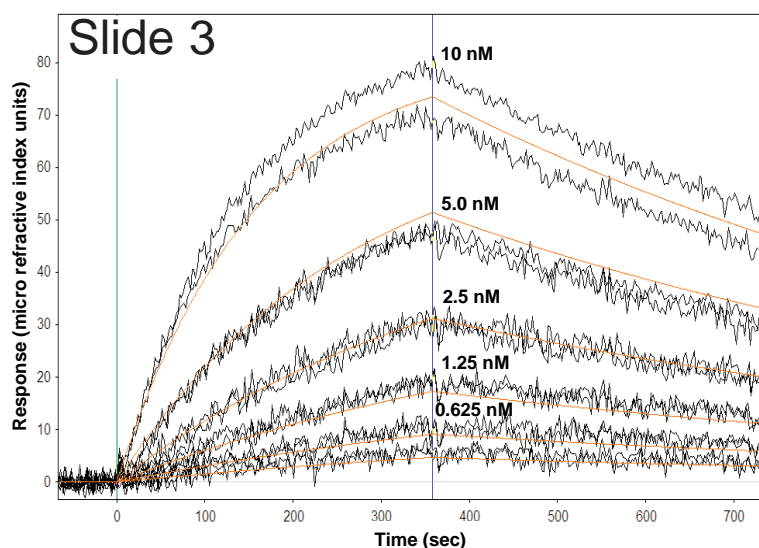
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Moderate anti-HSA surface immobilization level (2000  $\mu$ RIU immobilized)

Reaction Model:  
 $A + B \leftrightarrow AB$

$k_a$ ( $M^{-1} s^{-1}$ )	$k_d$ ( $s^{-1}$ )	$R_{max}$ ( $\mu$ RIU)	$K_D$ (nM)
$5.272 \times 10^5$	$1.062 \times 10^{-3}$	226.9	2.017



Low anti-HSA surface immobilization level (700  $\mu$ RIU immobilized)

Reaction Model:  
 $A + B \leftrightarrow AB$

$k_a$ ( $M^{-1} s^{-1}$ )	$k_d$ ( $s^{-1}$ )	$R_{max}$ ( $\mu$ RIU)	$K_D$ (nM)
$5.290 \times 10^5$	$1.100 \times 10^{-3}$	599.6	2.220

## $K_D$ Summary

Slide	$K_D$ (nM)
1 (High)	1.980
2 (Moderate)	2.017
3 (Low)	2.220

