

# Kinetic Analysis of Fab Binding to Immobilized Antigen Poster

Common  
Revision A.01

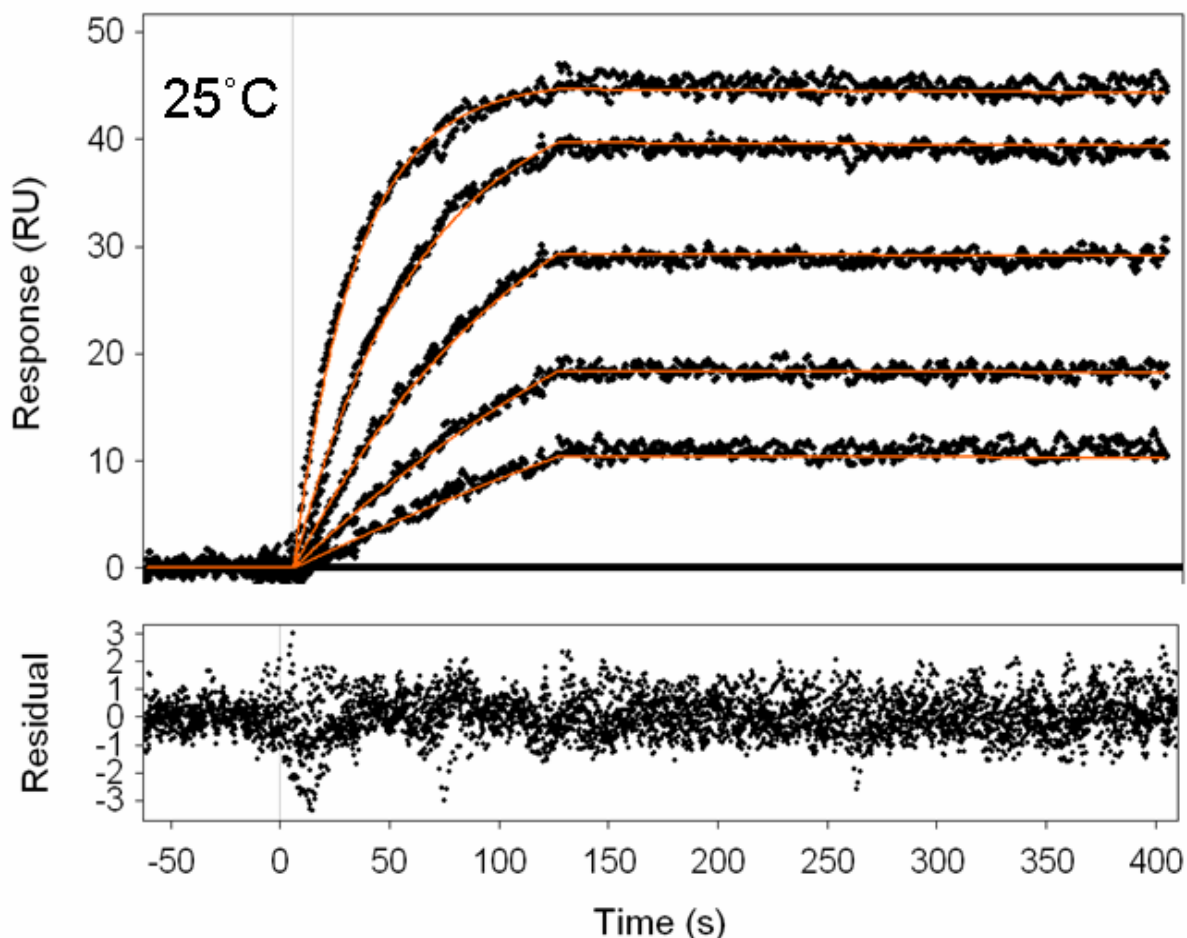


Figure 1: Global fit to a simple 1:1 Interaction Model

## Experiment

A GST-Ag fusion protein (60kDa) was immobilized by amine coupling onto a single channel of a planar carboxylated surface. Channel 2 was uncoated and used for real-time reference curve subtraction. Blank buffer injections were employed to allow double referencing of the data set.

Serial doubling dilutions of Fab (50kDa) from 200nM to 12.5nM were prepared in running buffer (i.e. 10mM HEPES, pH7.4, 150mM NaCl, 0.005% Tween 20). Samples

were randomized and analyzed in duplicate. The flow rate was held constant at 50 $\mu$ l/min. Samples were injected for 2 min, allowed to dissociate for 6 min, and then regenerated with 50 $\mu$ l of 20mM phosphoric acid.

Data processing and kinetic model fitting were performed using Qdat, derived from Scrubber2 and developed by BioLogic Software.

|                      |   |
|----------------------|---|
| <b>k<sub>a</sub></b> | <b>1.72 x 10<sup>5</sup> (M<sup>-1</sup>s<sup>-1</sup>)</b> |
| <b>k<sub>d</sub></b> | <b>3.79 X 10<sup>-5</sup> (s<sup>-1</sup>)</b>              |
| <b>K<sub>D</sub></b> | <b>0.22 nM</b>  |
| <b>Rmax</b>          | <b>45 (RU)</b>  |
| <b>ResSD</b>         | <b>0.77 (RU)</b>  |

### **Conclusion**

SensíQ enables high quality kinetic analysis of biomolecular interactions.