

Analysis of Biomolecular Interactions with SensiQ Discovery

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Introduction

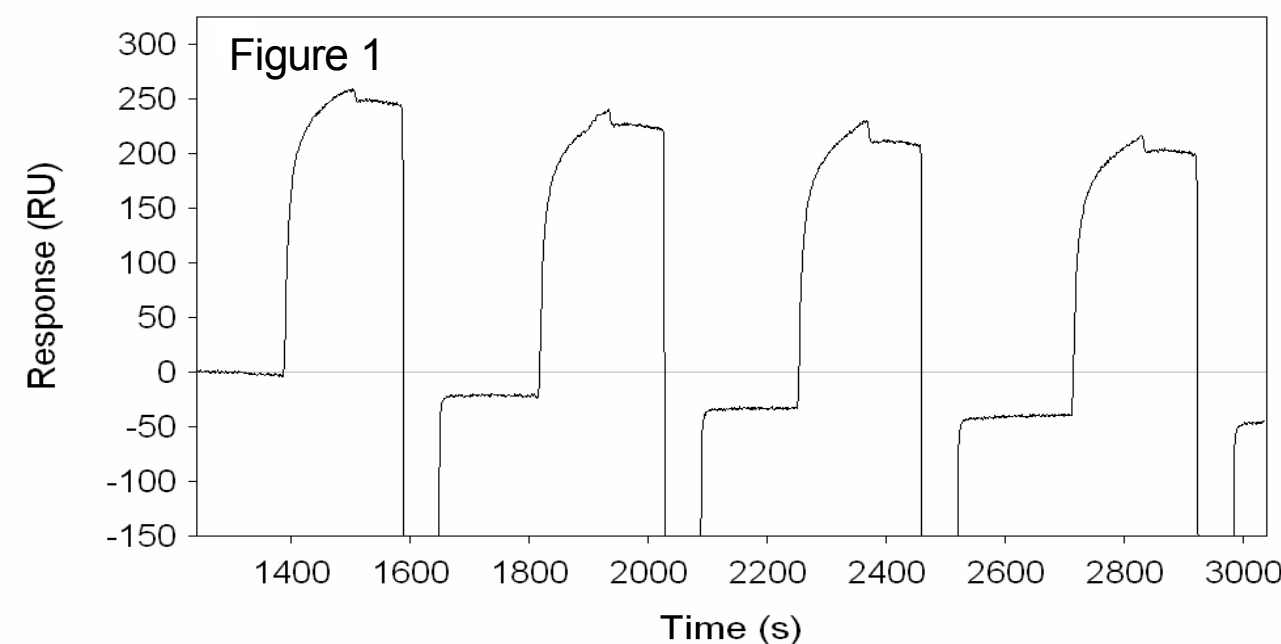
SensiQ Discovery is a dual channel SPR-based instrument for concentration, affinity and kinetic analyses. In the following we present data that demonstrate high quality data for kinetic analysis and concentration determination. The system employs two injection loops in a flow injection analysis configuration for simultaneous injection over both sensing channels. The software allows a preconfigured assay cycle consisting of up to six injections to be entered. The flow rate is controlled from a touch pad control interface. Each loop is manually loaded with a standard sample loading syringe. The user is then prompted by the software to actuate the injection valve to begin, and end, an injection. It is recommended that one of the channels is used as a reference where ligand is not immobilized. An enormous variety of assay formats are possible. When beginning assay development it is useful to run binding response curves in manual mode where injections are performed at any time.

Experimental

The running buffer for all experiments was HBS buffer, pH 7.4, containing 10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, and 0.005% (v/v) Tween 20. The flow rate was set at 50 $\mu\text{L}/\text{min}$ at all times unless otherwise stated. Polyclonal rabbit anti-mouse Fc antibody was immobilized onto the planar carboxylated sensor surface by conventional amine coupling giving a yield of 1800RU.

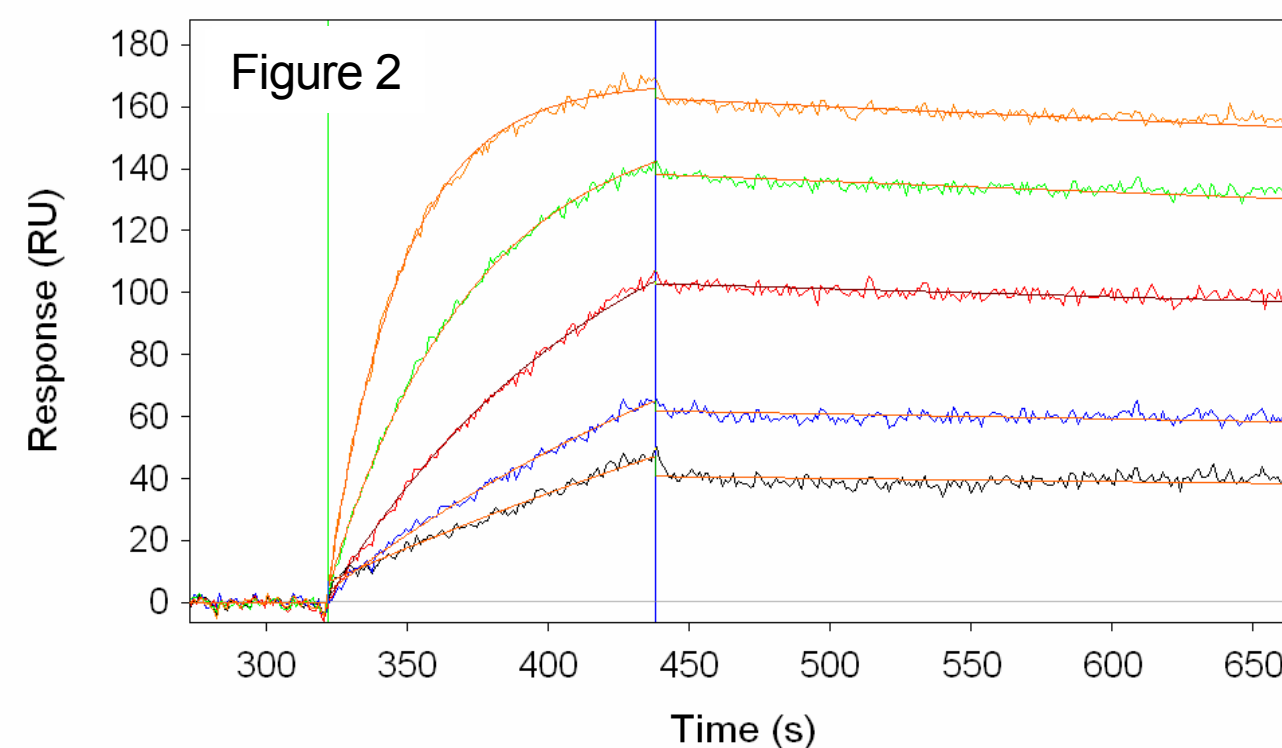
Seasoning the Surface

In order to establish a reproducible affinity-capture surface we condition, or season, the surface by running a sequence of affinity capture binding cycles. Monoclonal mouse IgG1 (100nM) was injected for 2 min and the surface was then regenerated with a 1 min injection of 0.1 M phosphoric acid. This sequence was repeated four times. The covalently immobilized polyclonal capture antibody possesses a heterogeneous distribution of high, medium and low affinity antibodies and the objective of this seasoning process is to block all non-regenerable high affinity sites. This results in accumulation of this “non-sense” monoclonal on the surface with a concomitant decrease in binding capacity. Importantly, after 5 to 10 binding-regeneration cycles are completed the high affinity sites are filled and the binding-regeneration cycles become reproducible allowing high quality kinetic analysis to be conducted.



Kinetic Analysis

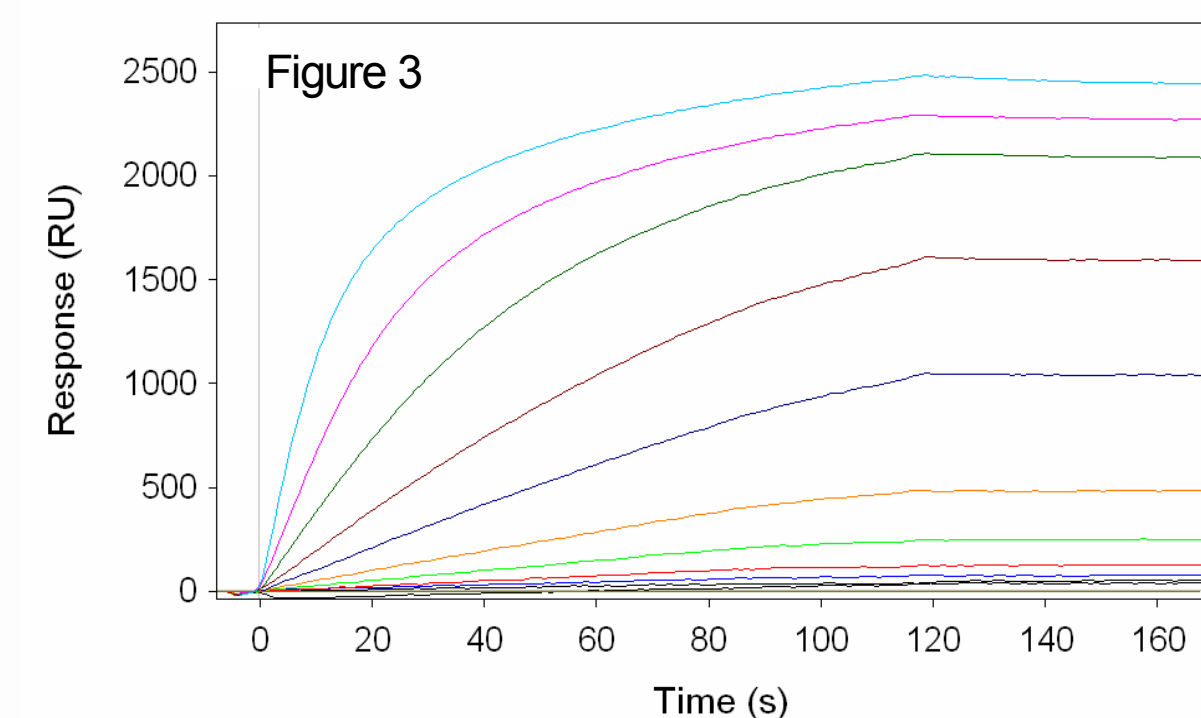
The affinity capture format of figure 1 was repeated for a lower capacity surface over a range of neutravidin concentrations from 12.5nM to 0.78nM. The curves were imported into Qdat (a model fitting program for kinetic and affinity analyses) and referenced against the control surface (i.e. surface with no capture antibody immobilized). A simple pseudo-first-order binding interaction model was fitted (superimposed red curves) to the actual binding curves (Figure 2). Global fitting of the on-rate (k_a) and the off-rate (k_d) to the complete data set constrains the fit rigorously. The Rmax was fitted locally.



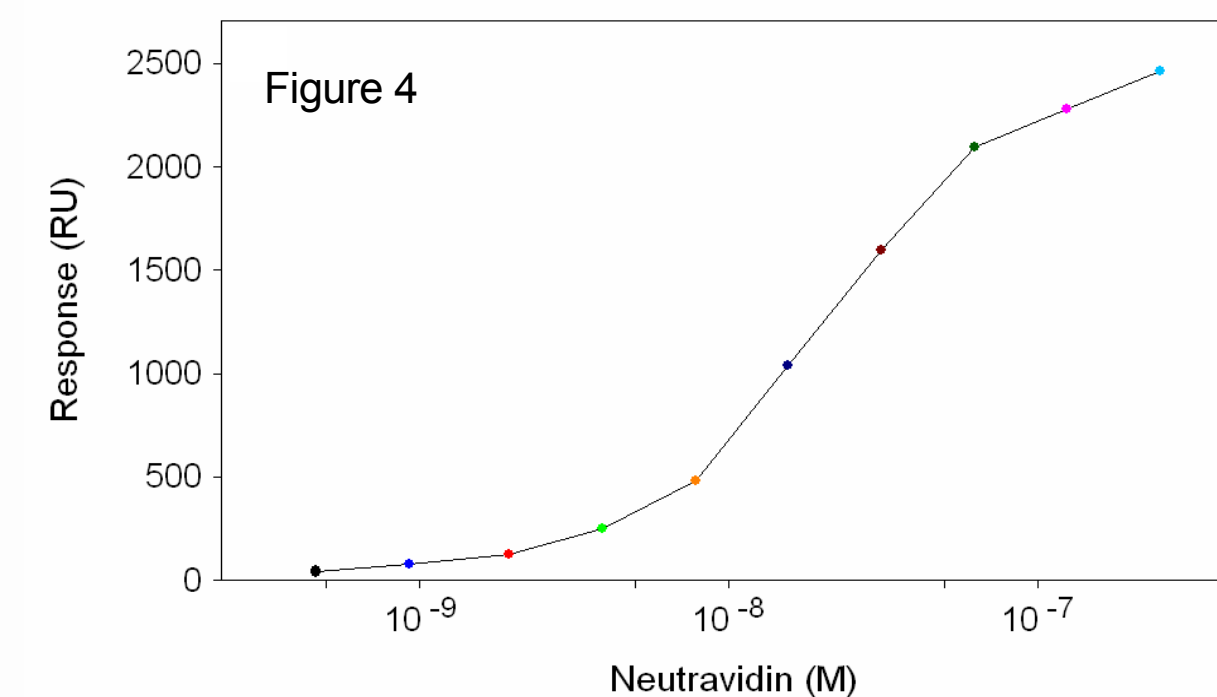
The goodness of fit was measured as the average residual standard deviation of the fitted curves from the actual curves. A value of 1.78RU was observed and confirms that the data does indeed obey the pseudo-first-order binding interaction model and validates the extracted kinetic and affinity constants. The k_a was $1.52 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, the k_d was $2.6 \times 10^{-4} \text{ s}^{-1}$.

Dose Response Curve

Figure 3 are overlaid response curves for the injection (100 μL) of monoclonal anti-neutravidin antibody (from 250nM to 0.46nM) over a neutravidin-coated surface at 50 $\mu\text{L}/\text{min}$. The surface was then regenerated with 20mM phosphoric acid leaving the surface ready for the next antibody sample. The data was imported into Qdat, reference curve subtracted, and plotted.



The maximum response (i.e. average of 5 points at the end of each injection) was then plotted as a dose response curve in figure 4. This curve is suitable for the determination of unknown sample concentrations.



Conclusion

We have demonstrated that SensiQ Discovery provides high quality kinetic data for an antibody-antigen interaction. A simple dose response curve was also constructed for this interaction where the Rmax was ~2500RU thereby providing good sensitivity.

