

Kinetic Analysis of High Affinity Biomolecular Interactions Poster

Common
Revision A.01

Introduction

SensiQ is a dual channel SPR-based instrument for accurate kinetic and affinity analysis. Here we demonstrate that reliable kinetic measurements may be recorded for interactions that are extremely difficult to regenerate and without consuming a large number of sensor surfaces or requiring a high channel count.

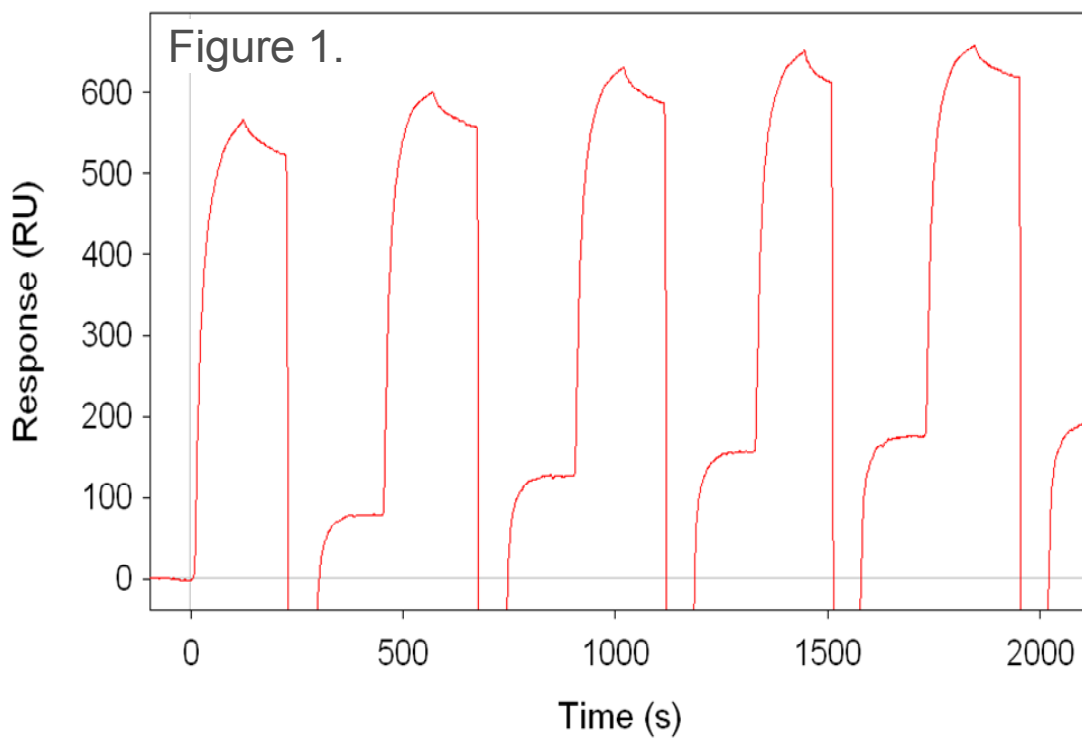
As is the case for any solid phase affinity system regeneration conditions must become more aggressive with increasing affinity. Therefore, high affinity monoclonal antibody-antigen interactions are often difficult to regenerate without incurring large decreases in binding capacity. This denaturation of the immobilized monoclonal antibody can be avoided if one simply replaces the immobilized monoclonal antibody with each cycle. This can be achieved by reversible affinity-capture of the monoclonal antibody. It is important to ensure that the captured antibody does not dissociate appreciably as this gives rise to a drifting baseline that distorts kinetic analysis of the resulting data.

Experimental

The running buffer for all experiments was HBS buffer, pH 7.4, containing 42 mM HEPES, 150 mM NaCl, 0.7 mM EDTA, and 0.001% (v/v) Tween 20. The flow rate was set at 50 μ L/min and the temperature was 25°C. Polyclonal rabbit anti-mouse Fc antibody was immobilized onto the planar carboxylated sensor surface by conventional amine coupling giving a yield of 2500RU.

Conditioning the Surface

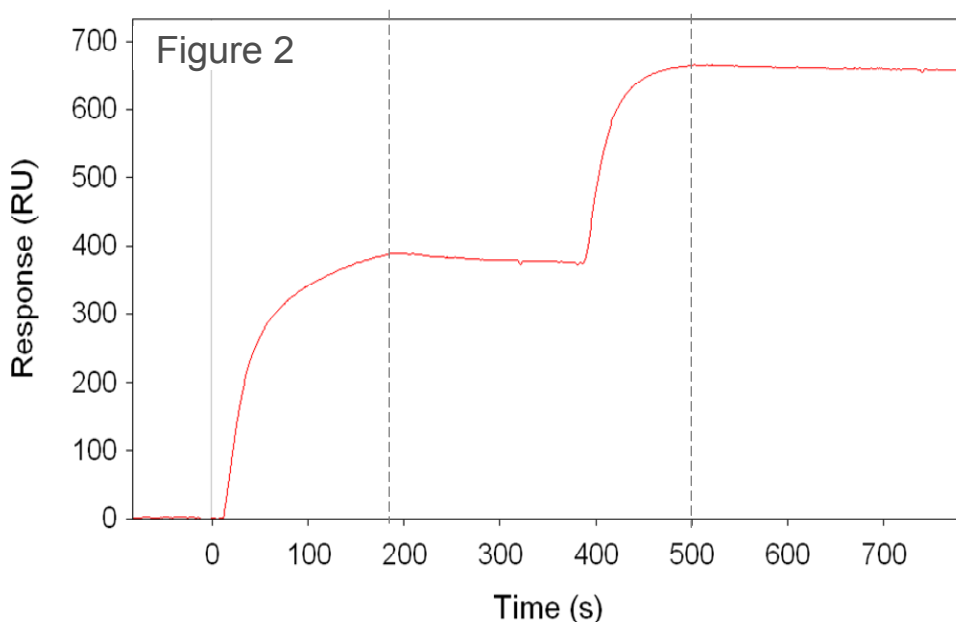
In order to establish a reproducible affinity-capture surface, we condition the surface by running a sequence of affinity capture binding cycles. Importantly, we capture a monoclonal antibody of the same subtype as that required in the kinetic analysis but with a different specificity (or no specificity). The response curve shown in figure 1 shows a typical conditioning sequence. 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 5 times. The covalently immobilized polyclonal capture antibody possesses a heterogeneous distribution of high, medium and low affinity antibodies and the objective of this conditioning process is to block all non-regenerable high affinity sites.



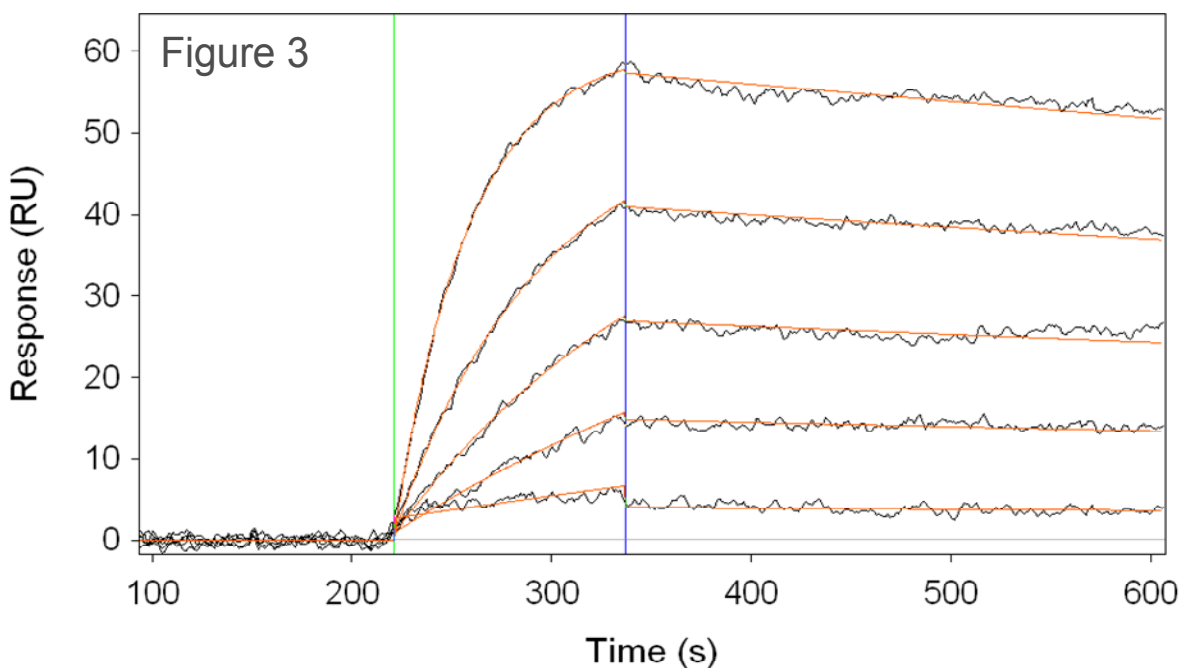
This results in accumulation of this “nonsense” monoclonal antibody on the surface with a concomitant decrease in binding capacity. After 5 to 10 binding-regeneration cycles are completed, the high affinity sites are filled and the binding-regeneration cycles become reproducible allowing high quality kinetic analysis to be conducted.

Estimation of Binding Activity

Retention of binding activity is the most important consideration when immobilizing a biomolecule and can be measured by comparing the relative binding responses recorded as follows. Figure 2 is a response curve for the sequential injection of monoclonal anti-avidin antibody and neutravidin. The dotted lines mark the end of each injection. At saturation a binding ratio of 0.83 is expected assuming a molecular weight of 145kDa for the bivalent mouse IgG1 (100nM) and 60kDa for neutravidin and assuming 100% retention of binding activity. Figure 2 shows a binding response of 348RU for affinity captured monoclonal antibody and a neutravidin (50nM) saturation response of 289RU.



Thus a binding ratio of 0.83 is indeed observed thereby confirming ideal presentation of antibody binding sites at the surface. A neutravidin binding response of 289RU is excessive for kinetic analysis therefore the amount of affinity captured anti-avidin monoclonal antibody was reduced to give an expected neutravidin saturation (Rmax) of <60RU.



The affinity capture format of figure 2 was repeated for a lower capacity surface over a range of neutravidin concentrations from 50nM to 3.1nM. The curves were imported into Qdat 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 3). 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.

Conclusion

The goodness of fit was measured as the average residual standard deviation of the fitted curves from the actual curves. A value of 0.55RU 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 returned Rmax values were in agreement with experiment. The k_a was $5.3 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, the k_d was $4.1 \times 10^{-4} \text{ s}^{-1}$, giving an affinity constant (K_D) of 0.77nM. This demonstrates a universal approach for the characterization of any interaction where a polyclonal antibody is available to affinity-capture the ligand at a site other than the analyte binding site.