

## Conventional Approaches

**Kinetics.** We created a set of model curves for a complex 1:2 interaction ( $K_{D1}$  2  $\mu\text{M}$ ,  $K_{D2}$  100  $\mu\text{M}$ ) and fitted an inappropriate 1:1 kinetic model (Fig 1) obtaining a reasonable fit (average squared residual of 0.37 RU) but inaccurate kinetics/affinity constants (5-fold relative error) The common practice of fitting a bulk refractive index term allows the model to appear well fit. The affinity constant ( $K_D$  29  $\mu\text{M}$ ) was within approximately 3-fold for the more prevalent weak sites and within 10-fold for the less abundant high affinity sites indicating a tendency to deemphasize the less abundant site. It is probable that high affinity sites can remain masked by the presence of a dominant weak affinity component.

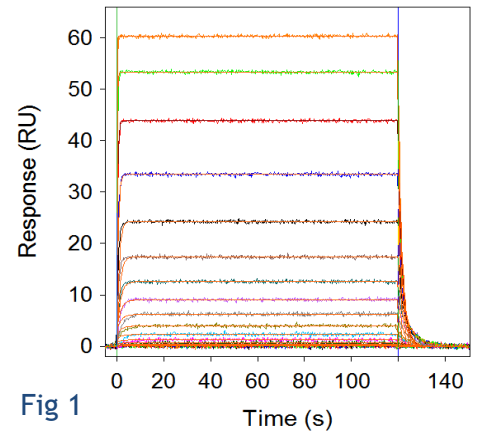


Fig 1

**Steady-State Affinity.** At steady-state, binding response curves are flat and the response can be plotted as a function of concentration and fitted with an affinity isotherm model (Fig. 2-3). The less abundant high affinity site is readily resolved in a steady-state analysis but general application of this approach is limited by the need for many primary response curves. A minimal set of curves (e.g. 6) can suffice in the case of simple 1:1 interactions.

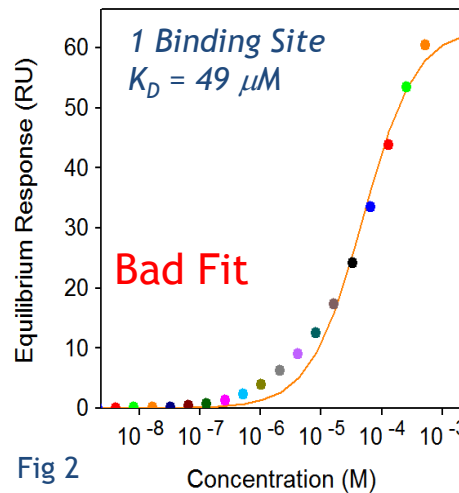


Fig 2

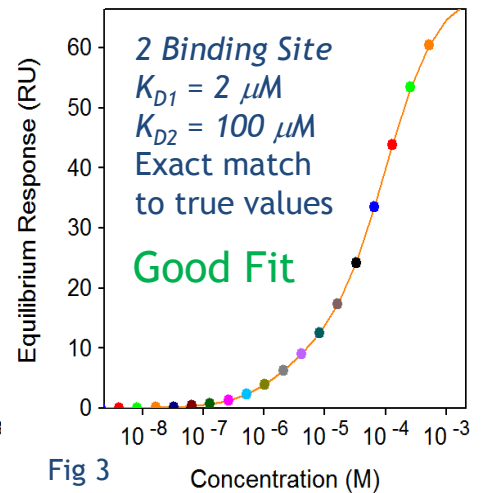


Fig 3

## Real-time Kinetic/Affinity Model for TDi Curves

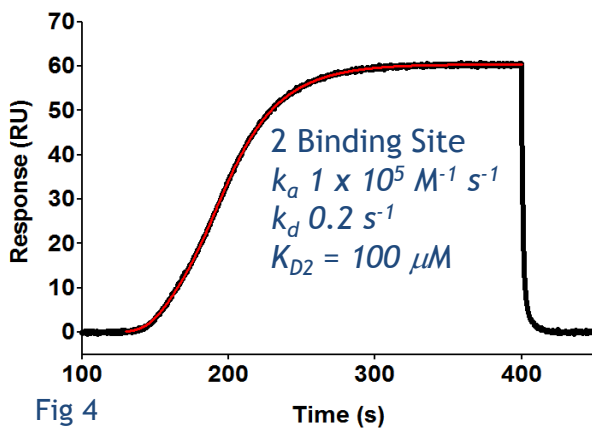


Fig 4

TDi provides literally thousands of points from a single injection that define the complex affinity interaction. Any interaction model can be fitted directly to a single TDi binding curve. For example, a TDi curve representing the complex binding interaction of Fig 1 is shown with a fitted model (Fig. 4). The high affinity component is best defined by a kinetic term while the weak affinity component is preferably lumped into a single affinity term. A second TDi curve recorded at a different flow rate can be included in a global fit when fitting the analyte diffusion coefficient. The apparent diffusion coefficient can be used to detect analyte aggregation such as micelle formation, denaturation and other heterogeneous analyte artifacts.

The biphasic association phase resembles the biphasic steady-state isotherm (Fig 3) because both are analyte titrations.