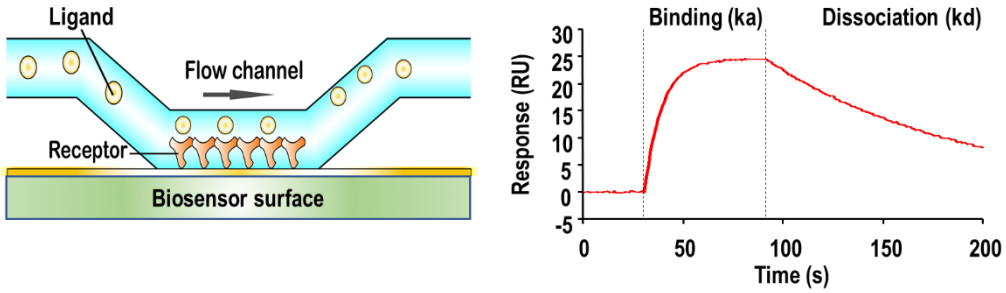
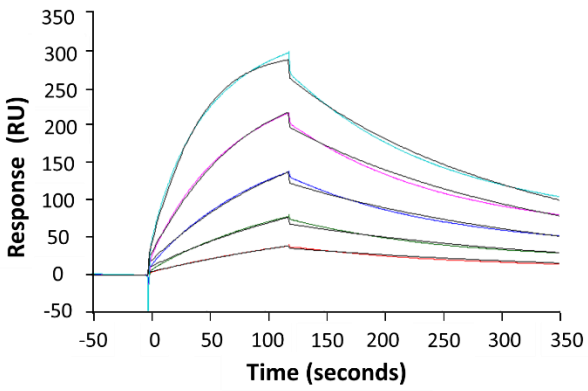
KINETIC AFFINITY

Kinetic affinity assays provide a method for determining the affinity of a ligand for its receptor by measuring the real-time binding association and dissociation rates using Surface Plasmon Resonance (SPR). In this method, one of the binding partners (e.g. the receptor protein) is immobilised onto a biosensor surface. The second partner (e.g. the drug ligand) is then continuously flowed across the biosensor surface, where it binds to the immobilised receptor. Binding is measured as an increase in resonance units (RUs) on the biosensor surface. Measuring the increase in binding over time for a given ligand concentration gives the association rate (ka or Kon). By ceasing to flow drug ligand and changing to buffer alone, then allows the ligand to wash off the receptor. Measuring the decrease in bound ligand over time gives the ligand dissociation rate (kd or K_{off}). The affinity of the ligand for the receptor (the equilibrium dissociation constant, K_D) is calculated from the kinetic association and dissociation rates (kd/ka) for several different ligand concentrations.

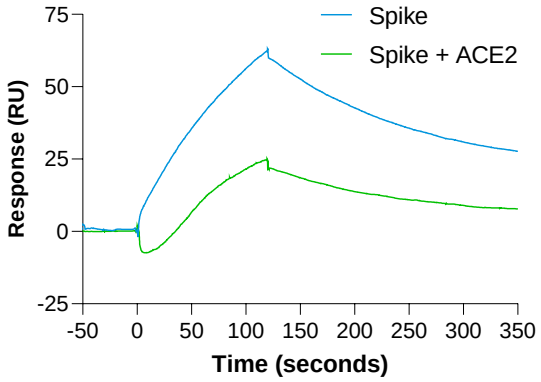


COVID-19 Kinetic Affinity Example

The virus infects human lung cells through the binding of the SARS-CoV-2 S 'spike' protein to the trans-membrane receptor Angiotensin Converting Enzyme 2 (ACE2) expressed on the cells. SPR analysis (Biacore T200) was used to determine the affinity of the spike protein for human ACE2 receptor and to demonstrate partial blockade of the binding interaction by masking the spike protein with a non-membrane-bound form of ACE2.



Binding of recombinant SARS-CoV-2 S spike protein to biotinylated human ACE2 protein captured onto a streptavidin-coated sensor chip. The spike protein was applied at 12.5 to 200 nM and gave a kinetic-derived affinity constant (K_D) of 35.6 nM.



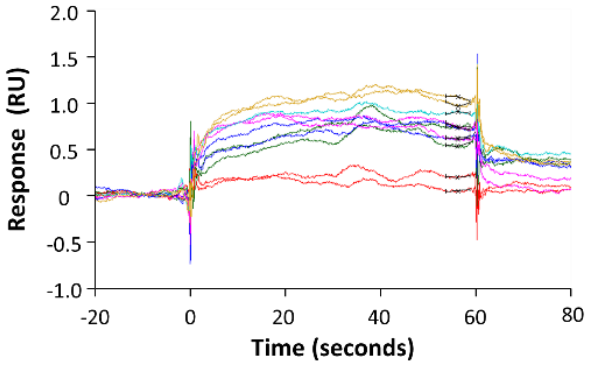
Binding of recombinant spike protein (20 nM) to surface-bound (streptavidin-captured) biotinylated ACE2 is attenuated by pre-incubation of the spike protein with a 10-fold excess of soluble, non-biotinylated ACE2 (200 nM).

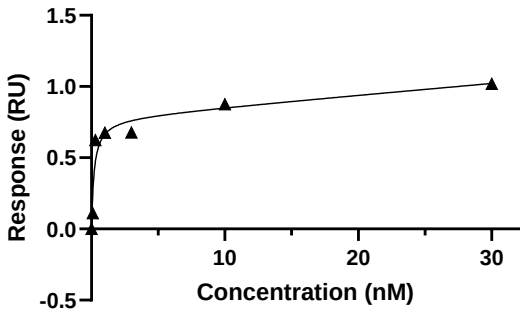
EQUILIBRIUM BINDING

Receptor-ligand kinetic affinity determinations are usually successful for receptors with larger ligands that have slower association and dissociation rates, as shown in the example above. For receptor-ligand interactions with faster association and dissociation rates, obtaining accurate kinetic parameters can be difficult. For these situations, equilibrium binding affinity is more appropriate.

Equilibrium Binding Example

Equilibrium binding of the endogenous estrogen, 17β-estradiol, to human estrogen receptor α (ERα).





Recombinant his-tagged human ERα was captured onto a nickel-treated NTA biosensor chip surface, then 17β-estradiol from 0.1 to 30 nM was bound to the ERα for 60 seconds, followed by 180 seconds dissociation (only part is shown). Blank-subtracted average binding responses were fitted to a saturation equilibrium binding model to generate a K_D of 0.18 nM. (IUPHAR reference K_D for 17β-estradiol binding to ERα = 0.16 nM).

LABEL-FREE AFFINITY MEASUREMENT

Surface plasmon resonance is label-free; it does not require either of the binding partners to be labelled with a radioisotopic or fluorescent label. It therefore provides an alternative to a radioligand binding assay where a [3H]-radioligand is unavailable or custom radiolabelling is impractical. It also avoids problems with incorporation of a bulky isotope (e.g. [125I]) adversely affecting the ligand structure and hence its affinity for the receptor.

Kinetic affinity measurements can also be complementary to radioligand equilibrium binding affinity, providing additional binding dynamics. For instance, two ligands may have the same binding affinity, but kinetic measurements can show differences in dissociation rates that may lead to selection of a ligand with a slower off-rate as a more effective receptor blocker.