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SPR Binding Assay
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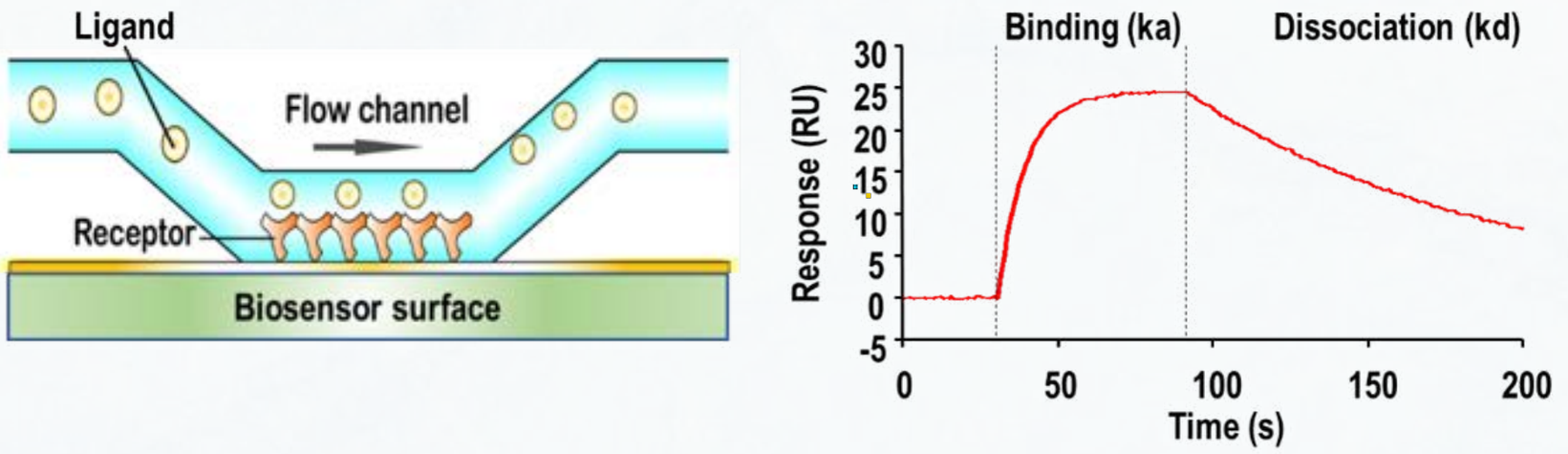
KINETIC AFFINITY

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 (k_a or K_{on}). 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 (k_d 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 (k_d/k_a) for several different ligand concentrations.



COVID-19 Kinetic Affinity Examples

Binding of the SARS-CoV-2 virus / COVID-19 S 'spike' protein to the trans-membrane receptor Angiotensin Converting Enzyme 2 (ACE2) expressed on human lung cells allows the virus to infect the cells. SPR analysis (Biacore T200) was used to determine the affinity of the spike protein for human ACE2 receptor captured onto a streptavidin-coated sensor surface or immobilized onto a CM5 sensor surface.

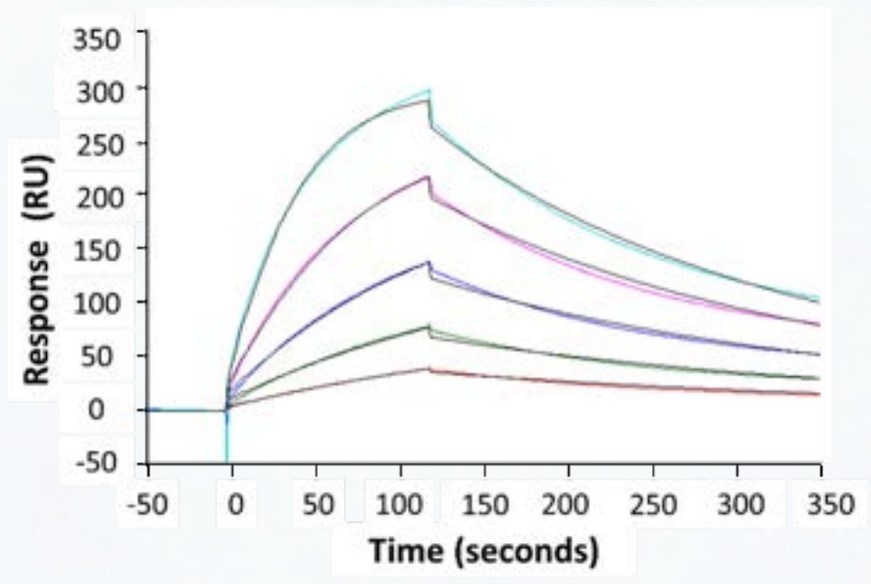


Figure 1: Binding of recombinant SARS-CoV-2 S spike protein to biotinylated human ACE2 protein captured onto a streptavidin-coated sensor chip. The spike protein was applied at 12.5 to 200 nM and gave a kinetic affinity constant (K_D) of 35.6 nM.

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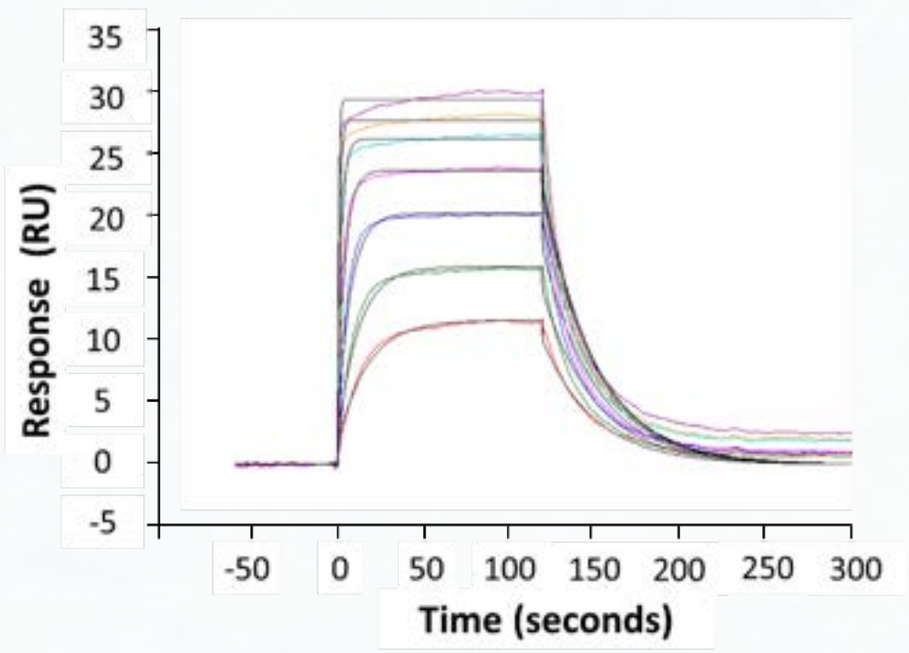

Figure 2: Binding of recombinant Angiotensin II (AT2) to FC-tagged human ACE2 protein immobilised onto a CM5 sensor chip. Angiotensin II was applied at 9.8 to 625 nM and gave a kinetic affinity constant (K_D) of 12.8 nM.

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 SARS-CoV-2 spike binding example above (Fig. 1). For receptor-ligand interactions with faster association and dissociation rates (similar to the Angiotensin II example above, Fig. 2), obtaining accurate kinetic parameters can be difficult. For these situations, equilibrium binding affinity is more appropriate.

Equilibrium binding (or steady state binding) requires a test compound (ligand) to stably bind to its immobilised receptor, for the majority of the duration of sample injection (e.g. 2 minutes) before dissociation occurs during the buffer 'wash' stage. Measurements are taken at the end of the sample injection period (indicated by the black crosses on the example curves below, Fig. 3) for each curve in a test compound concentration range. These measurements are then used by the Biacore software to plot a saturation response curve, from which the K_D is determined as the concentration at half R_{max}.

COVID-19 Equilibrium Binding Example

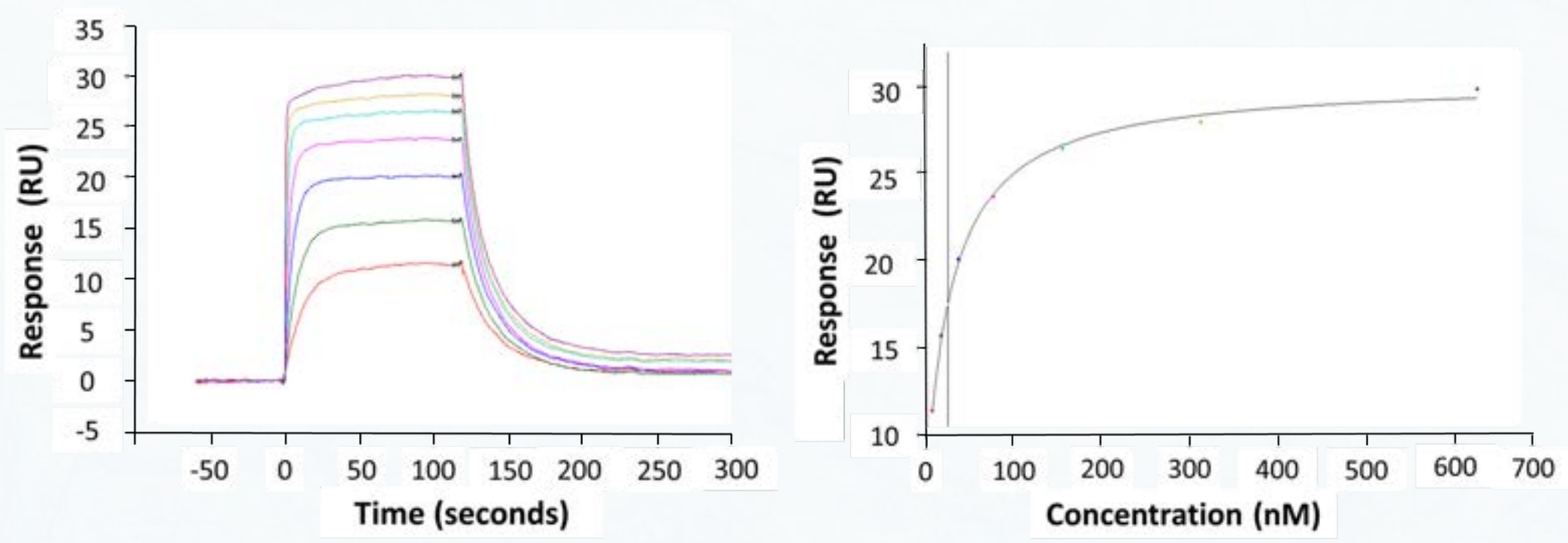

Figure 3: Binding of recombinant Angiotensin II (AT2) to FC-tagged human ACE2 protein immobilised onto a CM5 sensor surface. Angiotensin II was applied at 9.8 to 625 nM and gave an equilibrium affinity constant (K_D) of 26.7 nM.

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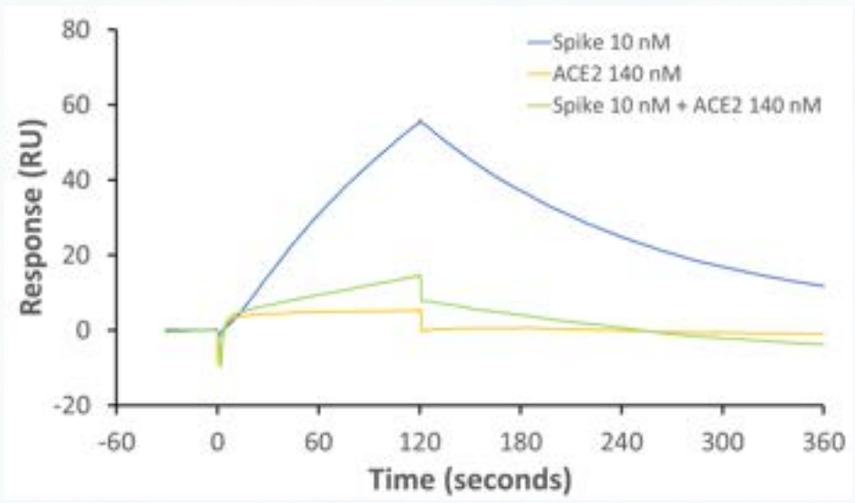
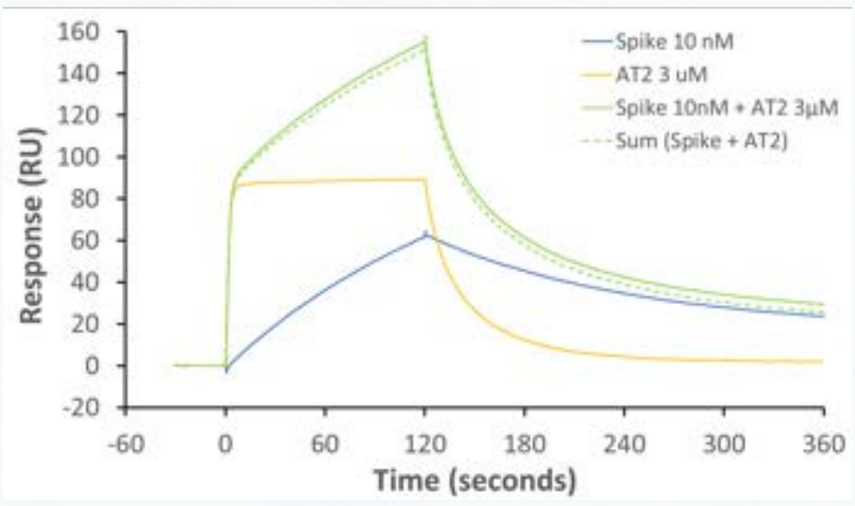
SOLUTION PHASE COMPETITION BINDING

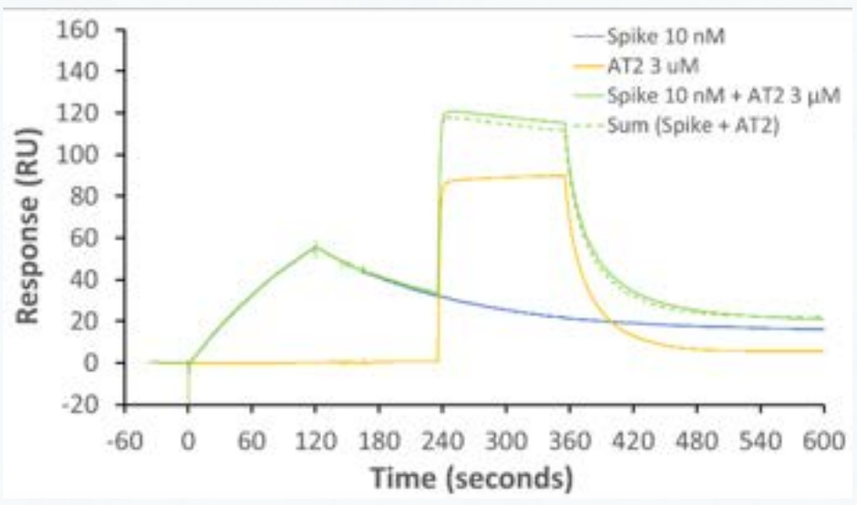
Solution phase binding is a means for evaluating binding interactions without one of the partners being bound to the sensor surface. Two binding partners are mixed and allowed to bind in solution, then the mixture is injected onto a sensor coated with a receptor that also binds one of the partners. The level of binding to the sensor reflects the proportion of free, unbound ligand in solution.

COVID-19 Solution Phase Competition Binding Example

A competition binding assay for spike protein binding to ACE2 in solution, then injecting the mixture onto ACE2 immobilised onto a sensor surface, was used to show competition for spike binding between ACE2 in solution and surface bound ACE2. Markedly reduced binding of spike protein to the surface-immobilised ACE2 was observed. The assay demonstrates the principle of 'masking' or 'decoying' the viral spike protein with a non-membrane-bound form of ACE2 to prevent the virus binding to the cell surface form of ACE2 to trigger infection.







Solution phase binding of COVID-19 spike protein (10 nM) with ACE2 (140 nM) prior to injection of the sample onto an ACE2-conjugated sensor significantly reduces spike binding in the presence of ACE2 (green line) compared with binding of spike alone (blue line). ACE2 does not bind to ACE2 on the sensor (only a small bulk shift; yellow line).

In contrast, injection of a mixture of angiotensin II (AT2, 3 µM) and spike (10 nM) onto the ACE2-conjugated sensor (solid green line) shows no difference from the sum of the responses (dotted green line) to AT2 alone (yellow) and spike alone (blue). Lack of attenuation of spike binding in the presence of AT2 compared with spike alone indicates no interaction in solution.

Furthermore, sequential injection of spike (10 nM) followed by AT2 (3 µM) reveals that, even when spike is bound to ACE2, there is no inhibition of AT2 binding and dissociation in the presence of spike, compared with binding of AT2 alone. This suggests that spike and AT2 bind to distinct, non-overlapping sites on ACE2.

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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 [^{3}H]-radioligand is unavailable or custom radiolabeling is impractical. It also avoids problems with incorporation of a bulky isotope (e.g. [^{125}I]) 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.

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