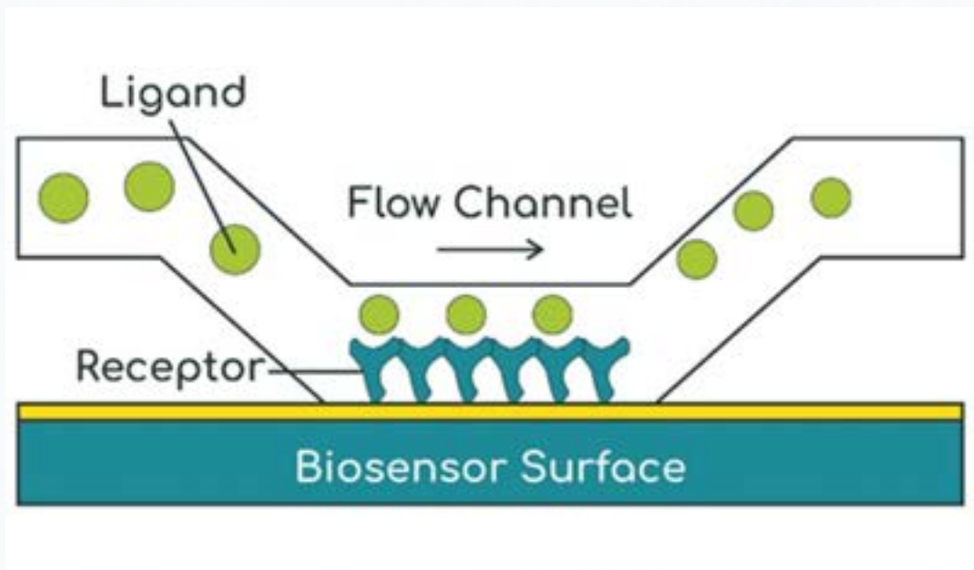
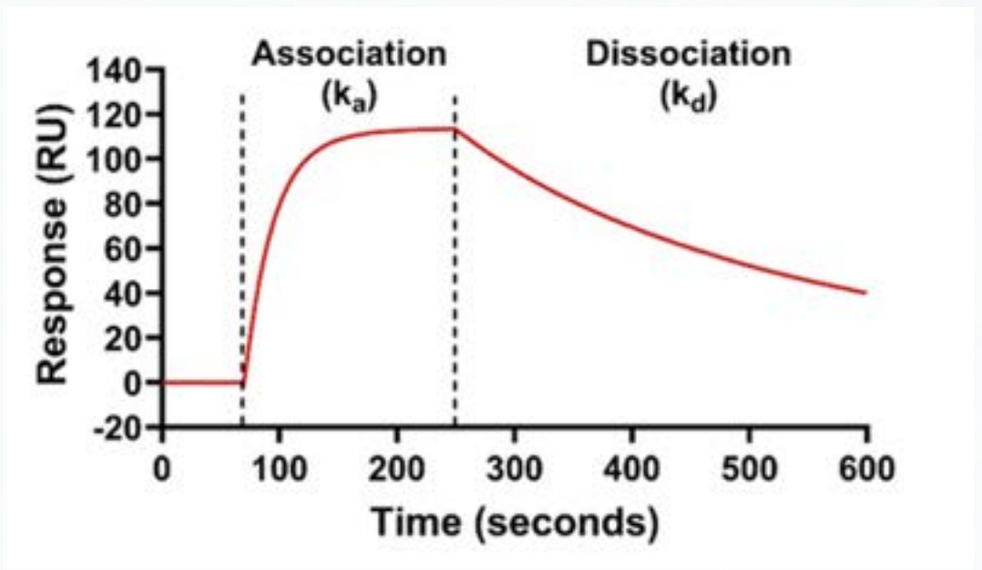
Data Sheet
SPR Binding Assay
Examples

PRINCIPLES OF SPR

• SPR (Biacore) measures the refractive index of the material within a ~100 nm layer immediately above the gold-coated biosensor surface.
• A target protein (receptor) is first immobilised / captured onto the biosensor surface.
• A test substance (ligand) is then flowed over the surface to bind to the receptor.
• As the ligand binds to the receptor, the refractive index increases in real-time – this allows the ligand binding association rate (ka) to be measured.
• The ligand is then washed off the receptor with buffer and the refractive index falls in real time – this allows the dissociation rate (kd) to be measured.
• The kinetic binding affinity constant (K_D) is then calculated as k_d / k_a.





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.

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EXAMPLE DATA

Protein – Protein Interactions

Measuring the interaction of two proteins using SPR is straight-forward. The interaction can often be measured with either partner immobilised onto the surface and the other partner in solution. There are a choice of methods to immobilised the proteins onto the sensor surface. Solution-phase competition allows the interaction of a third binding component to be analysed. Two analytes are mixed and allowed to bind in solution. The pre-bound components are then passed over the immobilised target on the sensor to reveal if one component inhibits the binding of the second component to the immobilised target.

Typical binding interactions studies include:

- Receptor – exogenous protein binding
- Receptor – endogenous protein ligand binding
- Protein complex binding partner interactions

Example data: kinetic affinity (K_D) and solution-phase competition (IC_{50} and K_i) analysis

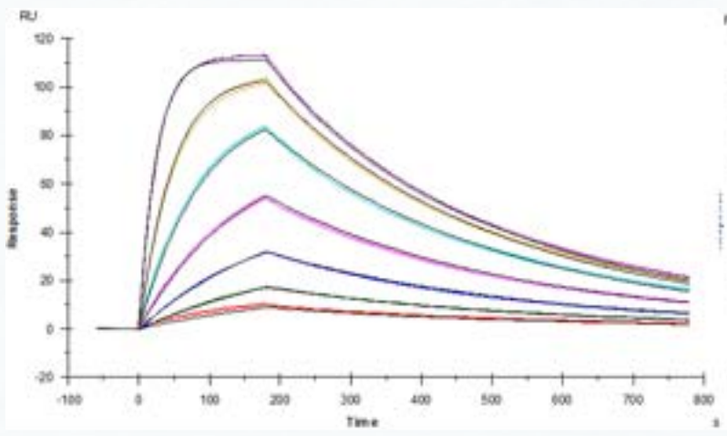

Figure 1: Angiotensin converting enzyme 2 (ACE2) covalently coupled onto a CM5 sensor. SARS-CoV2 Spike RBD protein binds to ACE2 with a kinetic affinity K_D of 30 nM.

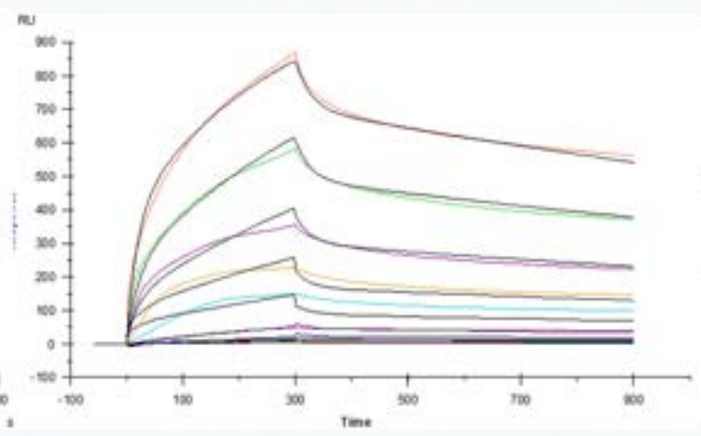

Figure 2: Insulin-like growth factor 2 receptor (IGF2R) covalently- immobilised onto a CM5 sensor. Stanniocalcin 1 (STC1) binds to IGF2R with a kinetic affinity K_D of 16.8 nM.

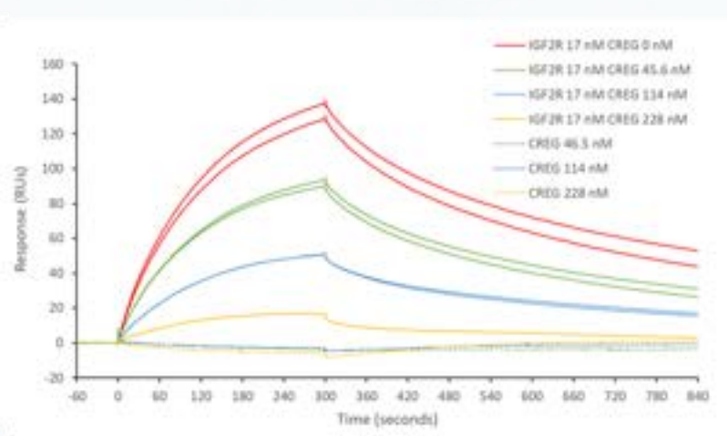

Figure 3: STC1 covalently-immobilised onto a CM5 sensor. Preincubating IGF2R (17 nM) with CREG (0 – 228 nM) inhibits IGF2R binding to STC1 with an IC_{50} of 102 nM and K_i of 50.7 nM.

Antibody – Antigen Interactions and Quantitation in Biological Samples

As for protein – protein interactions, measuring antibody – antigen interactions using SPR is routine. Similarly, the interaction can often be measured with either partner immobilised onto the surface and the other partner in solution. There are a choice of methods to immobilised the proteins onto the sensor surface

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The approach can be applied to full antibodies, fAb and ScFv antibody fragments. Monoclonal antibodies will give single-site binding kinetics; polyclonal antibodies may give composite binding kinetics from 2 or more binding species in the polyclonal serum.

Divalent antibodies binding to an immobilised antigen can give spurious, very high affinity binding kinetics. This is due to an avidity effect, when both arms of an antibody bind to separate immobilised antigens – the dissociation rate becomes dramatically slowed because the antibody must dissociate from both antigens simultaneously. This can be reduced by immobilizing the antigen very sparsely, or avoided by configuring the assay with the antibody immobilised on the sensor.

The binding interactions can also be measured in complex biological matrixes, e.g. serum or plasma, provided the sample is diluted to reduce non-specific binding from bulk protein in the biological sample.

Example data: SARS-CoV2 spike RBD – anti-spike antibody binding (in buffer and 10% serum)

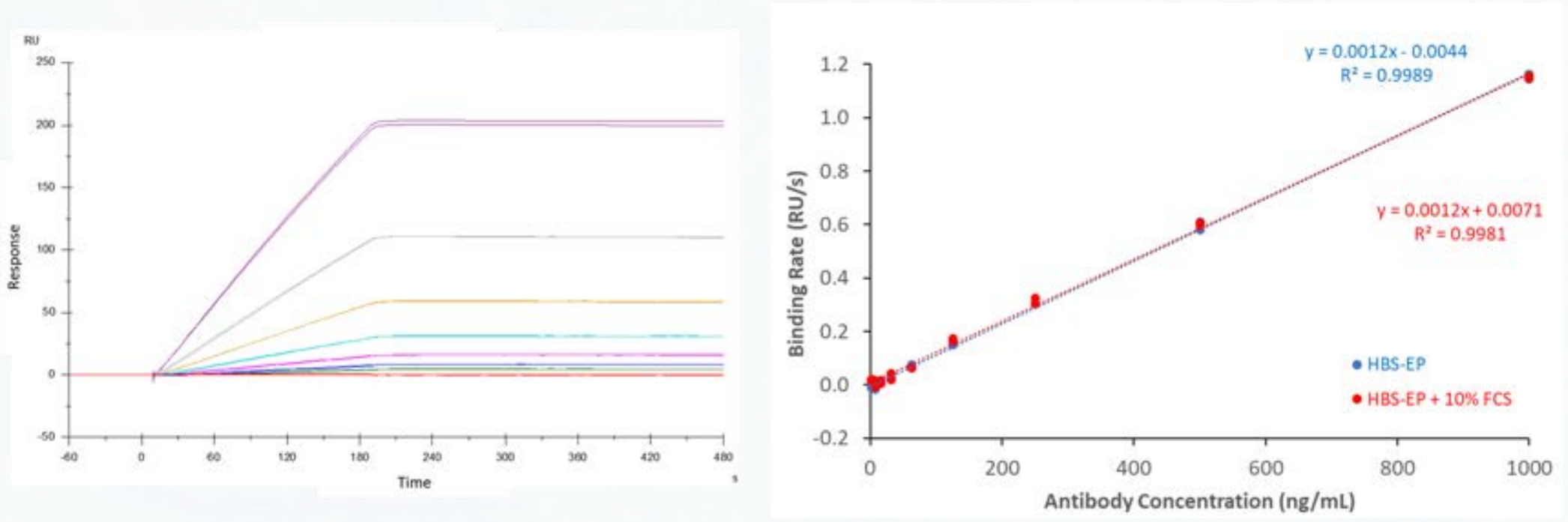

Figure 4: His-tagged SARS-CoV2 spike receptor binding domain (RBD) protein covalently coupled to CM5 sensor surface. Rabbit anti-spike binding responses in buffer (HBS-EP) or buffer + 10% serum generated antibody calibration curves. Lower limit of detection (LLD): 3.9 ng/mL; lower limit of quantitation (LLQ): 15.6 ng/mL.

Receptor – Ligand Interactions

Measuring binding of endogenous ligands to a native receptor allows the binding affinity to be determined. Usually, a kinetic affinity binding model can be used to calculate K_D, as for protein – protein interactions. However, some ligands bind to their receptor with much faster association and dissociation rates (k_a and k_d) than is typical for protein – protein interactions. This can make kinetic affinity measurements problematic . In these cases, stable equilibrium binding response amplitudes over a range of ligand concentrations can be fitted to a saturation binding model to determine the K_D as the concentration giving a half-maximal binding response.

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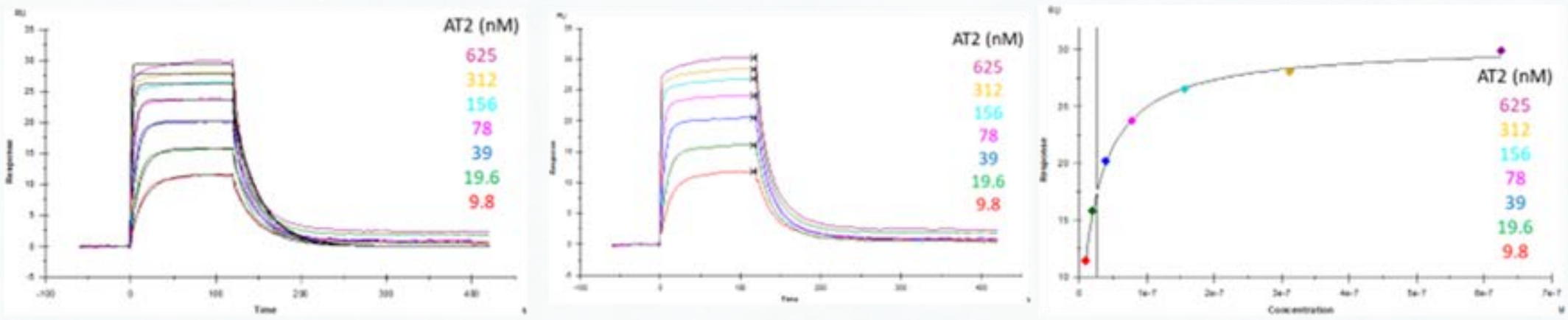
Example data: ACE2 + AT2 – kinetic affinity and equilibrium binding analysis



Analyte	Ligand	Model	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	Rmax (RU)	Chi^2 (RU^2)	Offset
AT2 (kinetic affinity)	ACE2	Kinetics - Single Site	2.57E+06	3.30E-02	1.28E-08	22.9	1.12	
AT2 (equilibrium)	ACE2	Equilibrium			2.67E-08	25.8	0.97	4.6

Figure 5: ACE2 covalently immobilised onto a CM5 sensor. Angiotensin II (AT2; 1046.2 Da) binding responses to ACE2 were fitted to a single-site kinetic affinity model (left) to give a K_D of 12.8 nM. Fitting the same data to an equilibrium binding model (centre) using the response amplitudes marked at 'x', plotted on a linear saturation binding model (right), gives a K_D (concentration at half-maximal response) of 26.7 nM.

Receptor - Small Molecule Interactions

Measuring small molecule ligands binding to immobilised protein receptor targets is dependent on the ratio of the molecular weights of the target and the ligand and the level of receptor immobilisation on the sensor surface. The theoretical binding response amplitude is calculated from:

Ligand binding (RUs) = Protein immobilisation level (RUs) / Protein MW (Da) x Ligand MW (Da)

Hence, binding of a relatively large ligand (e.g. 500 – 1000 Da) to a relatively small protein (e.g 25,000 – 50,000 Da), with 10,000 RUs immobilised on the sensor, will give a theoretical binding response of 100 – 400 RUs. This gives sufficient range to measure concentration-dependent responses to calculate a K_D.

Conversely, binding of a small ligand (e.g. 200 Da) to a large protein (e.g. 200,000 Da) with a low immobilisation level (2,000 RUs) will give a response of 2 RUs. This is insufficient to be able to calculate the binding K_D.

These calculations are performed routinely to determine the feasibility of an SPR study.

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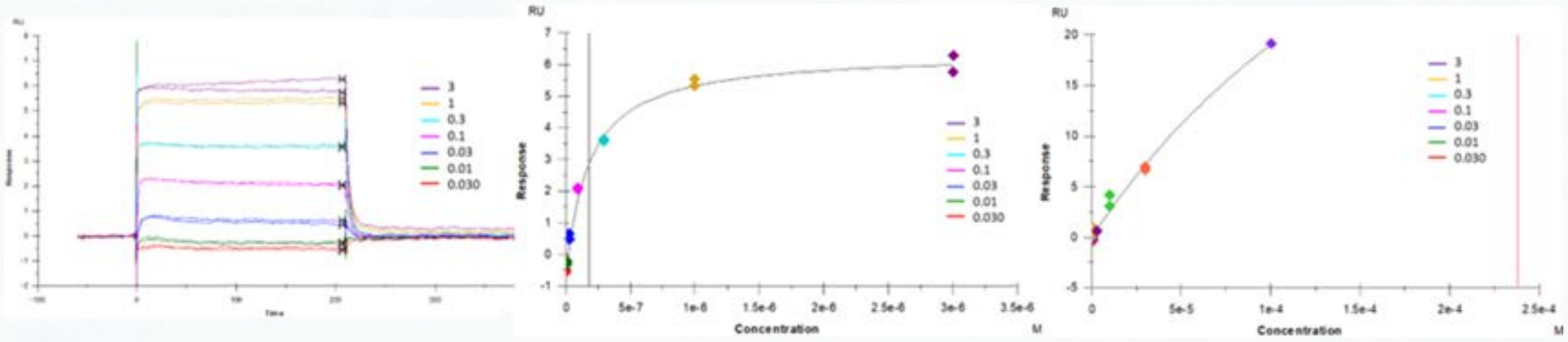
Example data: Protac ligand binding to Von Hippel–Lindau (VHL) protein



Analyte	Ligand	Model	K_D (M)	Rmax (RU)	Chi^2 (RU^2)	Offset
MZ1	VHL	Equilibrium - single site	1.78E-07	7.0	0.0319	-0.5925
cis-MZ1	VHL	Equilibrium - single site	2.38E-04	64.3	0.01633	0.223

Figure 6: Protac ligand MZ1 (1002.64 Da) binding to covalently-immobilised Von Hippel-Lindau (VHL) protein (25,800 Da) gives a saturable binding response of 7.0 RUs with a K_D of 178 nM. In contrast, the non-binding control, cis-MZ1 (1002.64 Da) gives a non-saturable, non-specific binding response with an estimated K_D > 240 µM.

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