UNDERSTANDING SPR DATA

K_D (i.e. affinity) values can be measured with two methods: steady-state and kinetic fitting. Typically, compounds predicted in CACHE challenges are relatively weak binders, with rapid association (k_a) and dissociation (k_d) and do not contain sufficient kinetic information for reliable binding evaluation by kinetic fitting. Instead, the steady-state method is applied, where K_D is determined by plotting RU values measured at stead-state versus compound concentration (Fig. 1).

RU: response unit = binding signal

R_{max}: Maximum RU (extrapolated by the software if steady state was not reached).

Expected R_{max}: Maximum expected RU value, based on the amount of protein immobilized on the chip and a 1:1 binding mode. R_{max} > expected R_{max} indicates multiple binding sites or non-specific binding.

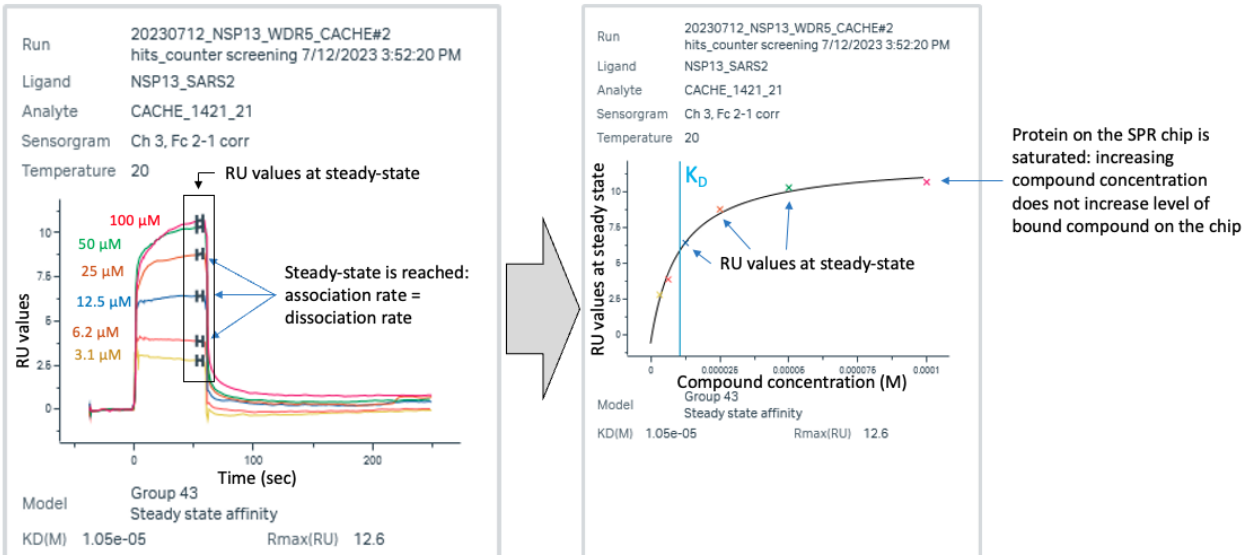

Figure 1: Steady-state affinity measurement.

<u>Primary screening</u>

Primary screening of a large chemical library is typically conducted at one (or two) concentration(s), and binding is evaluated based on the SPR sensorgram (Fig 1, left) at that concentration.

Binding is evaluated as an "adjusted relative" response that represents a relative binding value (in %) of binding to the protein: R_{max} values are subtracted by the response in a blank sensorgram (no compound), divided by the compound's molecular weight and divided by protein capture level.

The following parameters are used to evaluate the reliability of the binding signal:

"Slope" and "slow dissociation" indicate that the compound is not coming off from the immobilized protein/reference surface, a potential sign of non-specific binding.
R_{max} > Expected R_{max} indicates that the measured binding signal (RU) is greater than the expected signal.

<u>Dose response experiments</u>

Binding affinity (K_D) is measured as shown in Fig. 1

A number of parameters are available to evaluate data quality for steady-state affinity measurement (K_D):

Measured K_D < 0.5 x max compound concentration. Good indication that saturation was reached in the titration experiment (Fig.1, right panel). If saturation was not reached, K_D value is inaccurate.

Chi^2: Squared difference between experimental data and fitted curve (Fig.1, right panel).
Chi^2 < 2% R_{max}: good fit
Chi^2 > 10% R_{max}: bad fit

T-value: evaluates the significance of a descriptor. Obtained by dividing the parameter value by the standard error (significance of a fitted parameter).
T < 1: not significant
T > 10: is significant for the fitting and the value may be regarded as reliable

Offset: represents the response at zero analyte concentration. It should be close to 0

HOW TO USE THIS DATA?

<u>Primary screening (single concentration) experiments:</u>
"Adjusted relative" should be used as the binding signal. As a rule of thumb, a value > 0.5 (i.e. > 50% of expected binding) is a reasonable cut-off. "Adjusted relative" values are reliable if the following red flags are not raised:

- Slope = yes
- Slow dissociation = yes
- R_{max} > Expected R_{max} = yes

Dose-response experiments:

K_D is the affinity of the compound for the protein and is reliable if none of the following red flags are raised:

- R_{max} > expected R_{max}
- K_D > 0.5 x max compound concentration
- Affinity Chi^2 > 10% R_{max}
- T(K_D) < 1
- Binds with measurable K_D to an unrelated target (if data provided)
- Is not 100% soluble or aggregates at concentrations ranges < 2 x K_D
- Offset is not between -3 and 3 RUs