New tools for the rapid evaluation of Fragment Based Drug Discovery Campaigns by SPR

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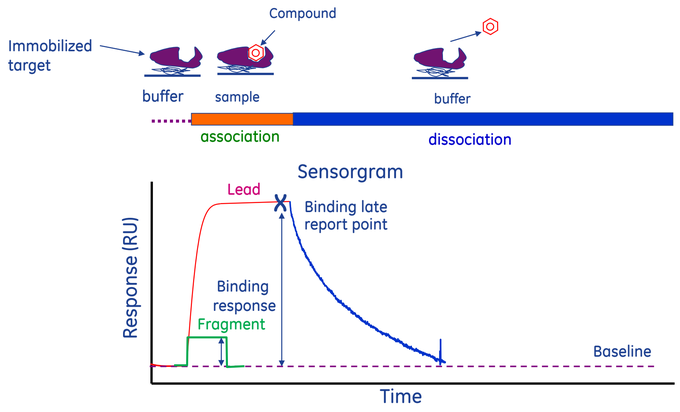
SPR based biosensors have become a powerful tool for rapidly screening and characterizing large libraries of fragments –requiring minimal protein consumption (25-50 µg) and rapid assay development whilst still providing information-rich data. The high sensitivity of Biacore™ T200 brings the data quality needed for confident hit prioritization. However, one common hurdle has been how to efficiently evaluate the data and select the best candidates for follow-up validation and determination of affinity. Until now, these steps required hours of qualified manpower as well as special algorithms and equations.
Here we present a new software capability and workflow for the rapid evaluation of single concentration and affinity screens to alleviate the bottlenecks in FBDD campaigns.
All examples are from screening PARP15 with the iNovacia fragment library using Biacore T200 software 2.0.

What do Biacore Measure

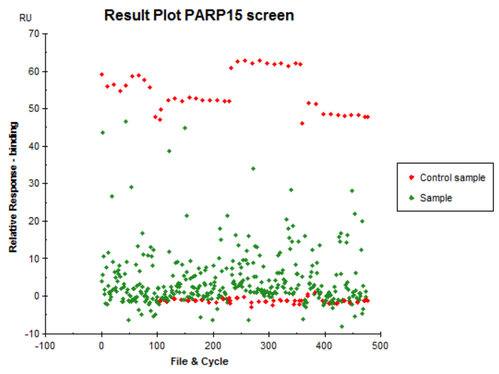


The sensorgram provides real time information about the entire interaction. Kinetics can be calculated from the curvature during injection (orange) and dissociation (blue).
Fragment binding rarely contains kinetic information, instead the response at equilibrium binding is used for affinity assessment (green). Sensorgrams from fragment binding that shows curvature most often indicate secondary binding.

Single Concentration Screening

Co-evaluation of several runs

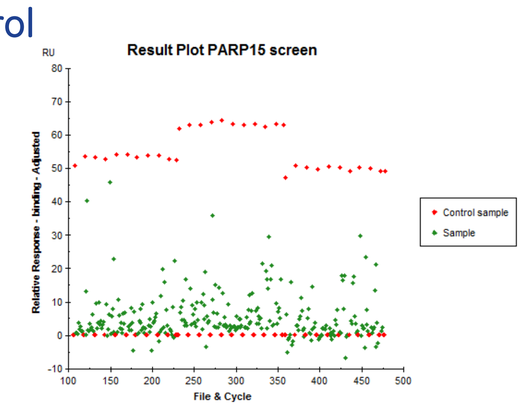
Screening of a fragment library requires multiple runs with Biacore T200. In order to avoid run to run bias in the prioritization of fragments several runs are handled in the same result plot giving better comparability.
Up to 5000 samples can be combined in the same plot.
In the diagram four separate 96-well runs are plotted together, which can be seen from the different levels of the positive control



Alignment of baseline - Negative control subtraction

The responses for non-binders can vary from run to run or drift within runs. The software offers the option of subtracting blanks neighboring in run order. This will efficiently eliminate jumps and drifts in baseline.

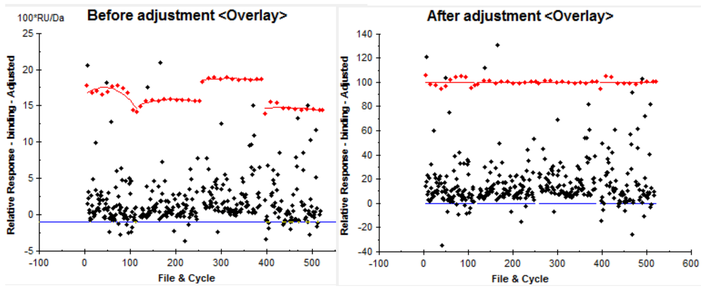
Nearest, preceding, following or average of two nearest blanks can be subtracted. Controls or samples with zero concentration can be used as blanks.



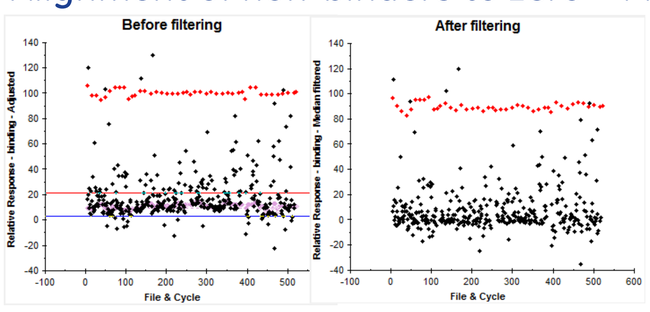
Alignment of sample responses - Mw – adjustment and Normalization to control responses

The signal corresponds to mass concentration changes and the molecular weight can vary four fold among fragments. Dividing the responses with Mw make them more proportional to binding strength on a molar basis.

The control sample variations for each run are fitted to an equation that is used to calculate the normalization factor for each sample



Alignment of non-binders to zero – Median filtration



A median is subtracted from each sample defining a new non-binder level around zero. This is very useful when negative controls are missing or do not accurately represent non-binding samples .

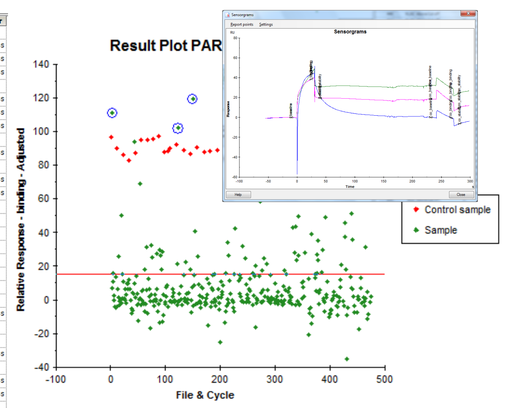
The median (purple points) is calculated from neighboring samples (user set x-axis window) that falls within a user set y-axis window.

Cut-off setting and Binding behavior assessment

If the negative control has become irrelevant for statistics based cut-off (due to its properties or adjustments), cut-off is placed to give as many positives as desired downstream. Binding behaviors are assessed and annotated.
40 fragments with typical binding were selected.

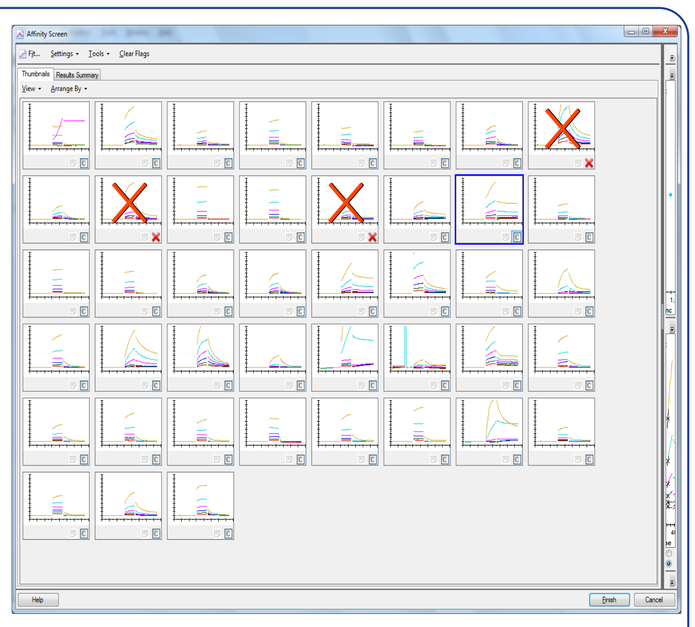
The Cut-off stringency can be set a free no of SD from the average of a control.

Y-Value	Cut-off	Assay Step	Sample	Annot:Binding behavior
78,66	Above Cut-off	Sample	F_4	
71,6	Above Cut-off	Sample	I_5_box1	Slow diss
70,18	Above Cut-off	Sample	I_2	Slow diss
68,86	Above Cut-off	Sample	E_7	Slow diss
63,21	Above Cut-off	Sample	H_5	
58,49	Above Cut-off	Sample	C_9	Slow bind & diss
51,06	Above Cut-off	Sample	G_2	Slow bind & diss
49,74	Above Cut-off	Sample	B_6	Slow bind & diss
49,59	Above Cut-off	Sample	I_5	
49,05	Above Cut-off	Sample	C_5	Slow bind & diss
45,31	Above Cut-off	Sample	F_1	
43,44	Above Cut-off	Sample	H_9	Slow bind & diss
42,83	Above Cut-off	Sample	H_7	Slow bind & diss
42,06	Above Cut-off	Sample	T_10	
40,36	Above Cut-off	Sample	D_6	
38,71	Above Cut-off	Sample	A_4	
37,16	Above Cut-off	Sample	F_2	
35,81	Above Cut-off	Sample	G_3	
34,83	Above Cut-off	Sample	B_4	
33,7	Above Cut-off	Sample	I_2	
32,44	Above Cut-off	Sample	G_5	
31,95	Above Cut-off	Sample	A_6	Slow bind & diss
31,1	Above Cut-off	Sample	F_8	
31,05	Above Cut-off	Sample	I_4_box1	
29,61	Above Cut-off	Sample	I_1	Slow bind & diss
29,46	Above Cut-off	Sample	B_10	
28,28	Above Cut-off	Sample	I_5	Slow bind & diss
28,1	Above Cut-off	Sample	B_6	Slow bind & diss
27,69	Above Cut-off	Sample	H_8	



Affinity Screening

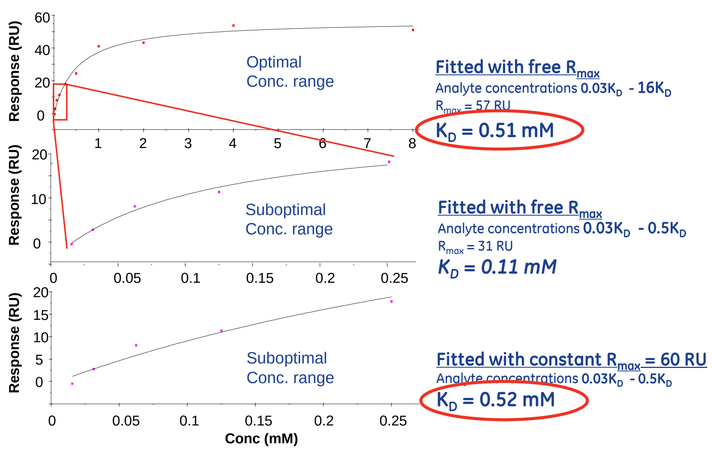
Steady state responses from target binding of concentration series of each chosen fragment are used to estimate the affinity.



Assess screen quality - Overview the series

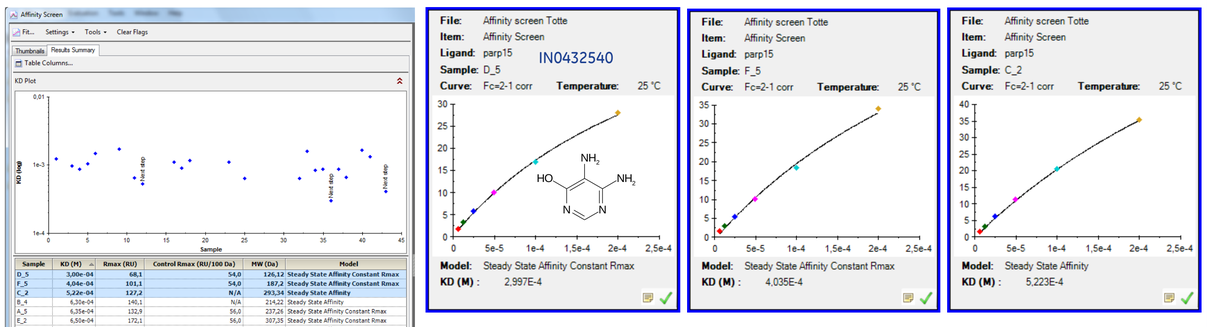
The software supports a rapid and visual overview of the data prior to fitting to allow the identification and flagging of compound series that do not show dose dependent behavior – e.g. concentration dependent aggregates.

Fit with constant R_{max}



Fragment solubility limitations force the use of suboptimal concentrations. Therefor free fitted R_{max} often renders unreasonable values (of R_{max} & K_D) due to lack of curvature in binding data.
By using a positive control we can determine the response when all binding sites are occupied (R_{max}). Using this value (Mw-adjusted) as a constant parameter in fitting compensates for the lack of site occupancy by the fragments. Thereby significantly improving the affinity determination.

Prioritization based on Affinity and Quality



After fitting the affinities are plotted and overviewed in order to aid in the selection of the best candidates to advance by assessing with respect to affinity, fitting quality and secondary binding.
A high dispersion of the data points indicates uncertainties in the K_D-value.
Secondary binding is often revealed by non-stoichiometric response levels and by slow on- and off-rates. A binding competition experiment with the positive control can quantify the degree of secondary binding from 0 – 100% as well as providing identification of allosteric binders. Fragments not showing secondary binding in single concentration screens might do so in concentrations series.
Many fragment screeners avoid fragments showing secondary binding in SPR "they are troublemakers with other techniques and downstream", adding value to the information from SPR.

All affinity plots and/or sensorgram overlays can be exported as separate pictures along with the result table. Plots and table can also be copied in to the clip board.

Conclusions

The new dedicated evaluation tools in Biacore T200, together with its outstanding sensitivity, have improved the ability to select fragments based on their affinity through the entire workflow

- The workflow provide true fragment screening tools reducing time consumption and needs for external software.
- Improved data alignment reduce the risk for run to run biases in selection of candidates
- The impact of experimental drifts and noice is significantly reduced
- Overviewing the Affinity screen data makes data quality based prioritization more easy and reliable
- Control based constant R_{max} fitting facilitates more accurate determinations of low affinities

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