Clean Screen: Optimizing a commercially available fragment library by identifying promiscuous binders with SPR using Biacore systems

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Introduction

Fragment-based drug design is an established approach to identify suitable scaffolds in drug discovery. SPR (Surface plasmon resonance) is an attractive biophysical method for fragment screening due to its high sensitivity, low target consumption, and generation of high-quality, information-rich data.

As fragments exhibit low affinities, screening assays typically require high concentrations. This is why many fragment libraries are designed for solubility at high concentrations. However, this does not remove the possibility of sticky substances that can persistently aggregate on target molecules, disturbing and lowering data quality of subsequent sample cycles. Some fragments also show stickiness to sensor surfaces. To avoid repeating experiments, you can identify and remove these compounds before binding and affinity analysis. Biacore™ systems provide a dedicated ***Clean Screen*** tool for efficient identification and pre-analysis elimination of undesirable sticky compounds. Here we present data in collaboration with Maybridge describing ***Clean Screen*** campaigns of the Maybridge Ro3 fragment library analyzing three Biacore sensor chips.

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Biacore systems fragment screening workflow

Clean Screen
- Screen of library against all targets and dextran
- Goal: Removal of troublemaking samples that impact subsequent cycles and can lead to false negatives

Binding Level Screen
- Single concentration screen against target and reference
- Goal: Rapid prioritization of fragments in the library

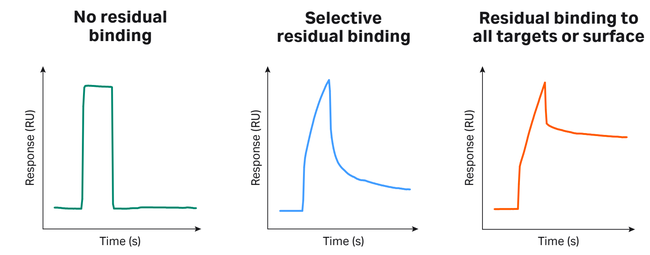
Affinity Screen
- Steady state analysis based on concentration series
- Goal: Estimate affinity and verify binders

Competition assays
- Usage of inhibitor in samples and buffer
- Goal: Validation of screen hits and binding site mapping

Clean Screen on Biacore systems

Clean Screen is typically run once per library and sensor chip type. You also need to run ***Clean Screen*** for each individual target protein. The samples are run over target(s) and a blank dextran surface at one concentration.

Clean Screen evaluation automatically identifies samples that show residual binding to all targets and surfaces (general binders); residual binding to some targets or surfaces (selective binders); or no residual binding at all (nonresidual binders). All these samples have the potential to disturb subsequent assay cycles by blockage or drifting data, and could cause false negatives.



Clean Screen of Maybridge Ro3 2500 diversity fragment library

We ran ***Clean Screen*** campaigns involving > 2700 fragments from the Maybridge Ro3 diversity library using the three most commonly used Biacore sensor chips in Fragment Based Drug Discovery (FBDD) campaigns:

Series S Sensor Chip CM5
The most versatile sensor chip available — the first choice for immobilization via -NH2, -SH, -CHO, -OH or -COOH groups

Series S Sensor Chip CM7
Used to study interactions involving small molecules and when achieving the required immobilization level is a challenge

Series S Sensor Chip SA
Used for immobilization of biotinylated peptides, proteins, nucleic acids, or carbohydrates

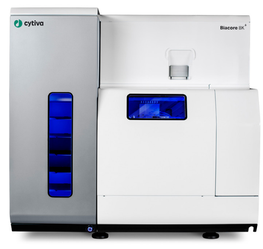
By running ***Clean Screen*** campaigns on these sensor chips, we established a fragment library that is better prepared for FBDD campaigns using Biacore systems.

Experimental setup

All experiments were run on Biacore 4000. However, the approach can be applied also on the current Biacore platforms supporting fragment screening, Biacore 8K, Biacore 8K+ and Biacore S200.

In each screen 2740 fragments were loaded in 384 well PP plates and screened at 1 mM in PBS-P+ with 2% DMSO.


Biacore S200

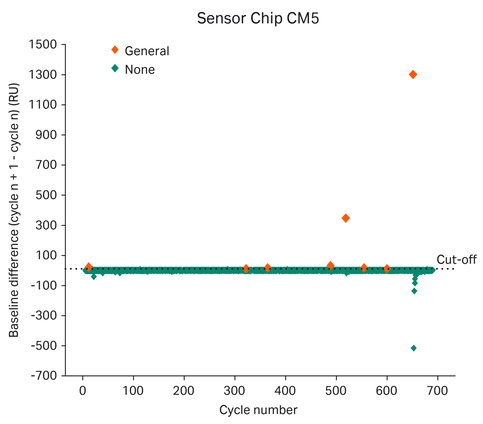

Biacore 8K+

Results of Clean Screen

Clean Screen identified approximately 1% of the fragments as sticky with respect to the Biacore sensor chips used.

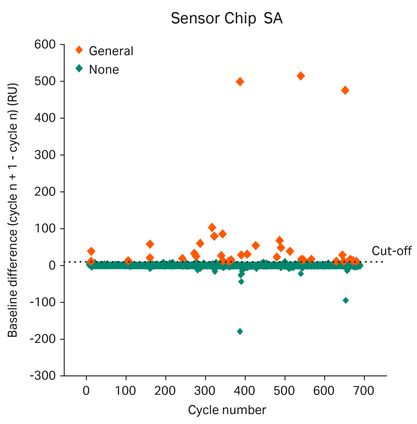
Sensor Chip CM5

On Sensor Chip CM5, the majority of the fragments are well-behaved with square-shaped curves and do not show any blockage or residual binding. We saw no effect on subsequent samples. ***Clean Screen*** identified 11 fragments (0.4%) as sticky with respect to the sensor surface.



Sensor Chip SA

This sensor chip is preimmobilized with Streptavidin and the number of sticky substances are therefore higher. ***Clean Screen*** identified 37 fragments (1.4%) as sticky.



Sensor Chip CM7

Compared to Sensor Chip CM5, we saw a slightly higher degree of sticky binders for Sensor Chip CM7, mostly likely due to higher charge density. ***Clean Screen*** identified 16 fragments (0.6%) as sticky. The sensorgram below (cycle n) shows an extremely sticky fragment that generates very large disturbances in the subsequent sample cycle (cycle n + 1).

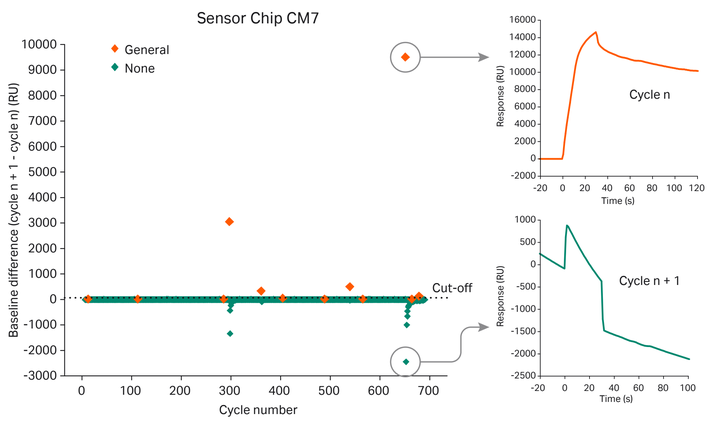


Table 1. Fragments identified as sticky for Biacore sensor chips

Fragment ID	Sensor Chip CM5	Sensor Chip CM7	Sensor Chip SA
AC10033	•		
AC16190	•		•
AC23506	•		
AC24866			•
AC42568		•	•
AC42849	•	•	•
BR00046			•
BTB01058			•
BTB03435			•
BTB09252			•
BTB10716		•	•
CC08601			•
CC11601			•
CC24201			•
CC33116			•
CC34301			•
CC39801			•
CC39901			•
CC40009			•
CC40996	•		•
CC41309			•
CC46201	•		
CC48713		•	
CC50513	•		
CC51509			•
CC52909			•
CC52916	•	•	•
CC55714		•	•
CC58701			•
CC60313		•	•
CC60364	•	•	•
CD08880		•	
DP01095			•
DP01601	•		•
GK02514			•
HTS01520			•
HTS07422		•	
JFD02085	•	•	
KM00452			•
KM09503			•
MO00072		•	
MO00397			•
MO07699			•
MO08161			•
SB01680			•
TL00355			•
XBX00167		•	

Conclusions

- We ran complete clean screens of Maybridge Ro3 2500 diversity fragment library on Sensor Chip CM5, Sensor Chip CM7 and Sensor Chip SA, and identified undesirable sticky fragments to the sensor surfaces.
- The dedicated software tools for fragment screening on Biacore systems efficiently and quickly identified fragments with unwanted binding properties.
- The optimization of the Maybridge library has established a fragment library that is better prepared for FBDD campaigns, and will reduce assay optimization when working with Biacore systems.

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