GE Healthcare

Clean Screen: Optimization of a commercially available fragment library by identification of promiscuous binders by SPR using Biacore™ systems

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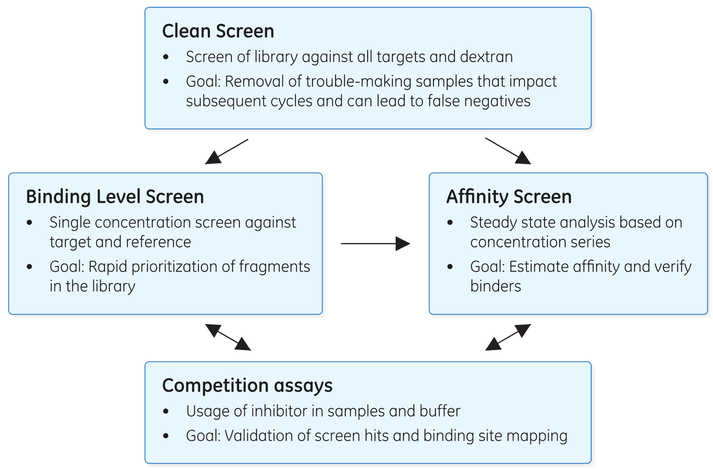
Introduction

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, high concentrations are typically required in screening assays. Many fragment libraries are therefore designed for solubility at high concentrations (mM). However, this does not remove the possibility of sticky substances that may persistently aggregate on target molecules, thereby disturbing and lowering data quality of subsequent sample cycles. Some fragments also show stickiness to sensor surfaces, and to minimize the need for repeat experiments, it is important to identify and remove these compounds prior to binding and/or affinity analysis. Biacore systems provides 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.

MAYBRIDGE

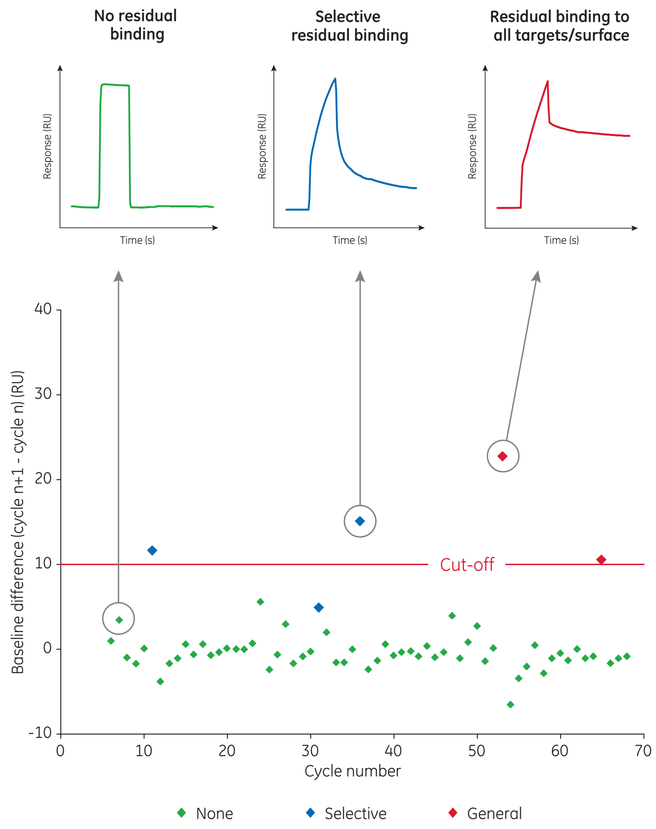
Biacore systems fragment screening workflow



Clean Screen on Biacore systems

Clean Screen is typically run once per library and Sensor Chip type, but need also to be assessed for each individual target protein. The samples are run over target(s) and blank dextran surface at one concentration.

Clean Screen evaluation automatically indicates samples that show residual binding to all targets and surfaces (general binders); residual binding to some targets/surfaces (selective binders); or no residual binding at all (nonresidual binders). All these samples can potentially disturb subsequent assay cycles by blockage and/or drifting data and could therefore result in false negatives.



Clean Screen of Maybridge Ro3 2500 diversity fragment library

Clean Screen campaigns involving > 2700 fragments from the Maybridge Ro3 diversity library were run, using the three most commonly used Biacore Sensor Chips in Fragment Based Drug Discovery (FBDD) campaigns:

Series S Sensor Chip CM5

The most versatile chip available — the first choice for immobilization via -NH2, -SH, -CHO, -OH or -COOH groups

Series S Sensor Chip CM7

Use to study interactions involving small molecules and when achieving the required immobilization level is a challenge

Series S Sensor Chip SA

Use for immobilization of biotinylated peptides, proteins, nucleic acids or carbohydrates.

By running Clean Screen campaigns on these Sensor Chips we have established a fragment library that is better prepared for FBDD campaigns using Biacore Systems.

Experimental setup

All experiments were run on Biacore 4000, which allows for analysis of four samples in parallel. In each screen 2740 fragments were loaded in eight 384 well PP microplates.

The fragments were screened at 1 mM in PBS-P+ buffer with 2% DMSO.

Biacore 4000

Results of Clean Screen

Approximately 1% of the fragments were identified 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/residual binding. No effect is seen on subsequent samples. 11 fragments (0.4%) were identified 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. 37 fragments (1.4%) were identified as sticky.

Sensor Chip CM7

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

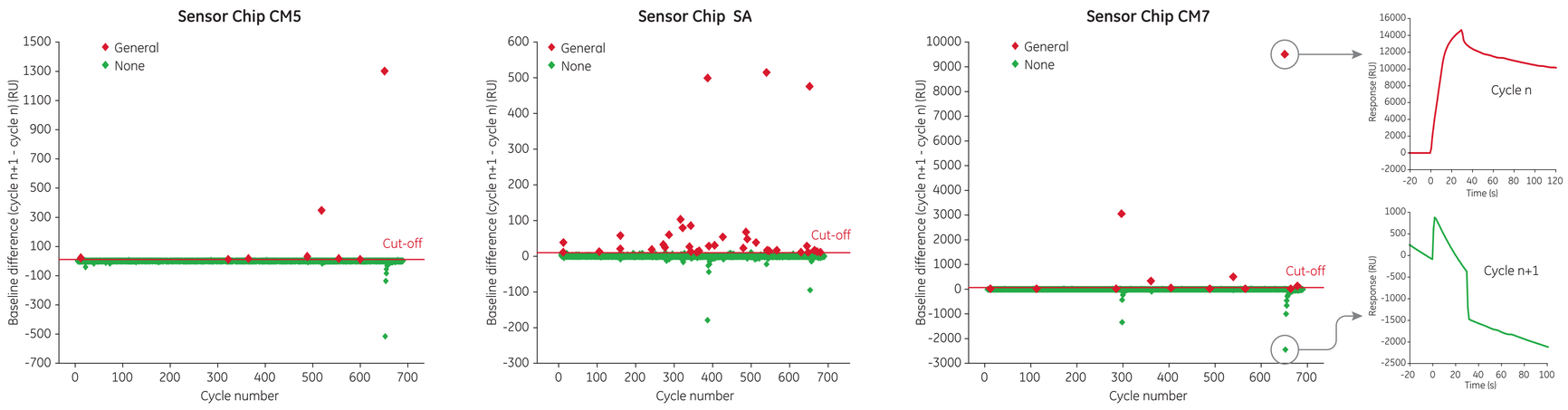


Table 1. Fragments identified as sticky for Biacore Sensor Chips

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 have run complete Clean Screens of Maybridge Ro3 2500 diversity fragment library on Biacore Sensor Chips CM5, CM7 and 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 manner.
- 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.

29155462 AC 06/2016