Parallel, label-free fragment screening with Biacore 4000

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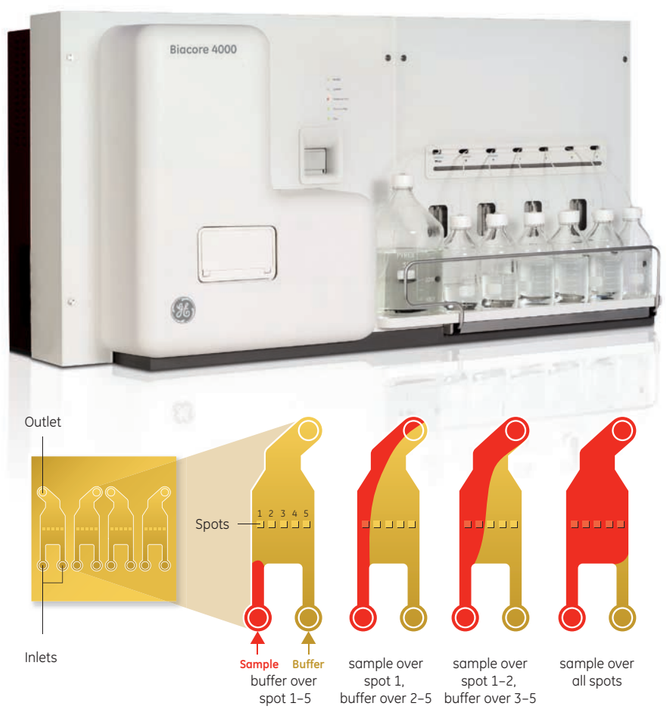
Introduction

Surface plasmon resonance (SPR) biosensors can be used to screen and affinity-rank thousands of fragments consuming only microgram amounts of protein for a complete screen. With Biacore™ 4000, we have improved the efficiency of the SPR-based fragment screening by the development of new, powerful methodologies including early identification and elimination of super-stoichiometric sticky binders and focused prioritization of promising binders. Most importantly, affinity based ranking is enhanced with novel tools for affinity determination at suboptimal fragment concentrations and in the presence of secondary, multi-site binding.

Biacore 4000

- Powerful data processing for large-scale studies
- Screening and characterization with the same instrument
- Parallel analysis enhances throughput and assay development



- Four parallel flow cells with 5 addressable spots in each
- Measure 4 fragments binding up to 4 proteins in parallel
- Affinity ranking of > 200 fragments/24 h

Fragment-related challenges

- **Low affinities (0.1 to 10 mM)**
  High sample concentration required
  Suboptimal sample concentrations in relation to K_D and R_{max}
- **Low molecular weight (80 to 300 Da)**
  Mass-dependent detection gives low signals
- **No binding kinetics**
  Square pulses (fast on, fast off) allow steady state affinity analysis only
- **Secondary interactions – multi site binding**
  Binding not related to the addressed binding site induced by high compound concentrations

Concept overview

Clean Screen
- Rapid screen of entire library against each target
- Goal: Removal of fragments that could potentially disturb subsequent assays

Binding Level Screen
- Single concentration screen
- Goal: Rapid prioritization of fragments

Affinity Screen
- Steady state analysis based on concentration series
- Goal: Affinity ranking and verification

Competition assay
- Use site specific inhibitor
- Goal: Validation and binding site mapping

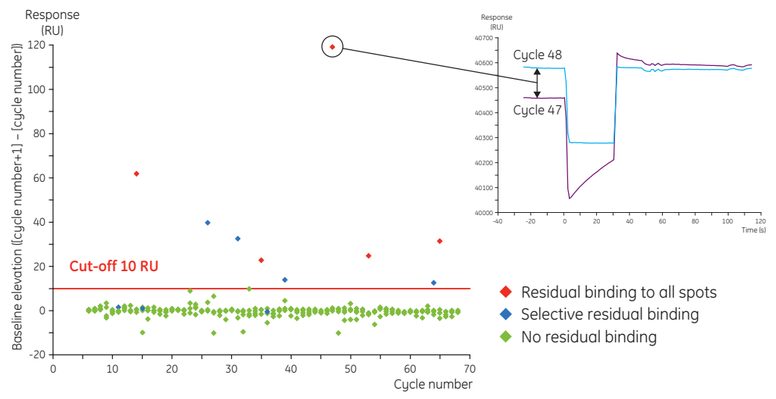
Model system

500 fragments from Maybridge with thrombin as target and carbonic anhydrase and GST as control targets.
Buffer: 10 mM PBS, 0.05% P20 and 5% DMSO.

Novel methodology

Clean Screen

Removal of fragments that may disturb subsequent assays.

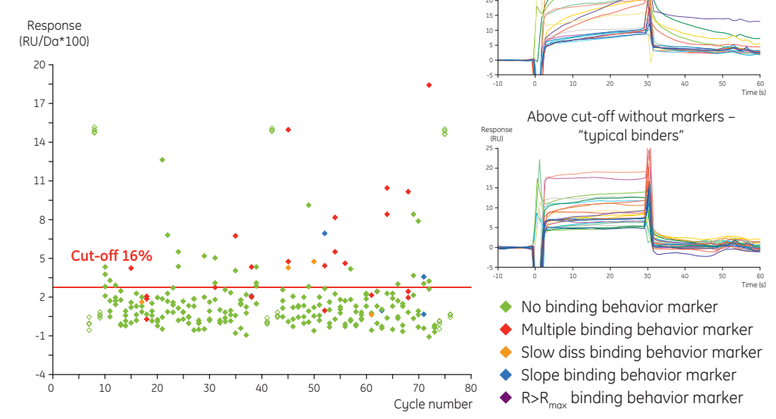


Clean screen is run at the highest sample concentration to be used in subsequent assays, with the simplest possible set-up: No reference spot and no controls.

Fragment "stickyness" is often target selective, thus Clean Screen is recommended for every new target.

Binding Level Screen

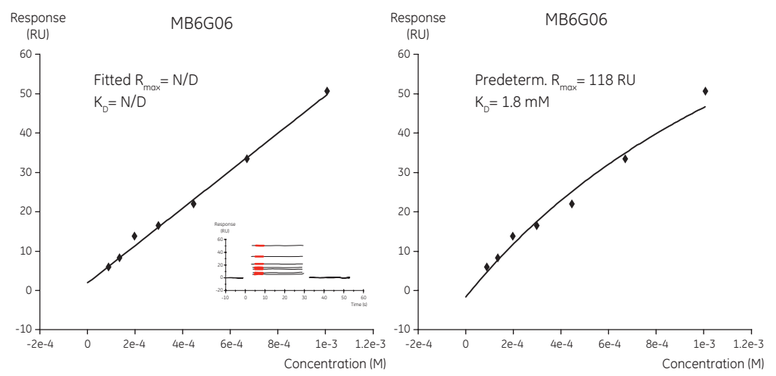
Prioritize well-behaved binders.



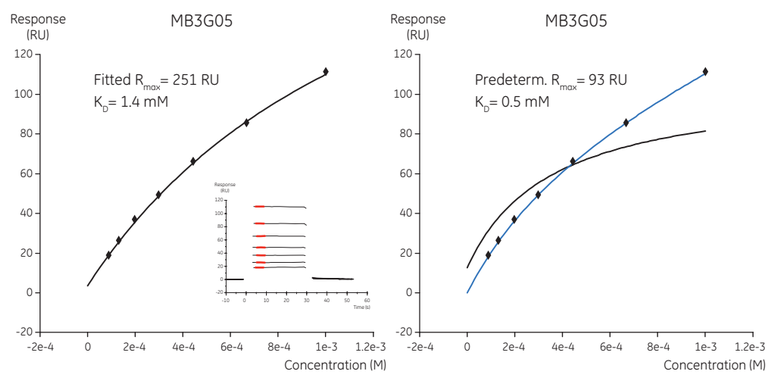
Prioritize fragments based on binding level at one single concentration. The binding behavior markers aid in focusing on typical binders.

Affinity Screen

Affinity ranking in the mM range also in the presence of multi-site binding.



Positive control predetermined R_{max} gives K_D also from data lacking information about R_{max}.

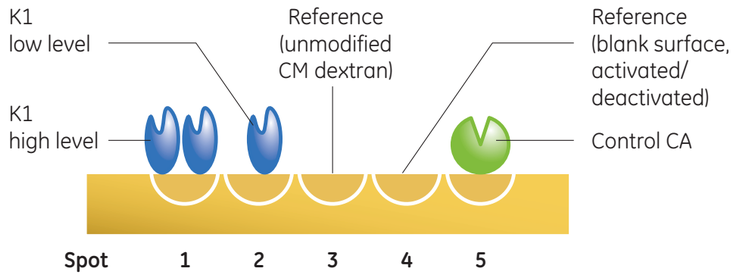


Multi-site binding evaluation (blue line) with predetermined R_{max} gives K_D determination also in the presence of secondary binding. Usage is suggested by the software.

Fragment screen study

Screen setup

Target: Kinase domain of tyrosine kinase: K1 (Mw 32 kDa).



Fragment library

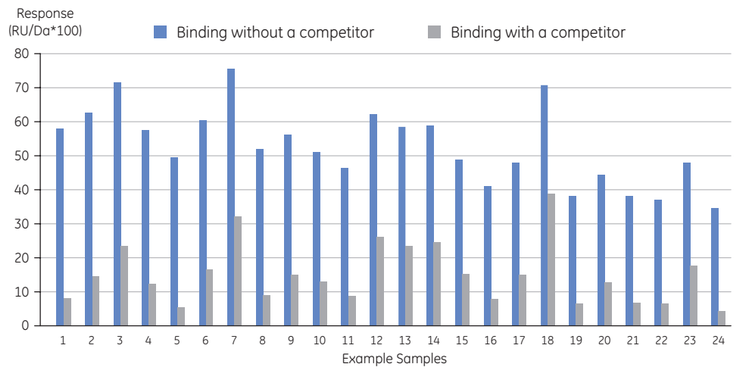
Unbiased, in-house library of **1920 fragments** selected according to "the rule of 3" with a particular emphasis on chemical diversity and solubility. M_W between 100 and 260 Da, average 220 Da.

Clean Screen at 2 mM

12 fragments, potentially assay disturbing, out of 1920 were removed (0.6%).

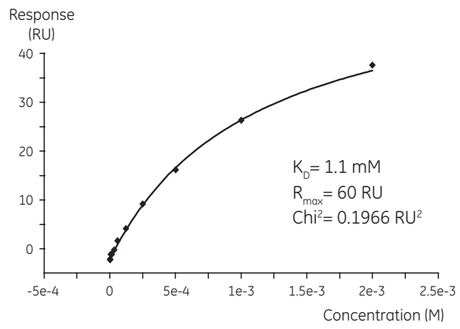
Binding Level Screen at 2 mM

With and without competition.



180 fragments (~10%) were selected for further analysis on the criteria:
– Difference in binding with and without site specific competitor > 20 RU
– Binding in presence of site specific competitor < 10 RU

Affinity Screen, 2 µM to 2 mM



105 fragments bound with 1:1 stoichiometry with K_D from 13 µM to 3 mM.

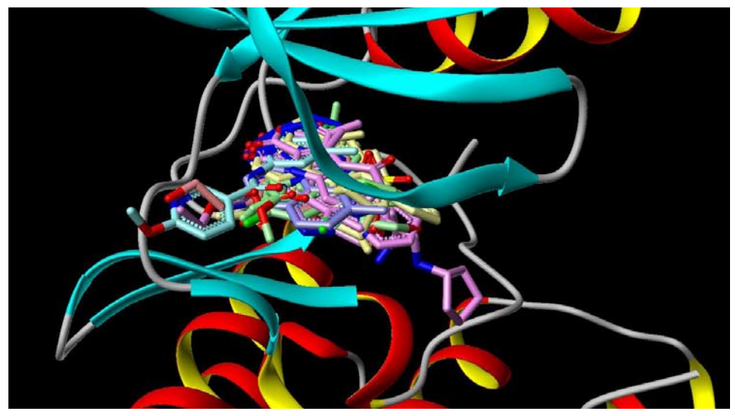
The high solubility criterion used in library selection allowed use of wide concentration ranges.

This gave good agreement between fitted R_{max} and predetermined R_{max}.

MicroCal™ ITC confirmed **80%** of the hits.

X-ray crystallography

- 48 high quality fragments were selected for crystallization based on chemical diversity, ligand efficiency and affinity
- For 41 fragments X-ray structures were solved
- **X-Ray success rate > 85%**
- Affinity range 43 µM to > 3 mM, resolution 2.0 to 2.9 Å



Overlay of 41 fragment structures in the active site of K1 showing various chemical subtypes including unusual pharmacophores.

Conclusions

- **Biacore 4000 provides an efficient and informative fragment screening methodology**
- **Provides throughput adequate for screen campaigns of 1000 to 10 000 fragments including affinity ranking, binding site identification and competition experiments**
- **Target consumption is very low (~24 µg protein/2000 fragments)**
- **Clean Screen meets the challenge of high sample concentrations by rapidly identifying aggregative and sticky binders**
- **Binding Level Screen meets the challenge of low affinity fragments by high assay sensitivity and efficient hit selection tools**
- **Affinity Screen meets the challenges of suboptimal sample concentrations, and secondary multi-site binding by using predetermined R_{max} and the multi-site model**

imagination at work

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