Target-based drug discovery and low-binding affinities using Biacore™ 8K

GE Healthcare

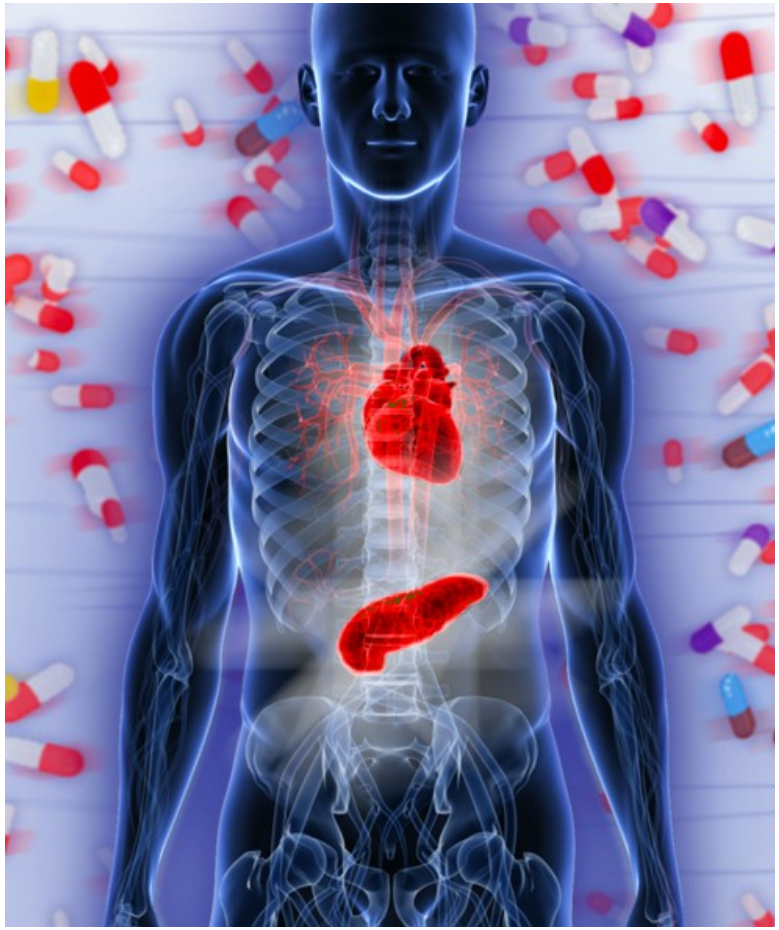
October 2016

Imagination at work

Drug discovery trends

Fragment-based approaches widely established

- Moving towards new targets
- Use of traditional targets in novel ways
- Development of small-molecule inhibitors against protein-protein interactions



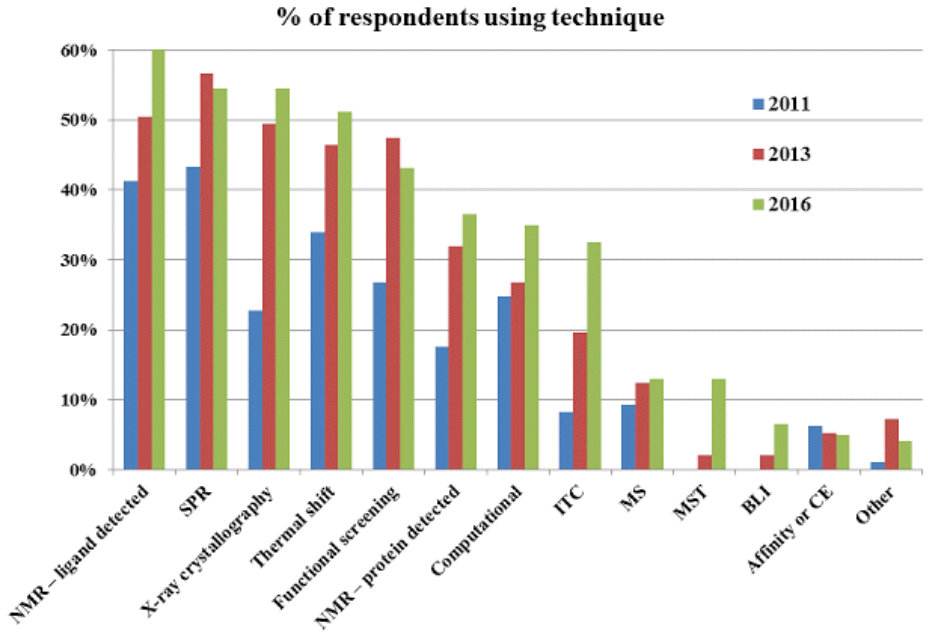
Use multiple techniques to cross-correlate results for increased reliability and confidence

Number of enabling techniques for FBDD increases

No single technology provides all the answers

Label-free SPR one of the most used techniques

- Increased sensitivity, robustness, throughput



Permission to use from D. Erlandson

Biacore™ 8K — It’s a hit. For sure

Biacore™ 8K – a single solution for interaction analysis in both screening and characterization

Increase operational efficiency

- Eight separate channels with two flow cells each
- Capacity for four 96- or 384-well microplates
- Queuing capability of methods, cleaning procedures, temperature changes etc.

Get started in minutes and obtain quality results

- Flexible control software backed up with intuitive evaluation software

Ensure the integrity of samples in long runs

- Temperature controlled analysis and sample storage, 4°C to 40°C

Biacore™ 8K developed together with users

Six external collaborators participated in the project

-involved to test and contribute to development

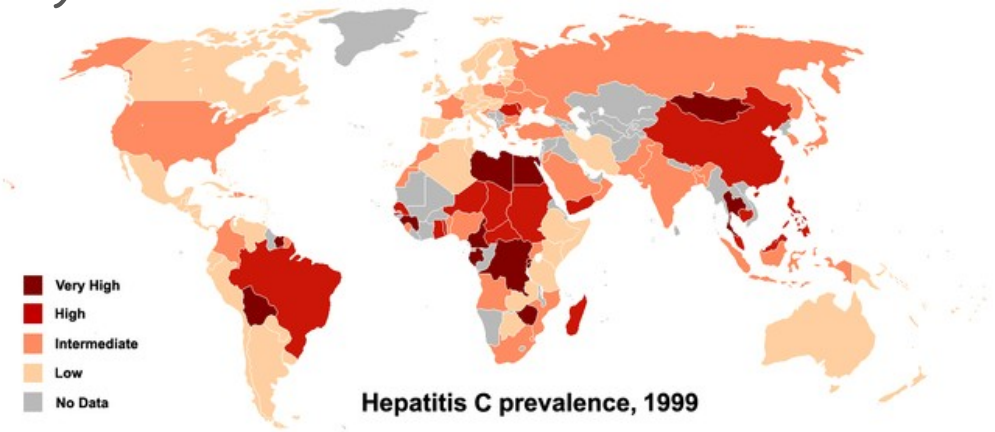
	Application examples
GPCR characterization	*-capture from crude membrane preps*
Antibody screening	*-differentiation of high-affinity binders*
Antibody characterization	*-kinetics with minimized avidity effects*
LMW screening	*-inhibition of protein-protein interaction*
Fragment screening	*-against solubilized GPCR target* *-identification of allosteric site binders*

Fragment screening on Biacore™ 8K against NS5B 1b, a Hepatitis C drug target

Collaboration with Prof. H. Danielsson at Uppsala University, Sweden

Hepatitis C virus (HCV)—an important drug target

- Causes acute or chronic liver disease
- Globally, around 150 million people chronically infected
- Estimated 700 000 people die each year
- No vaccine against HCV
- Current antiviral drugs have ~ 90% treatment success
  - ➢ but problems with drug resistance



Geographic distribution of Hepatitis C prevalence, 1999. Source: WHO –Guide: Hepatitis C, 2002. Author: PhilipN. This image is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license

Need for new drugs against HCV

Search for novel allosteric binders

Target

- NS5B, a RNA polymerase that plays a fundamental role in replication of Hepatitis C virus

Purpose

- Identify fragments that bind the *allosteric Thumb pocket II site of NS5B 1b*

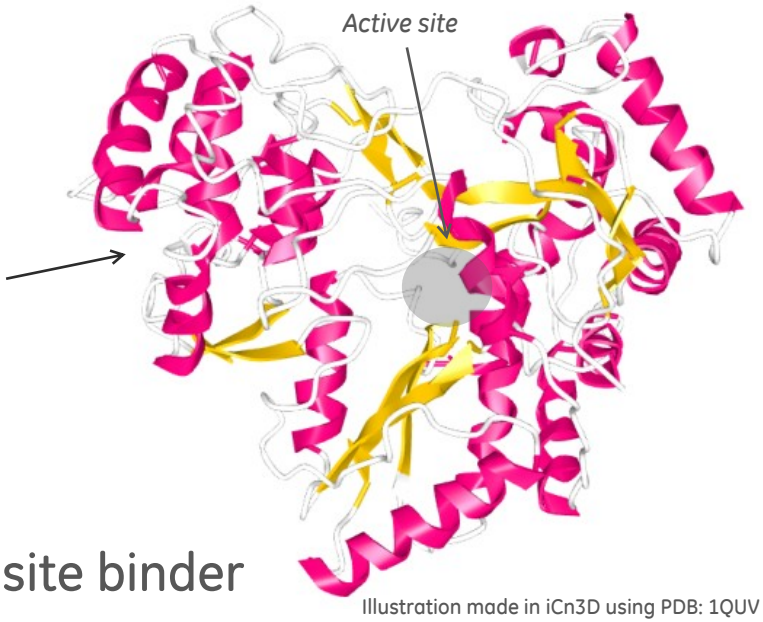


Illustration made in iCn3D using PDB: 1QUV

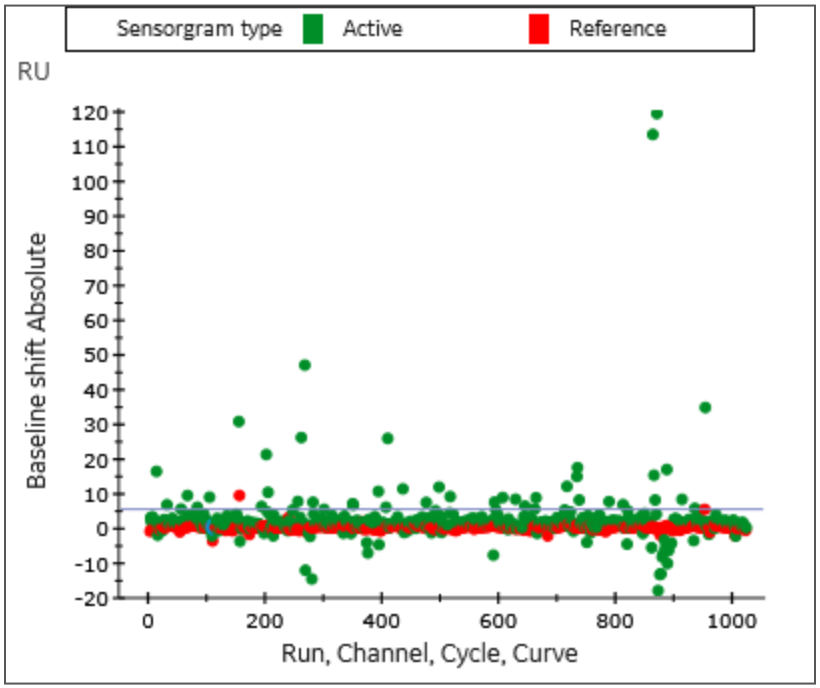
Assay setup

- Immobilization of recombinant NS5B 1b
- Positive control: Filibuvir, known Thumb II site binder
- Fragment screening of Maybridge 500 Ro3 library

Rapid assessment of fragment library qualities using Biacore™ 8K *Fast Injection*:

Screen for promiscous fragments performed at 1 mM against target protein and reference surface

Out of the 500, 44 fragments were regarded sticky and omitted from subsequent screens



500 fragments checked for stickiness against target/reference surface in 1 h

Screen for binders against target performed in a single run with minimized systematic effects

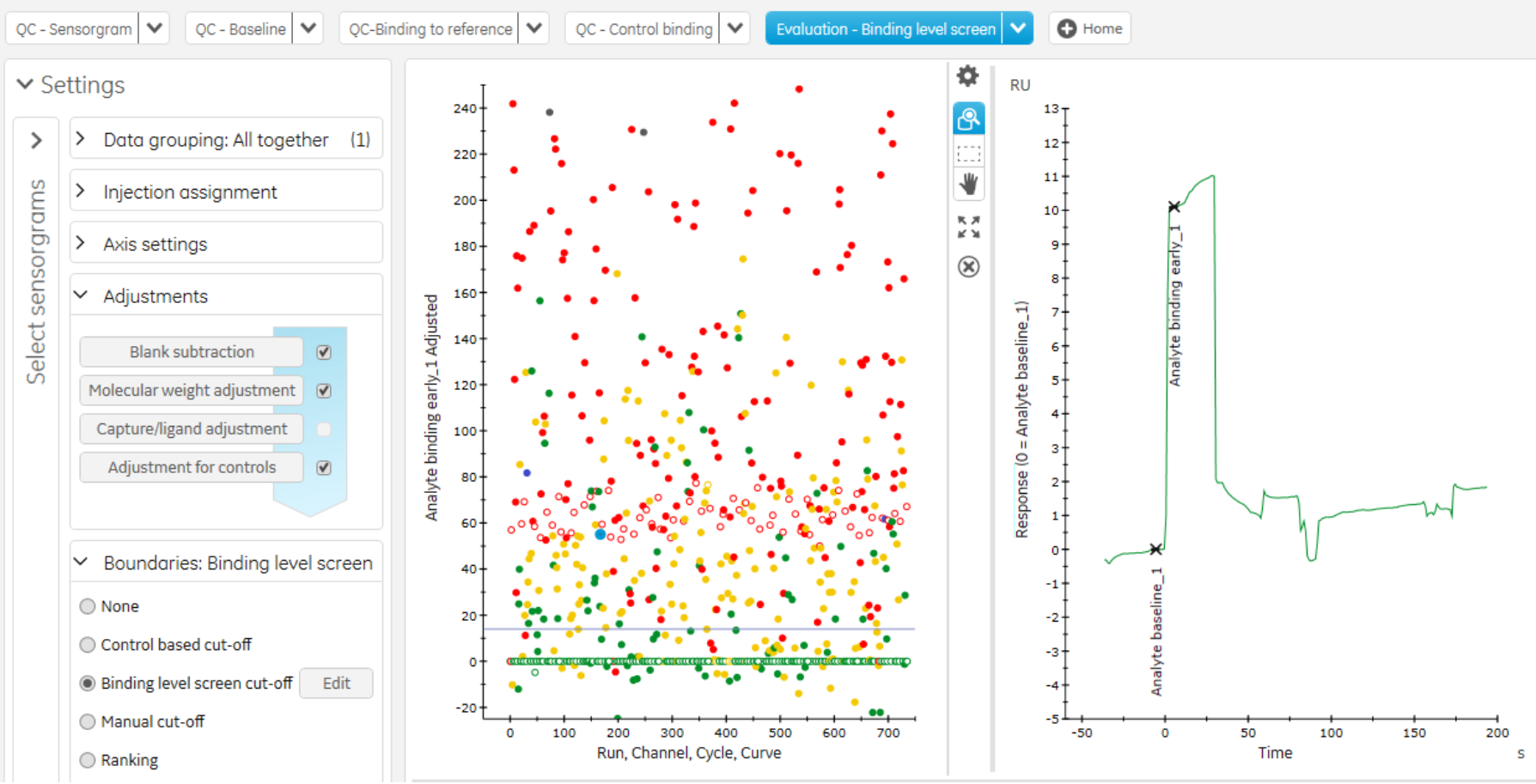
Positive controls

Fragments at 1 mM

Negative controls

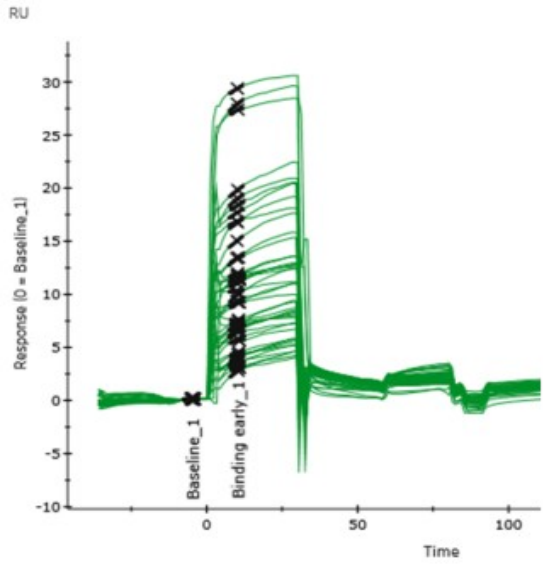
New SW functionality allows controls to be defined and run with samples

Evaluation of binding level screen data simplified using predefined evaluation methods

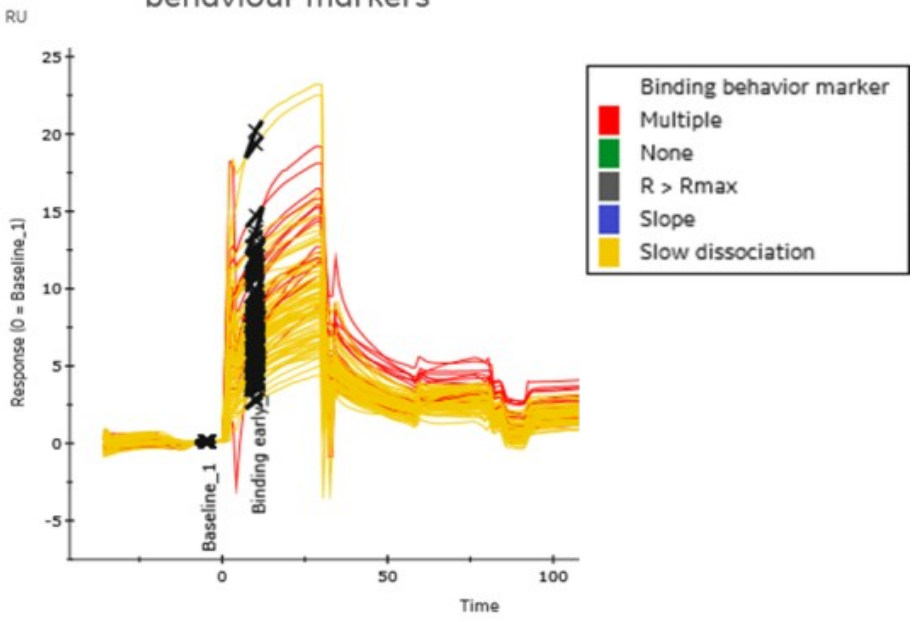


Analysis of sensorgram shape provides deeper insights into binder characteristics

Above cut-off without binding behaviour markers



Above cut-off with binding behaviour markers



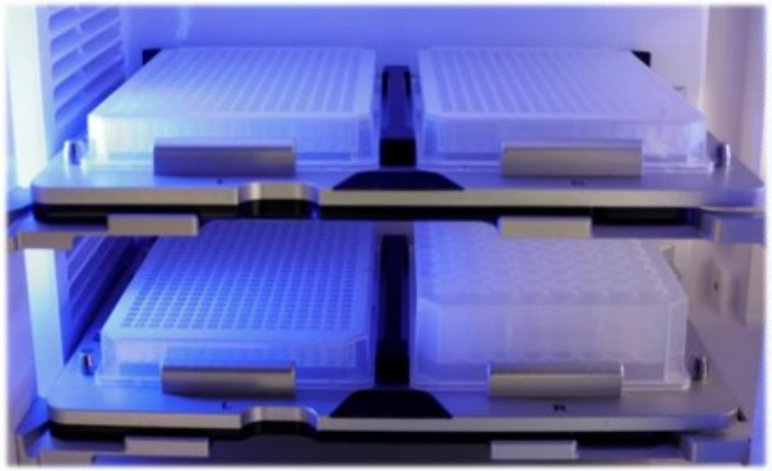
Binding level screen of libraray performed in one run in 8 h 25 min.
48 fragments identified with well-behaved binding characteristics

Concentration series of fragments easily set up in 96-well plates utilizing the hotel functionality

Steady-state analysis of the 48 selected fragment against NS5B 1b, the Hepatitis C drug target, were performed in 2 runs

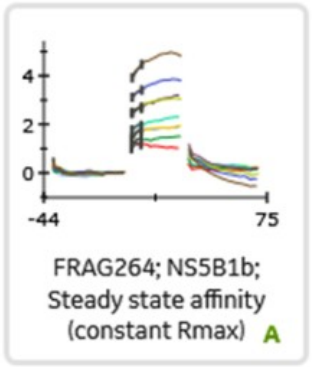
Each fragment analyzed at nine concentrations (39 to 1000 µM)

Time per run: 5 h 8 min

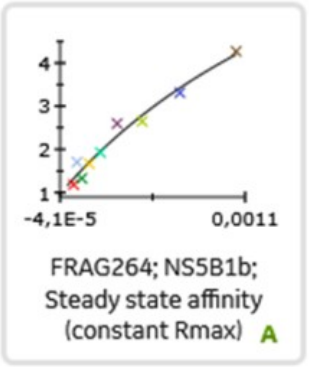


Hotel capacity:
Four 96- or 384-well microplates
Plates can be loaded during run

Improved reliability of steady state results using constant R_{max} during data fitting



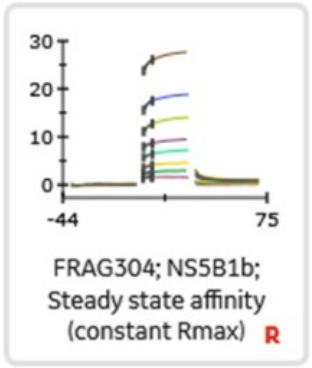
FRAG264; NS5B1b; Steady state affinity (constant Rmax) **A**



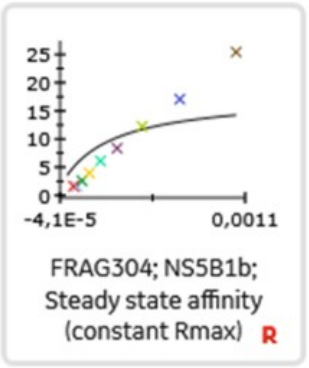
FRAG264; NS5B1b; Steady state affinity (constant Rmax) **A**

Example of fragment with good fit following steaty state analysis using constant Rmax

K_D= 2.2 mM



FRAG304; NS5B1b; Steady state affinity (constant Rmax) **R**

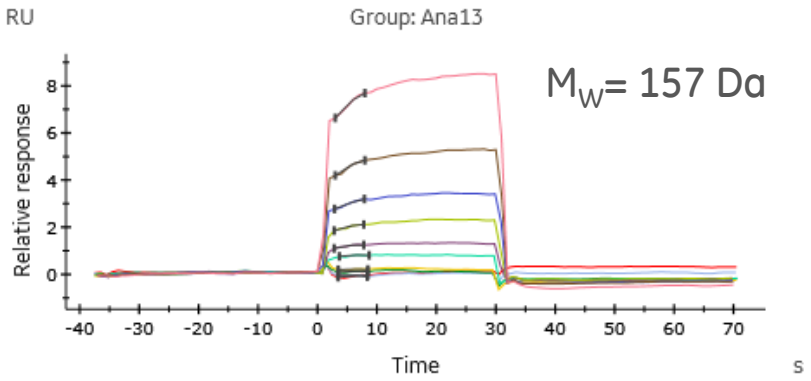


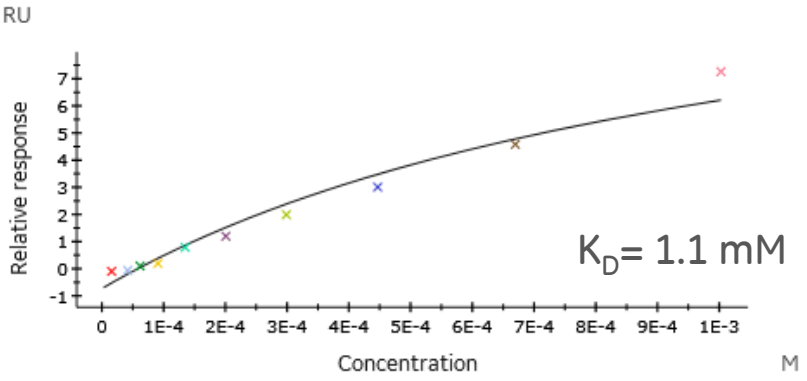
FRAG304; NS5B1b; Steady state affinity (constant Rmax) **R**

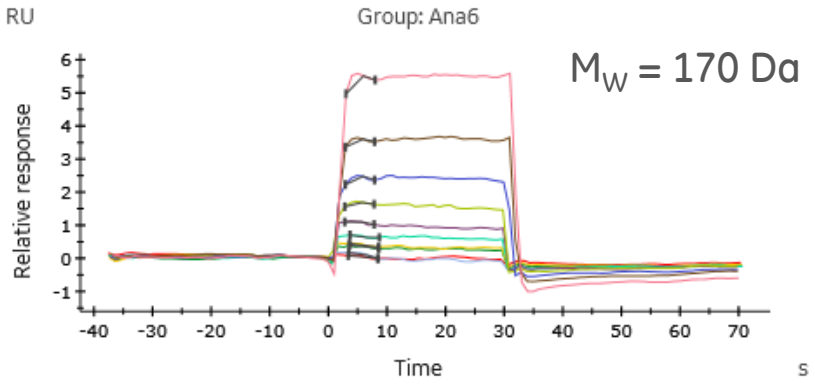
Example of fragment with superstoichiometric data detected by poor fit with constant Rmax.

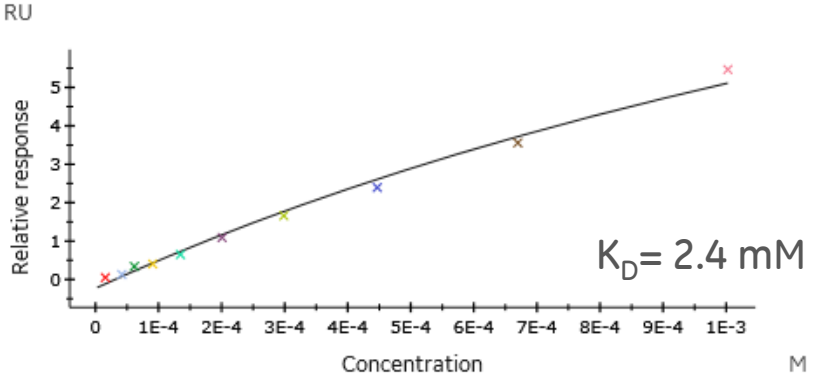
16 fragments with affinities between 0.5 to 5.8 mM and with good fit revealing no interfering secondary interactions identified

High sensitivity allows for analysis of small, weakly bound fragment analogs









Promising Structural Activity Relationship (SAR) study initiated

LMW screening on Biacore™ 8K against a PPI target

Collaboration with Dr Satoru Nagatoishi and Prof Kouhei Tsumoto, University of Tokyo, Japan

Inhibition of protein-protein interactions

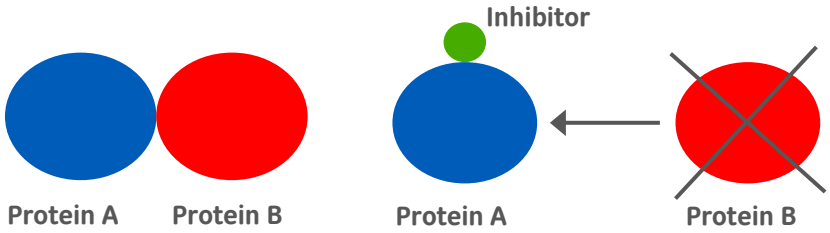
Hot research area in drug discovery

The number of interesting targets are increasing by the day

Challenges

- Identification of how to inhibit the specific PPI
- Large interaction areas
- Problems with specificity and low affinity

Advanced instrumentation and intuitive, dedicated software needed for efficient screening and confident selection of candidates.



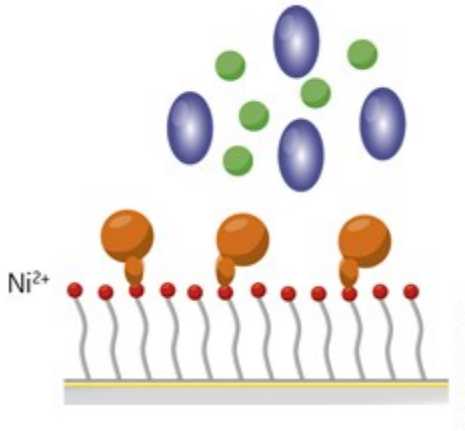
Screening of LMW compounds for inhibition of a protein-protein interaction

Purpose

- Find suitable candidate for inhibition of the IDP X - Protein Y interaction

Assay setup

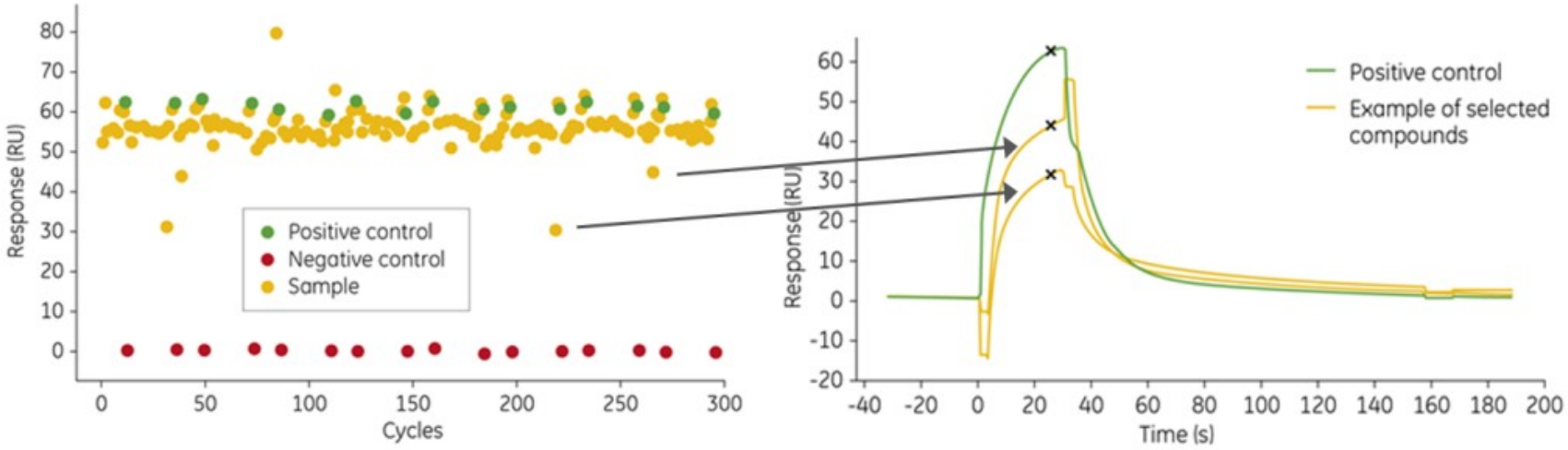
- Capture of IDP X on Sensor Chip NTA
- Protein Y is mixed 1:1 with LMW compound and injected over IDP X
- Positive control: Protein Y mixed 1:1 with buffer



Output

- Responses lower than the control response indicates inhibition

Rapid PPI inhibition screening using Biacore™ 8K



352 compounds screened in 3.5 h.
Built-in evaluation tools applied for confident selection of hits

Acknowledgements

Prof. Helena Danielsson at Uppsala University, Sweden

Dr Satoru Nagatoishi and Prof. Kouhei Tsumoto, University of Tokyo, Japan

We acknowledge all customers that kindly participated in prototype evaluations of Biacore 8K, thereby providing us with invaluable feedback on hardware, software, and user experience as well as data from numerous interesting applications that demonstrates the versatility of Biacore 8K.

Thank you all!

Biacore™ 8K

Discover more, more efficiently

Sensitivity - Meeting the toughest challenges in small molecule and biotherapeutic screening and characterization

Speed - Eight-channel parallel setup that boosts efficiency regardless of the number of samples

Interactivity and ease of use - Get started in minutes and obtain quality results in a single click for rapid decision-making

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GE Healthcare Bio-Sciences Corp.
100 Results Way
Marlborough, MA 01752
USA