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The search for novel allosteric drug leads: fragment screening against the NS5B 1b Hepatitis C drug target using SPR

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Introduction

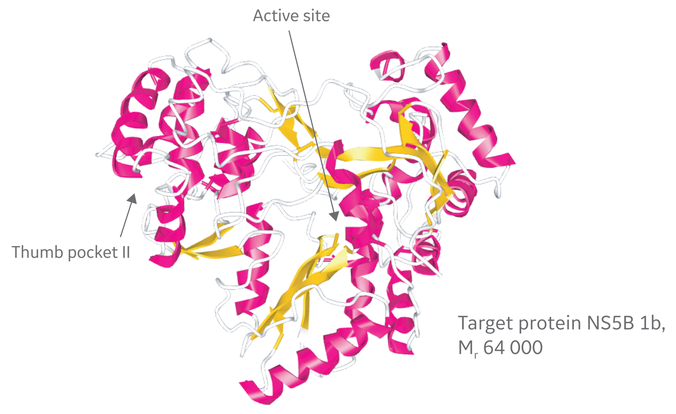
The intense effort to develop drugs against Hepatitis C virus (HCV) has been successful with several antivirals now available for patients. However, the current drugs are not effective against all genotypic variants of the virus and resistance against drugs in clinical use will ultimately emerge. Therefore, the search for new drugs is a constant mission.

In collaboration with Uppsala University, Sweden, we conducted a fragment-based screening study against NS5B, a viral polymerase that plays a fundamental role in the replication of Hepatitis C virus. We selected NS5B genotype 1b, a globally prevalent variant of HCV, with the overall aim to prospect for novel allosteric drug leads.

This poster outlines the fragment screening workflow conducted on Biacore™ 8K surface plasmon resonance (SPR) system and how challenges with target instability encountered during assay development were overcome by maximizing the throughput of the instrument.

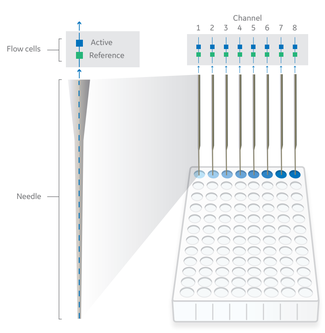
Experimental setup

Purpose: identify fragments that bind to the allosteric Thumb pocket II site of NS5B 1b.



- Biacore 8K was used for assay development and screening.

- Prior to each run, recombinant NS5B 1b was amine coupled (~10 000 RU) to Series S Sensor Chip CM5.
- Immobilization in all eight active flow cells enabled high throughput with high-quality reference-subtracted data from each channel.
- 500 fragments of the Maybridge Ro3 library (Maybridge) were screened in 2% dimethyl sulfoxide (DMSO) buffer.



- Positive control Filibuvir, a known Thumb pocket II site binder, was used as positive control, allowing estimation of R_{max} and monitoring of surface binding capacity over time.

Acknowledgment

We wish to thank the group of Prof. Helena Danielson at Uppsala University, Sweden, for a fruitful collaboration and for providing the target protein.

Reference

1. Application note: Biacore 8K. GE Healthcare, 29245694 Edition AA (2017).

Fragment screening workflow on Biacore 8K SPR system

A three step-analysis approach was undertaken.

	Experiment	Purpose	Inject type	Contact time (s)	Dissociation time (s)	Including controls, SC*, 50% DMSO wash	Fragment concentration (mM)
1.	*Clean screen*	Library curation	Fast injection	10	0	No	1.0
2.	*Binding level screen*	Identification of binders	High performance	30	12	Yes	1.0
3.	*Affinity screen*	Validation of binders	High performance	30	30	Yes	0.013 to 1.0

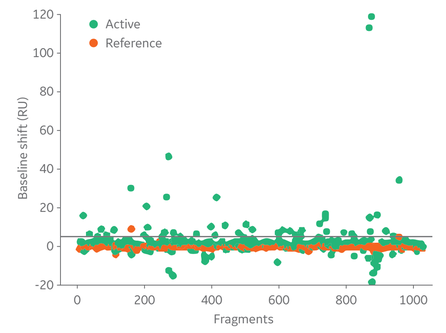
*SC = Solvent correction

Results

1. Clean screen

500 fragments checked for stickiness against the sensor chip surface in 1 h.

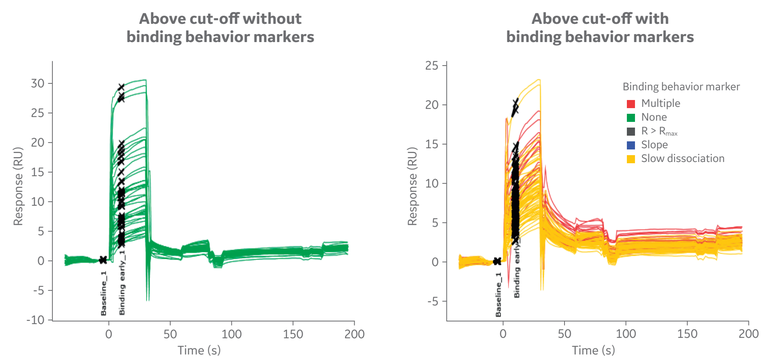
- 43 fragments found to be sticky against the immobilized NS5B 1b target (active flow cell).
- Only one fragment found to be sticky against the unmodified surface (reference flow cell)
- Thus, 44 fragments omitted for the subsequent screens.



2. Binding level screen

456 fragments screened in one run in 8 h 25 min.

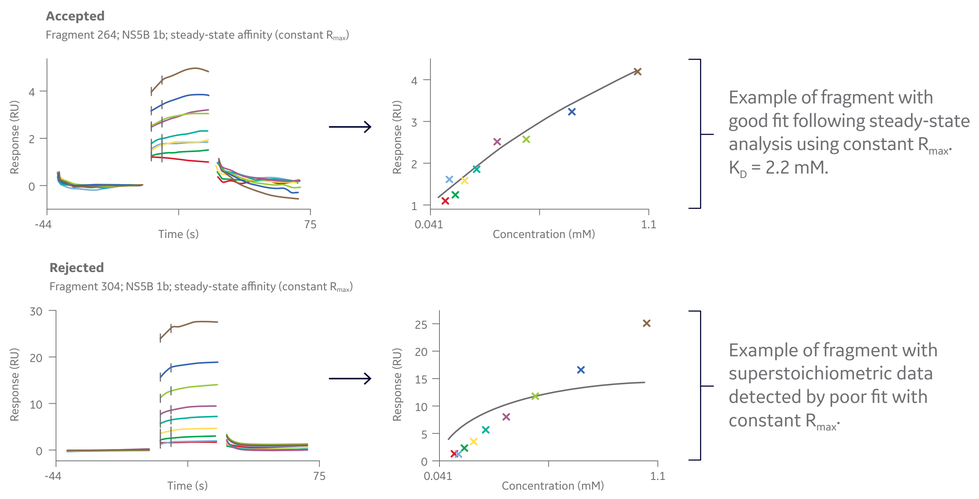
- Automatic analysis of sensorgram shape provided deeper insights into binder characteristics.
- 48 fragments identified with well-behaved binding characteristics (green). These were taken forward to the next screen.



3. Affinity screen

Steady-state analysis of the 48 selected fragment against NS5B 1b were performed in two runs of 5 h 8 min each.

- Concentration series setup in 96-well plates using the hotel functionality of Biacore 8K.
- Each fragment analyzed at nine concentrations.
- 16 fragments with affinities between 0.5 and 5.8 mM and good fits were identified. No interfering secondary interactions were identified.



Conclusions

- A complete fragment screen workflow was conducted on Biacore 8K SPR system against the Hepatic C target NS5B 1b.
- The novel features of Biacore 8K enabled fast screening procedures and reduced risk of artifacts resulting from limited protein stability encountered during assay development.
- The total run time for screening of 500 fragments in three analysis steps was 20 h.
- The screening identified 16 well-behaved fragments with affinities in the low millimolar range. Follow-up experiments have demonstrated that six of these fragments compete with filibuvir for binding, indicating that they might be allosteric site binders. These fragments have since been the focus of Structure Activity Relationship (SAR) studies using hit analogs.

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