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Fragment Library Screening Using the FórteBio Octet Red

Shirley Li*, David Mark*, Yingsi Chen*, Kuo-sen Huang*, Charles Wartchow+, Frank Podlaski*

*Discovery Technologies, Hoffman La Roche, Nutley, NJ , +FórteBio, Menlo Park, CA

Abstract

We describe, here, the first use of the FórteBio Octet Red and its label-free biolayer interferometry technology for successfully completing a fragment library screening campaign for a protein-protein interaction target. The unique features of the system allowed for rapid screening against immobilized target, minimal protein degradation, and the sensitivity to detect the binding of small molecules in the 200 MW range. Compounds were screened at a single concentration, followed by three point dose response measurements against the primary and a secondary protein target. Parallel biochemical functional assays were also run to gather supporting data that hits were targeting the correct binding sites of the proteins. Evaluation of all data allowed the selection of prospective candidates for more critical characterization by full dose response on Biacore and lower throughput technologies such as NMR.

The Octet Red offered fast method development, sufficient throughput (>300 cpds/day), and low target protein consumption. The use of disposable sensors minimized problems associated with target degradation and the non-fluidic dip and read configuration minimized typical problems associated with insoluble compounds, yet allowed for profiling of partially precipitated samples.

Introduction

We had previously evaluated and established that the Octet Red system can produce comparable data to the Biacore for hit validation with our general Pharma library of compounds 300-500 daltons (presented as a tutorial at the SBS Label-Free Technologies in Drug Discovery Symposium June 10-11, 2008, Dresden, Germany). We now extend this evaluation to include screening of our fragment library for compounds sized <300 daltons.

Materials and Methods

❖Biotinylated target protein

In vivo biotinylated target protein was generated in E.Coli by co-expression of a biotin-tag (GLNDIFEAQKIEWR) appended target with E.Coli biotin ligase (BirA), resulting in protein that is modified specifically at the Lysine residue in the tag (underlined). The biotinylation group was used for both protein purification and biosensor capture.

❖Preparation of Super Streptavidin (SSA) Biosensors for Target Capture

Biotinylated target at 1µM was incubated offline with SSA biosensors overnight at 4˚C. This protocol yielded binding activities similar to those obtained with saturated target densities shown with online capture.

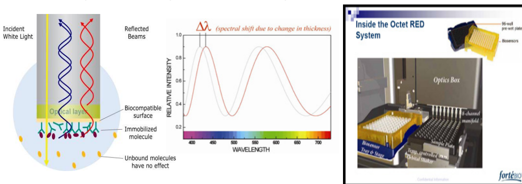
Reference SSA (no protein) was blocked with biocytin at 10µg/ml overnight at 4˚C.

❖Biochemical Assay

Three point dose responses against two protein targets were run using a standard TR-FRET format to monitor the binding of biotinylated peptide substrates to His6 tagged proteins. Energy transfer between the Europium streptavidin donor and APC-antiHis6 acceptor was quantified using an Envision reader (615/665nm).

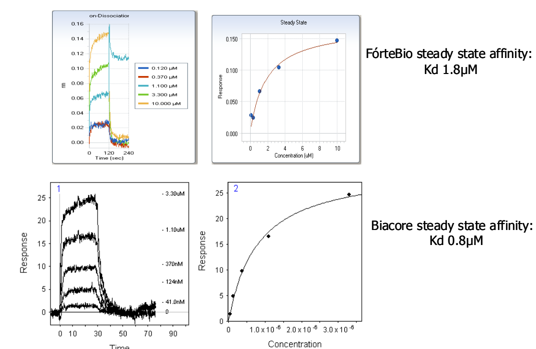
BioLayer Interferometry

The FortéBio Octet instrument platforms detect the binding of molecules to the tip of a disposable fiber-optic biosensor with biolayer interferometry (BLI). This technique generates an interference pattern by monitoring visible light reflected from two surfaces within the fiber-optic biosensor. When binding events occur at the tip of the biosensor in solution, the “optical thickness” of this layer changes, and the interference pattern shifts to higher wavelengths. These changes are monitored in real time.

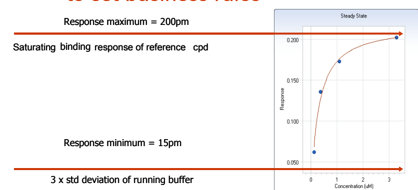


Results

Identification of reference compound by FórteBio Octet Red and confirmation by Biacore

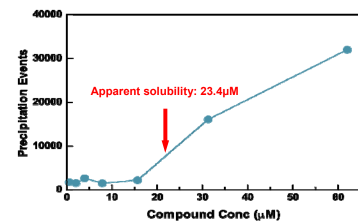


Response window of reference compound used to set business rules



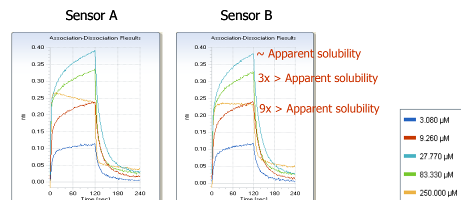
Setting the screening concentration: Consequences of compound precipitation?

- Apparent solubility of Cpd X determined by nephrometry (BD Solubility Scanner)
- Tested in FórteBio buffer and within FórteBio assay time frames



Binding of partially precipitated compound can be detected

- Results are reproducible and correlate with solubility measurements
- Non-fluidic configuration of system eliminates clogging problems



Fragment Screening Platemap
68 Unknowns + 2 Reference Compounds

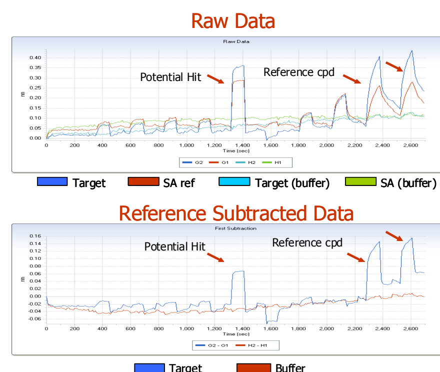
Column 1 = Equilibration and baseline for entire compound row
Column 2 = Dissociation for entire compound row

Each compound screened at a single concentration (100 µM)

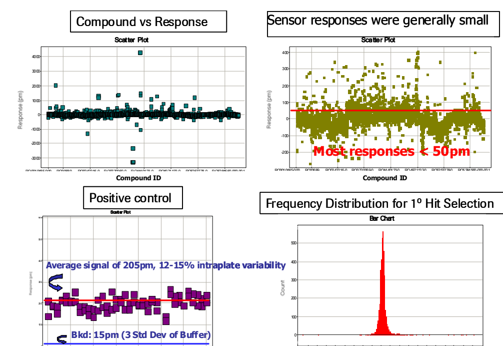
Average throughput : 1.75-2 hrs per plate; 4-5 plates per day (272-340 cpds)

Maximum throughput: 420 cpds/day

Turnaround time between plates: 5-10 minutes



HTS Statistics



Confirmation Assays: 21 Compound Platemap

Reference cpd

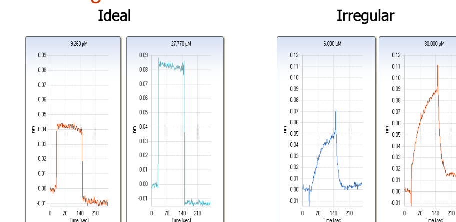
Conc 1: 33 µM Conc 2: 100 µM Conc 3: 300 µM

§ Rule 1: Equilibrium binding signal (Read 1) is between 15-200 pm. The lower limit represents 3 standard deviations of the buffer signal. The upper limit was set at 200 pm based on the maximum experimental response generated by a reference compound

§ Rule 2: Dissociation response (Read 2) returns to a value of <50pm

§ Rule 3: Binding responses: Conc 1 < Conc 2 *and/or* Conc 2 < Conc 3

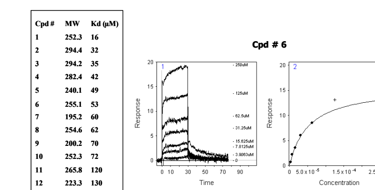
Sensorgram evaluation as final selection criteria



Biochemical Functional Assays

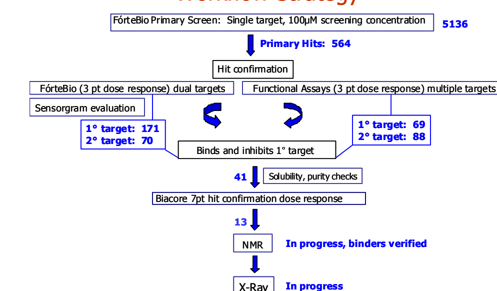
❖ Assays were run to provide supporting data that the fragments were binding to the correct sites of the target protein. A cut-off of ~50% inhibition (primary target only), at the maximum concentration tested, was assigned to identify candidates for further characterization.

Biacore identification of true stoichiometric binders



Analysis performed on the Biacore S51 utilizing the same biotinylated target captured on a SA sensor chip and evaluated with Scrubber (BioLogic Software)

Workflow Strategy



Conclusions

We have successfully identified fragment compounds for a protein-protein interaction target using the Octet Red.

❖Octet Red’s dip and read configuration allows quick assay development
❖Sensitivity is enough to detect the binding of 200 dalton fragments
❖Has sufficient throughput (272-340 cpds/day)
❖Does not consume target protein
❖Use of disposable sensors maintains integrity of target protein
❖Able to assay precipitated samples without the complications connected with a fluidic system