Small Molecule Fragment Screening on the Octet Platform

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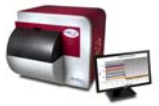

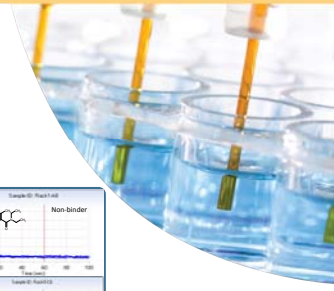

INTRODUCTION

The Octet RED384 instrument uses biolayer interferometry (BLI) to measure molecular interactions and is well-suited for label-free screening of small molecule fragments. It is a 16-channel robot-friendly instrument that is compatible with 384- and 96-well plates, is capable of screening thousands of compounds per day by measuring the interaction of a compound with protein target that is attached to the tips of biosensors. This instrument measures interactions directly in a microtiter plate without the use of microfluidics, and generates binding profiles, responses, and kinetic constants that correlate with SPR including k_{on}, k_{off}, and K_D.

BIO-LAYER INTERFEROMETRY

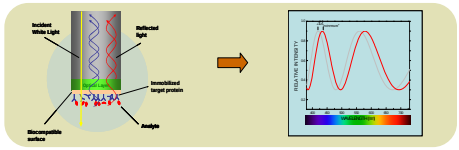

Figure 1. Bio-Layer Interferometry illustration.

- A layer of molecules attached to the tip of an optic fiber creates an interference pattern at the detector
- Any change in the number of molecules bound causes a measured shift in the pattern

METHODS

Materials: The Maybridge library consists of 500 compounds (94-345 Da) with solubility > 1mM and was obtained through Fisher Scientific. The compounds were diluted to 100 µM in a 96-well polypropylene microtiter plate in 200 µl of PBS (with 5%DMSO). The compounds were transferred to a 384-well black, polypropylene Greiner plate for analysis.

Biosensors: Biotinylated carbonic anhydrase or biotinylated streptavidin (reference protein blocked with biotin) were loaded onto Super Streptavidin Biosensors (ForteBio Inc.) at 50 µg/mL in PBS to give loading responses of 10 and 4 nm, respectively.

Analysis: Rows A and B of the sample plate contained assay buffer for drift corrections, and Rows C-P contained samples or controls. The sample plate was loaded onto station one, and a second plate containing assay buffer was placed in station two. The second plate is used for baselines and dissociations, and association data is taken in the sample plate. Reference biosensors (N = 16) were equilibrated in station two for 15 min. Baseline, association, and dissociation data were taken with durations of 30, 30, and 60 seconds, respectively. The target sensors (N = 16) containing biotinylated carbonic anhydrase were subsequently run using the same protocol. The reference data set is subtracted from the target data set to correct for systematic optical artifacts, and the drift is corrected by subtracting the data sets for sensors in column A, which collect data in buffer only, in parallel to acquisition of sample data. The cut off for identifying a "hit" is determined by running a separate experiment with assay buffer or PBS + 5% DMSO prepared using the same dilutions as those performed for samples. Both of these negative controls give similar results, and the cut off is equal to the average + 3SD (3.4 + (3 x 6.4) = 23 pm, N = 336).

REFERENCES

- "Fragment-based Screening Using Surface Plasmon Resonance Technology" S. Perspicace, D. Banner, J Benz, F Müller, D Schlatter, W Huber *Journal of Biomolecular Screening* 14(4), 337-349, 2009.
- "Fragment-based Discovery of Hepatitis C Virus NS5b RNA Inhibitors" S Antonysamy et al *Bioorganic and Medicinal Chemistry Letters* 18(9), 2990-5, 2008.
- Label-free Primary Screening and Affinity Ranking of Fragment Libraries Using Parallel Analysis of Protein Panels *Journal of Biomolecular Screening* 13(3), 202-209, 2009.

TYPICAL ANALYSIS

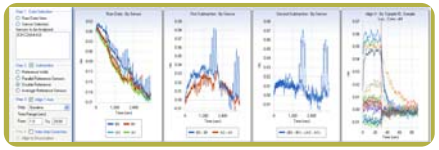

Figure 2. (A) Raw data for reference and target biosensors (B) Data corrected for optical artifacts (C) Data corrected for drift (D) Sensorgrams showing association and dissociation for controls and samples (E) Each biosensor analyzes 24 wells in this experiment in series.

RESULTS

- Analysis of negative control (PBS+5% DMSO)
  - Average response for association is 3.4 ± 6.4 pm (N = 336)
  - Hits have responses greater than 3.4 + 3(6.4) = 23 pm
- All 500 compounds were successfully analyzed on two 384-well plates. Each run is ~3h per plate.
- Ten compounds have responses > 23 pm
- Follow-up characterization with a 2X or 3X dilution series confirms that 5/10 compounds bind to carbonic anhydrase in a specific and concentration-dependent manner, including three sulfonamides, which are known inhibitors of carbonic anhydrase.
- Two compounds were aggregators, and three compounds did not show a concentration dependent response from 1 - 1000 µM.

MAYBRIDGE Ro3 FRAGMENT LIBRARY ANALYSIS

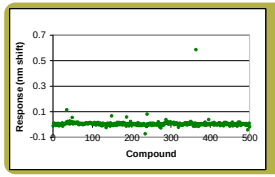

Figure 3. Analysis of the Maybridge Ro3 500 Fragment Library with Carbonic Anhydrase

CHARACTERIZATION OF HITS

Entry	Description	ID	Structure	Name
1	Non-binding	E6		3-(2-hydroxyphenyl)propanoic acid (166 Da)
2	Non-binding	A8		N2,N2-diethyl-3,4,5-trimethyl-1H-pyrrole-2-carboxamide (208 Da)
3	Hit	D10		9-oxo-9H-fluorene-4-carboxamide (223 Da)
4	Hit	F5		3,5-dichlorobenzene-1-sulfonamide (226 Da)
5	Hit	H5		6-methyl-2,3,4,5-tetrahydropyridazin-3-one (112 Da)
6	Hit	1-C6		2,5-dichlorothiophene-3-sulfonamide (232 Da)
7	Non-specific aggregator	G8		3-pyridin-3-ylaniline (170 Da)
8	Hit	H3		3-chloro-5-fluoro-2-methylbenzene-Sulfonamide (223 Da)
9	Non-specific aggregator	5-C6		6,7,8,9-Tetrahydro-5h-carbazole-3-carboxylic acid (215 Da)
10	Non-binding	G3		pyridin-4-amine (94 Da)

Table 1. List of hit compounds and their physical structures.

HIT VALIDATION: GLOBAL FITTING

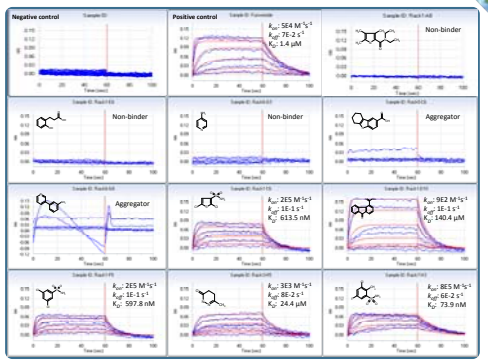

Figure 4. Hit Validation data. Note that the compound corresponding Sample ID: Rack 1-D10 from another vendor did not show a response and may indicate a contamination in the Maybridge compound library.

HIT VALIDATION: STEADY-STATE ANALYSIS

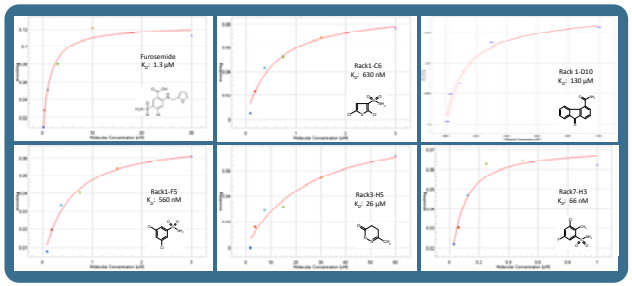

Figure 5. Steady-state analysis to follow up on the kinetic analysis showed good correlation of the affinity constant measured by both methods.

DISCUSSION

Screening the Maybridge Ro3 500 fragment library at 100 µM with the Octet RED384 instrument resulted in the identification of 10 compounds with responses higher than negative controls, and 3 compounds showed responses <0.02 nm as a result of preferential binding to the reference sensor. This screen was completed with two 384-well plates in two separate runs using ~6 hours of run time for the entire screen of 500 compounds at 100 µM in PBS containing 5% DMSO. The positive responses were characterized with a 2X or 3X dilution series; five compounds showed behavior consistent with a 1:1 interaction with carbonic anhydrase, two compounds were aggregators at 1 mM, and three compounds did not show a response from 1-1000 µM. Non-specific binding of the compounds was not an issue in these studies since 487/500 compounds did not bind to the sensors at 100 µM.