

B209 Rapid Fragment Screening with a Robotically-Automated ForteBio Octet RED384

Frank Podlaski, Charles Warchow, Lin Gao, Shirley Li, Sean Walker, Sevan Ibabekci, Kuo-Sen Huang, David Mark
Discovery Technologies, Roche, Inc., 340 Kingsland Street, Nutley, NJ 07110



Abstract

We report the use of ForteBio's label-free Octet RED384 instrument in a fragment screening campaign with a kinase that contains a known allosteric binding site. These studies were achieved by integrating the RED384 instrument with robotics and a liquid dispensing station. System validation, including well-to-well and plate-to-plate precision, accuracy, and instrument noise, was established with two targets: the kinase and a 238 Dalton fragment that binds to the ATP binding site, and a second target protein with a 500 Dalton reference compound. After validating the automated system, a fragment screen was performed against the kinase target with a throughput of ~100 compounds per hour.

Introduction

Biosensor-based fragment screening is an emerging tool in the drug discovery process. Several screens with proteases have been reported for SPR biosensors and strategies for identifying primary hits include specificity studies with mutant proteins, unrelated proteins, mathematical models that consider ligand efficiency, and upper limits based on receptor binding capacity and binding stoichiometry. Proteins that contain allosteric binding sites pose a unique challenge for label-free analytical methods because compounds potentially bind to one or more sites.

Small molecule detection with biolayer interferometry (BLI) is generally comparable to SPR methods, and the kinetic constants obtained with BLI correlate well with SPR methods for molecules as small as 150-200 Daltons. In one study, fragment screening with BLI was demonstrated with 5000 compounds with the Octet RED instrument, which is compatible with 96-well plates. In this study, hits were confirmed with the Biacore S51 instrument, which has ~5X better S/N.

The RED384 instrument is a higher-throughput, 16-channel instrument that is compatible with 384-well plates. This instrument was integrated with a Hudson Plate Crane and a Multidrop dispenser for plate handling and buffer dispensing, respectively. The integrated system is capable of analyzing ~100 compounds per hour.

Materials and Methods

Sensor Preparation Target proteins were biotinylated *in vivo* with the biotin ligase recognition sequence GLNDIFEAQKIEWR. Superstreptavidin biosensors (ForteBio Inc) were placed in a solution of 1 μM protein and incubated for a minimum of six hours at 4°C. Sensors with target protein were blocked with biocytin, and sensor function was verified with a control compound.

Sample Plate Preparation Sample plates containing (+) and (-) controls and compounds were prepared by adding 6μL of DMSO solution. Dilutions with buffer were performed using the Multidrop dispenser and the final DMSO concentration was 5%. For the fragment screen, the (+) control was 20μM, which is below the KD.

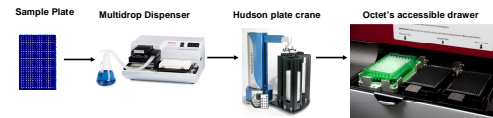
Accuracy and Precision Studies Accuracy for the ForteBio RED384 instrument was established with a 6-point dilution series of a 501 Dalton compound with a protein-protein interaction target, and also with a 238 Dalton compound with a kinase target with an allosteric binding site. Precision was determined at three concentrations for each compound with the respective protein target.

Automated fragment analysis on the ForteBio RED384 instrument A typical automated run occurs as follows: sample plates containing 6μL of sample in DMSO are placed in a plate tower, and a plate is transferred to the Multidrop station with the Hudson Crane. Buffer is added to the plate, and the plate is transferred to the instrument for analysis using 16 sensors with target protein and 16 reference sensors, run serially. The fragment concentration was 200μM. After analysis, the plate is removed, and the cycle is repeated with the same sensor set. A typical fragment screening cycle involves 3-5 plates that are analyzed with a single sensor set, with a maximum throughput of 8 plates per day. The fragment screen was completed in seven days with 47 plates.

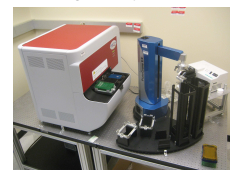
Data Analysis Double reference subtraction was performed with ForteBio data analysis software. This method removes drift and reduces well-to-well variability. Hits were established by measuring a plate of buffer, and a lower limit of 30pm was chosen.

Automation of the Octet RED384

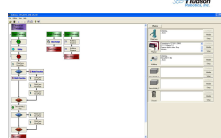
Automated Plate Handling



The Integrated System

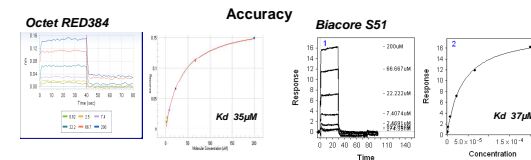


System control with SoftLinX

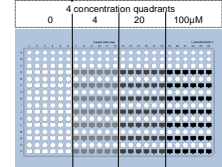


Validation

Kinase Target and a 238 Dalton Reference Compound



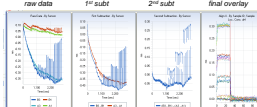
Platemap



Test platemap is divided into 4 concentration quadrants. 8 sensors are read column by column for 7 rows of samples + 1 row of running buffer (red). The alternating rows are reserved for baseline and dissociation measurements.

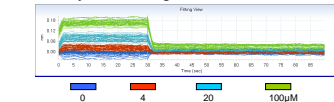
Precision

ForteBio's Double Subtraction Sequence



1st subtraction: Responses from biocytin blocked streptavidin sensors are subtracted from responses of the target sensor. Concurrently, buffer responses from each sensor are subtracted. 2nd subtraction: corrected running buffer responses are subtracted to correct for drift.

Overlay of all sensorgrams

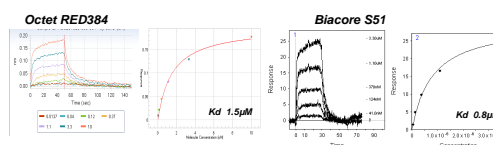


Conc (μM)	Average (nm)	SD (nm)
0	-0.009	0.010
4	0.022	0.010
20	0.078	0.015
100	0.167	0.014

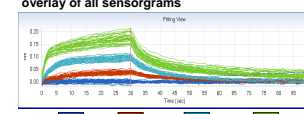
4 concentrations x 6 replicates x 7 sensors = 168 analyses, Z' = 0.64

Protein/Protein Interaction Target and a 501 Dalton Reference Compound

Accuracy



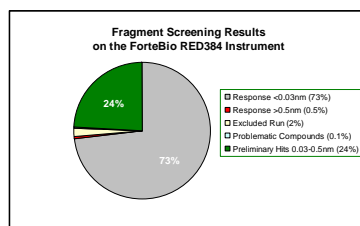
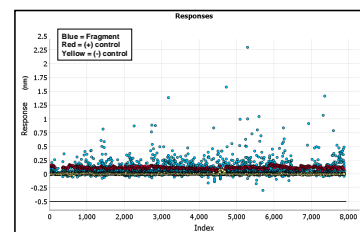
Precision



Conc (μM)	Average (nm)	SD (nm)
0	0.001	0.005
0.4	0.034	0.005
2	0.090	0.008
10	0.161	0.015

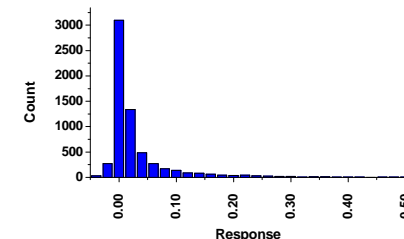
4 concentrations x 6 replicates x 7 sensors = 168 analyses, Z' = 0.63

Screening Results for a Kinase with Two Sites



Problematic compounds have responses >0.5nm and dissociate less than 50% in one minute. One run was excluded based on high standard deviation for the negative controls (SD=0.033nm).

Distribution of Responses for Fragments



Summary

- The ForteBio RED384 instrument is precise, and results for control compounds are in agreement with the Biacore S51 instrument.
- Binding profiles are similar on the ForteBio and Biacore instruments.
- Automation of the ForteBio RED384 instrument allows for analysis of a maximum of eight plates per day. A screen of compounds in 47 plates was completed in seven days.
- The hit rate for a kinase with an allosteric binding site, screened in the absence of ATP, was 24%.

References

Small molecule analysis on ForteBio platforms

"Label-free detection of biomolecular interactions using Biolayer interferometry for kinetic characterization" J Conception et al. *Comb Chem High Throughput Screen* 2009 12(8) p791.

"Label-Free Determination of Kinetic Constants for Small Molecule Binding to Proteins Using ForteBio's Octet RED Multi-Channel System" C Warchow et al. *SBS 14th Annual Conference* 2008 P202.

"Fragment library screening using the ForteBio Octet Red" S Li et al. *SBS 15th Annual Conference* 2009 P704.

SPR-based fragment screens

"Fragment-based screening using surface plasmon resonance Technology" S Perspicace et al. *J Biomol Sci* 14(4) 2009 p337.

"Fragment-based discovery of hepatitis C virus NS5b RNA polymerase inhibitors" S Antonsyamy et al. *Bioorg & Med Chem Lett* 18 2008 p 2990.

"Label-Free primary screening and affinity ranking of fragment libraries using parallel analysis of protein panels" M Hämäläinen et al. *J Biomol Sci* 13(3) 2008 p202.

"Fragment Screening by Surface Plasmon Resonance" I Navratilova and A Hopkins *ACS Med Chem Lett* 2010.