Fragment Screening on the FortéBio RED384 Instrument

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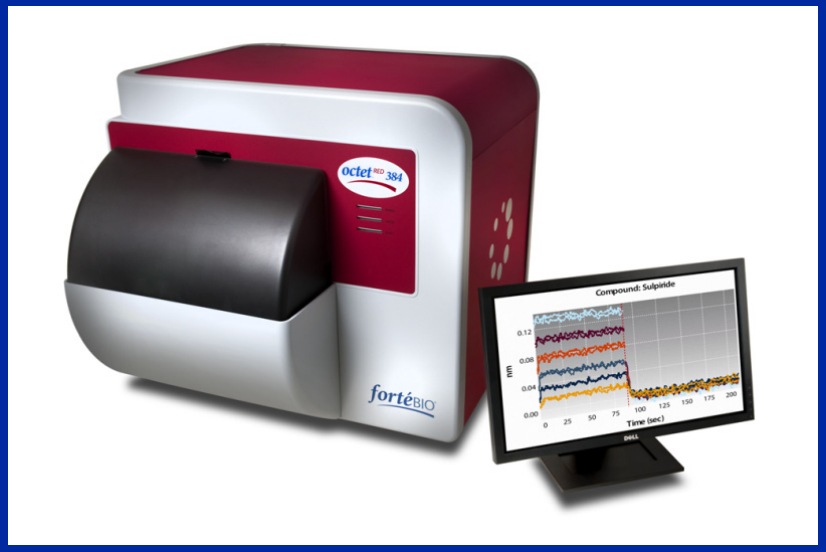


FBLD 2010

Applications of FortéBio Fragment Detection in Drug Discovery

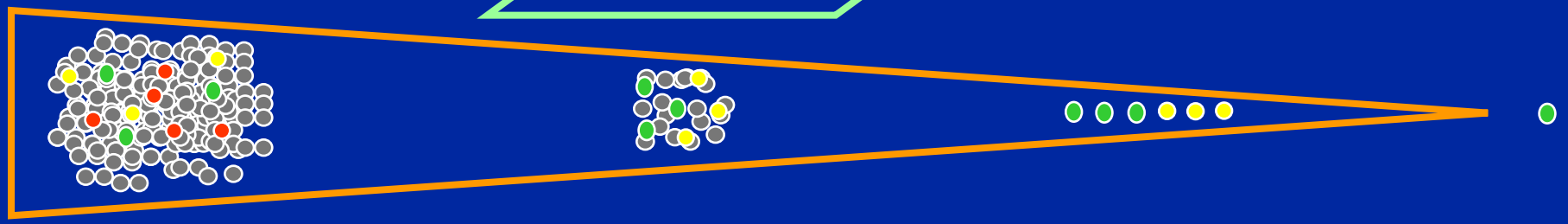
Current work-flow

Primary HTS Biochemical Screen

Biacore Hit Confirmation

FortéBio Hit Confirmation

NMR and X-ray Studies



Primary FortéBio Screen

FortéBio Hit Confirmation

Biochemical Assay

NMR and X-ray Studies

In progress

The Octet RED384 System

Integration with a plate handler and a liquid dispensing station

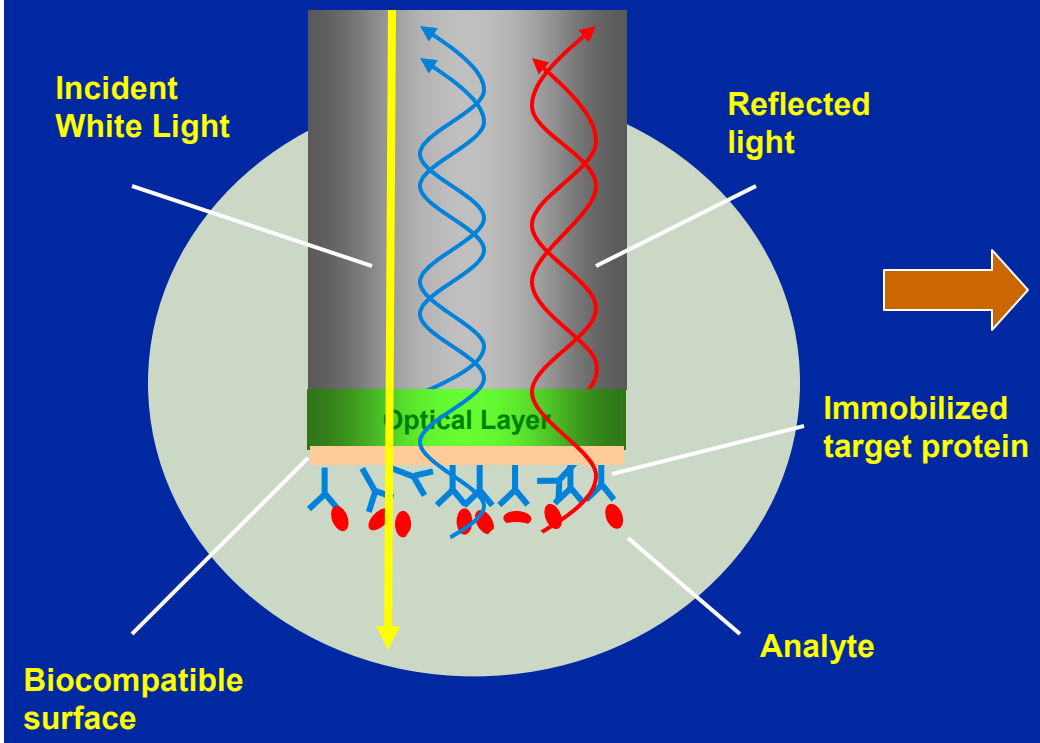
Sensor tray and sample plates

FortéBio Small Molecule Detection 101

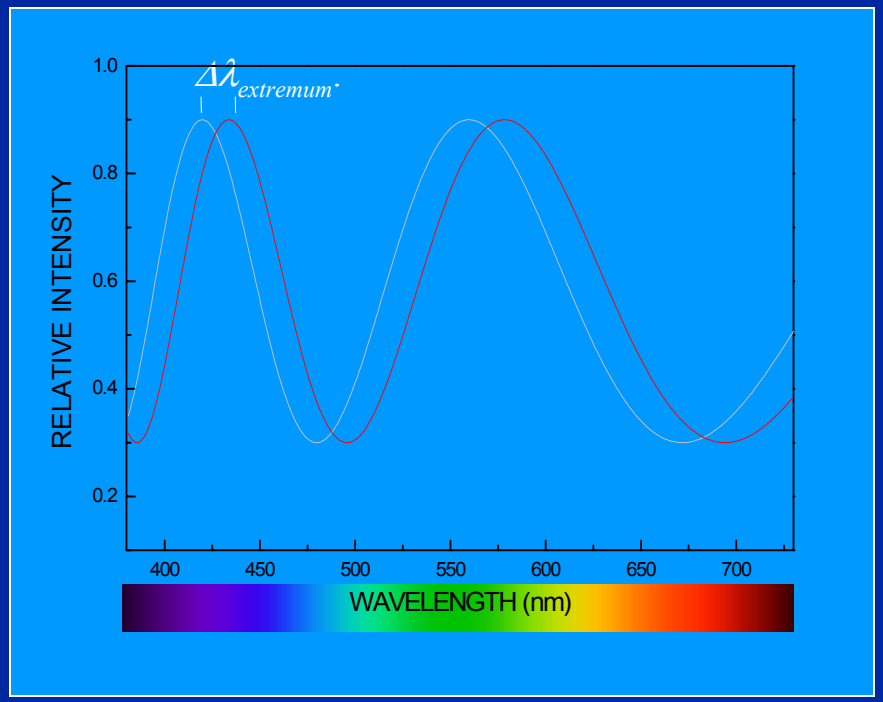
- **Detection**
  - White-light biolayer interferometry on a disposable fiber-optic surface
  - Microtiter plate (96 or 384)
  - No fluidics
- **Sensor preparation**
  - High density steptavidin sensor
  - *in-vivo* and *in-vitro* biotinylation of protein
  - On-line loading and off-line loading
- **Output**
  - Responses in association, dissociation
  - Small molecule kinetics (K_D, k_{on}, k_{off})
  - Binding profiles
    - Classical binding (Myszka's SPR studies, Analytical Biochem 2004)
    - Atypical binding (Gianetti et al J Med Chem 2008)
    - Non-binding
- **FortéBio RED384 Instrument**
  - 16-channels
    - reduced risk of sensor fouling/inactivation by problematic compound
  - Robot friendly
    - Run multiple plates
  - Throughput
    - 140 compounds + 16 positive controls + 16 negative controls in 62 minutes
    - Potentially advantageous for protein targets with stability issues
    - ~1000 compounds per day in a typical run with robotics

Bio-Layer Interferometry (BLI)

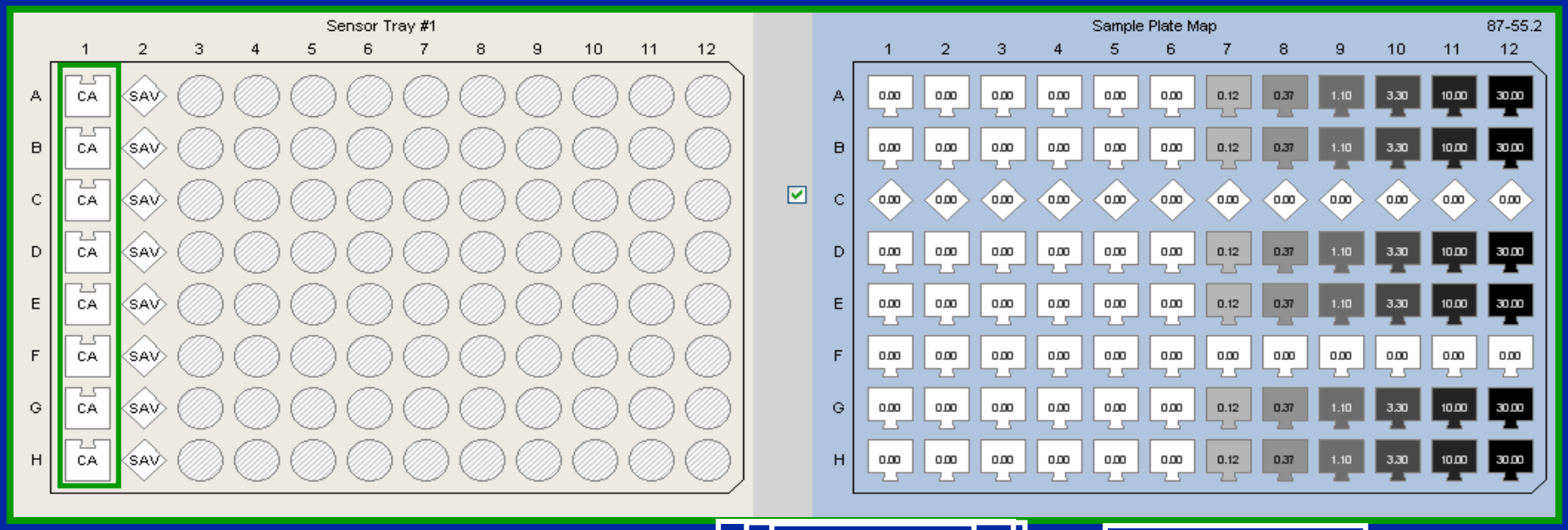
- A layer of molecules attached to the tip of an optic fiber creates an interference pattern at the detector
- A change in the number of molecules bound causes a measurable shift in the pattern



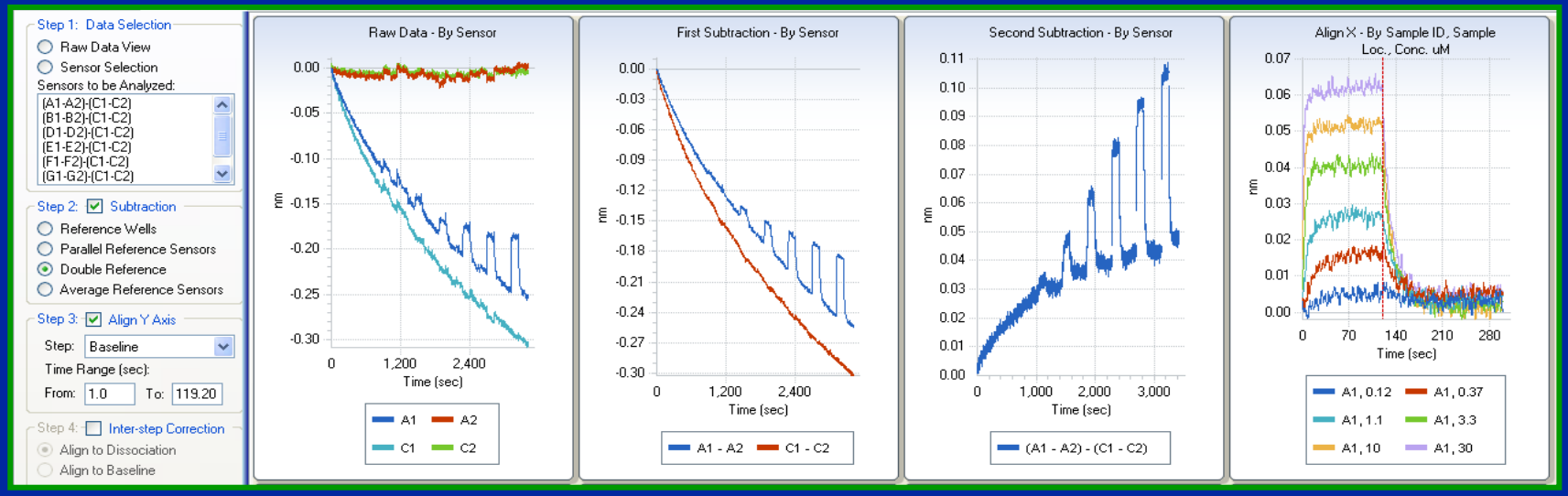
Interference spectrum



A Typical Experiment



Association



Instrument Validation Studies

- **Carbonic Anhydrase Model (SBS 2008, Wartchow et al. Poster P2072)**
  - Kinetic constants are similar to those reported for SPR (Myszka, Analytical Biochem 2004)
  - Detection limit is ~150 Daltons
  - Rmax correlates with molecular weight (R^2 0.9)
  - Small molecule signal for furosemide is predictable based on protein loading signal
  - Precision for responses is 10-15%

- **Hit Validation with multiple well-characterized targets**
  - PPI target and kinase target (SBS 2010, Podlaski et al., Poster B208)
    - Binding profiles and K_Ds agree with Biacore T100 results
    - Good precision for K_D determinations

- **Fragment library Screening**
  - Carbonic anhydrase screen with the Maybridge Ro500 Library (SBS 2010, Wartchow et al., Poster B271)
    - 10 Hits, 5 confirmed
    - Minimal binding to reference sensor
  - PPI Targets 1,2 (SBS 2009, Li et al., Poster 7024)
    - 10% Hit rate, one hit validated by NMR
    - MW limit ~200Da
  - PPI Target 3 (SBS 2010, Podlaski et al., Poster B208)
    - 24% hit rate
    - Minimal binding to reference sensor
  - PPI Target 4
    - 3.4% hit rate

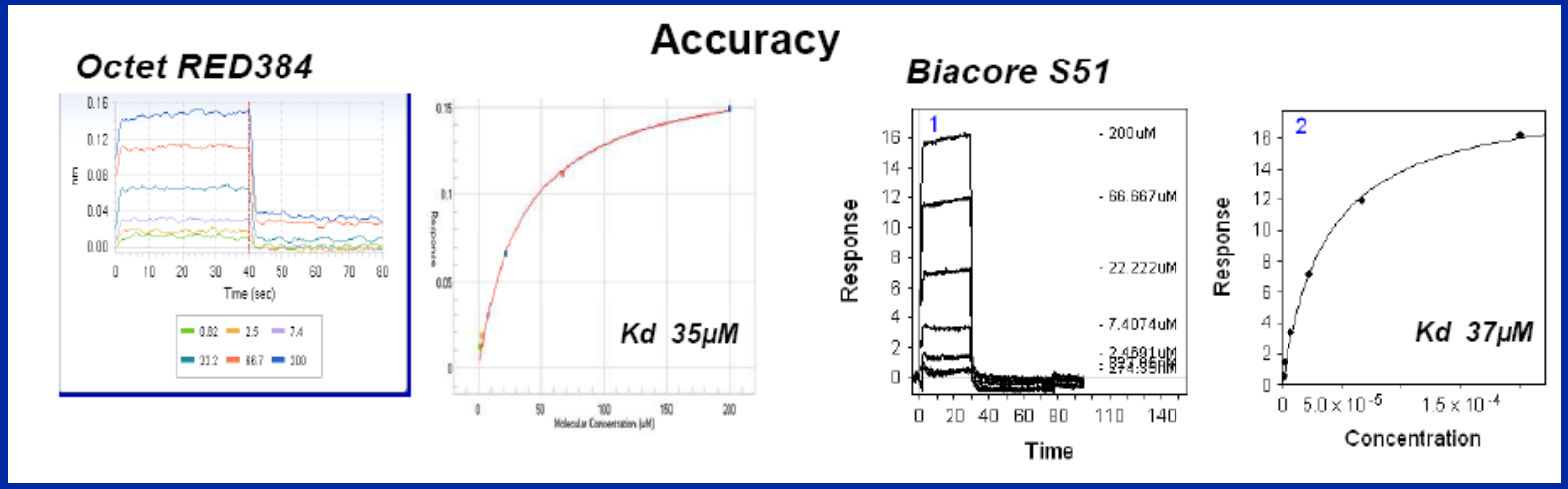
Octet RED Results with Carbonic Anhydrase Correlate to Biacore

Compound	ForteBio[1]	Biacore[2,3]
Acetazolamide (222 Daltons)	K_D = 0.039 µM k_{on} = 8.2E5 $M^{-1}s^{-1}$ k_{off} = 0.033 s^{-1}	K_D = 0.039, 0.019 µM k_{on} = 3.0E6, 2.9E6 $M^{-1}s^{-1}$ k_{off} = 0.079, 0.056 s^{-1}
Benzenesulfonamide (157 Daltons)	K_D = 2.4 µM k_{on} = 1.1E5 $M^{-1}s^{-1}$ k_{off} = 0.26 s^{-1}	K_D = 0.8, 0.85 µM k_{on} = 1.7E5, 1.7E5 $M^{-1}s^{-1}$ k_{off} = 0.12, 0.14 s^{-1}
Furosemide (330 Daltons)	K_D = 1.2 µM k_{on} = 6.4E4 $M^{-1}s^{-1}$ k_{off} = 0.078 s^{-1}	K_D = 1.0, 0.51 µM k_{on} = 6.3E4, 9.7E4 $M^{-1}s^{-1}$ k_{off} = 0.061, 0.05 s^{-1}
Sulpiride (341 Daltons)	K_D = 239 µM k_{on} = 3.9E3 $M^{-1}s^{-1}$ k_{off} = 0.93 s^{-1}	K_D = 48, 186 µM k_{on} = 8.0E3, 3.4E3 $M^{-1}s^{-1}$ k_{off} = 0.38, 0.64 s^{-1}

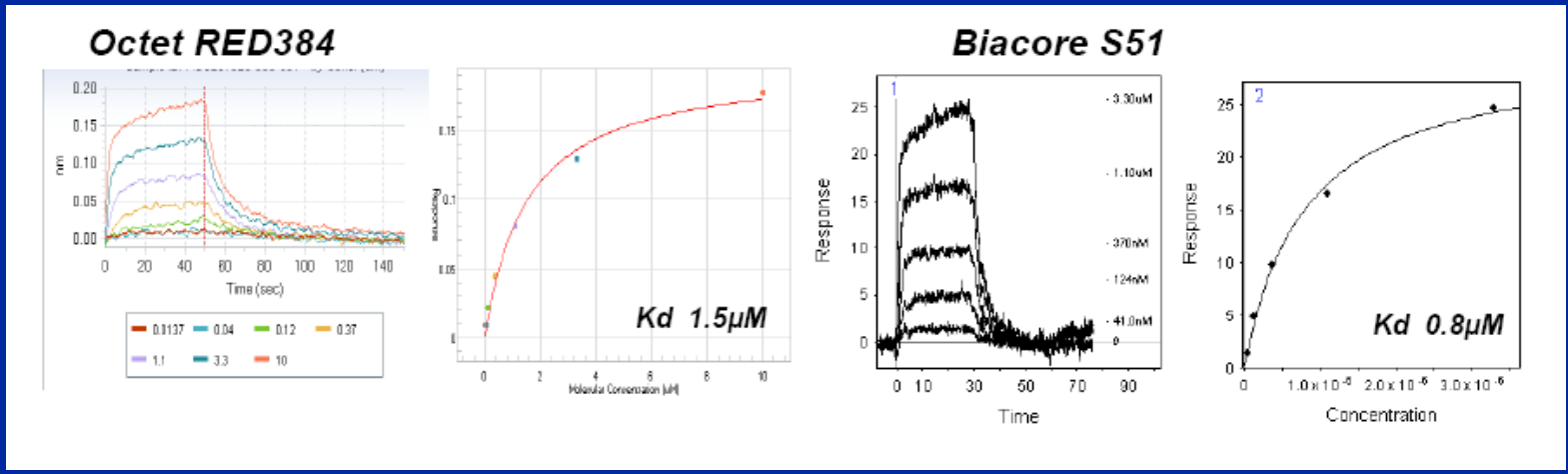
[1]Wartchow et al., SBS 2008, Poster P2072
[2]Papalia et al., Analytical Biochem 359 (2006), 94-105
[3]Myszka, Analytical Biochem 329 (2004), 316-323

BLI results generally correlate with SPR

Kinase Target with a 238 Da compound



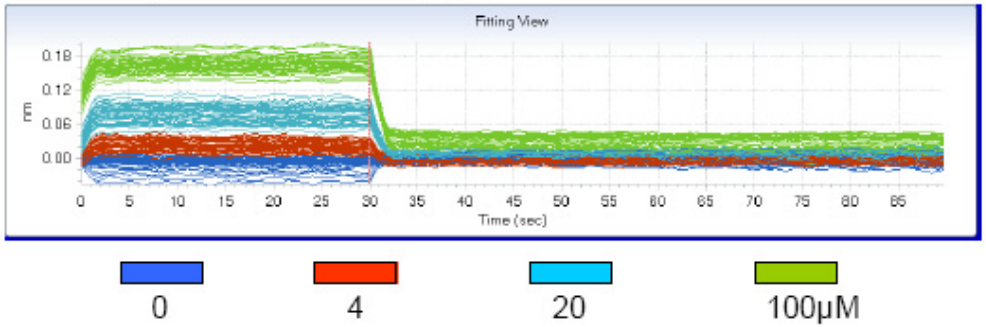
PPI Target with a 501 Da compound



Podlaski, SBS 2010, Poster B208

Precision

Overlay of all sensorgrams



0 4 20 100µM

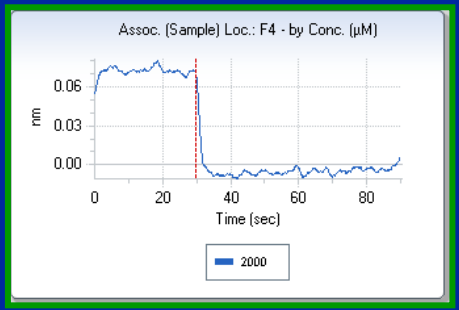
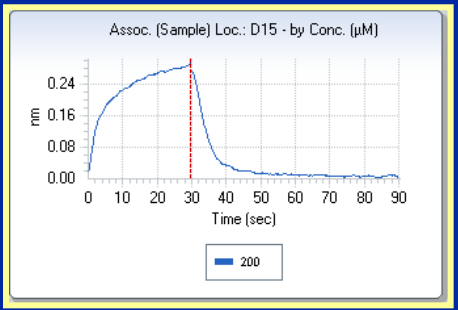
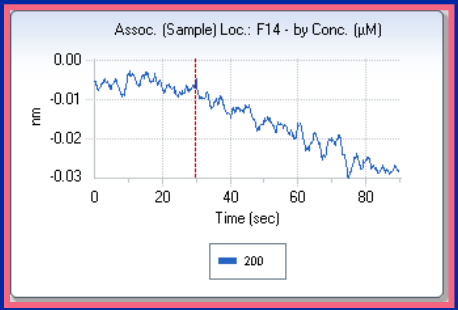
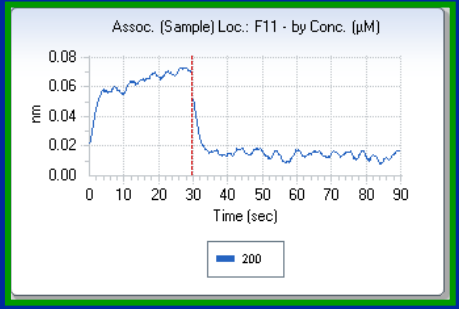
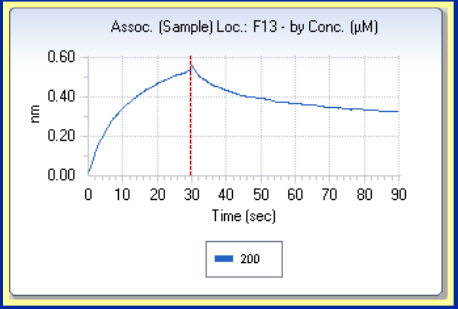
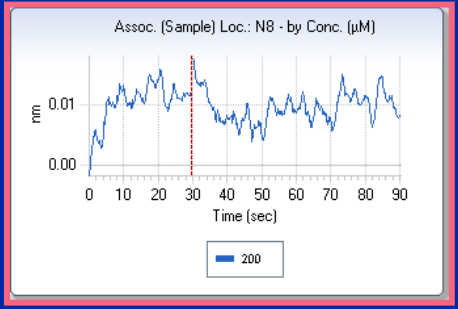
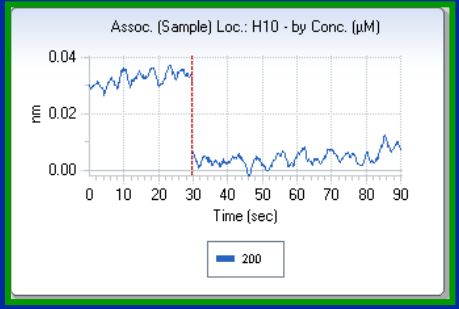
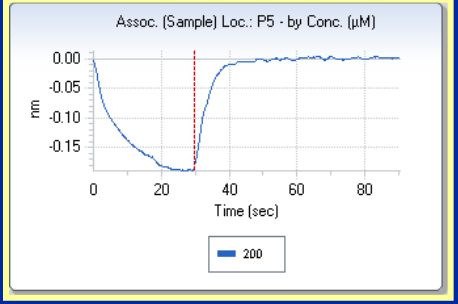
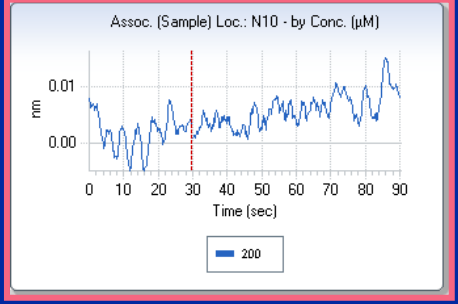
Conc (uM)	Average (nm)	SD (nm)
0	-0.009	0.010
4	0.022	0.010
20	0.078	0.015
100	0.167	0.014

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

Variability includes well-to-well variability, and sensor-to-sensor variability

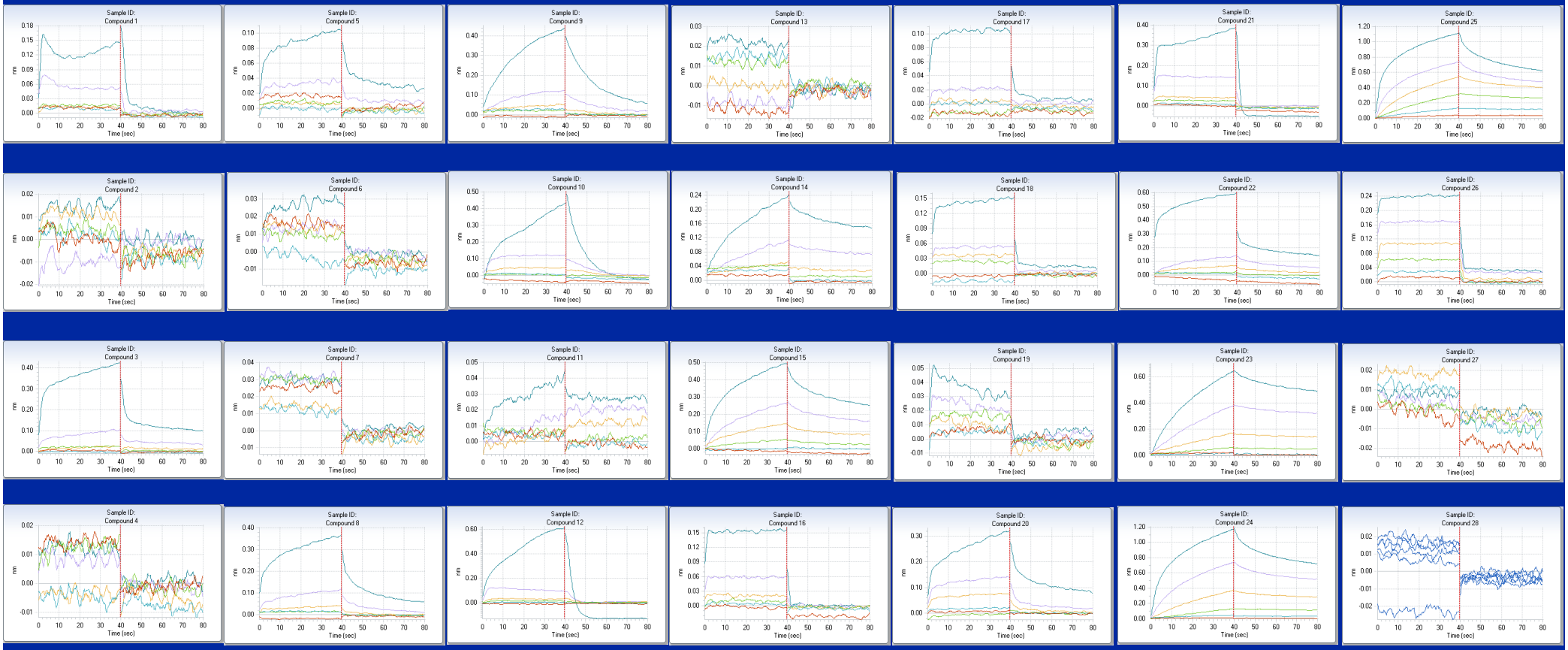
Podlaski, SBS 2010, Poster B208

Quantitative *and* Qualitative Assessment of Binding Profiles

Primary Hits	*Atypical*	*Non-binders*
		
		
		

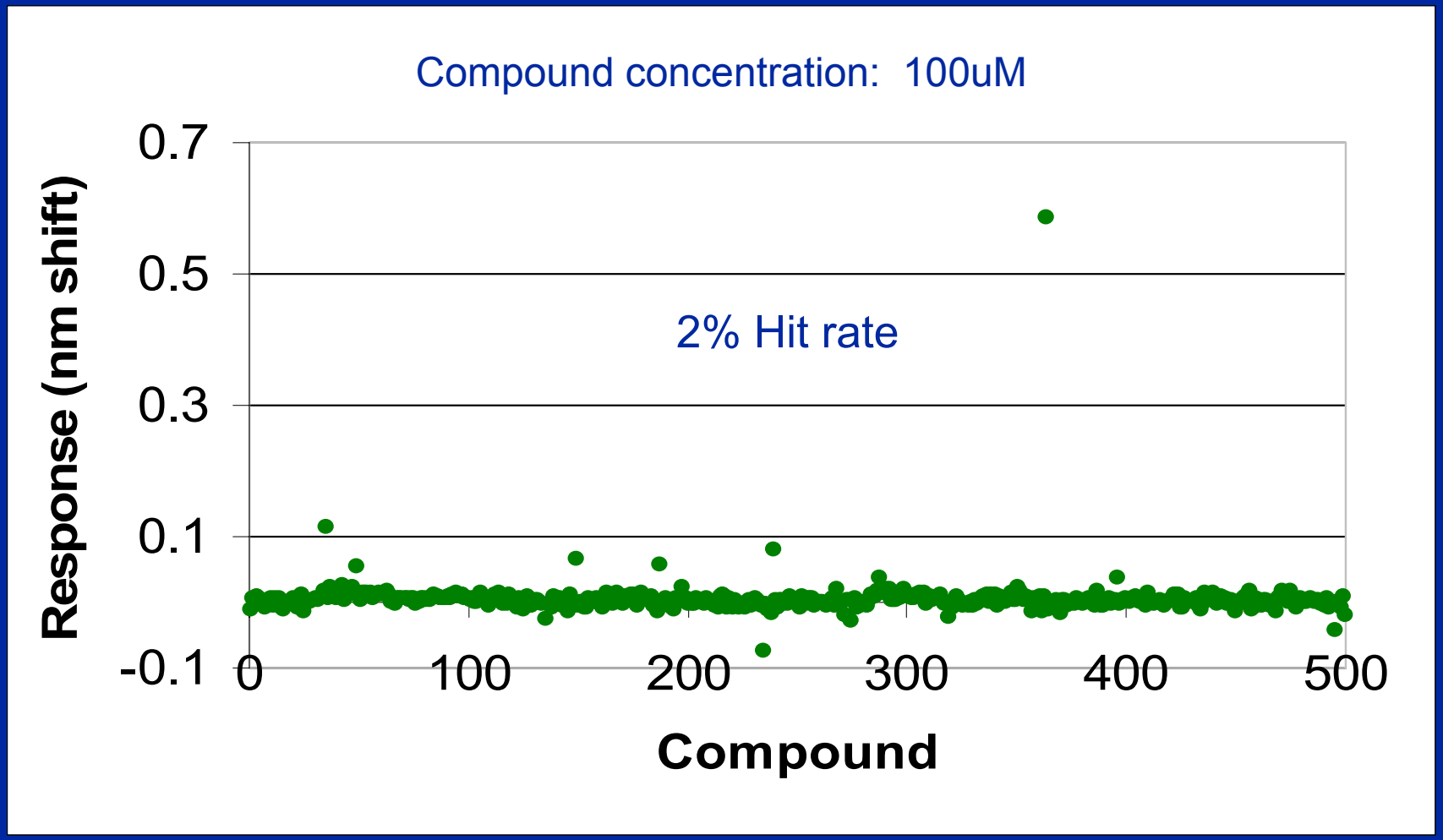
Profiles are generally similar on Biacore

Characterization of PPI hits from a biochemical assay on RED384



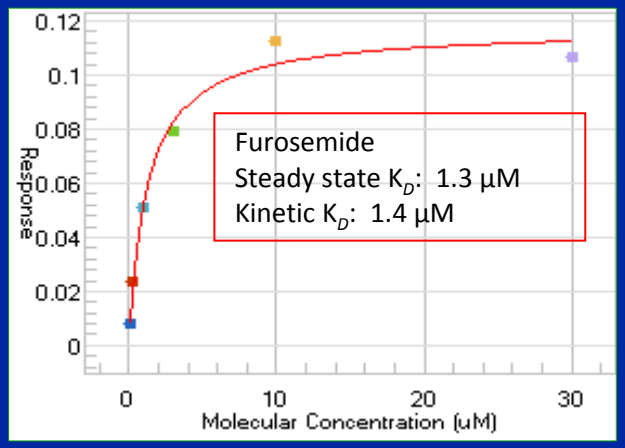
- 28 compounds
- 6pt concentration series
- 3 h

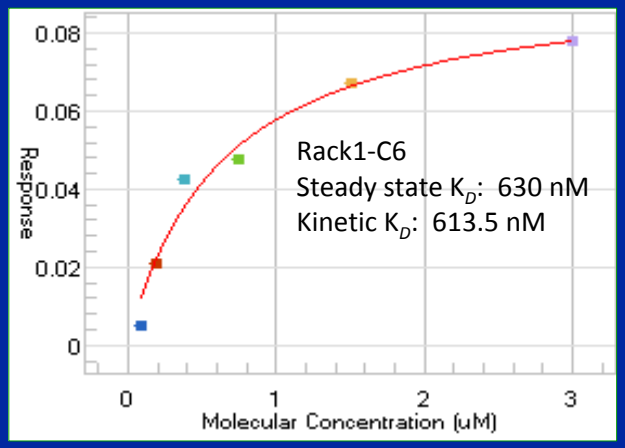
Maybridge Ro3 500 Library Screen with Carbonic Anhydrase

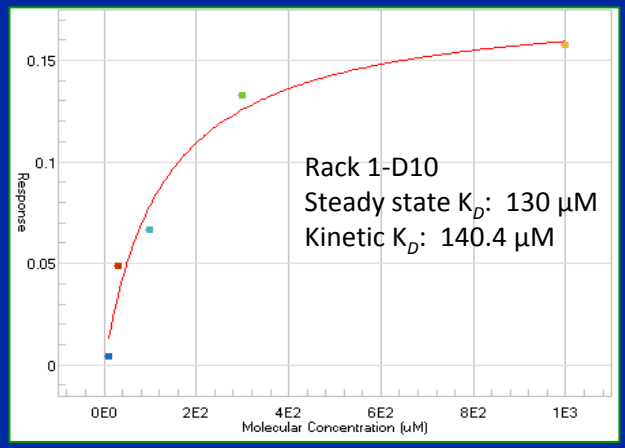


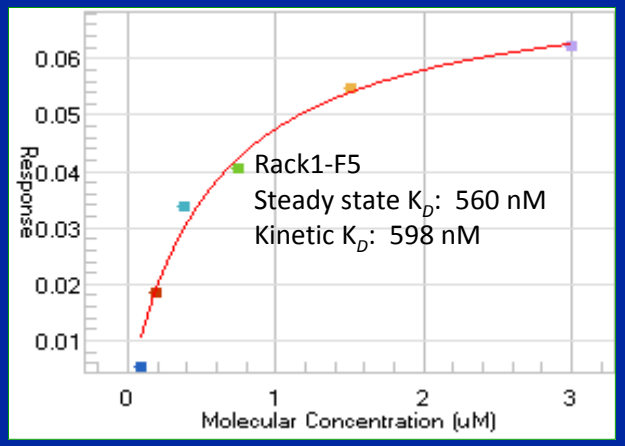
Wartchow et al., SBS 2010, Poster B271

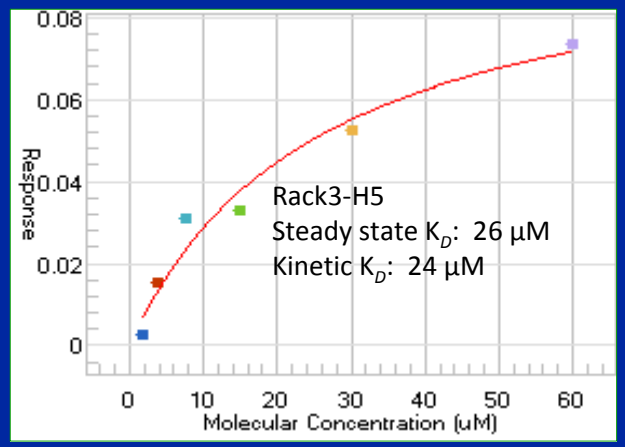
Steady-state Analysis of Confirmed Hits

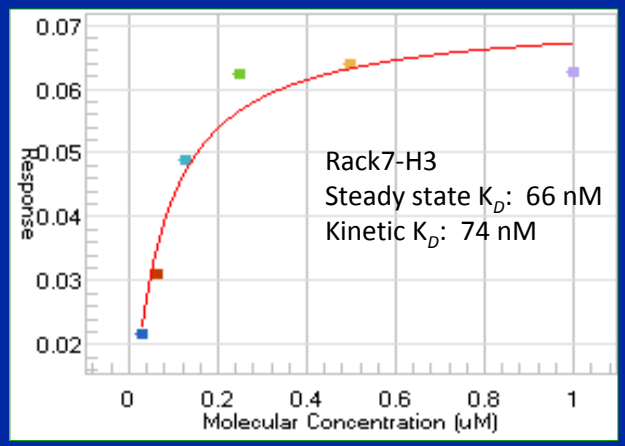












- **10 Hits identified, 5 hits confirmed**
- **3/5 compounds are sulfonamides**
- **Steady-state analysis is in agreement with Kinetic analysis**
- ***Wartchow et al., SBS 2010, Poster B271***

Recent Fragment Screen Results

- PPI Target 4 with multiple binding sites, stability issue
- Method
  - 200uM, N=1
  - 5% DMSO
  - 47 plates screened in 10 days
  - 0.9 mg protein (un-optimized)
- FortéBio assay
  - 3.5% primary hit rate
    - 3 scaffolds were observed repeatedly, one scaffold gave “atypical” binding profiles
    - 17% of primary hits were “atypical”
    - 11% of primary hits had responses >2X of positive control
    - 88% of primary hits are unique to FortéBio assay, and were not found in Biochemical assays
  - Confirmation *in progress*
    - 44% of primary hits were confirmed in a follow-up FortéBio assay (N=1)
  - 30% loss in activity noted for 2nd plate, normalization would increase hit rate
  - K_D cut-off at 460±153µM (a conservative estimate, calculated from responses at the end of plates 1 and 2)
  - Responses on reference sensor noted for many hits
- Overlap with biochemical assays
  - Site 1: 52% overlap with FortéBio assay
  - Site 2: 38% overlap with FortéBio assay, atypical binders found

Summary

- **The FortéBio RED384 instrument is a valuable tool for drug discovery**
  - **General findings**
    - Highly reliable, robust (minimal maintenance, consistent responses over time, moderate sensitivity to RI mismatches)
    - Efficient tool for the identification of problematic compounds
    - Sensitivity is sufficient for small molecule characterization, and fragment detection
    - Throughput of 140 compounds/hr is advantageous for proteins with limited stability
    - 16-channel format reduces risk of run failure due to target inactivation
    - Improvements in sensitivity, precision will increase hit rate for low MW compounds and/or compounds with high K_D
    - Non-specific binding to the sensor is a minor issue, but can complicate analysis occasionally
    - Valuable tool for developing methods for new targets
  - **Hit Validation**
    - Kinetic constants and binding profiles generally correlate with Biacore results
  - **Fragment Screening**
    - A work in progress…….
    - Early results are promising
    - Complimentary to biochemical HTS

Acknowledgements

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  - *Kuo-Sen Huang*
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