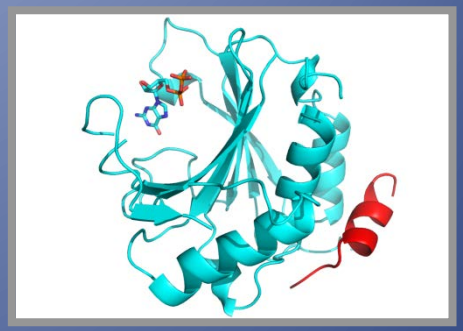
Biosensor-based Fragment Screening and Hit Confirmation with Protein-protein Interaction (PPI) Targets

Charles Wartchow, PhD



Outline

- Introduction
  - Targeting protein-protein interactions (PPI)
  - Fragment-based drug discovery
  - FortéBIO biolayer interferometry (BLI) best practices
  - Method development and challenges
- eIF4E Fragment Screen and Hit Confirmation
  - Confirmation of hits from HTS assays, FortéBIO Fragment Screen
  - Eliminating false positive results through formulation with 1%PEG/1%Hydroxy-propyl cyclodextrin
- FortéBIO Ras Screen
  - Screening summary
  - Hits and removal of false positive results due to metal contamination by addition of TPEN

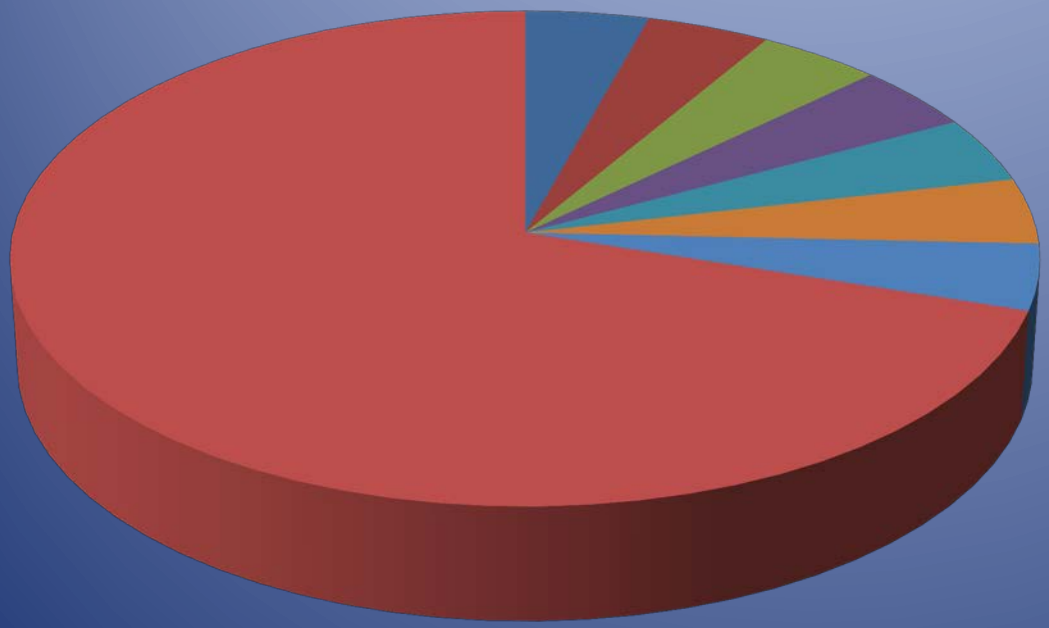
Protein-Protein Interaction (PPI) Targets

- Protein-protein interactions are key regulators of disease biology
  - "Biological gold mine".....Asher Mullard *Nature Rev Drug Disc 2012*
- PPI targets are challenging because they lack well-defined pockets, but they contain ligandable "hot spots"
  - "……progressing towards the dream" Michelle Arkin and Jim Wells in *Nature Rev Drug Disc* 2004
- BH3 mimetic and Bcl-2 inhibitor ABT-737 Mark van Delft et al. *Cancer Cell* 2006
  - Bcl-2 is a regulator of apoptosis, an anti-apoptotic protein
- Nutlins: Inhibit p53/MDM2 interactions
  - Inhibitors of MDM2 block interaction with p53, increasing pro-apoptotic p53 activity
  - Fry, Wartchow, Graves, Janson, Lukacs, Kammlott, Belunis, Palme, Klein, Vu ACS Med Chem Lett 2013

Fragment-based Drug Discovery

- Compounds are RO3 (Congreve et al. Drug Disc Today 2003)
  - MW <300, rotatable bonds <3, H-bond donors/acceptors <3, PSA<60
  - Remove undesired functionality
  - Fragments are “well-behaved” relative to larger compounds
- Advantages of FBLD (Murray et al. Trends in Pharm Sci 2012)
  - Poor physico-chemical properties are a major source of attrition
  - Fragments form high quality interactions (later stage compounds differ by <0.8Å)
  - Ligand efficiency (LE) is a guide for selection of lead fragments
  - Fragments sample a large fraction of chemical space

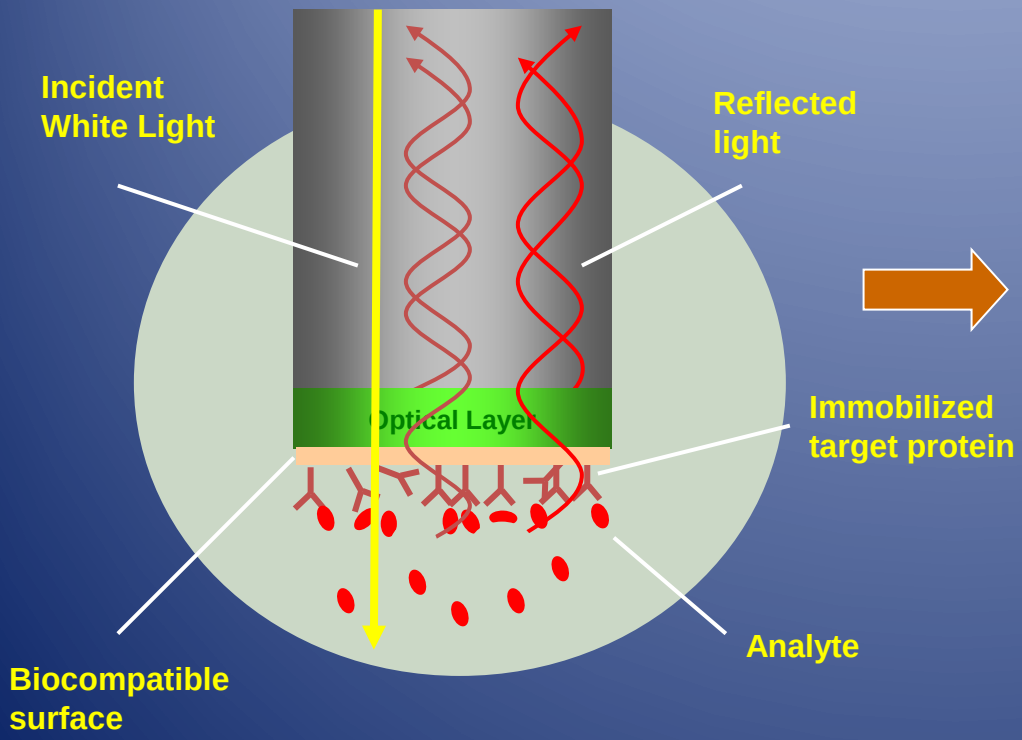
A Successful Biosensor Screen



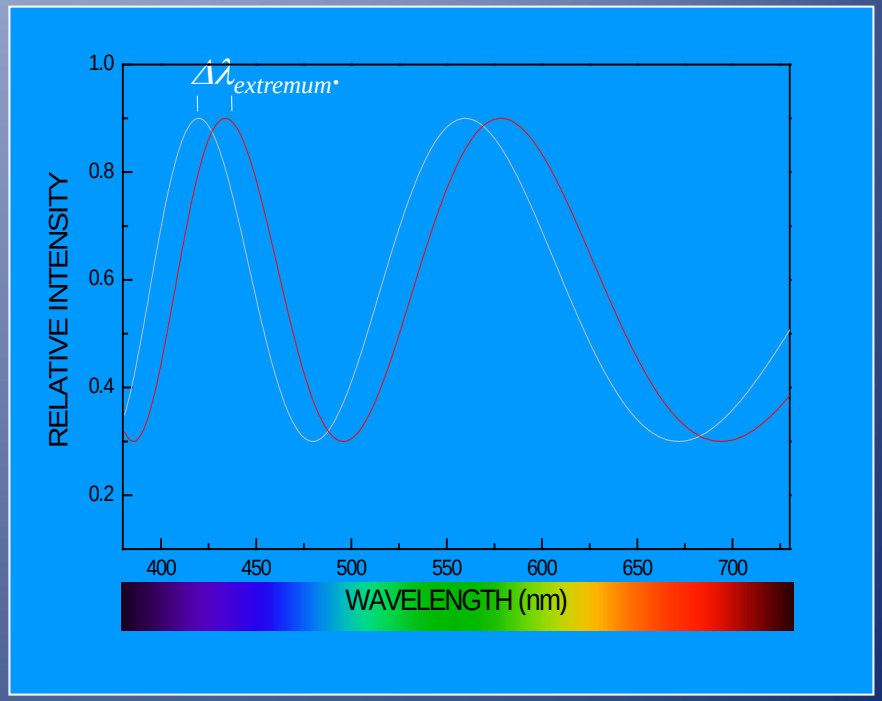
- Target Stability
- Target Activity
- Target Ligandability
- Optimization
- Compound Behavior
- Technique
- Library Composition
- Correlation to NMR or Crystallography

FortéBIO 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



Charles Wartchow, Frank Podlaski, Shirley Li, Karen Rowan, Xiaolei Zhang, David Mark, Kuo-Sen Huang "Biosensor-based Small Molecule Fragment Screening with Biolayer Interferometry: *J Comput Aided Mol Des* **2011**, 25(7), 669-76.

Eric Martin, John Wang, Isabel Zaror, Jiamin Yu, Kelly Yan, Mike Doyle, Paul Feucht, Kevin Shoemaker, Bob Warne, Mike Chin, Blisseth Sy, Lukas Leder, Marco Meyerhofer, Charles Wartchow, Danfeng Yao, "Novartis Evaluation of the ForteBio Octet RED: A versatile instrument for direct-binding experiments" in *Label-free Technologies for Drug Discovery*, Chapter 15, Cooper, M. and Mayr, L. M. (editors), Wiley Press, 2011.

Octet RED-based Screening Method Development

- Best practices
  - 384-well tilted well plate (**FortéBIO**)
  - 140 compounds per plate, single station protocol
  - SuperStreptavidin biosensors (**FortéBIO**)
- Qualify protein with validated controls (small molecules, peptides, proteins)
  - Monitor signals, sensorgram quality, stability of target
  - Proteins are typically biotinylated *in vitro* or contain AVI tag
- Optimize
  - Harmonize buffer with other methods if possible
  - Detergent is typically 0.05%P20
  - DTT, TCEP concentrations (T Giannetti Methods in Enzymology 2011)
    - These agents can generate peroxide with some compounds (Johnston, Curr Opin Chem Biol 2011)
  - pH, ionic strength, 2-5% DMSO
- Controls
  - Run (+) and (-) control throughout the run, monitor stability
  - Run standard curve for (+) control at beginning and end of run
  - Run test library (~200 compounds, N=1, 200uM) X N=3

The Challenges of LMW Biosensor-based Screening

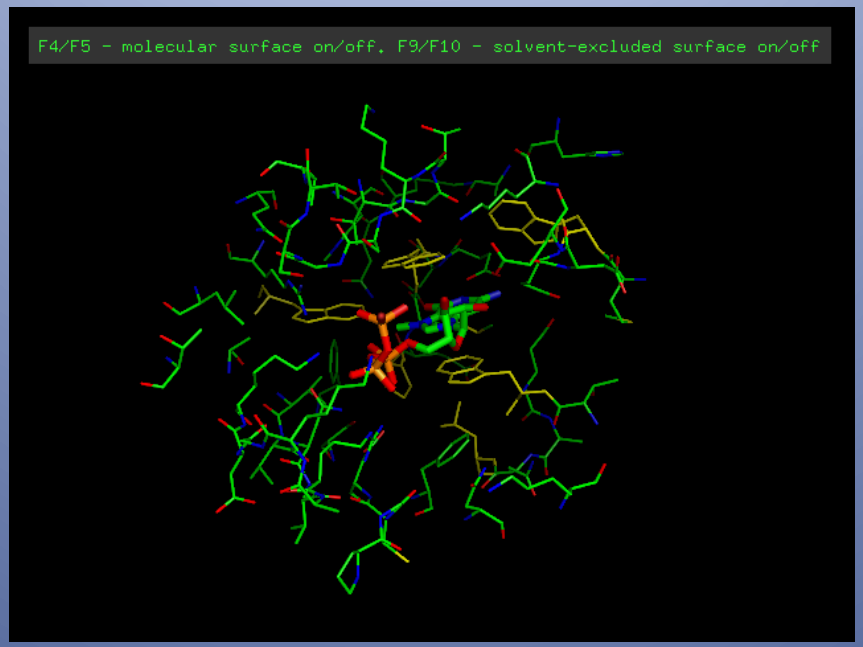
LMW=low molecular weight

- Differentiating true responses from noise
  - Average+3SD typically removes many false-positives
- Identifying false-positives due to aggregating compounds
  - Unreasonably high responses
  - Matrix (buffer) studies
- Eliminating false-positives due to impurities

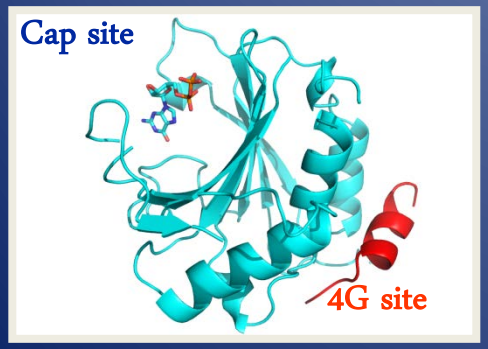
Case Study: eIF4E

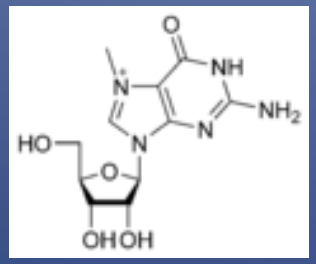
eIF4E: Eukaryotic translation initiation factor

-an important oncology target



Cap site guanosine binding region is hydrophobic, and phosphate binding region contains numerous lysines and arginines. 4G peptide binding site is "shallow".





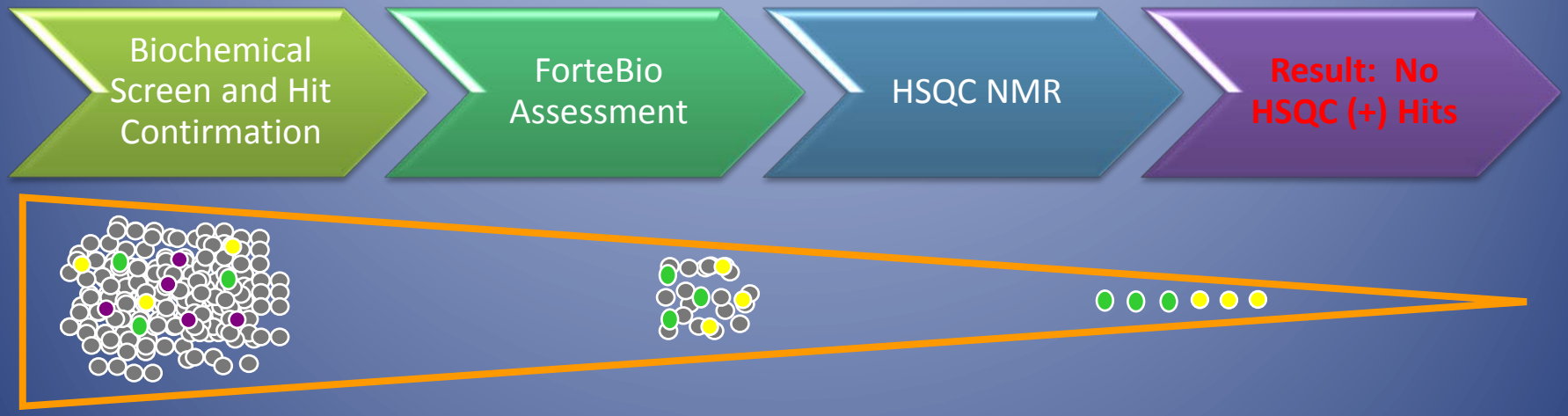
M7G

- 4G binding-site pocket is shallow, which makes this a difficult target
- Cap site is well-defined, binds 7-methyl guanosine triphosphate at the 5' end of mRNA

Brown et al. Cell Cycle 2009

HTS-based eIF4E Fragment Screen

Confirmation of eIF4E Cap-site and 4G site biochemical fragment hits



- 4G PPI site HTS assay results
  - No “ideal” results in FortéBIO analysis
  - Many 4G hit responses were high, and linear with increasing concentration
- Cap-site HTS assay results
  - Some curvature noted in FortéBIO DR plots, but no saturation

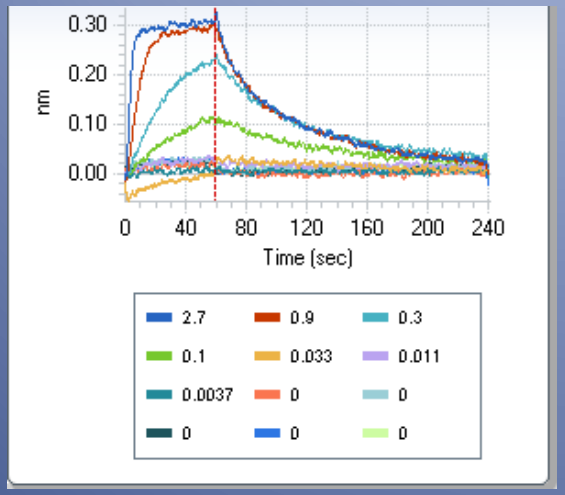
- *HSQC NMR analysis of “hits” was negative*

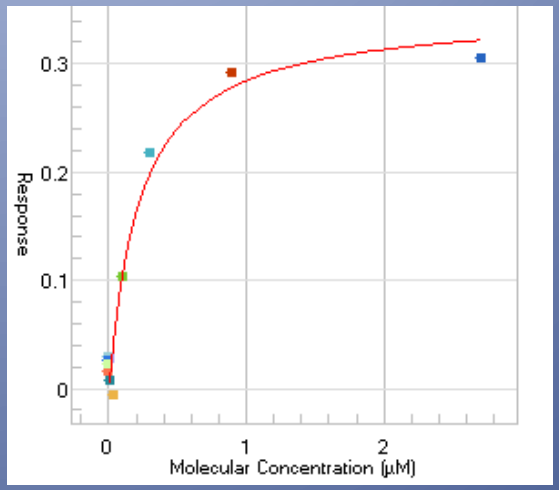
How do we identify authentic binders in PPI fragment screens?

- Biochemical assays produced false-positive results
  - What is the correlation between biosensor results with 2D NMR, a highly reliable method for hit validation?
- ForteBio eIF4E fragment screen: a better assay?
- Formulation studies (more detergent, novel excipients)
- Examine 28 representative compounds
  - NMR(+) control compound, 7MG, cap-site and 4G site biochem hits, ForteBio fragment screen hits
  - Top conc =200uM

Binding of a 4G peptide to eIF4E

-a positive control



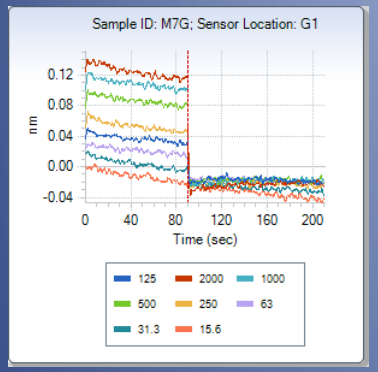


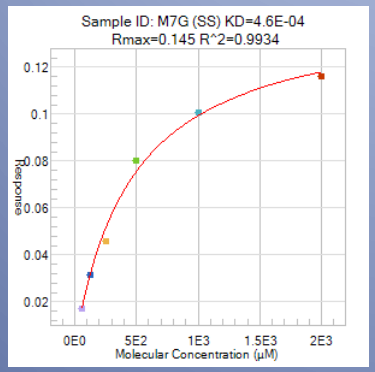
K_D 230nM

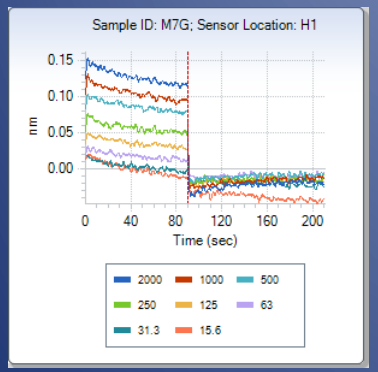
- A well-behaved 4G peptide shows ideal responses
- Experiment is not perfect: need longer association time

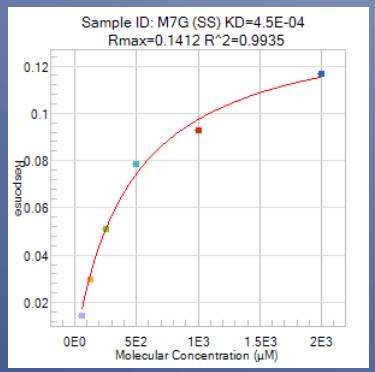
Analysis of NMR (+) 7MG

ForteBio Analysis

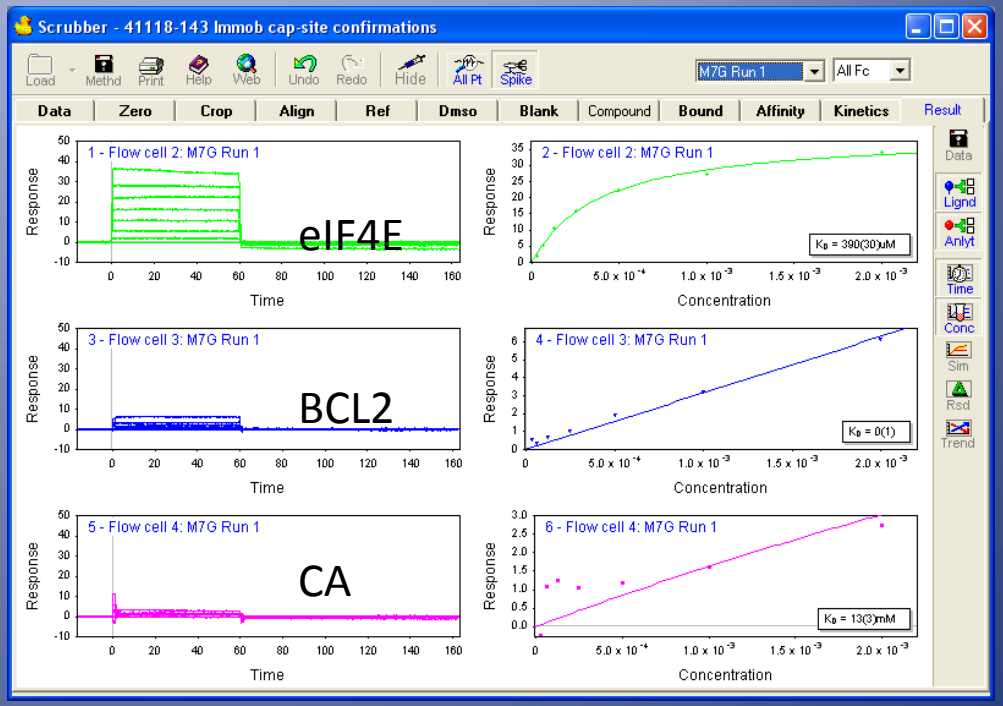


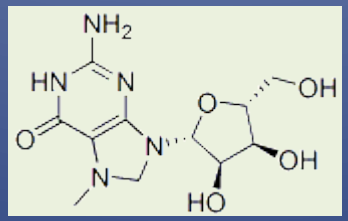






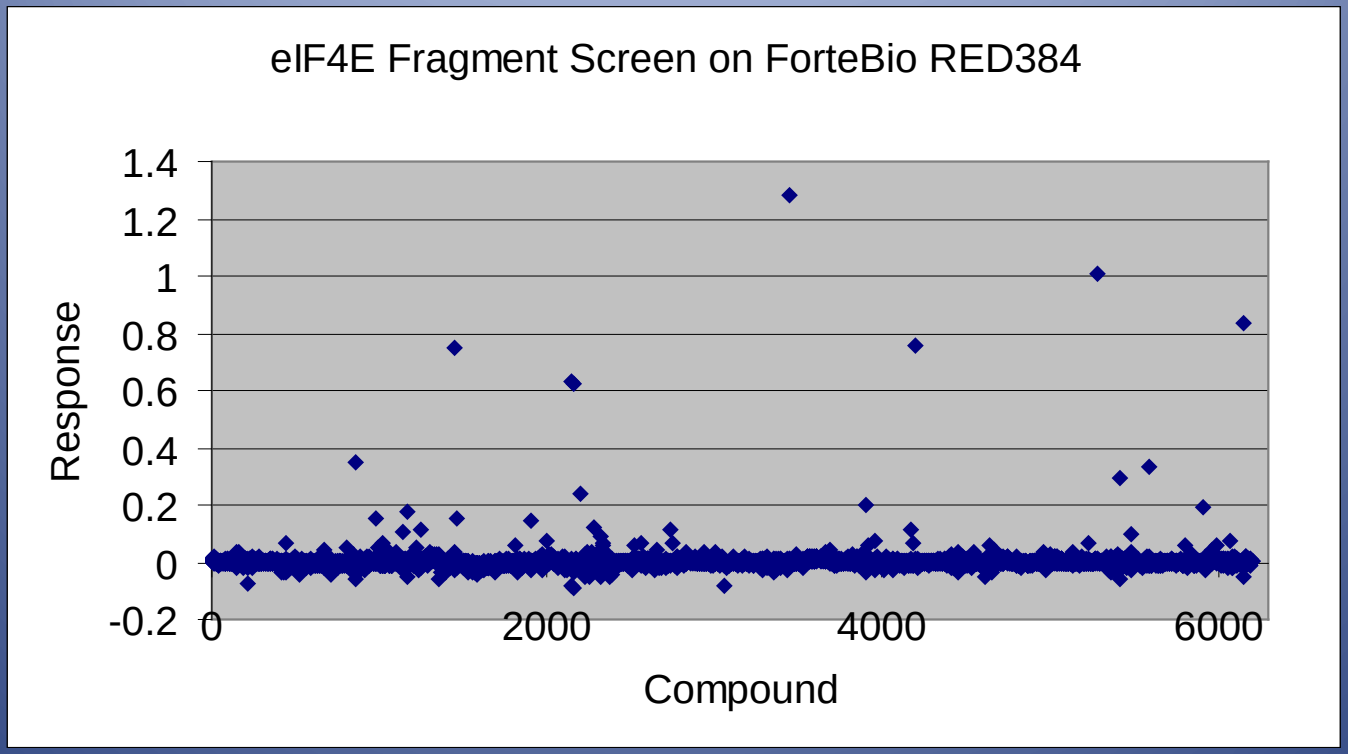
Biacore Analysis





7MG (A known literature compound)

eIF4E ForteBio Fragment Screening Results



Hit Rate 224/6359 = 3.5%

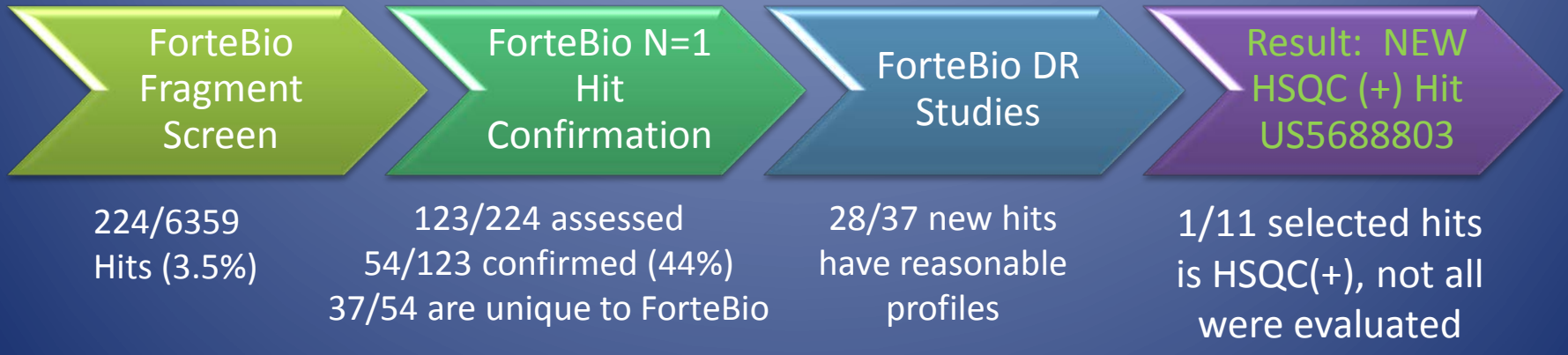
Formulation Reduces NSB Dramatically

- Aggregates bind to targets: Brian Shoichet
- Aggregates interfere with Biacore studies (Giannetti et al. J Med Chem 2008)
- P20 (Tween-20), other detergents give mixed results
- New solution: Non-detergent excipients (1% PEG+1% beta-hydroxypropyl cyclodextrin, Nandi, Pharm Sci Tech 2003)

ForteBio Fragment Screen with eIF4E
Can this method identify authentic binders?

Post-program method development

- Optimization on ForteBio platform: Reformulate buffer to reduce false positives
- Eliminate non-binders, compounds with high signals, de-prioritize non-selective compounds



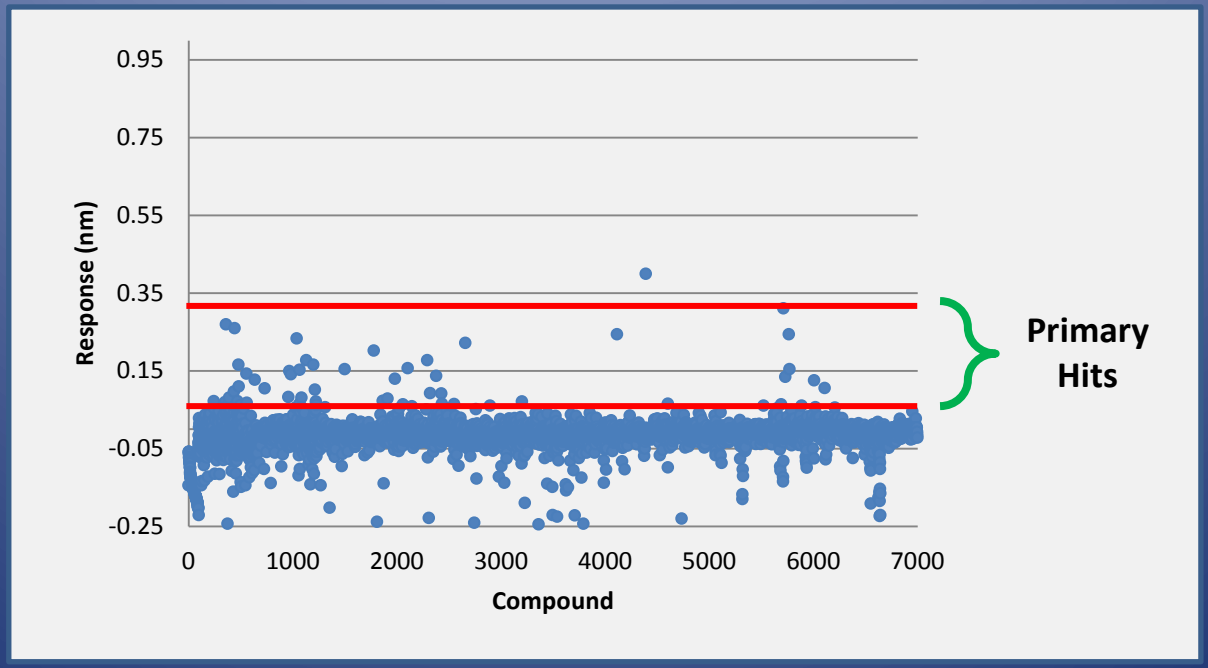
Case Study: Ras

Ras: an Important Oncology Target

- Mutated in ~30% of all cancers
- Gly12 mutation results in accumulation of the active Ras:GTP form of the enzyme
- Ras:GTP interacts with Raf
- In RasG12V, the GAP effector protein cannot aid in hydrolysis of GTP to generate inactive Ras:GDP
- Goal: identify binders to Ras, study mechanism

FortéBIO Fragment Screening Results with Kras G12V

- Fragment library screen (6900 compounds)
  - Novel zinc interference identified in FortéBIO methods, several compounds are hits after removal of interference
  - K_Ds are high micromolar to mM
  - 3-7 primary hits overlap with biochemical assay results, but had atypical features.



Shirley Li and Charles Wartchow

Many Hits Contain Interfering Zinc

- Five compounds are “clean”
- Five compounds are compromised by metal interference (Hermann, ACS Med Chem Lett 2013, 4(2) pp197-200
- Corrected responses were obtained in the presence of zinc chelator TPEN

Correlation of 2D NMR with Biosensor-based Methods

Compound	Structure Type	ForteBio Status	Biacore Status	HSQC NMR Status
1 (DCAI, *Maurer et al, PNAS* 2012)	indole	KD 0.5mM	Hit	Positive at 1mM
2 CAS# 2731-06-8	indole	Non-ideal Hit	ND	Negative at 1mM
3 CAS# 61-54-1	indole	Non-ideal Hit	Hit	ND
4	Indole	Weak Hit (200uM) No Interference	Hit	Negative at 1mM Weakly positive at 6mM

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