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

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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

• 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


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”.

- 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*

- **Biochemical Screen and Hit Confirmation**
- **ForteBio Assessment**
- **HSQC NMR**
- Result: No HSQC (+) 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 =200μM
Binding of a 4G peptide to eIF4E - *a positive control*

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

$K_D$ 230nM
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

ForteBio Fragment Screen
224/6359 Hits (3.5%)

ForteBio N=1 Hit Confirmation
123/224 assessed
54/123 confirmed (44%)
37/54 are unique to ForteBio

ForteBio DR Studies
28/37 new hits have reasonable profiles

Result: NEW HSQC (+) Hit US5688803
1/11 selected hits is HSQC(+), not all were evaluated
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
  - $K_D$s are high micromolar to mM
  - 3-7 primary hits overlap with biochemical assay results, but had atypical features.
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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure Type</th>
<th>ForteBio Status</th>
<th>Biacore Status</th>
<th>HSQC NMR Status</th>
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<tbody>
<tr>
<td>1</td>
<td>indole</td>
<td>KD 0.5mM</td>
<td>Hit</td>
<td>Positive at 1mM</td>
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<tr>
<td>(DCAI, Maurer et al, PNAS 2012)</td>
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<td>2</td>
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<td>Negative at 1mM</td>
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<td>Non-ideal Hit</td>
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<tr>
<td>4</td>
<td>Indole</td>
<td>Weak Hit (200uM) No Interference</td>
<td>Hit</td>
<td>Negative at 1mM Weakly positive at 6mM</td>
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