Integration of Octet RED384 system into the Emory Chemical Biology Discovery Center for Small Molecule Discovery

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Outline

- Emory Chemical Biology Discovery Center (ECBDC)
- Integration of Octet RED384 into ECBDC for small molecule discovery
- Applications of Octet RED384
  - Characterization of small molecule 14-3-3 inhibitors
  - Mechanism of action studies for 14-3-3 hit compounds
  - Assay optimization for challenging enzyme inhibitor study
Mission

• To discover novel chemical leads targeted to disease-related proteins for research tools and therapeutics;

• To train the next generation of drug discovery scientists
Emory Chemical Biology Discovery Center (ECBDC) a member of the NIH MLSCN (2005 - 2009) & the NCI’s Chemical Biology Consortium (since 2009 - ) NCI’s CTD² (Cancer Target Discovery & Development Network) for Functional Genomics (2012 - )
Two HTS/HCS Platforms for Diverse Screens

- Chemical libraries: 365,000 compounds
- CambridgeSoft database
Emory Chemical Biology Discovery Center

Two HTS/HCS Platforms for Diverse Screens

- **High Throughput Screening (uHTS)**

- **High Content Screening (uHCS)**

- **Label-free Screening**
  - Biochemical
  - Cell-base

- **In silico screening**

Small molecule Modulator discovery

Systems biology
Octet RED384 System

Key Features:
- High sensitivity detection
- 16-well simultaneous sampling
- 384-well and 96-well formats
- Two plate locations maximize throughput and improve workflow
- Biosensor re-racking allows maximum flexibility and operational cost savings
Case study 1: the use of Octet RED384 for characterization of 14-3-3 protein inhibitors
Targeting 14-3-3 protein-protein interactions in cell survival signaling

- 14-3-3 suppresses cell death and promotes cell survival

- 14-3-3ζ overexpression correlates with poor survival of lung cancer patients

Small molecule 14-3-3 inhibitor discovery -
Identification of new scaffolds of 14-3-3 inhibitors

TR-FRET assay in 1536-well uHTS format:

14-3-3ζ/Bad uHTS: 217,515
Primary active: 1128
IC50 <20 uM: 200
Chemical classes:
21 chemical structural clusters

Development of the direct binding assay for 14-3-3 protein inhibitors

- Immobilize protein into SSA biosensor

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\[ 14-3-3 \text{ζ} (\text{WT}) \]
\[ 14-3-3 \text{ζ} K49E (\text{MT}) \]

Max
Biocytin

Labeling protein with Biotin

Equilibrium (Buffer)
Loading (Biotinlated protein)
Quenching (Biocytin)
Wash (Buffer)

14-3-3 K49E (MT)

14-3-3 ζ (WT)

Max
Biocytin

Good protein immobilization signal
Validate the specificity of the 14-3-3 protein binding assay with peptides

pBad peptide is derived from a well studied 14-3-3 binding protein, Bad.

- 14-3-3ζ WT showed higher binding signal than mutant 14-3-3 protein to pBad peptide.
- The binding signal of pBad to 14-3-3 was higher than its binding to Bad peptide.
- Negative controls, Max protein and Biocytin, did not give rise to any binding signal.
Optimize conditions for 14-3-3/compound binding

Increasing DMSO and Tween-20 in the buffer for compound 42# at 50 µM

- Higher percentage of DMSO may increase compound solubility decrease non specific binding (NSB).
- 0.01% Tween-20 may decrease NSB
- Use 20% DMSO, 0.01% Tween-20 as assay buffer for next binding study.
Identification of 14-3-3 binding compounds from HTS hits

- Series of compounds have shown to bind 14-3-3 protein directly.
Challenges: non-specific binding of the compounds

Major cause: compound solubility?

- Non-ideal heterogeneous curve occurs at higher concentrations of compound.
- Buffer optimization to increase compound solubility is necessary to decrease the non-specific binding of the compound.

Typical non-ideal curve!
Screening buffer conditions to increase compound solubility and minimize aggregate-induced non-specific binding.

- Tris buffer+20% DMSO+0.01% Tween-20: ~5 µM
- PBS buffer+20% DMSO+0.01% Tween-20: ~6 µM
- Kenetics buffer+20% DMSO+0.01% Tween-20: ~4 µM
- HEPES buffer+20% DMSO+0.01% Tween-20: ~8 µM

82# compound is soluble in HEPES buffer at upto ~8 µM.

Standard compound: Phenytoin
Reported solubility: 32 µg/ml

Phenytoin (µg/ml)
Counts
-10000 0 10000 20000 30000 40000 50000
0 5 10 15 20
0 2000 4000 6000 8000 10000 12000

Counts

Optimize conditions to increase compound solubility
Direct binding of cpd 82# to 14-3-3

Binding signal increases with increasing compound concentration within the solubility range in optimized HEPES buffer.

- 82# compound has shown the direct binding to 14-3-3 protein.
- 82# compound may serve as a lead class for validation and optimization.

**82# (µM)**

- 0
- 0.3
- 0.6
- 1.2
- 2.5
- 5
- 10

**HEPES buffer+20% DMSO+0.01% Tween-20**

**K_D=3.3 µM (82# with 14-3-3ζ WT)**
Case study 2: the use of Octet RED384 for mechanism of action study
Application of BLI for compound MOA study: 14-3-3 isoform selectivity

82# compound selectively binds to 5 of 7 isoforms of 14-3-3 proteins.

- 14-3-3ζ: KD = 0.55 µM
- 14-3-3η: KD = 0.8 µM
- 14-3-3τ: KD = 0.89 µM
- 14-3-3β: KD = 0.55 µM
- 14-3-3γ: KD = 0.65 µM

Assay buffer: PBS + 0.01% BSA, 0.002% Tween20, 0.005% sodium azide + 10% DMSO.

No detectable binding signal for 82# compound with 14-3-3σ and 14-3-3ε.

- 82# compound is not a pan 14-3-3 inhibitor.
- 82# compound does not bind to 14-3-3σ, which is the only 14-3-3 isoform with tumor suppressor function, indicating its potential therapeutic application.
82# compound series show distinct binding with different mutants of 14-3-3ζ protein.

L227 of 14-3-3 protein is likely the binding site for 82 compound series.
Case study 3: the use of Octet RED384 for characterization of challenging enzyme inhibitors
Production of biotinylated Active Enzyme

**Avi-Tag Cloning**

- HisSUMO
- 15-mer Avi-tag (GLNDIFEAQKIEWHE)

**Small-scale purification**

1. Ni²⁺-affinity
2. ULP1 cleavage
3. Ion exchange
4. S200 Exclusion

Fractions pooled.
Buffer optimization for enzyme/small molecule binding

Positive compound (1 µM)

50 µM HEPES, 5% Glycerol
1: pH 7.8
2: pH 7.8, 0.01% Tween-20
3: pH 8.0
4: pH 8.0, 0.01% Tween-20
5: pH 8.2
6: pH 8.2, 0.01% Tween-20

PBS
7: pH 7.2
8: pH 7.2, 0.01% Tween-20
9: pH 7.4
10: pH 7.4, 0.01% Tween-20
11: pH 7.6
12: pH 7.6, 0.01% Tween-20

300 mM NaCl, 100 mM MnCl₂, 2% DMSO
Monitoring the binding of inhibitors to active enzyme

After buffer optimization, we are able to detect weak small molecule binders to the active enzyme.

The data is consistent with the data from ITC.
Summary of application of OCTERED 384 for small molecule discovery

- ECBDC’s capability enables small molecule modulator discovery through large scale HTS.
- Integration of OCTET RED384 system allows characterization of the direct binding of small molecules to target proteins.
- Increase solubility of small molecule minimizes non-specific binding to the biosensor for binding selectivity and specificity.
- OCTET system enables small molecule MOA study and cost effective discovery of challenging enzyme inhibitors.
- Caution: data interpretation
ECBDC technologies for Small Molecule Discovery

- **Protein-protein interaction**
  - Ligand-receptor binding (in vitro)
  - Ligand-induced binding
  - Enzyme assays
    - Ligand-receptor binding (cell-based or membrane-based)
    - Affinity assays
  - Reporter gene assays
    - Secondary message
    - Cell-based ELISA
    - Cell-based physiological assay
    - Cell growth/proliferation

- **Cellular image (HCS)**

- **Multi-label plate reader (96/384/1536 well)**
  - Absorbance
  - Luminescence
  - Fluorescence Intensity (FI)
  - Fluorescence Polarization (FP)
  - Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
  - AlphaScreen
  - BRET

- **Automated image reader (96/384/1536 well)**
  - Protein translocation (e.g., receptor internalization)
  - Clustering of cell-surface membrane proteins
  - Morphometric measurements (e.g., neurite outgrowth)
  - Calcium imaging in living cells
  - Target identification via arrayed siRNA libraries

- **Label-free detection (384 well)**
  - System 1: Corning Epic
  - System 2. Octet RED 384 (ForteBio)
Emory Chemical Biology Discovery Center (ECBDC)

Integrated operation with other academic units

We are committed to enabling translational research!

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