Research Resources 101:

"Emory Chemical Biology Discovery Center (ECB D C)"

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Overview

• Introduction – druggable genome
• Emory Chemical Biology Discovery Center
  “Enabling Academic HTS, HCS, and HT Biology”
• HTS & HCS platforms
• Converting an assay from the bench to HTS
• HTS Project examples
• Collaborative model and contact info

Learning Objectives

1. To recognize capabilities and resources for small molecule modulator discovery through high throughput screening
2. To understand basic principles to convert a conventional assay to a high throughput assay for large scale screening
Chemical Biology & Drug Discovery: interaction of biological target space with chemical space

*Forward* pharmacology – active drugs to MOA/targets

*Reverse* pharmacology - targets to compounds/drugs

β tubulin

**Meyerkord & Fu (2012) Harnessing the power of chemistry for biology & Medicine.**

*In “Chemical Genomics” (Ed. H Fu), p1-9.*
Current drug targets encoded by the human genome


- GPCR (36% drugs-357/989)
- Ion channels
- RTK
- NR

- Transporters 15%
- Enzymes 29%
- Others 51%
- Receptors 44%

Number of targets

Current targets and targets in clinical trials

Johns, Russ, Fu (2012) “Current Drug Targets & the Druggable Genome” In CHEMICAL GENOMICS (Ed. Fu, H; Cambridge U Press; pp320-331)
The changing landscape of drug targets

Ave 18 new drugs/yr; (4 NTD/yr) 60% small molecule agents

NTDs approved per year

NTD: Novel target drugs
Chemical biology & drug discovery

Use chemical tools to study biology
Use chemicals for drug discovery—targets
**Mechanism-based Drug Discovery Process**

1. **Target identification & validation**
2. **Screening of small molecules for target modulators (HTS & HCS)**
3. **Validation bioassays**
4. **Identify potential chemical leads**
5. **Chemical lead optimization**

**Preclinical development**

- **IND & Clinic development**
  - ADME (absorption, distribution, metabolism, elimination)
  - Toxicology
  - Drug interactions
  - Animal model
Target identification & validation

Screening of small molecules for target modulators (HTS & HCS)

Validation bioassays

Identify potential chemical leads

Chemical lead optimization

Preclinical development

IND & Clinic development

Old paradigm

Universities

Pharma

Biotech

ADME (Absorption, Distribution, Metabolism, Elimination)

Toxicology

Drug interactions

Animal model
Target identification & validation

Screening of small molecules for target modulators (HTS & HCS)

Validation bioassays

Identify potential chemical leads

Chemical lead optimization

Preclinical development

IND & Clinic development

Universities

ADME (absorption, distribution, metabolism, elimination)
Toxicology
Drug interactions
Animal models

Pharma

Biotech
In the old days (up to ~year 2000)

- Academics studied drug targets
- Pharmaceutical companies developed drugs from these targets

Now

- Human genome project and advances in biology revealed large number of potential targets
- Cost of high throughput screening instrumentation has come down & technology advanced
- Small molecule libraries are commercially available

What developments make HTS/HCS in universities possible?
Mission

• To discover novel chemical leads targeted to disease-related proteins for research tools and therapeutics
• To enable high throughput biology
• To train the next generation of drug discovery scientists
Emory Chemical Biology Discovery Center

Hit optimization

ChemINFORMATICS

High throughput screening

Assay adaptation

Target identification

IP

Research tool
Emory Chemical Biology Discovery Center: HTS/HCS platforms

HTS, HCS assays ➔ Integrated HTS/HCS operations ➔ Lead discovery & development

External projects

Internal Projects

High Throughput Label-free Biosensor System 384-plate format

Small molecule compound libraries:
Natural products & synthetic compounds
Integrated HTS instrument

Barcode reader

Liquid dispenser

Liquid handler

Computer

Robotic arm

Multimode plate reader

Incubator/plate hotel

Image reader (HCS)

Washer
Conversion from bench to HTS assays

Assay adaptation

Protein
(x µl/well)

Peptide
(Rhodamine-peptide)
(x µl/well)

Screening:
add compound to each well

Signal output

Setting: Fluorescence Polarization
Ex: 545 nm
Em: 610-75 nm

Analyst HT
## Features of HTS assays vs “bench top” assays

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bench top</th>
<th>HTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protocol</strong></td>
<td>May be complex with numerous steps, aspirations, washes</td>
<td>Few (5–10) steps, simple operations, addition only preferred</td>
</tr>
<tr>
<td><strong>Assay volume</strong></td>
<td>0.1 ml to 1 ml</td>
<td>&lt;1 µl to 100 µl</td>
</tr>
<tr>
<td><strong>Reagents</strong></td>
<td>Quantity often limited, batch variation acceptable, may be unstable</td>
<td>Sufficient quantity, single batch, must be stable over prolonged period</td>
</tr>
<tr>
<td><strong>Handling</strong></td>
<td>Manual</td>
<td>Robotic</td>
</tr>
<tr>
<td><strong>Variables</strong></td>
<td>Many—for example, time, substrate/ligand concentration, compound, cell type</td>
<td>Compound, compound concentration</td>
</tr>
<tr>
<td><strong>Assay container</strong></td>
<td>Varied—tube, slide, microtiter plate, Petri dish, cuvette, animal</td>
<td>Microtiter plate</td>
</tr>
<tr>
<td><strong>Time of measurement</strong></td>
<td>ms to months. Measurements as endpoint, multiple time points, or continuous</td>
<td>Minutes to hours. Measurements typically endpoint, but also pre-read and kinetic</td>
</tr>
<tr>
<td><strong>Output formats</strong></td>
<td>Plate reader, radioactivity, size separation, object enumeration, images interpreted by human visual inspection</td>
<td>Plate reader—mostly fluorescence, luminescence and absorbance</td>
</tr>
<tr>
<td><strong>Reporting format</strong></td>
<td>“Representative” data; statistical analysis of manually curated dataset</td>
<td>Automated analysis of all data using statistical criteria</td>
</tr>
</tbody>
</table>

Types of HTS assays

Biochemical Assays
- Affinity /Binding
  - Functional

Cell-based Assays
- Affinity /Binding
  - Functional

Increasing molecular complexity

- 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
Detection technologies for HTS

- **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)**

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

Readout: automated image reader
HCS (96/384/1536 well plates)
Image-based High Content Screening (HCS) Assay

HCS cell-based assays:
- 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

Readout: Automated image-reader (96/384/1536 well plates)
- Fluorescence image of the cells

Automated image acquisition and data analysis software
Assay Development for HTS: What to consider & How to evaluate?

Criteria for HTS assay

- good target sensitivity and specificity
- robust readout
- day-to-day reproducibility
- technical simplicity
- suitability for automation
- low cost

Evaluation of the assay performance for HTS:

- \( Z' \): evaluate the quality of a HTS assay

\[
Z' = 1 - \frac{(3SD_{\text{Signal}} + 3SD_{\text{Background}})}{(F_{\text{Signal}} - F_{\text{Background}})}
\]

0.5 < \( Z' \) < 1 indicates robust assay for HTS. Zhang et. al (1999) J Biomol Screen 4(2):67

- S:B: signal-to-background ratio
- DMSO tolerance
- Stability
"Compound libraries"

- Collections of diverse small molecules (300-600)
- Greater than 90% of approved drugs have a molecular size in the range of 200-800.
- Commercially available: > 8,000,000 small molecules (ChemNavigator database)
- Cost: depends on the nature of the library
- Compound stock: powder or 10 mM in DMSO in 96- or 384-well format
- Compound purity: > 80-90%
Data Analysis and Informatics

Data Collection and Analysis:
- $Z'$: evaluate HTS assay quality
- S:B: signal-to-background ratio

Software Tools
- facilitate data collection and analysis
- organize compound libraries and search compound structures

Commercial available database
Fluorescence Polarization (FP) assay to monitor 14-3-3 protein-Raf-1 interaction

Case study 1

- Polarized excitation light
- Rapid rotation
- Macromolecules (> 10,000 Da)
- Slow rotation
- Low FP
- FP signal
14-3-3 binding to the phospho-Raf peptide leads to FP signal:

\[
\text{FP Assay window} = \mu_b - \mu_f
\]

\[
Z' \text{ factor} = 1 - \frac{3 \text{ SD}_b + 3 \text{ SD}_f}{\mu_b - \mu_f}
\]

\[
S:N = \frac{\mu_b - \mu_f}{(\text{SD}_b^2 + \text{SD}_f^2)^{0.5}}
\]

SD\(_b\) and SD\(_f\) are standard deviation for bound (\(b\)) and free (\(f\)) peptide, \(\mu_b - \mu_f\) is the difference in mean signals for bound and free peptide.

FP Assay Development for HTS of 14-3-3 inhibitors

- **Specificity**
  - GST-14-3-3γ
  - GST

- **Validation**
  - R18
  - R18Lys

- **Stability**
  - Assay window (mP)
  - GST-14-3-3γ (µM)

- **DMSO tolerance**
  - Assay window (mP)
  - DMSO (%)

Z’ > 0.7
S/N > 8

FOBISINs (FOurteen-three-three BIInding Small molecule INhibitors)

- FOBISIN101 is a pan 14-3-3 inhibitor.
- FOBISIN101 is docked in the 14-3-3 ligand-binding site as revealed by co-crystal structure.
- FOBISIN101-like molecules may serve as lead compounds for therapeutic discovery.

Screening and characterization of HTS hits

Development of Cell-based TR-FRET Assay for cAMP Measurement in a 1536 well uHTS format

Case Study 2: cell based HTS

Status epilepticus

NMDAR → Ca²⁺ mobilization → cAMP

ERK → p38 → Cox-2

cPLA₂ → AA → PGH₂ → NFκB

IL1β → microglia

Ca²⁺ mobilization → cAMP

Ca²⁺ mobilization → nucleus

inflammation, neuron injury, synaptic plasticity, etc.

Cell-based cAMP competition TR-FRET assay

Donor cAMP → TR-FRET signal

Acceptor cAMP → TR-FRET signal

Donor cAMP → TR-FRET signal

Acceptor cAMP → TR-FRET signal

PGE₂ (nM)

EC₅₀ = 4.4 nM
uHTS of EP2 Allosteric Potentiators & Hit Confirmation Strategy

A. Potentiation (% max)

well number (thousands)

2352 hits

B. Secondary assays

- (a) Confirm: Repeat EP2 primary assay
- (b) Selectivity: Effect on EP4
- (c) False positives: effect on cAMP assay
- (d) Selectivity: Effect on β2-AR
- (e) False positives: Effect on parent C6 cells

Effect on PGE2 dose-response curve

Structural clustering

C. % induction of cAMP response

SID 14735057 cluster 45
SID 24797125 cluster 78

High throughput/content screening assays

High throughput assays (384-well plates):
• Receptor-ligand interaction
• Protein-protein interaction
• Enzyme assays
• Reporter assays (luciferase, GFP, etc)
• Viability and growth assays

Cell-based high content assays (96/384/1536-well plates):
• Protein translocation (e.g., receptor internalization)
• Clustering of cell-surface membrane proteins
• Morphometric measurements (eg, neurite outgrowth)
• Calcium imaging in living cells
• Target identification via arrayed siRNA libraries
Automated microscope for High Content Screening (HCS)
HCS Assay Development: NF-κB Translocation Assay using ImageXpress

Case study 3

Cytokine signals

IKK

Iκb

inactive NF-κB

degradation

Translocation of NFκB into nucleus

active NF-κB

Cancer
Inflammatory diseases,
Rheumatoid arthritis (RA)
Autoimmune disease

cytoplastic

nuclear

Project of Dr. Haian Fu; Emory University
Compounds that Block TNF\(\alpha\)-induced Nuclear Translocation of NF-\(\kappa\)B

Case study 3
3 parallel screens of 100,000 compounds have been completed.

Angiogenesis inhibitors in zebrafish embryos

Use of a reporter assay

Count intersomitic vessels

PTK787

water
Case study 4
Cell-based Pathway Analysis using Optical Biosensors

Micromotion of cells in response to a given signal:
• Dynamic movement
• Remodeling of cellular structure

Dynamic Mass Redistribution (DMR) signal
Evanescent wave
Detection zone (~150nm)
Broadband Light Source
Reflected Wavelength

Cell (layer 3)
Waveguide (layer 2)
Substrate (layer 1)

EGF
EGFR
Gefitinib
Erlotinib
LY294002
Wortmannin
HCTs for new Pathway Inhibitors

Δ Response (pm)

Time (sec)

UPCI-37B SCCHN cancer cells
A549 lung cancer cells
Gefitinib

### HTS Assay Examples at ECBDC

#### Biochemical HTS assays

### Protein-protein interactions
- Mcl1-Noxa/Bid 
  (Z. Nikolovska-Coleska)
- eIF4E-eIF4G (J. Pelletier)
- **14-3-3-Raf/Bad/Yap** (H. Fu)
- Estrogen Rα-coactivator 
  (J. Katzenellenbogen)
- p47-phox (D. Lambeth/S. Smith)
- BMP4-BMP4R (H. Jo)
- Hsp90 (C. Gabriela)
- gp42-gH/gL (T. Jardetzky)
- TrkB – substrate (J. McNamara)
- GTPCH-1/GFRP (D. Harrison)
- Myc/Max (E. Prochownik)
- Also PRI, PDI

### Enzyme assays
- BAP1 (K. Wilkinson)
- Calpain II (G. Miller)
- ASK1 (H. Fu)
- DnaK (M. Sturgess)

### Protein folding
- Label-free biosensor
- Genetically transformed organoids 
  (C. Kuo/Y. Du)
- 3D culture
- Angiogenesis (D. Thanh)

### Target-directed pathway assays
- EP2 prostanoid receptor (R. Dingledine)
- CHOP pathway (R. Kaufman)
- KLF5 pathway (V. Yang)
- Measles infection (R. Plemper)
- HIV fusion/infection (G. Maliken)

### Cell
- EGF/IGF pathways (Y. Du)

### Organoids
- Genetically transformed organoids 
  (C. Kuo/Y. Du)

### Organism
- zebrafish/organism
### HTS is effective

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target Class</th>
<th>Indication</th>
<th>Year HTS was run</th>
<th>Year of FDA approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib</td>
<td>Tyr kinase</td>
<td>Cancer</td>
<td>~1993</td>
<td>2003</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Tyr kinase</td>
<td>Cancer</td>
<td>~1993</td>
<td>2004</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Tyr kinase</td>
<td>Cancer</td>
<td>1994</td>
<td>2005</td>
</tr>
<tr>
<td>Tipranavir</td>
<td>Protease</td>
<td>HIV</td>
<td>~1993</td>
<td>2005</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>Protease</td>
<td>Diabetes</td>
<td>~2000</td>
<td>2006</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>Tyr kinase</td>
<td>Cancer</td>
<td>1997</td>
<td>2006</td>
</tr>
<tr>
<td>Maraviroc</td>
<td>GPCR</td>
<td>HIV</td>
<td>1997</td>
<td>2007</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>Tyr kinase</td>
<td>Cancer</td>
<td>~1993</td>
<td>2007</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>GRCP</td>
<td>Pulmonary hypertension</td>
<td>~1995</td>
<td>2007</td>
</tr>
<tr>
<td>Etravirine</td>
<td>Reverse transcriptase</td>
<td>HIV</td>
<td>~1992</td>
<td>2008</td>
</tr>
<tr>
<td>Tolvaptan</td>
<td>GPCR</td>
<td>Hyponatremia</td>
<td>~1990</td>
<td>2008</td>
</tr>
<tr>
<td>Eltrombopag</td>
<td>Cytokine receptor</td>
<td>Thrombocytopenia</td>
<td>1997</td>
<td>2009</td>
</tr>
</tbody>
</table>

ECBDC Collaborative Model – Recent Examples

**Federal Funding**

**NIH** R01 “Identification and Characterization of small molecule inhibitors of HIV-1 fusion” 2013-2016 Total Award: **$889,200**

- Greg Melikyan (PI) & Yuhong Du (mPI)

**High-Throughput HIV-Cell Fusion Assay for Discovery of Virus Entry Inhibitors**

Marina Marin,1,* Yuhong Du,2,3,* Charline Giroud,1

Jeong Hwa Kim,1 Min Qui,2,3 Haian Fu,2–4

and Gregory B. Melikyan1,5

VOL. 13 NO. 3 • APRIL 2015 ASSAY and Drug Development Technologies

**NIH** R33 “ArmR: a novel drug target and mediator of antibiotic resistance” 2014-2017 Total Award: **$1,569,981**

- David Weiss (PI) – Infectious Diseases

**Non-profit disease foundations**

**Fast Forward** National MS Society/Merck KgaA “Targeting the Oligodendrocyte-Enriched Receptors GPR37 and GPR37L1” 2015 Total Award for Phase 3: **$215,000**

- Randy Hall, PhD (PI) – Department of Pharmacology

**CURE Foundation** “Identification of novel therapeutics to restore chemotherapy response in high risk neuroblastoma” 2014-5 Total Award for Phase 3: **$105,000**

- Kelly Goldsmith, MD (PI) – Pediatrics (CHOA)

**Pilot funding**

**Emory-Udall Parkinson's Disease Center Pilot Grants Program** “Identification of DJ-1 substrates and DJ-1 targeting compounds for Parkinson’s disease therapeutic discovery” 2014-5 Total Award: **$30,000**

- Lih-Shen Chin, PhD (PI) – Department of Pharmacology
Emory Chemical Biology Discovery Center (ECBDC)

*Integrated operation with other academic units*

We are committed to enabling translational research at Emory!

contact us at [ECBDC@pharm.emory.edu](mailto:ECBDC@pharm.emory.edu)

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