CBC Tech Day: Optimizing Hits

July 9, 2012
Work Flow

**Hits**
- Structural Computation
- MedChem

**Leads**
- Pharmacokinetics
  - (Administration, Metabolism, Excretion)
  - Toxicity

**Candidates**
Today’s Presentations

- **Structural Approaches**
  - Dr. Wayne Anderson (Northwestern University)

- **Computational Approaches**
  - Dr. Jie Liang (UIC)
  - Dr. Pavel Petukov (UIC)

- **Medicinal Chemistry Approaches**
  - Dr. Karl Scheidt (Northwestern University)
  - Dr. Sergey Kozmin (University of Chicago)
  - Dr. Gregory Thatcher (UIC)

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"Optimizing Hits: Structural, Computational, and MedChem Approaches"

Drs. Wayne Anderson (NU), Jie Liang (UIC), Pavel Petukov (UIC), Sergey Kozmin (UofC), Karl Scheidt (NU), and Gregory Thatcher (UIC)

### Overview
- Once a library has been screened by high-throughput screening (HTS), ‘hits’ are optimized in a variety of manners:
  - The target can be crystallized in the presence of the hit, and the three-dimensional structure of the complex solved
  - A comparison of the chemical structures with activity can lead to a structural-activity relationship (SAR)
  - Analogizing of various side chains on a hit can identify positions that improve affinity and selectivity
- Once a hit has been improved, it can become a ‘lead’ for additional biological testing, and, if lucky, transitions into a ‘drug candidate’

### Structural Approaches
- Provide experimental data on how a small molecule ‘hit’ interacts with the target protein and suggest likely modifications to improve affinity and selectivity.
  - NMR and/or crystallographic methods can be used to determine the structures of protein-small molecule complexes
  - Crystalline complexes can be formed either by co-crystallization or crystal soaking
  - Initial hits can be low affinity ‘fragments’
  - Compounds with very low solubility in water can be a problem
- Chicago area institutions provide access to many resources and facilities for carrying out structure based optimization
  - The most important resource is the Advanced Photon Source (APS) at Argonne National Laboratory, where synchrotron beamlines focused on macromolecular crystallography make it possible to tackle difficult problems and apply high throughput methods

### Computational Approaches
- Computational analysis of the HTS hits
  - Typical scenarios – too many hits, too few hits, no hits
  - Typical false positives
  - Mining for other types of activities in Pubmed/PubChem
  - Similarity, dissimilarity, mining for common scaffolds
  - Pharmacophore modeling
  - Searching for analogs
  - Choice of libraries for follow-up
  - Methods for lead refinement and lead optimization
    - 2D and 3D QSAR
    - Docking, scoring
    - Computational fragment-based approach
    - ‘Hot spots’ in the binding sites
    - Receptor binding surface based compound searches
  - Binding surface calculation and evolutionary substitution calculation for promiscuity and specificity of enzyme functions.
    - Signature binding pockets for enzyme-class activities
    - Imprint of binding pocket generation and compound search
    - Model binding surface and perform large scale multiplex compound-receptor matching

### MedChem Approaches
- Hit is from HTS, “Sigma”, or “Merck” = non-proprietary
  - Database searches for structural IP space; SAR from literature
  - Synthesis of novel analogs including negative controls: screen for activity; NO-GO
  - Design of virtual library with MedChem groups to develop analogs using newer synthetic methodologies suitable for scale-up
  - In silico screening using docking or ligand-based approaches for triage
  - Iterative synthesis of analogs and testing on target protein and cell lines
  - Monitor absorption, distribution, metabolism, and excretion (ADME) and toxicity in animals
Structural Approaches

• Advantage is that you have experimental information on the position, orientation and interactions
  – If a modification of the compound results in it reorienting in the site, you will know

• NMR

• X-ray crystallography
  – Co-crystallization
    • Add compound to concentrated protein and set up crystallization screen
  – Crystal Soak
    • Use pregrown crystals and transfer to a solution containing the compound
Advantages and Disadvantages

• Reveal the atomic interactions
• See what changes to the compound may result in higher affinity
• Compare complexes with other protein structures to improve selectivity
• Can start with low affinity ‘fragments’ and rapidly optimize

• Lack of binding
• Ligand binding can disorder crystals
• Hydrophobic compounds
• Not *in vivo* conditions
Chicago Area Resources

- University facilities and resources

Advanced Photon Source
  - LS-CAT
  - SBC
  - BioCARS
  - GM/CA
  - SER-CAT

Advanced Protein Crystallization Facility
Structural Genomics Projects

Two Structural Genomics projects in Chicago Area that take requests from the scientific community in particular areas
1. CBC/CT-CMLD Cheminformatics infrastructure
   - Data storage and retrieval
   - Computing physical properties of compounds:
     • GPU implementation
   - Diversity management:
     • Descriptors, similarity, substructure searches

2. Biologically relevant chemical diversity
   - Enrichment from receptor surface:
     • Computing binding surfaces and
     • Surface comparison: sequence order independent alignment
     • Ruler: evolutionary patterns through Bayesian Monte Carlo
     • Signature of binding surface
   - Universe of receptor binding surfaces
     • Genome-wide receptor binding surface diversity
   - Comparatively modeled binding surfaces

3. Network based target validation
   - Emerging complex behavior from network.
   - Stochasticity: eg. cooperative binding
Universe of Receptor Binding Surfaces and Their Signatures

- Surface computation.
- Order independent surface alignment
- Substitution rate mapping
- Large scale mapping

\[ S_1 \]

MMP Binding Surface

Conserved Site

Kinase ATP binding sites

(Binkowski et al, 2006; Dundas et al, 2011, JMB; Tseng & Liang, 2006 MBE)
• Predicted binding surfaces
  – For signatures
• Imprint for compound enrichment

(Zhao et al, JSFG 2011; Ebalunode et al, J CIMD, 2008)
• Emerging behavior of complex network
  – Which protein is the right target?
  – Multiple critical control points?
  – Rare events

(Cao, Lu, and Liang, *Proc Natl Acad Sci USA*, 2010)
Flow of chemical information

Target (receptor)

Bioassay HTS

exploratory library

focused library

crude leads

refined leads

Chemical analogs

In-vivo safety

Optimized lead

μM potency

nM potency

Lead optimization

Lead refinement

Hit and lead identification

mine for diversity

mine for similarity

mine for specificity

In-vivo potency

Optimized lead

In-vivo safety

Chemical analogs

refined leads

focused library

exploratory library

Target (receptor)

Pan assay interference compounds. Specific mechanism of action of non-specific inhibitors

Hit and lead identification

mine for diversity

mine for similarity

mine for specificity
Typical outcomes and questions in HTS campaigns

• Outcomes
  – Too many hits
  – Too few hits
  – No hits

• Questions
  – What libraries are the best place to start?
  – How many compounds should we screen to find crude lead candidates?
  – What do we do if the chemistry is not easily amendable for analoging?
  – There are seem to be way too many (promiscuous) hits in my screening. Why were these compounds included in the libraries?
  – I have a pretty good idea about the macromolecular target but my assay is cell-based and I do not have an isolated enzyme. Is there a way to validate the target?
Hit and lead identification: Crude leads

Hit and lead identification: Specific mechanism of action of non-specific inhibitors

Hit and lead identification: Specific mechanism of action of non-specific inhibitors

Hit and lead identification: Specific mechanism of action of non-specific inhibitors

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<th>Assays Hit</th>
<th># Cpd</th>
<th>Total Cpd</th>
<th>Enrichment&lt;sup&gt;b&lt;/sup&gt;</th>
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Photoaffinity labeling as a way to validate the target/mechanism of action


Visualize the tagged protein target on the gel
Cut the spot and run protein ID by LC-MS/MS
ChemCore
Medicinal and Synthetic Chemistry Core

Center for Molecular Innovation & Drug Discovery
- Northwestern University Research Center enhancing research and training in interdisciplinary and translational medical research

ChemCore
- Provides medicinal chemistry, consulting, and instrumentation for academic drug discovery, chemistry, and chemical biology researchers

Services

Medicinal and Synthetic Chemistry
- Hit-to-Lead medicinal chemistry
- Synthesis of molecular probes

Molecular Modeling
- Virtual screening, docking, QSAR design, homology models, etc

Compound Purification
- Agilent A2Prep mass-directed preparative HPLC, etc

ChemCore is generously supported by the Chicago Biomedical Consortium with support from the Searle Funds at The Chicago Community Trust.

Support
Support from the following organizations gratefully acknowledged:

Contact
847-467-2629
drugdiscovery@northwestern.edu
www.cmidd.northwestern.edu/chemcore
- Academic research focuses on basic discoveries
  - Develops understanding of biological processes
  - Great at identifying potential new targets

- Industry develops new therapies for proven targets
  - Critical expertise in pre-clinical and clinical development
  - Great at optimizing compounds for known targets

**How to exploit new targets for new diseases?**

- Use medicinal chemistry to prove the viability of new targets

**Support**

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[Logos of supported institutions]

**Contact**

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www.cmidd.northwestern.edu/chemcore
Medicinal chemistry and cheminformatics for early stage drug discovery

- **Computational chemistry** for *in silico* screening (vHTS) and docking
- **Cheminformatics** to design novel compounds
- **Parallel synthesis** to carry out focused library production
- **Medicinal and synthetic chemistry** expertise to prepare novel molecules
- High-throughput mass-directed prep HPLC compound purification

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The mission of CENTRE is to stimulate and enhance the application of chemical, pharmaceutical, and translational knowledge to elevate biomedical discoveries at the University of Illinois to a level where the benefits of clinical application will enhance human health and benefit society.
**Project-Based Seed Grants**

### HTS Project

- Invention Disclosure with OTM
- CENTRE triage: PI+ Project Team Proposal
- Competitive Seed Grant for HTS
- Hit validated in PI’s assay
- Hit-to-Lead

### Stage 1 Project

- Client Invention Disclosure with OTM
- CENTRE triage: PI+ Project Team Proposal
- Competitive Seed Grant for Hit-to-Lead
- Druggable, proprietary, lead validated
- Lead to Drug Candidate

### Stage 2 Project

- Novel Composition IP
- PI+ Project Team Proposal
- Competitive Grant for Lead to Candidate
- Candidate with DMPK and Pretox
- IND enabling ADMET & API
The mission of CENTRE is to stimulate and enhance the application of chemical, pharmaceutical, and translational knowledge to elevate biomedical discoveries at the University of Illinois to a level where the benefits of clinical application will enhance human health and benefit society.
Hit is from HTS, "Sigma", or "Merck" = non-proprietary
1. Assay validated suitable for screening without artifacts and with controls: preferably cell-based with single protein back-up
2. Database searches for structural IP space; SAR from literature
4. Design of virtual library with Chem to develop analogues using newer synthetic methodologies suitable for scale-up
5. *In silico* screening using docking or ligand-based approaches for triage
6. Synthesis of 25-50 novel analogues iterative with screening; docking/SAR if possible; select using tPSA, etc.
7. Human liver microsomal stability; PK i.p. 30 min 1-10 mg/kg
8. Select bioavailable lead compound and back-up
9. Optional derivatization to identify targets with proteomics

Deliverable = druggable, proprietary Lead validated in client’s assays
Discussion