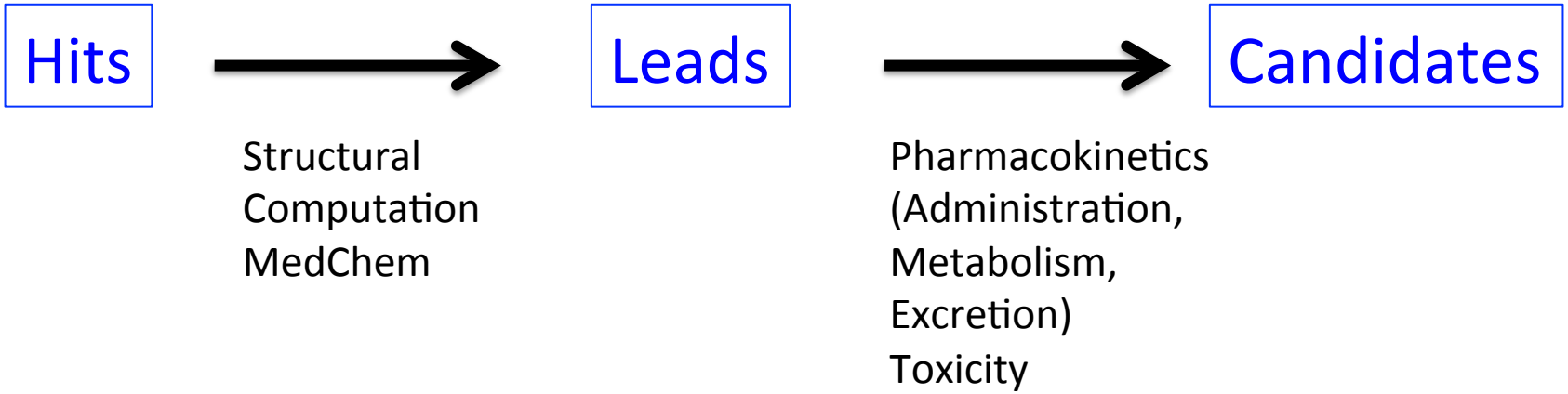
CBC Tech Day: Optimizing Hits

July 9, 2012

Work Flow


Hits
Leads
Candidates
Structural
Computation
MedChem
Pharmacokinetics
(Administration,
Metabolism,
Excretion)
Toxicity

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)

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

Drs. Wayne Anderson (NU), Jie Liang (UIC), Pavel Petukhov (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 becomes 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

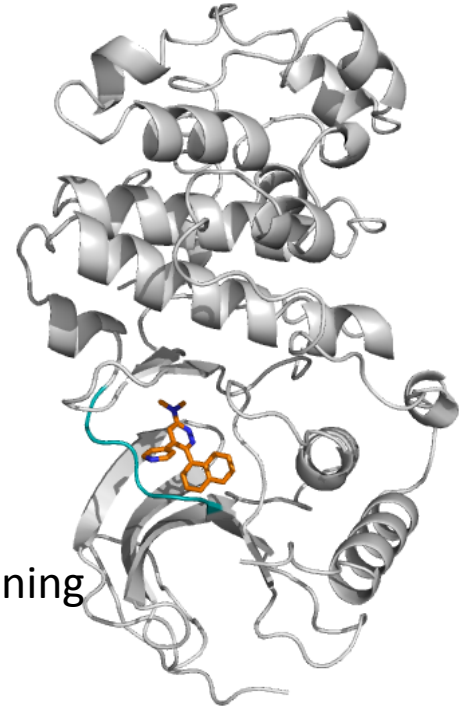
- Computational analysis of the HTS hits
  - Typical scenarios – too many hits, too few hits, no hits
  - Typical false positives
  - Mining for other types of activities in Pubmed/PubChem
  - Similarity, dissimilarity, mining for common scaffolds
  - Pharmacophore modeling
  - Searching for analogs
  - 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 calcuation for promiscuity and specificty 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

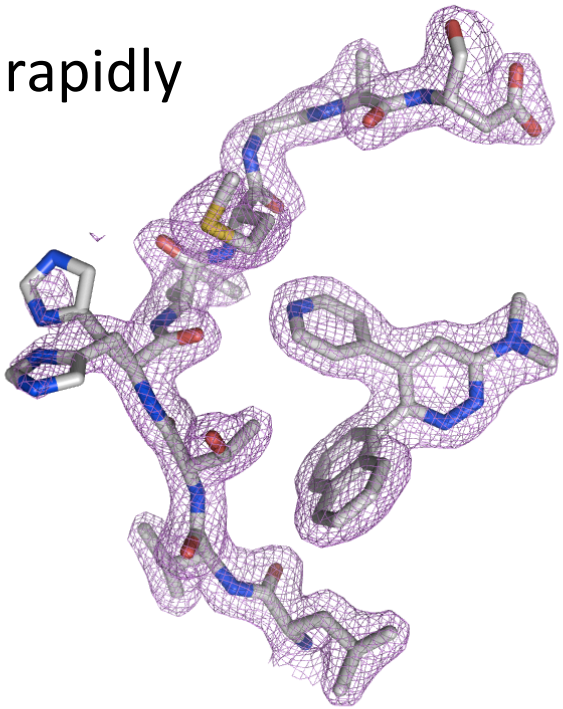
Structural Approaches

- 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

Center for Structural Genomics of Infectious Diseases

CSGID

Home | Target List | Selection | Community Requests | Clones | Screenings | XML files | Diffraction Images | Progress | Homolog Search | Clustering | Statistics | News | Help | Log In

National Institute of Allergy and Infectious Diseases
National Institutes of Health

Home | Target List | Selection | Community Requests | Clones | XML files | Diffraction Images | Progress | Homolog Search | Statistics | Log In | Help

Midwest Center for Structural Genomics

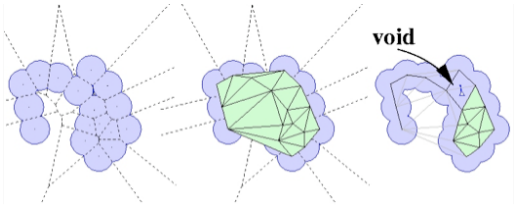
PSI
PSI:BIOLOGY

UIC Bioinformatics and Cheminformatics Colleges of Engineering and Medicine

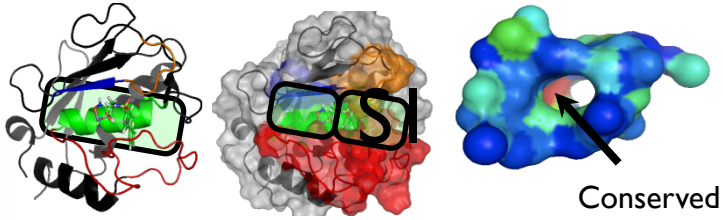
1. CBC/CT-CMLD Cheminformatics infrastructure
   - Data storage and retrieval
   - Computing physical properties of compounds:
     - GPU implementation
   - Diversity management:
     - Descriptors, similarity, substructure searches
2. Biologically relevant chemical diversity
   - Enrichment from receptor surface:
     - Computing binding surfaces and
     - Surface comparison: sequence order independent alignment
       - Ruler: evolutionary patterns through Bayesian Monte Carlo
     - Signature of binding surface
   - Universe of rececptor 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

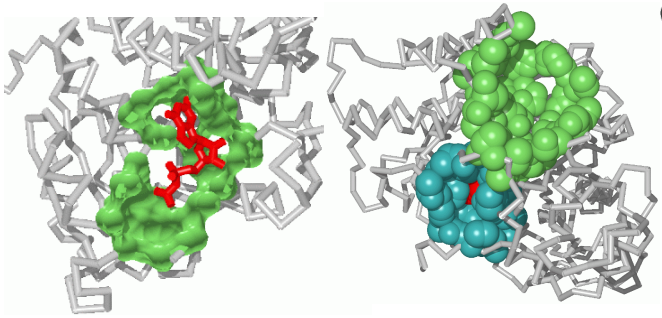
Universe of Receptor Binding Surfaces and Their Signatures

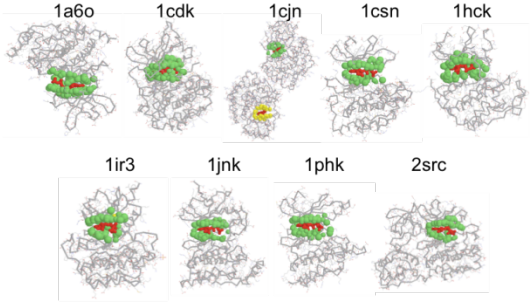
- Surface computation.
- Order independent surface alignment
- Substitution rate mapping
- Large scale mapping

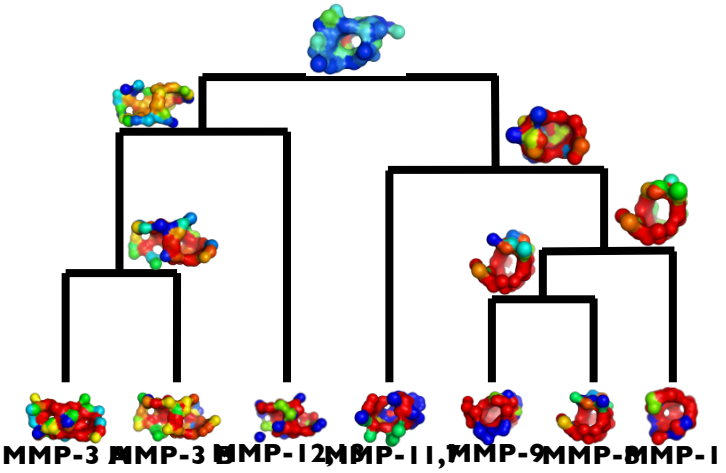


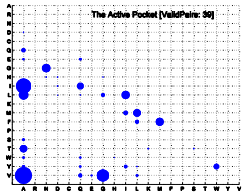
MMP Binding Surface

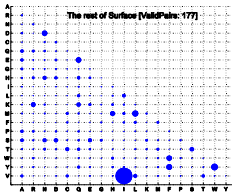










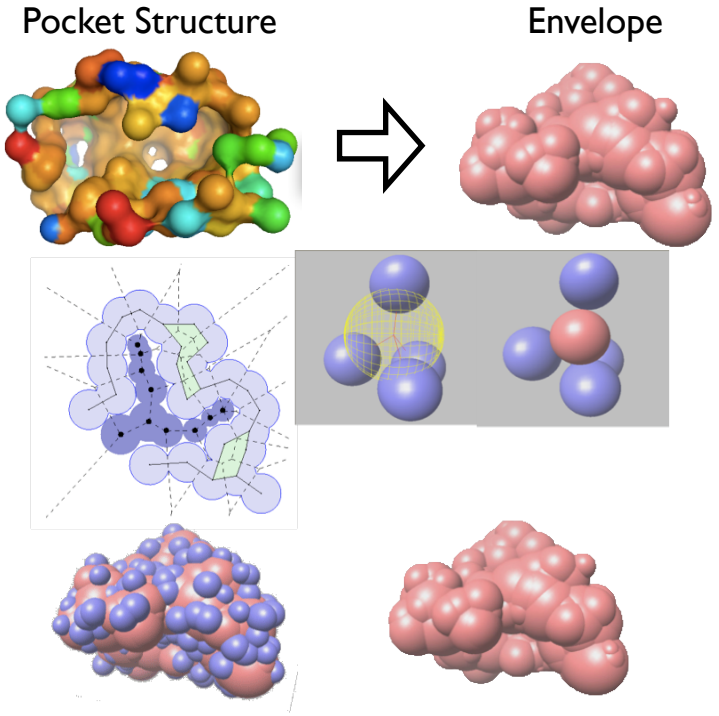


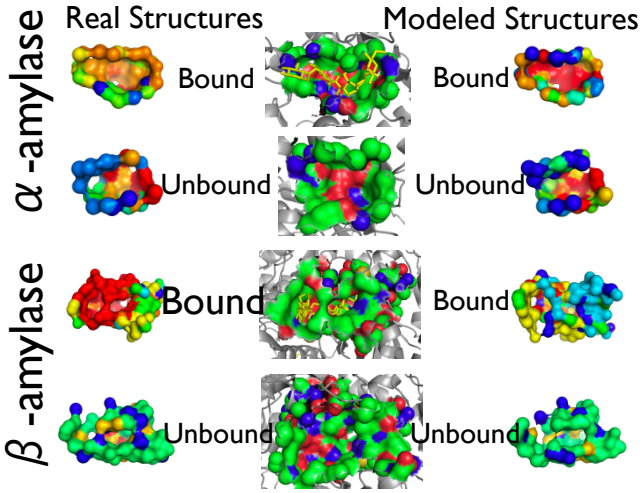
Kinase ATP binding sites

```
1ir3.A     LGQGSFG-VAKE--VMELMG--DR---M-D----- 21
2src.m     LGQGCFGEVAK---VTEYMGSDDRAN-LAD----- 26
1jnk.m     IGSGAQG-VAK---IMELMDANQKSNVLV------ 25
1phk.m     LGRGVSS-VAK---IFDLMGEDDKEN-LTDF---- 26
1csn.m     IGEGSFG-IAKEPEI-DLLGSDDKDN-LVDM---- 28
1hck.m     IGEGTYG-VAK---VFEFLD-KDKQN-LAD----- 24
1cdk.A     LGTGSFGRVAK---VMEYV---EKEN-LTDF---- 24
1a6o.m     -GRGKYS-VIKE--VFE-V---DKHN-MIDWGLAS 26
1cja.A     LSKGTL--FIKP---MELV---TKDN-MID----- 21
```

(Binkwoski et al, 2006; Dundas et al, 2011, JMB; Tseng & Liang, 2006 MBE)

- Predicted binding surfaces
  - For signatures
- Imprint for compound enrichment





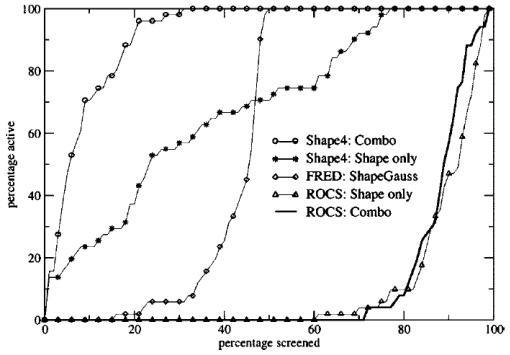
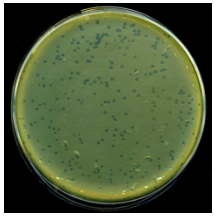
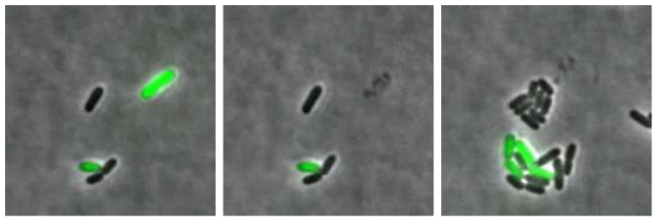


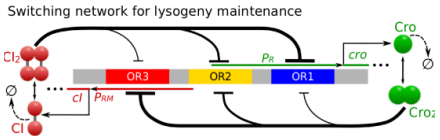
Figure 9. Enrichment plot for thymidine kinase (1KIM) virtual screening.

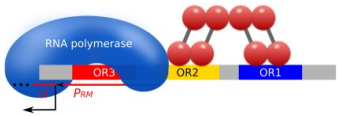
(Zhao et al, JSFG 2011; Ebalunode et al, *J CIMD, 2008*)

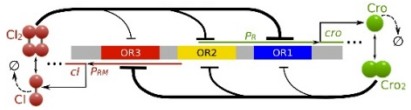
- Emerging behavior of complex network
  - Which protein is the right target?
  - Multiple critical control points?
  - Rare events

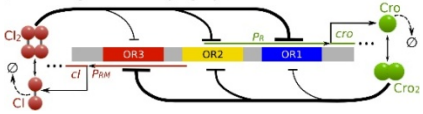


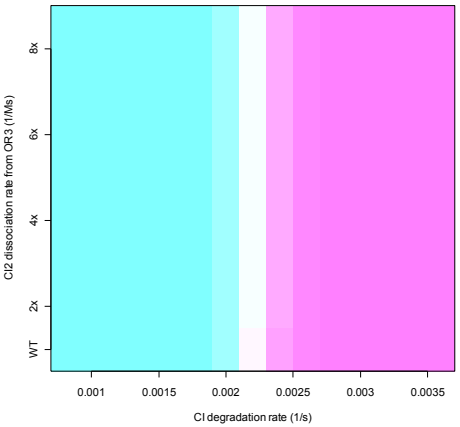


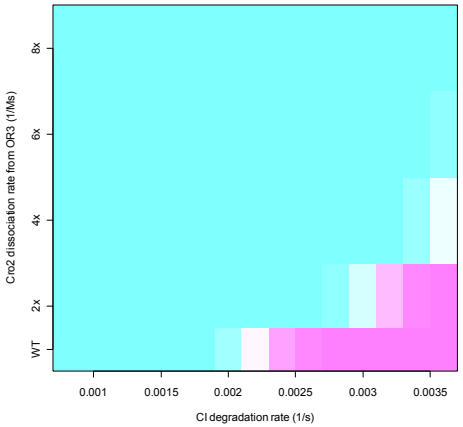


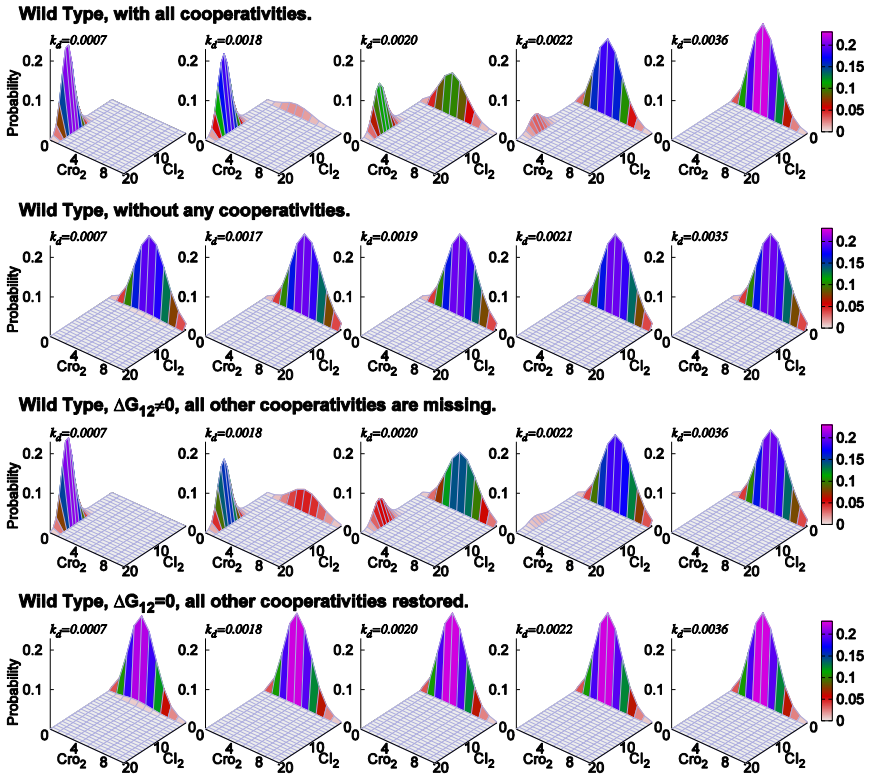












(Cao, Lu, and Liang, *Proc Natl Acad Sci USA*, 2010)

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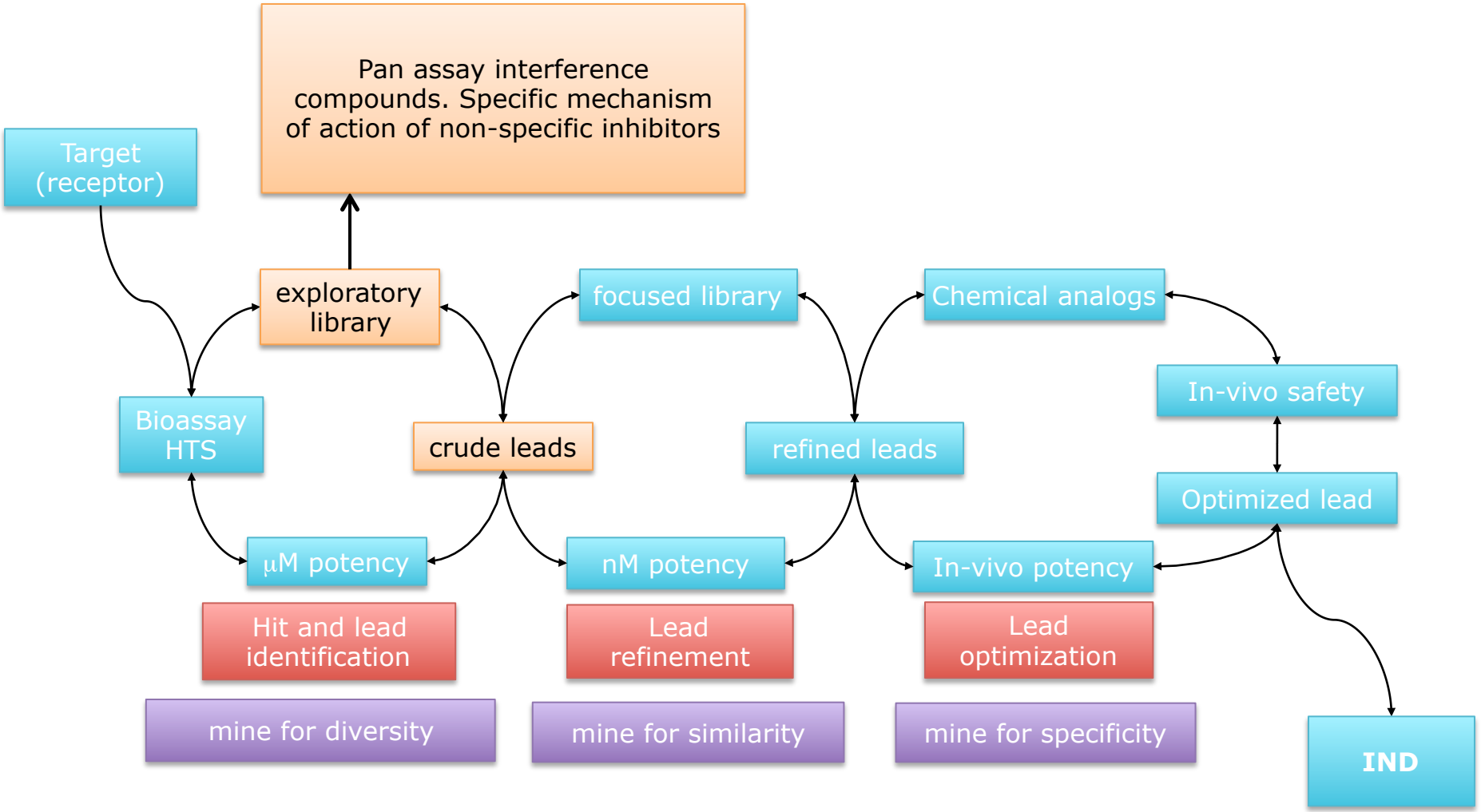
Flow of chemical information

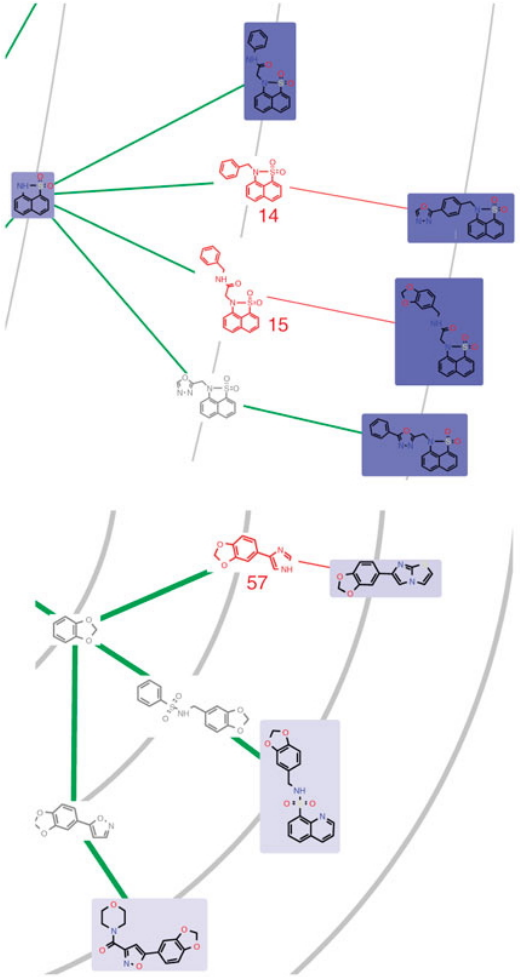


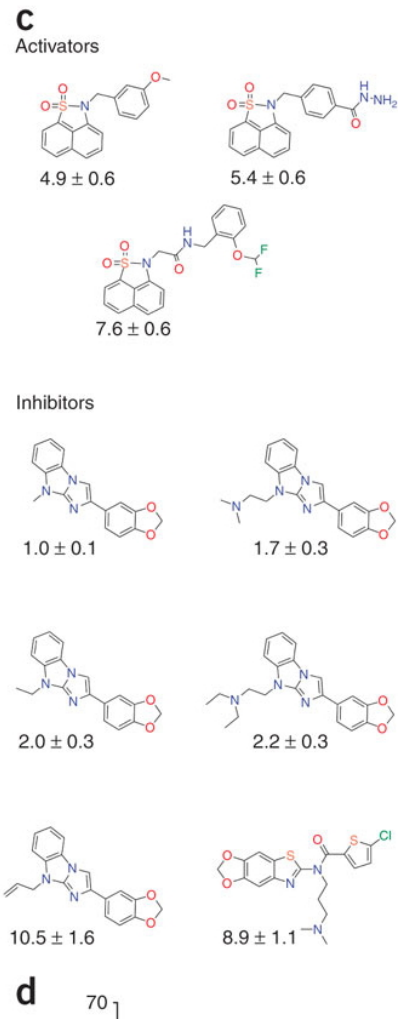
Typical outcomes and questions in HTS campaigns

- 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

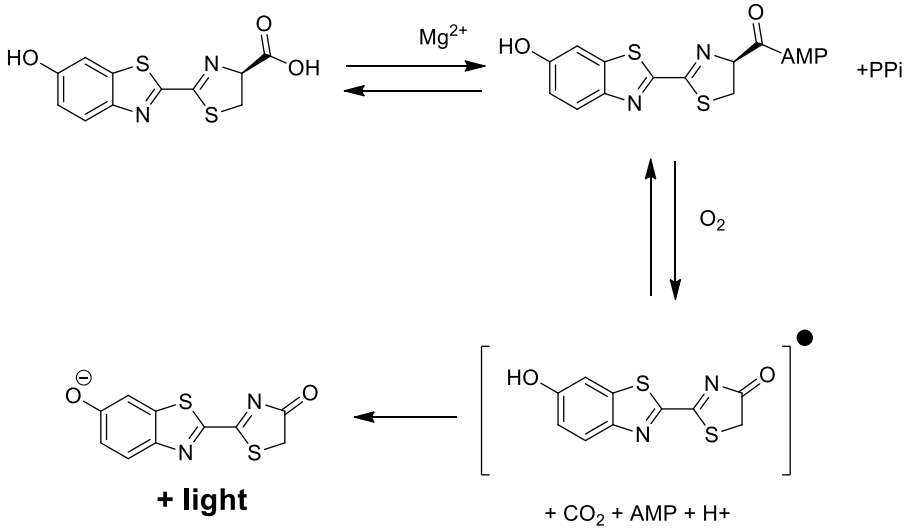
- Wetzel S, Klein K, Renner S, Rauh D, Oprea TI, Mutzel P, Waldmann H. Interactive exploration of chemical space with Scaffold Hunter. *Nat Chem Biol*. 2009;5(8):581-3. doi: 10.1038/nchembio.187.

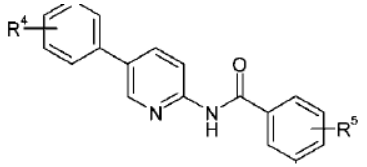




Hit and lead identification: Specific mechanism of action of non-specific inhibitors

- Heitman LH, van Veldhoven JP, Zweemer AM, Ye K, Brussee J, AP IJ. False positives in a reporter gene assay: identification and synthesis of substituted N-pyridin-2-ylbenzamides as competitive inhibitors of firefly luciferase. *J Med Chem*. 2008;51(15):4724-9. doi: 10.1021/jm8004509.

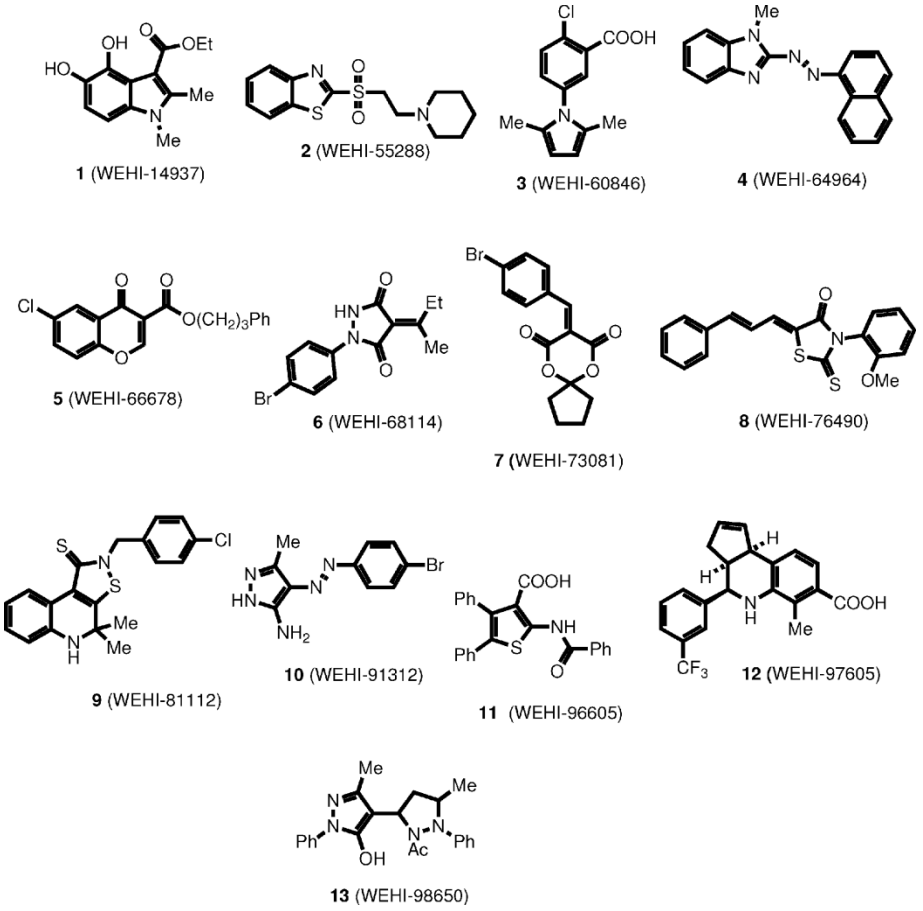




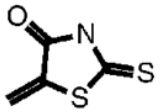
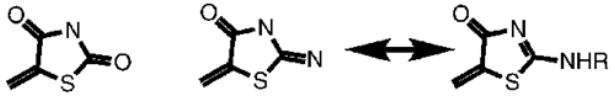
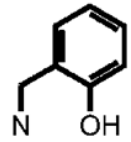
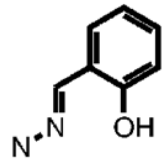
compd	R^4	R^5	IC_{50} (μM) or % inh[a]
6	H	H	0.069 ± 0.01
22	4-Cl	H	0.56 ± 0.02
23	4-OMe	H	0.31 ± 0.02
24	4-Me	H	51% (47−55)
25	3-Cl	H	38% (34−42)
26	4-$N(Me)_2$	H	14% (12−16)
27	4-O^{i}Pr	H	38% (27−48)
28	4-^{t}Bu	H	49% (46−53)
29	4-CF_3	H	39% (35−42)
30	3,4-diCl	H	4% (0−9)
31	H	4-Cl	0% (0−0)
32	H	4-OMe	28% (27−28)
33	H	4-Me	18% (17−19)
34	H	3-Cl	0.16 ± 0.01
35	H	3,4-diCl	13% (11−16)
36	H	2,4-diOMe	26% (22−30)
37	H	4-NH_2	1.2 ± 0.05
38	H	4-O^{i}Pr	12% (8−17)
39	H	4-$N(Me)_2$	35% (29−42)

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

- Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem*. 2010;53(7):2719-40. doi: 10.1021/jm901137j.

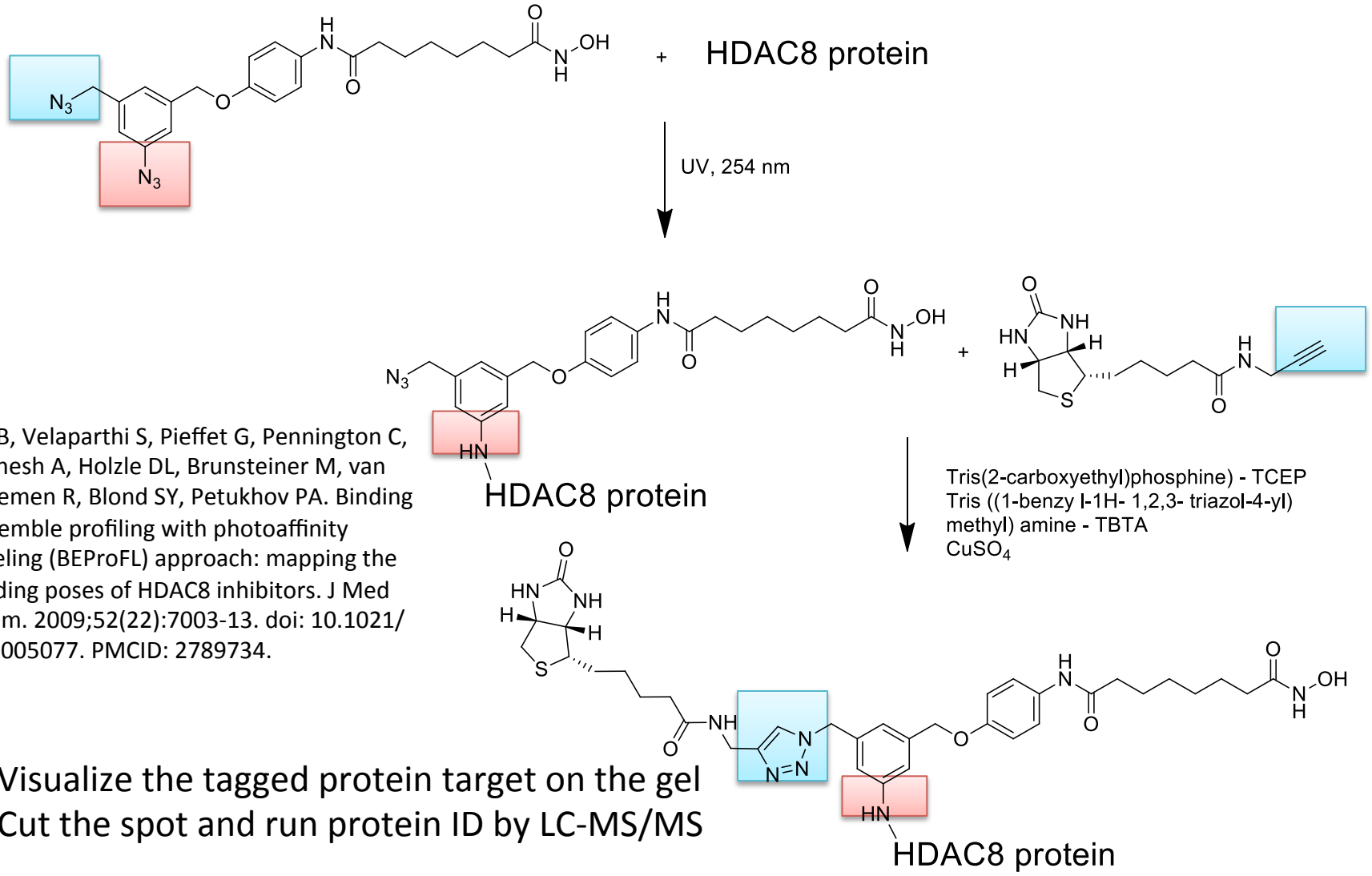


Hit and lead identification: Specific mechanism of action of non-specific inhibitors

Substructure[a]	Assays Hit	# Cpds	Total Cpds	Enrichment[b]	Refs for hits	Refs for MOA[c]
Rhodanines  ene_rhod_A	0	60	235	227%	21-55	Reactivity.[6,7,36-40] Chelation.[22]
	1	39				
	2	32				
	3	26				
	4	21				
	5	41				
	6	16				
Rhodanine-related  2-oxo 2-imino					21, 23, 25, 26, 38-42, 51, 52, 55, 56-77	Reactivity.[38,39]
Phenolic mannich bases  mannich_A	0	146	296	64%	78-83	Reactivity.[84,85,92] Chelation.[86-90] Cytotoxicity.[91]
	1	57				
	2	59				
	3	15				
	4	13				
	5	4				
	6	2				
2-Hydroxy-phenyl-hydrazone  hzone_phenol_A	0	156	479	154%	21, 41, 74, 75, 78, 91, 93-98. Also[d] 30, 75, 78-80, 91, 92, 94, 99, 100	Reactivity.[105] Spectroscopic.[101-102] Chelation.[103,104] Aggregates.[2]
	1	82				
	2	208				
	3	17				
	4	7				
	5	4				
	6	5				

Photoaffinity labeling as a way to validate the target/mechanism of action

UIC



He B, Velaparthi S, Pieffet G, Pennington C, Mahesh A, Holzle DL, Brunsteiner M, van Breemen R, Blond SY, Petukhov PA. Binding ensemble profiling with photoaffinity labeling (BEProFL) approach: mapping the binding poses of HDAC8 inhibitors. J Med Chem. 2009;52(22):7003-13. doi: 10.1021/jm9005077. PMCID: 2789734.

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

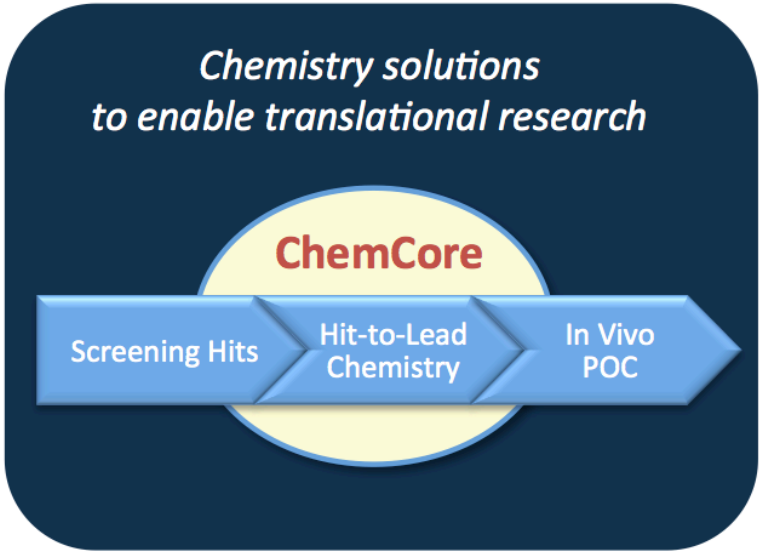
CMIDD
Center for Molecular Innovation and Drug Discovery
a catalyst for the integration of chemistry and biology

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 is gratefully acknowledged:

Contact

847-467-2629
drugdiscovery@northwestern.edu
www.cmidd.northwestern.edu/chemcore

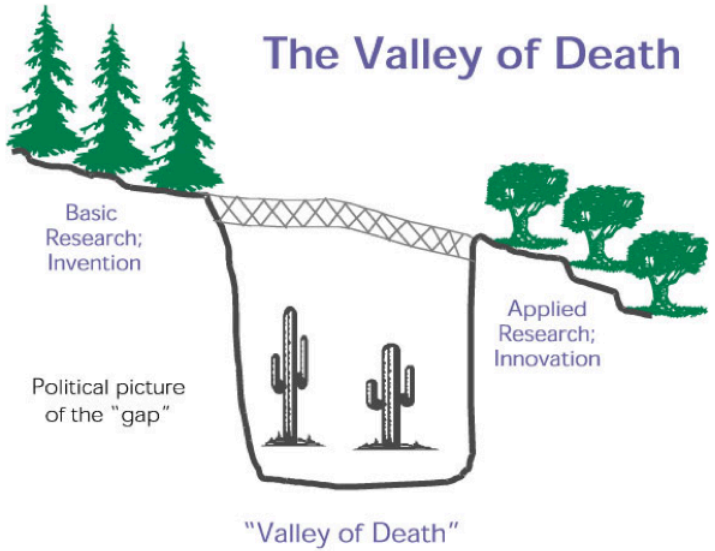
ChemCore
Medicinal and Synthetic Chemistry Core

CMIDD
Center for Molecular Innovation and Drug Discovery
a catalyst for the integration of chemistry and biology

- 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

Support from the following organizations is gratefully acknowledged:

Contact

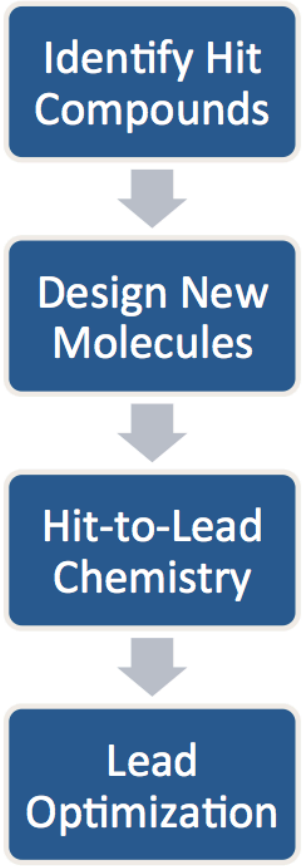
847-467-2629
drugdiscovery@northwestern.edu
www.cmidd.northwestern.edu/chemcore

ChemCore
Medicinal and Synthetic Chemistry Core

CMIDD
Center for Molecular Innovation and Drug Discovery
a catalyst for the integration of chemistry and biology

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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NORTHWESTERN UNIVERSITY · NATIONAL INSTITUTES OF HEALTH · CBC · NASA · clp

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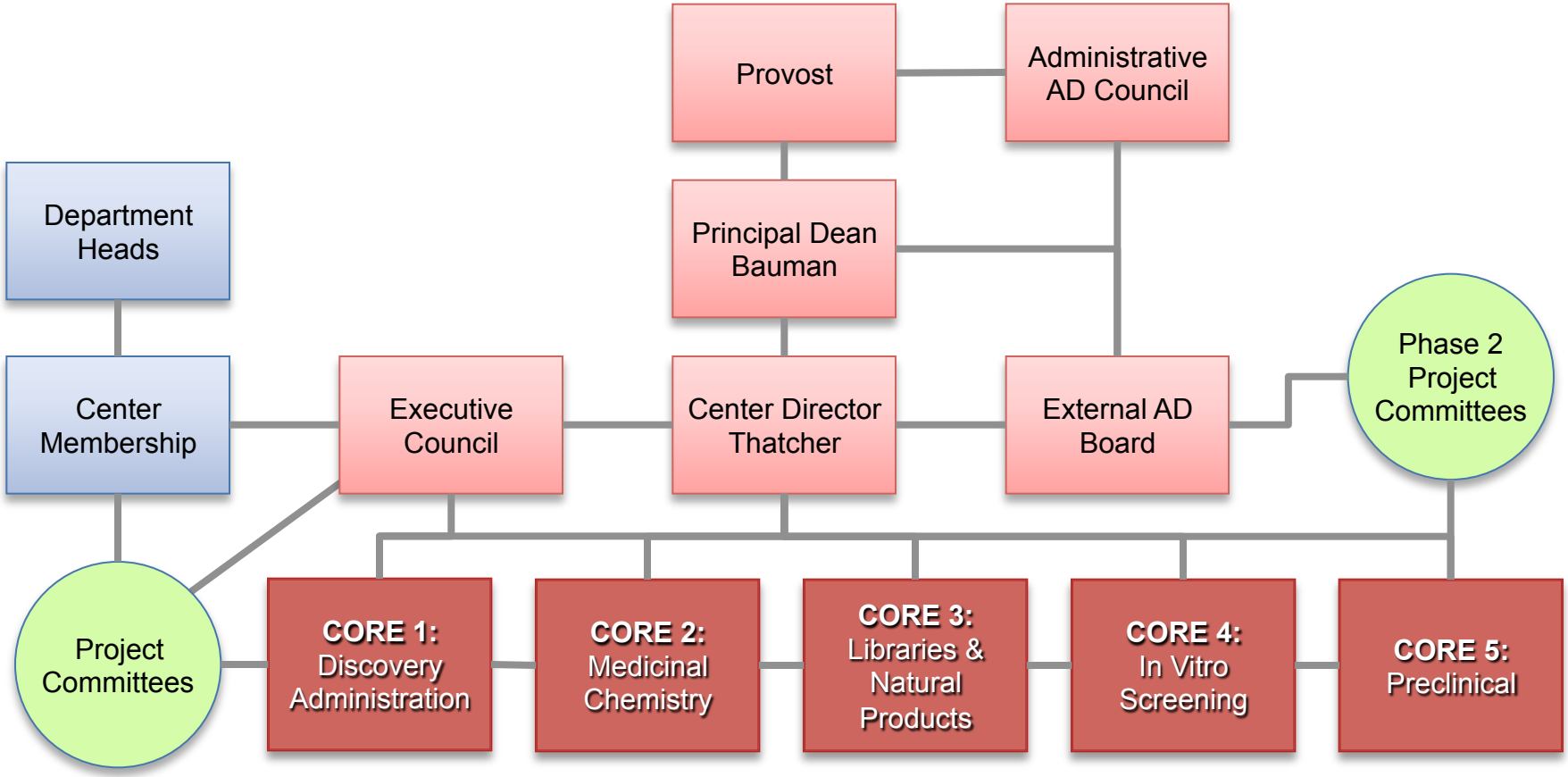


*The mission of **CENTRE** is to stimulate and enhance the application of chemical, pharmaceutical, and translational knowledge to elevate biomedical discoveries at the University of Illinois to a level where the benefits of clinical application will enhance human health and benefit society.*

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

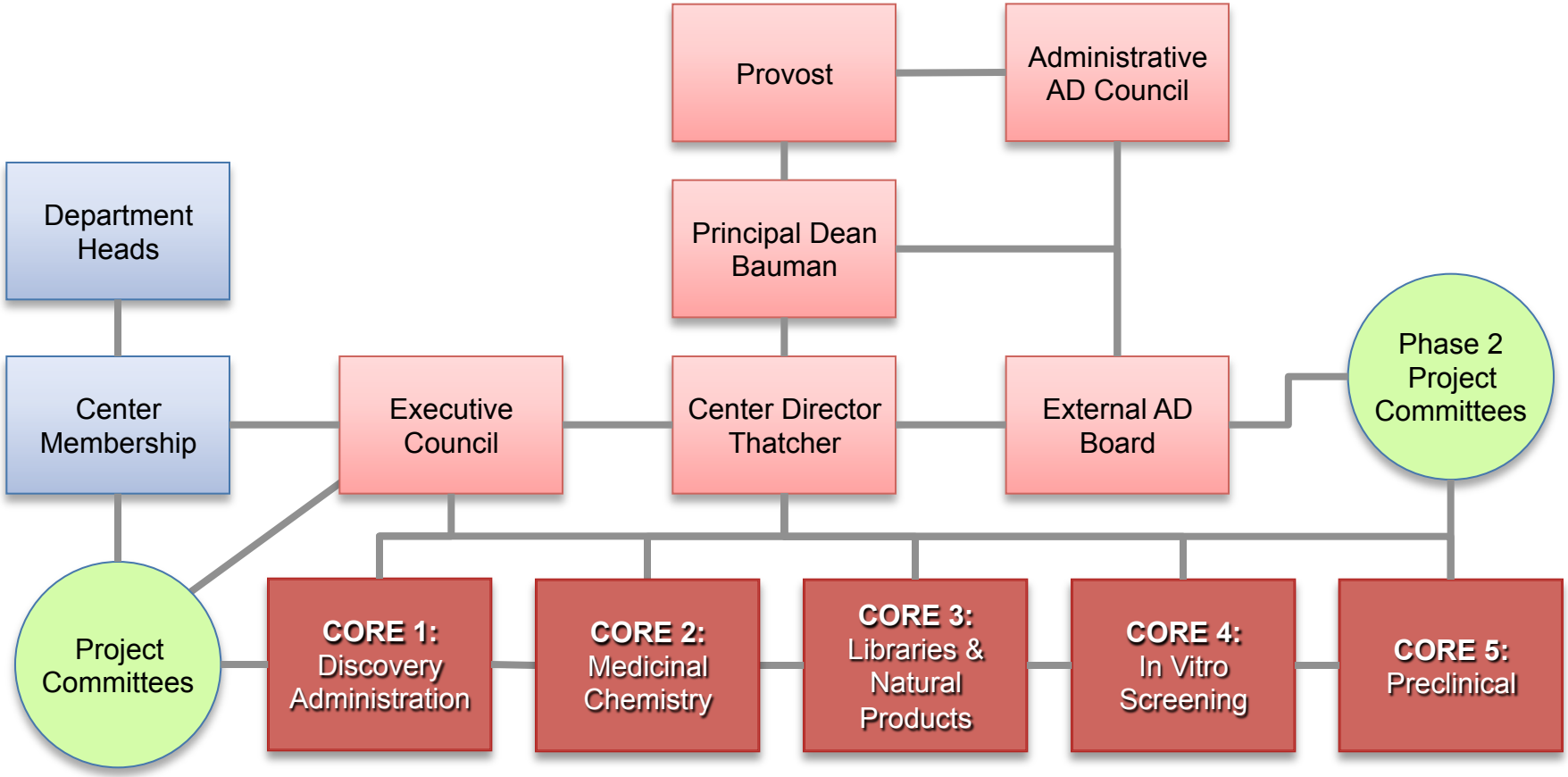
Stage 1 Project

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

UICENTRE



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UICentre

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
3. Synthesis of 15-25 novel analogues including negative controls: screening for activity versus Hit: NO-GO
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