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Tuning molecular specificity for design of medical countermeasures

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Controlling Binding Specificity

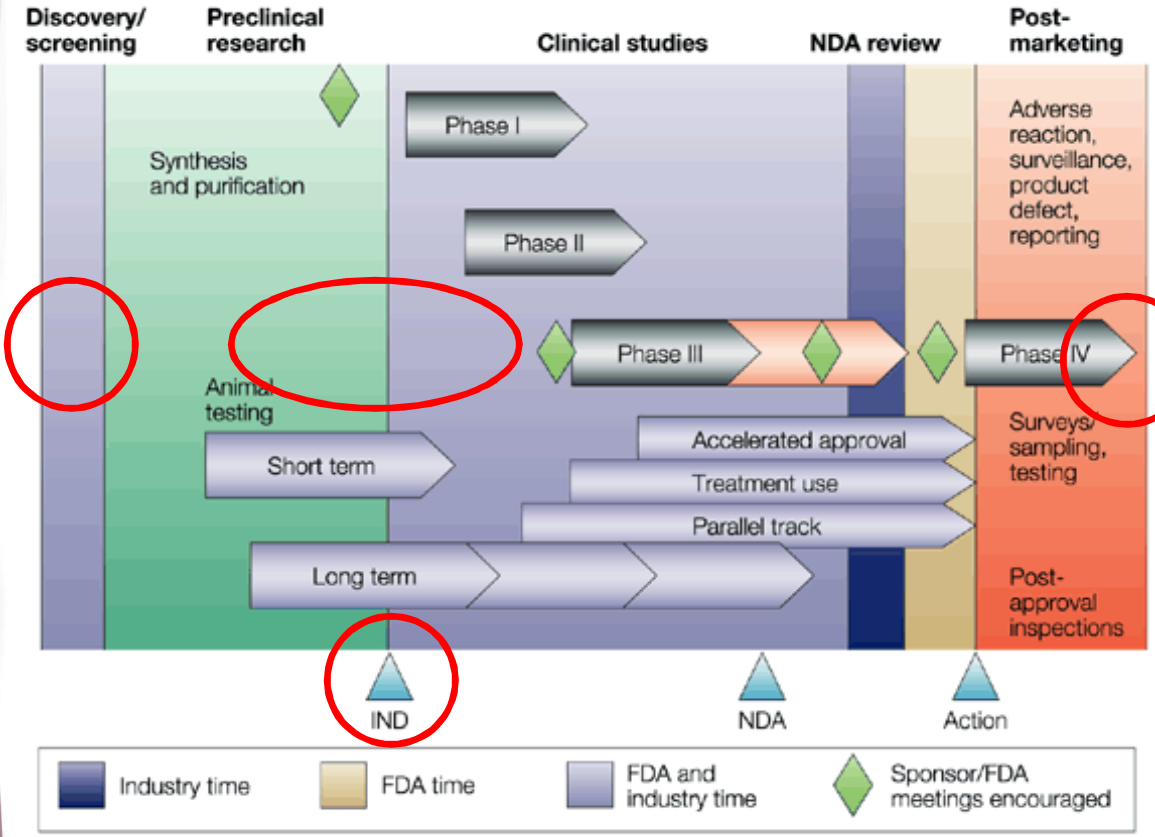
Our work is focused on classifying, understanding, predicting and controlling specificity of binding interactions across large classes of proteins and drugs.

- Combines computational, statistical, and experimental approaches to identify *Specificity Determining Features (SDFs)* of the molecular interaction interface.
- Can be used to answer questions like:
  - Can I find compounds that maintain antimicrobial activity but do not bind off-target host receptors?
  - How effective will my compounds be against new or genetically engineered strains?
  - Is resistance likely to emerge quickly?

Highlights: Specificity Profiling

- Developed pipeline for specificity profiling as part of an ongoing DTRA basic research project in studying molecular recognition.
- Integrates bioinformatics, structure-based drug design, experimental high-throughput screening, mutational analysis and next-generation sequencing.
- Produces intuitive, predictive models
- Key concept: probing specificity with ligand binding profiles
  - sequence- or structure-based predictions do not correlate well with experimental binding data.

Where should systematic specificity profiling be in the drug design cycle?



***Nature Reviews Drug Discovery* 2, 71-74 (January 2003)**

- To expand/tailor the specificity profile of a validated drug.
- *In development:* to explore salvage strategies.
- At the IND stage: to prioritize candidates with low off-target and resistance potential
- *In discovery*: to explore the possible breadth of activity across a target family.

Early use of specificity profiling can help determine which targets and lead compounds to move forward

Specificity Profiling: Finding specificity determining features (SDFs)

Binding Data (Ki, Kd)

Ligands

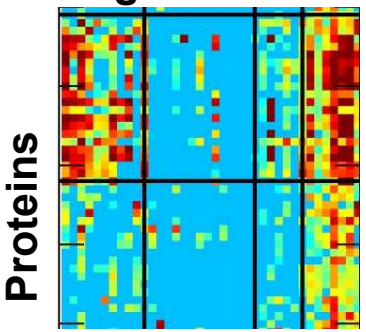
Proteins

Table of Binding Data

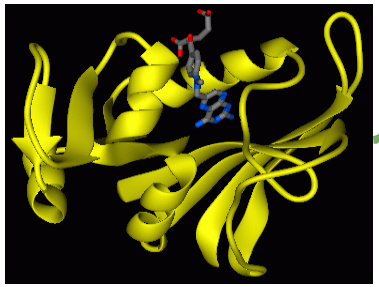
Classify

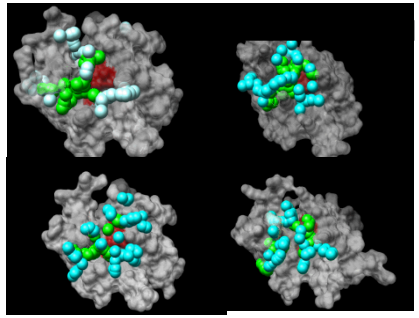
Ligands

Proteins

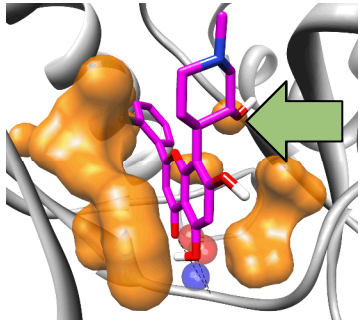


Simulation (Docking, MD)

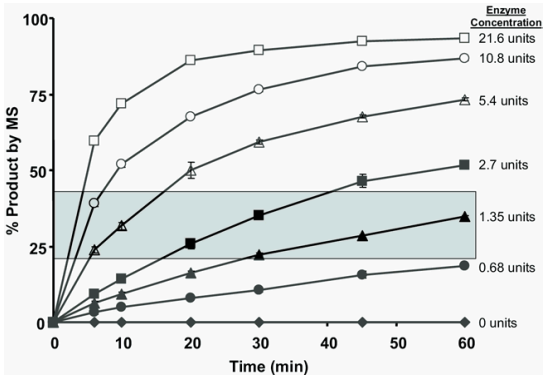




Extract SDFs



New predictions

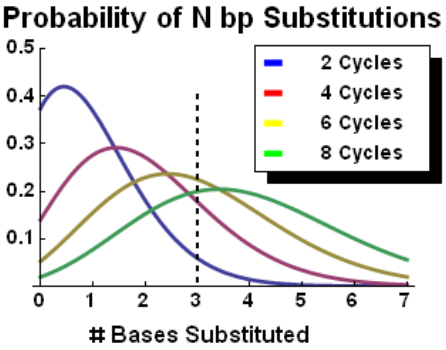


Experimental Validation

Mutational Profiling: Accelerated in-vitro approach to generate and evaluate ALL Triple Mutants of Binding Site

PCR out gene segment around the binding site (~ 70-130 bp)

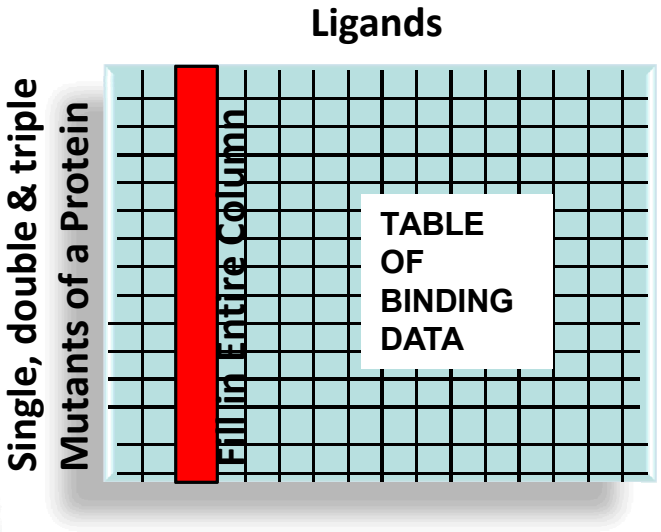
Amplify with Error-prone PCR to get all single, double and triple mutants



Reintegrate into gene & Express in phage protein display system (<= 1 protein copy per phage) to produce a library of triple (bp not aa) mutants.

Affinity chromatography versus immobilized ligands sorts out weak, medium and strong binders

Illumina Sequencing of each fraction determines which mutants are in it. 1-2 Million reads (~$200) provides >4x coverage of ALL triple mutants. Since coverage is complete, can be repeated for additional ligands.

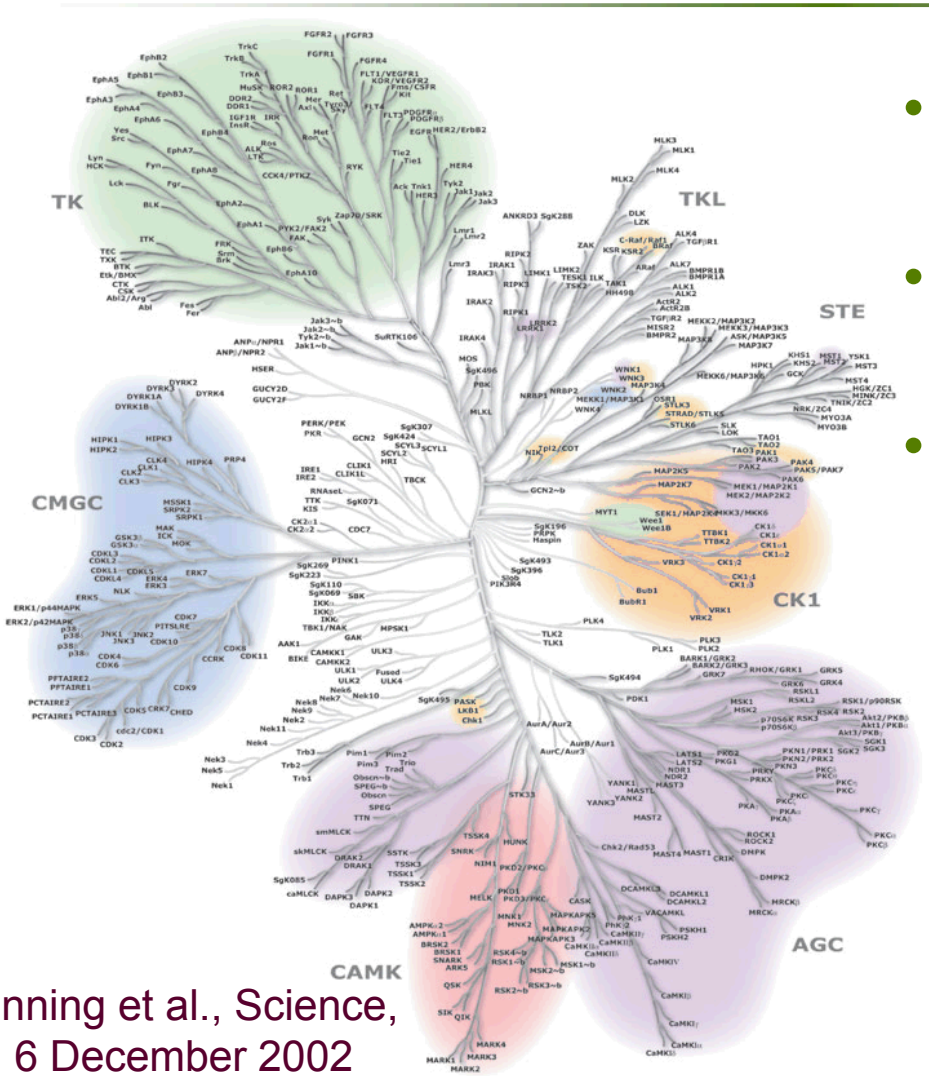


Current Test Systems

- **GOAL: Find specificity-determining features (SDFs) across protein target and ligand spaces**

Test System	Enzyme Source	Experimental Ligand Binding Data Available in literature
Protein Kinases	Human	>40,000
DHFR	Bacterial / fungal / protist	> 4000
HIV / HCV Proteases	Viral	>14,000 / >300

Specificity profiling protein kinases

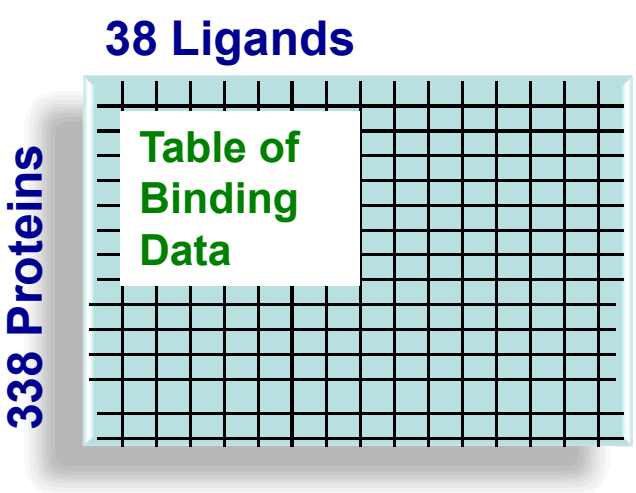


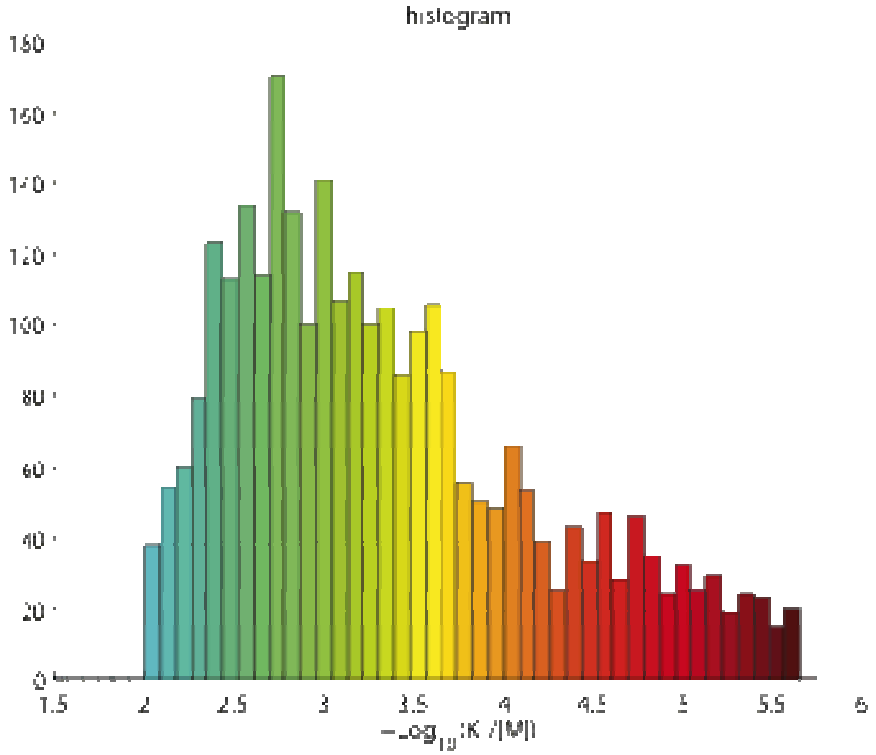
- Human kinome: over 518 proteins
- Most within cluster of >30% homology
- Known inhibitors can bind to the native fold (“type I”) or induced fit hydrophobic subpocket (“type II”)

Manning et al., Science, 6 December 2002

Starting TBD for the human kinome

Values for Kinase/Ligand TBD taken from a comprehensive experimental study in the literature.



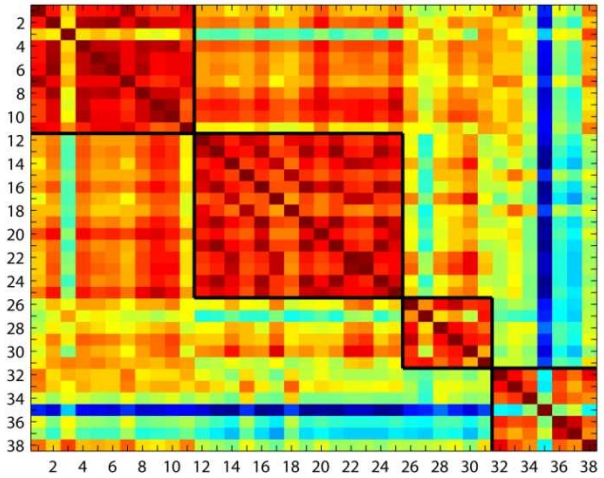


Karaman MW, et al, Nat. Biotechnol, 2008. 26 127-132.

Human Kinome Results: Cluster by ligand binding data

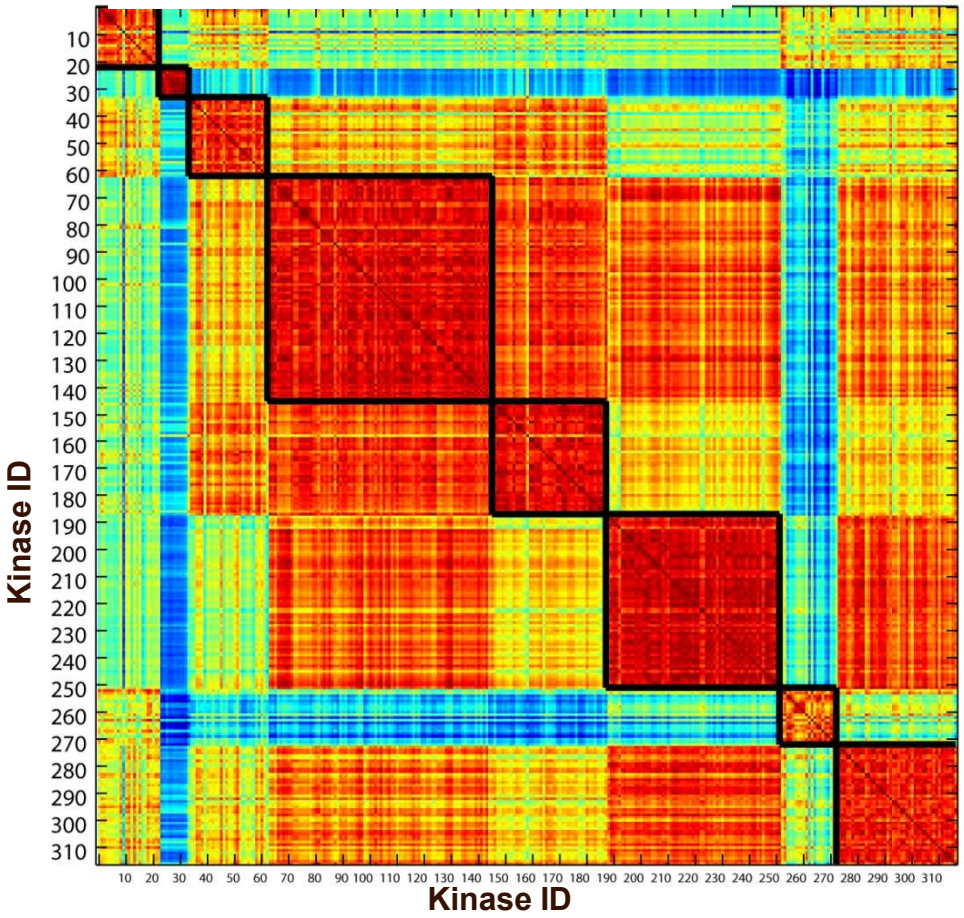
Ordered Heatmap showing kcenters clusterings

Ligand Clustering



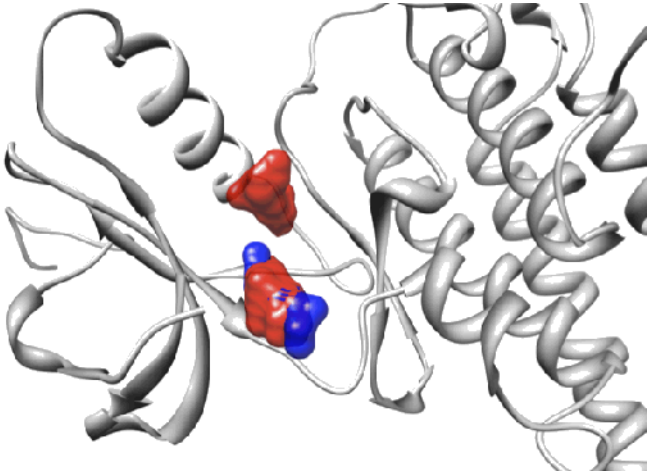
- "type-2" inhibitors in ligand cluster 1
- broad binders in ligand cluster 4

Protein Clustering

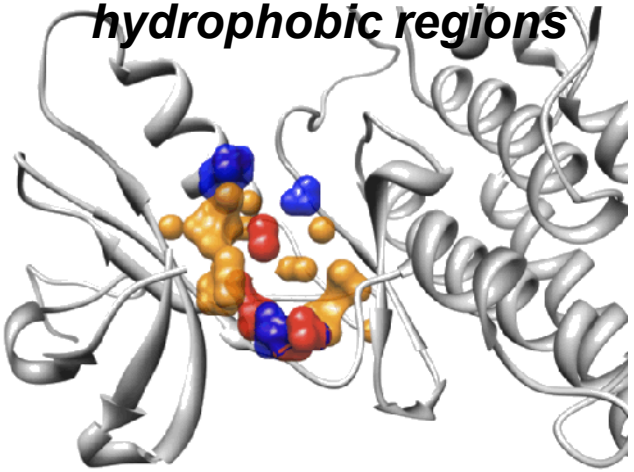


SDFs: Broad Binding Features (common among all clusters)

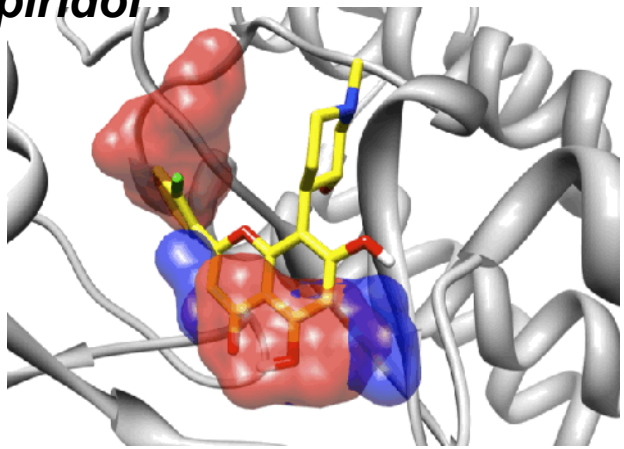
Ligand-space hbond regions



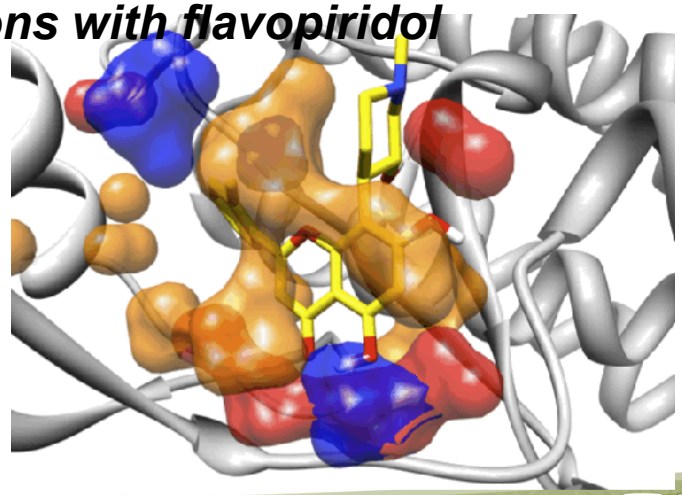
Protein-space hbond and hydrophobic regions



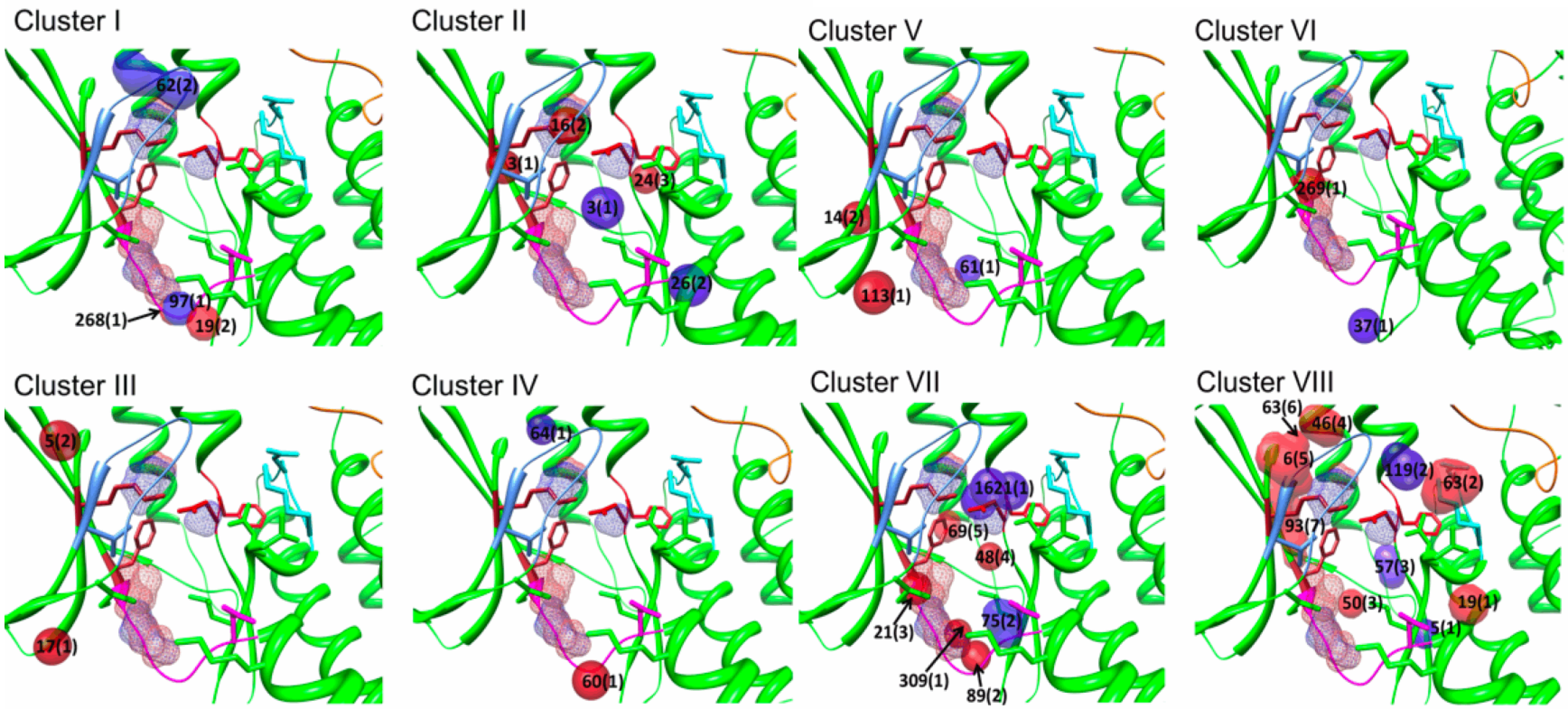
Ligand-space hbond regions with flavopiridol



Protein-space hbond and hydrophobic regions with flavopiridol



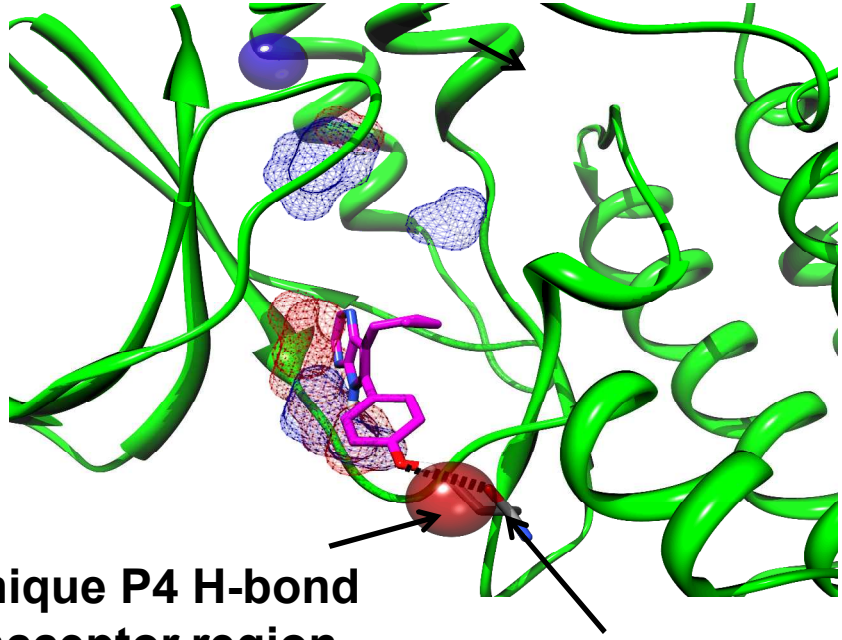
SDFs Unique to a Cluster



Note: individual ligands will have 1-2 SDFs from within this set which represents a superset of ligands

SDFs predictive for other datasets

CDK2 (IC_{50} = 880 nM)



Unique P4 H-bond acceptor region

H-bond acceptor for aloisine

- A prior study using traditional QSAR (Sheridan, 2009) produced different models for each dataset
- Preliminary data from SDF approach produces common SDF models between the Federov and Karaman data sets
- Example: unique H-bond acceptor SDF found in both datasets for cluster containing CDK2 & ZAP70. Compounds binding specifically to these proteins make this interaction.

Summary of Kinase Study

- Using ligand binding data is a robust way to cluster proteins and ligands and useful patterns of binding emerge from these clusterings.
- We can turn combine these clusters with docked poses to extract SDFs
- These SDFs match specificity features in ligands outside our initial data set.
- Predictive capability validated with ligands outside our dataset
- Experimental validation of novel predictions ongoing

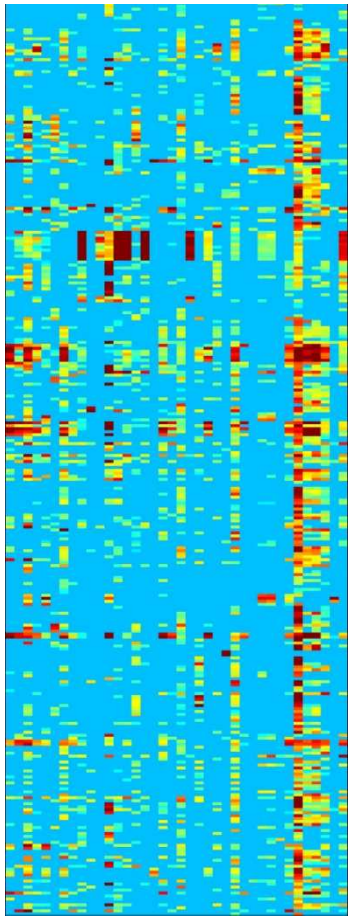
How can specificity profiling be used in potential TMT projects?

- *Emerging threats*: NDM-1 metallo β-lactamase – characterize structural features responsible for its resistance - mutational profiling to identify future potential variants, identification drug variants broadly binding to all variants (with Carol Zhou, Adam Zemla, LLNL)
- *Viruses*: Alphaviruses (or Flaviviruses) - identify broad spectrum inhibitors to viral proteases that are robust against resistance/genetic engineering, validated by experimental mutational profiling (with Stan Langevin, SNL; CDC)
- *Decision Tool :*Prioritize IND candidates with known structure-based targets (eg: viral proteases)
- *Host-directed therapeutics:* Immunopotentiation – RIG-1 pathway of innate immune system is a broad responder to many viral families. Find specific inhibitors to LGP2 to upregulate RIG-1 pathway (with Steve Branda, SNL)

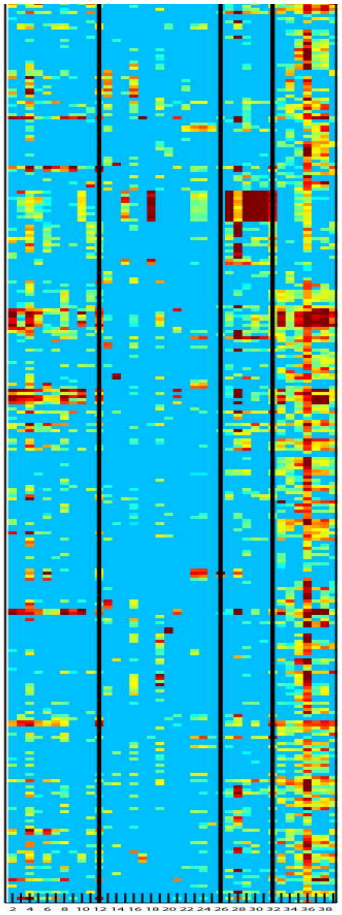
Extra Slides

Human Kinome Results: Binding Data Ordered by Clusters

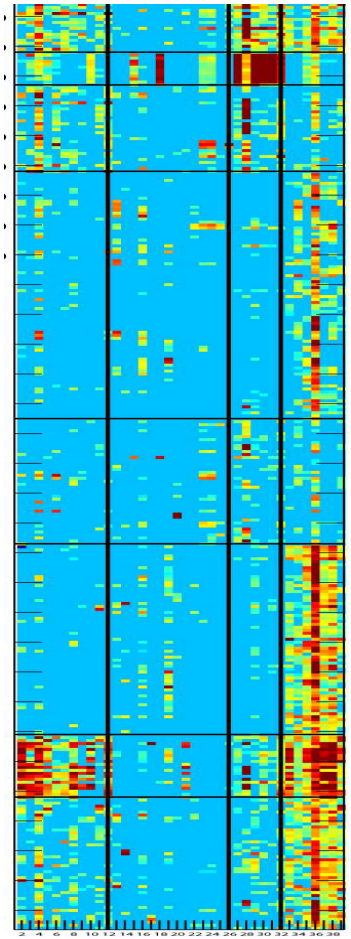
Unordered Binding Matrix



Binding Matrix Ordered by Ligand Clusters



Binding Matrix Ordered by Ligand and Protein Clusters

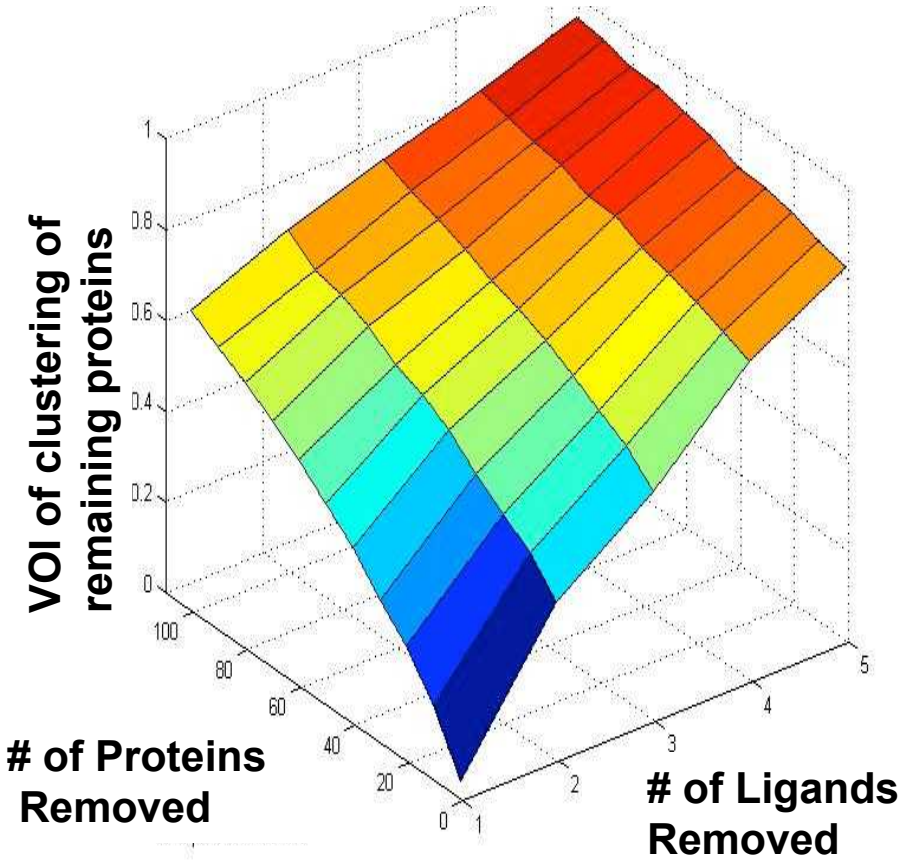


Robustness of Classifications

Leave 1-out analysis shows clustering robust for both ligands and proteins

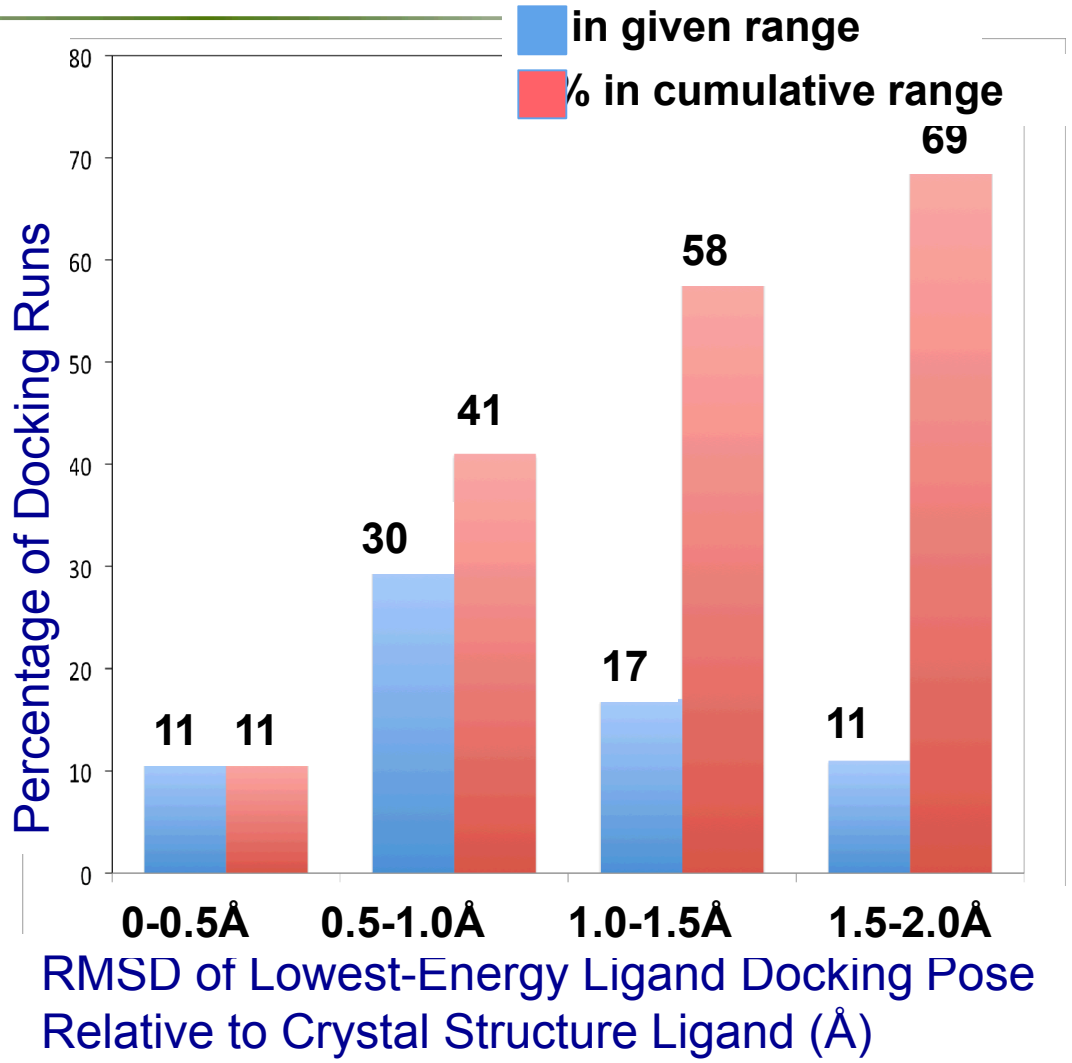
- Variation of information (VOI) Mathematical method to measure distance between 2 clusterings.
- *Clustering by sequence or structure do not capture the patterns in experimental data.*
  - VOI of random cluster is 3.7
  - VOI for clustering by sequences is 2.57
  - VOI for clustering by structure motifs is 2.73

Cluster Degradation with respect to protein and ligand removal



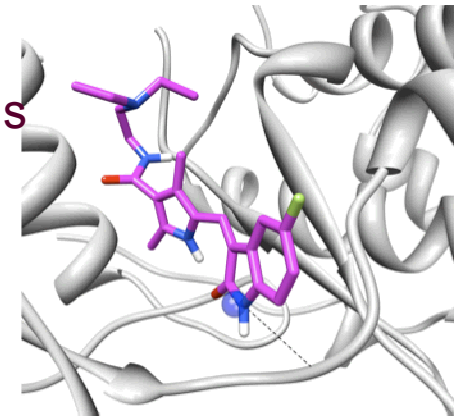
Docking to kinases and extracting specificity determining features (SDFs)

- Docked 38 ligands to 113 kinase structures using autodock 4 with flexible ligands
- Validated docking poses with crystallographic ones for those with co-crystals (figure)
- Features (h-bonds, polar, hydrophobic) extracted from docked poses using experimentally determined clusterings.
- Statistical approach to feature extraction– insenstive to “noise” from mis-docked features

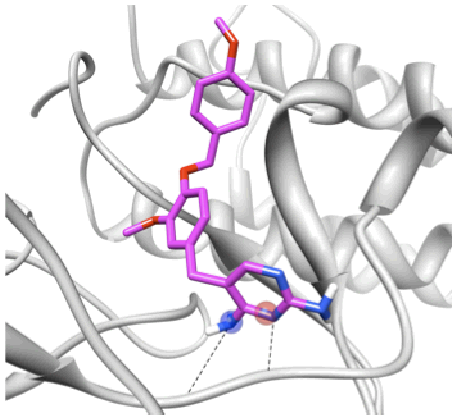


SDFs predictive for ligands outside our dataset

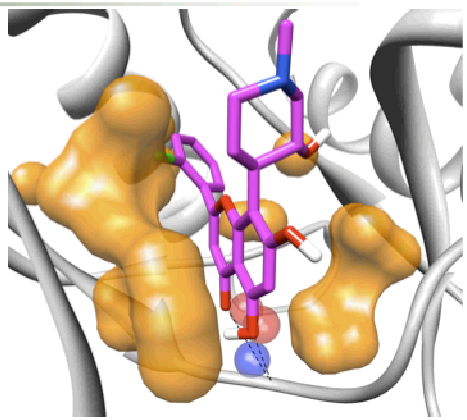
Top row: docked ligands from dataset



JNK1, sunitinib

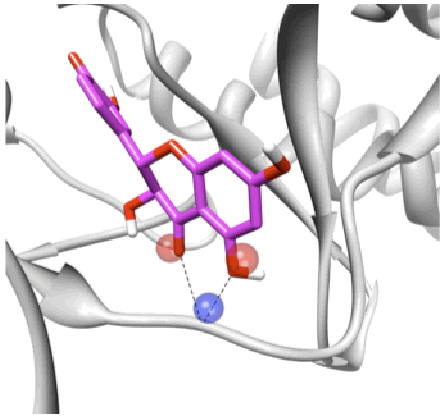


LYN, GW-2580

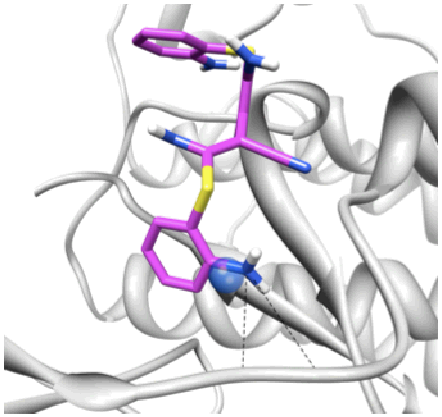


CLK1, flavopiridol

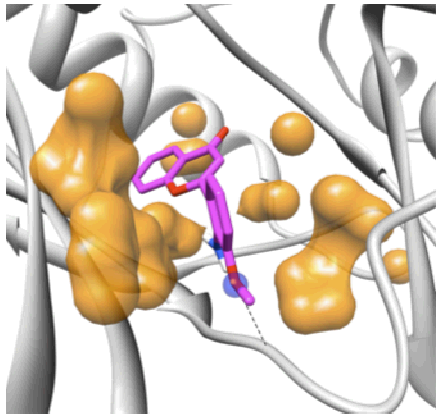
Bottom row: docked ligands from outside dataset



ITK, quercetin



ITK, U0126



KIT, PD98059

How to use SDFs in specificity tuning?

- To generate broad binding:
  - Design/optimize ligands to bind to globally present SDFs
  - Accelerated mutational analysis used to generate SDFs will help ensure ligands robust against simple strain variations
- To generate narrow binding:
  - Design ligands to bind to cluster-specific SDFs.
  - “Drill down” within cluster to find SDFs unique to target.
  - Use other members of cluster both computationally and experimentally
- To prioritize drug candidates: use SDFs to compare candidate’s specificity to desired specificity range (ie desired range of proteins).