

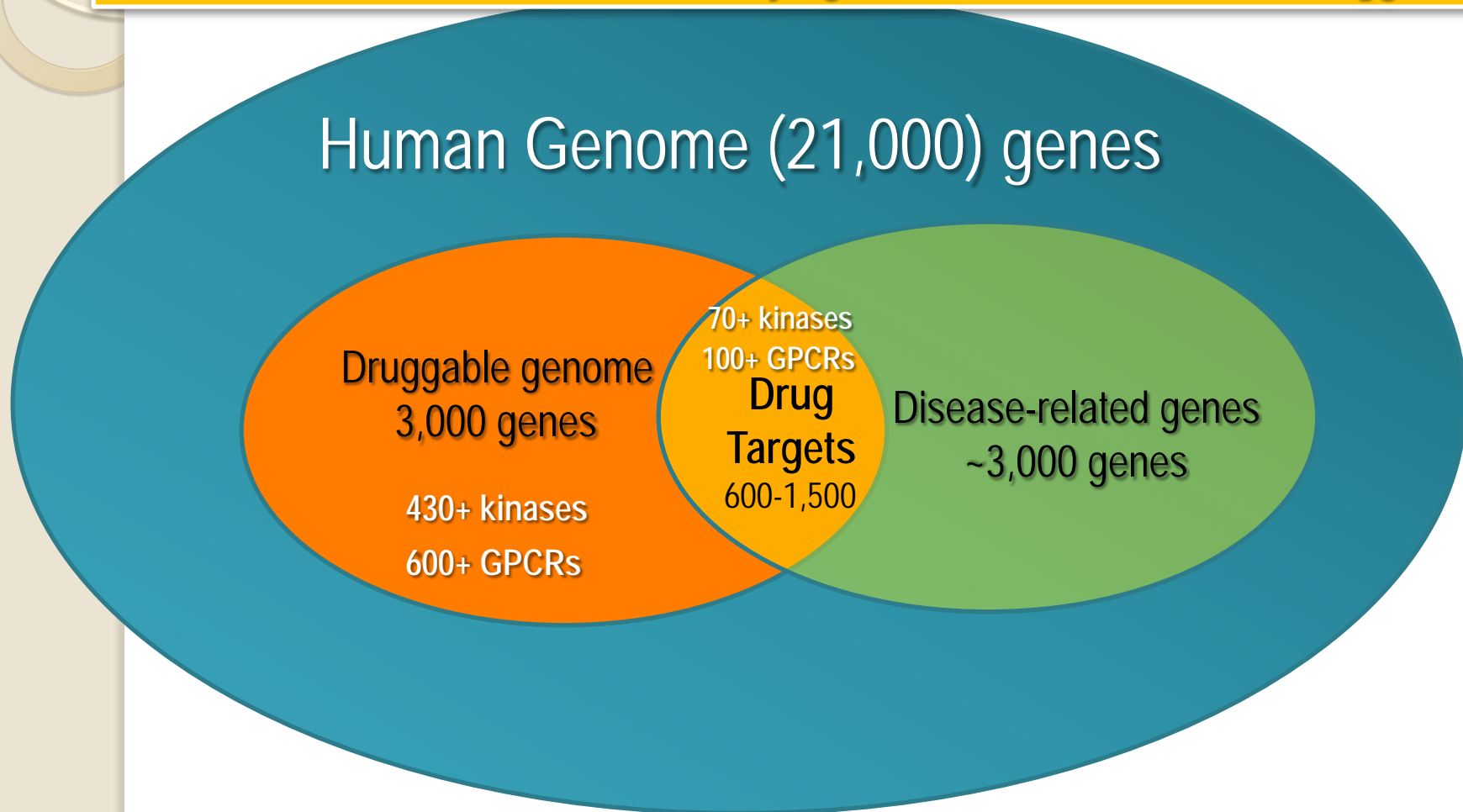


# SUMMARY

- 1 • Structure-encoded **flexibility** of drug targets and significance in drug discovery and design
- 2 • **Druggability** assessment: a first step before selecting a target
- 3 • Modularity and **promiscuity** of proteins and **quantitative systems pharmacology** methods

# Druggable Genome

A small subset of are 'disease-modifying' – and not all of them are druggable

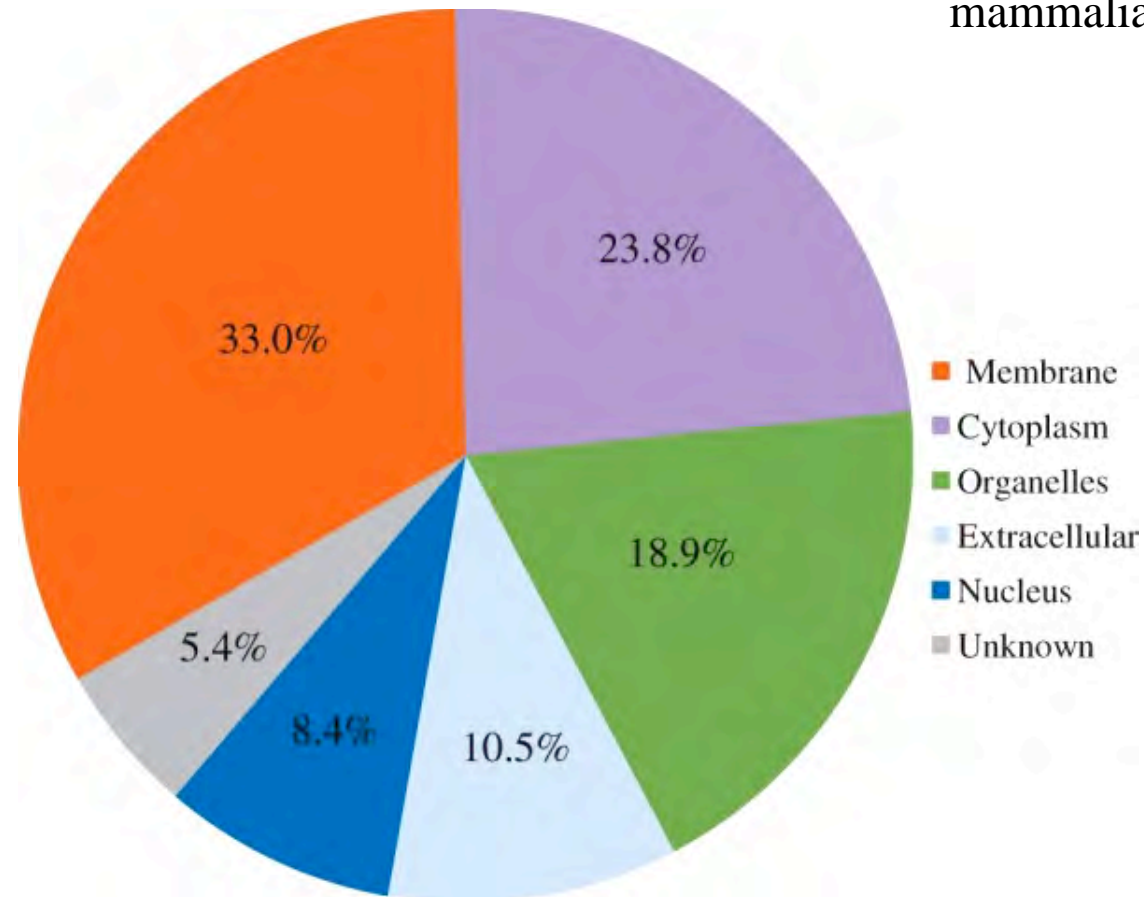


## A few numbers...

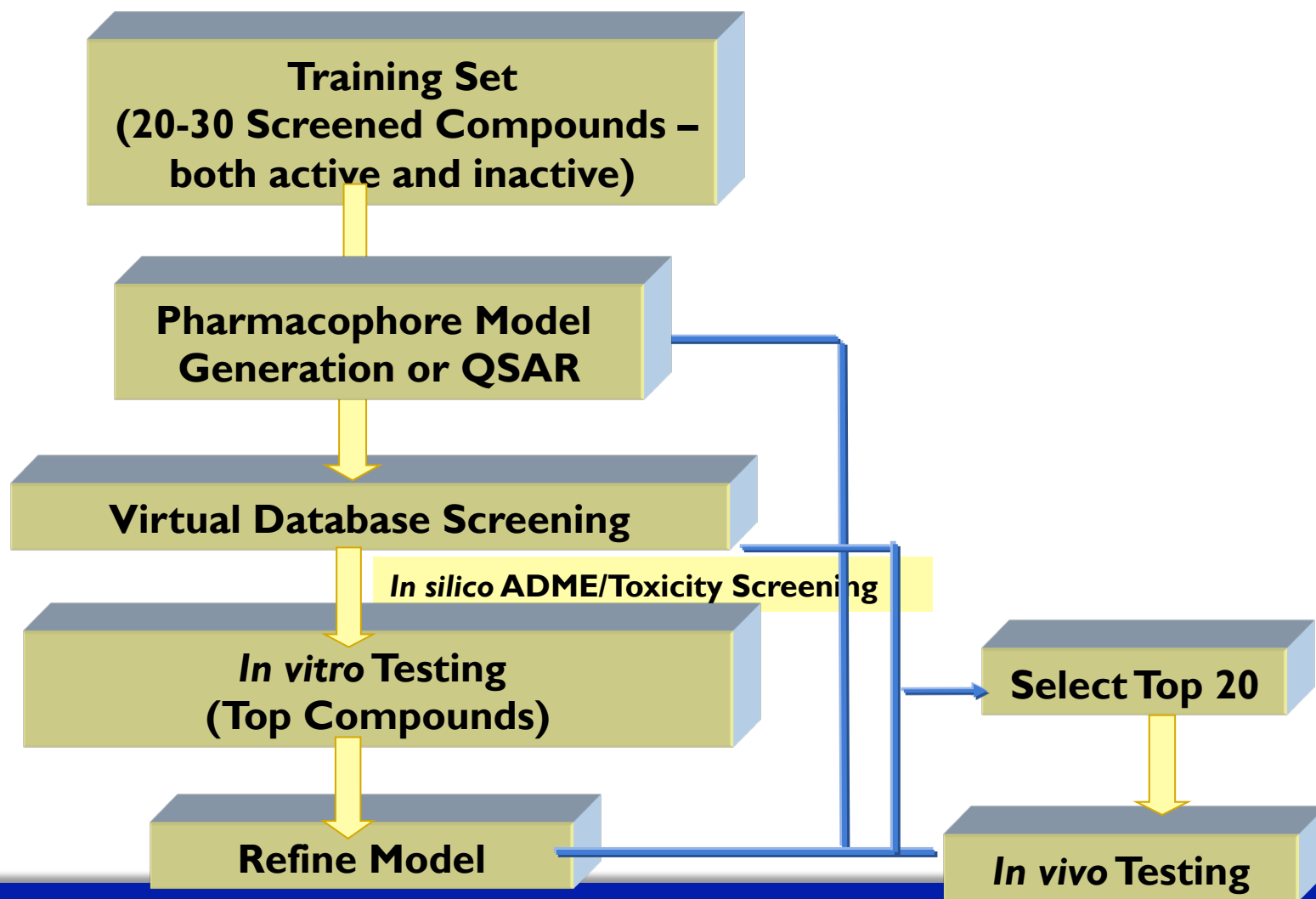
- Only 2% of human proteins interact with currently approved drugs.
- 10-15% of human proteins are disease-modifying
- 10-15% are druggable
- 5% are both disease-modifying and druggable

# Subcellular distribution of 1,362 druggable targets

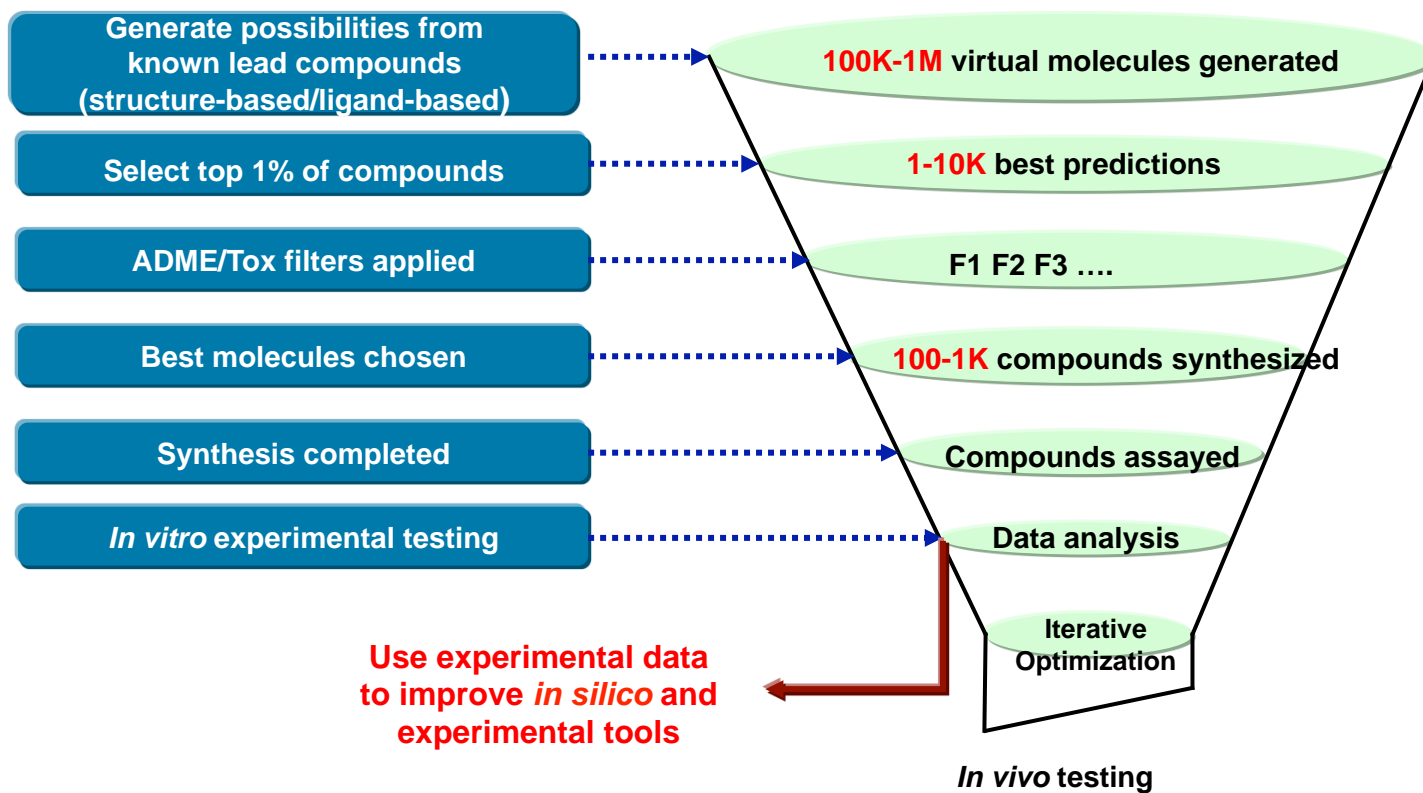
among four mammalian species.



# Ligand-Based Strategy



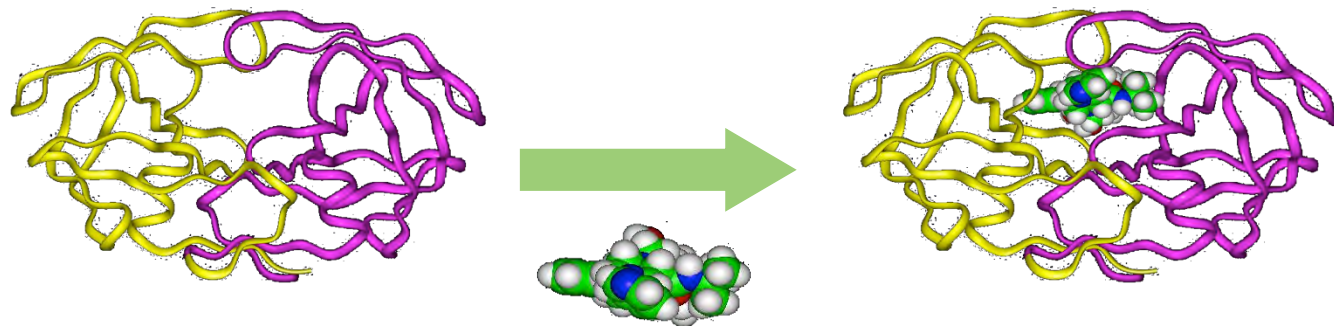
# Screening Cascade



# Rational Design of Inhibitors

3D structure of the target is used for

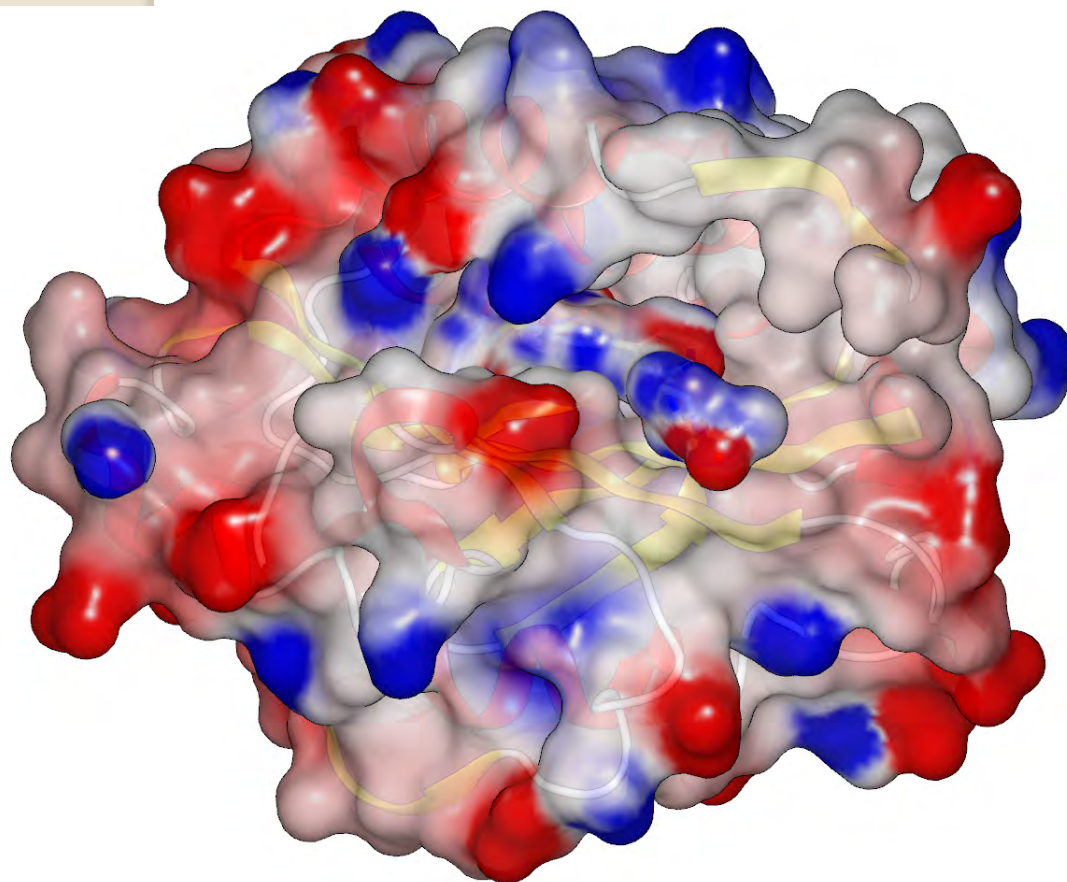
- Visual inspection/molecular graphics
- Docking (of both small molecules or fragments thereof)
- De novo methods
- Receptor properly mapping + database searching





2

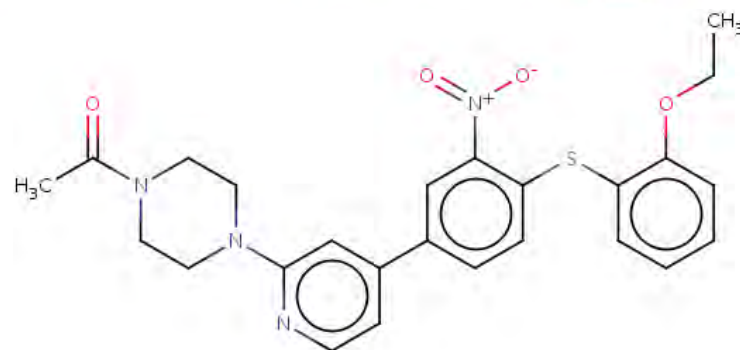
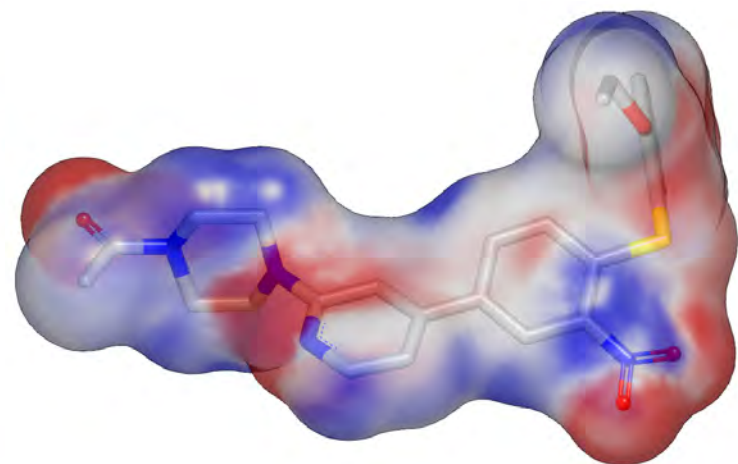
# Druggable or not?



Lfa1 - a leukocyte glycoprotein that promotes intercellular adhesion and binds intercellular adhesion molecule 1

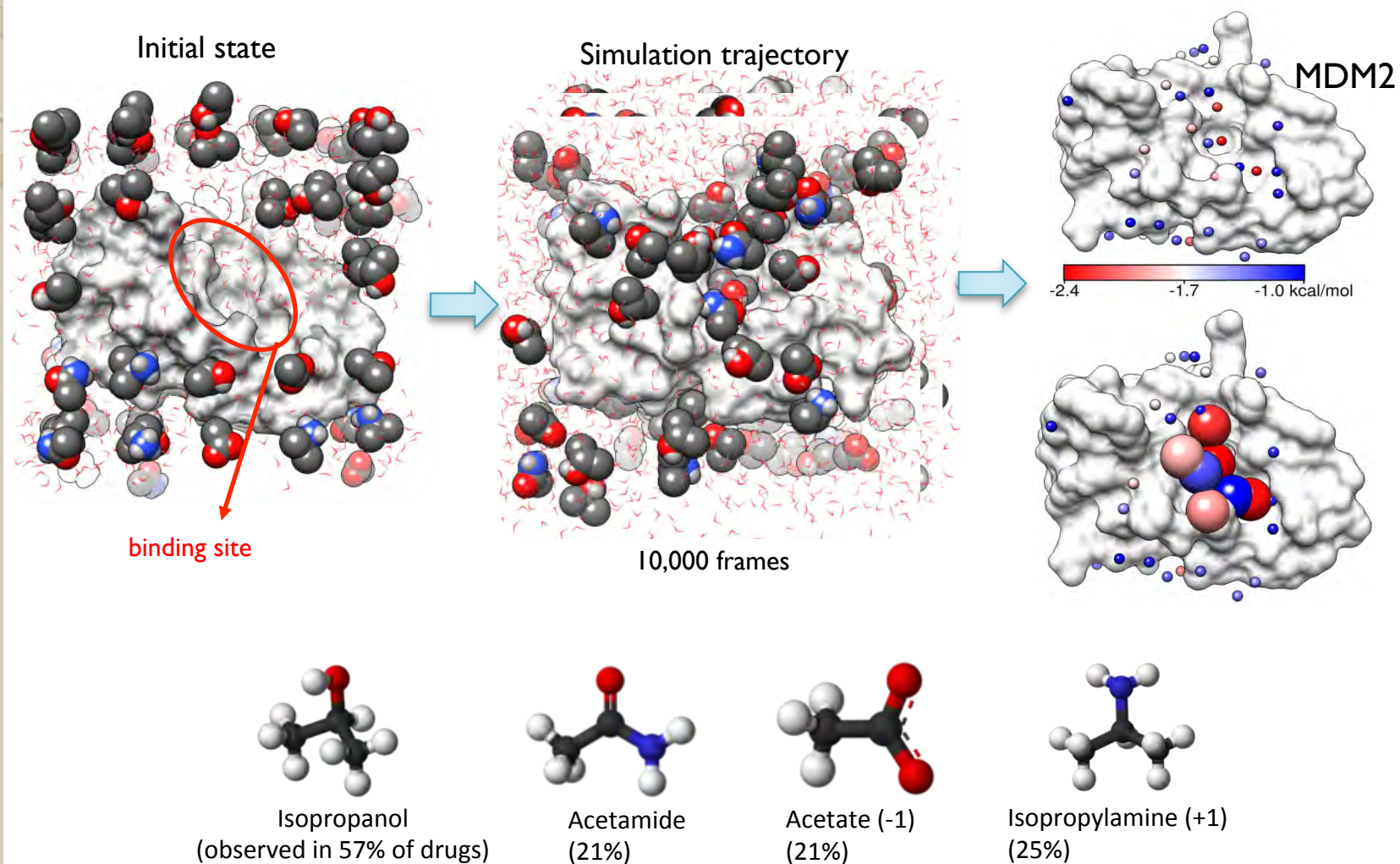
Active site druggability:

- Best known  $K_d$  18.3 nM
- Simulation 0.03-0.5 nM

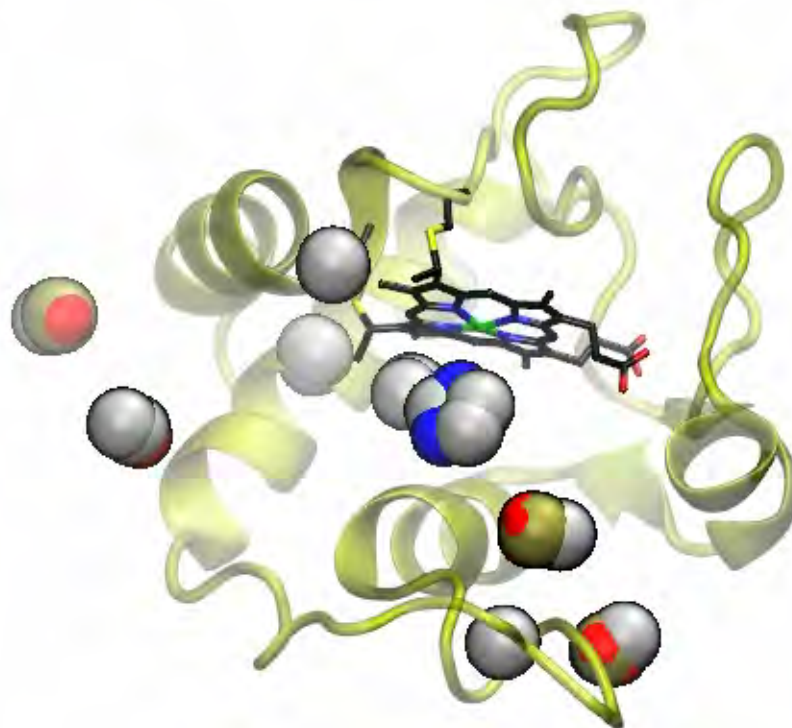




# Druggability Simulations

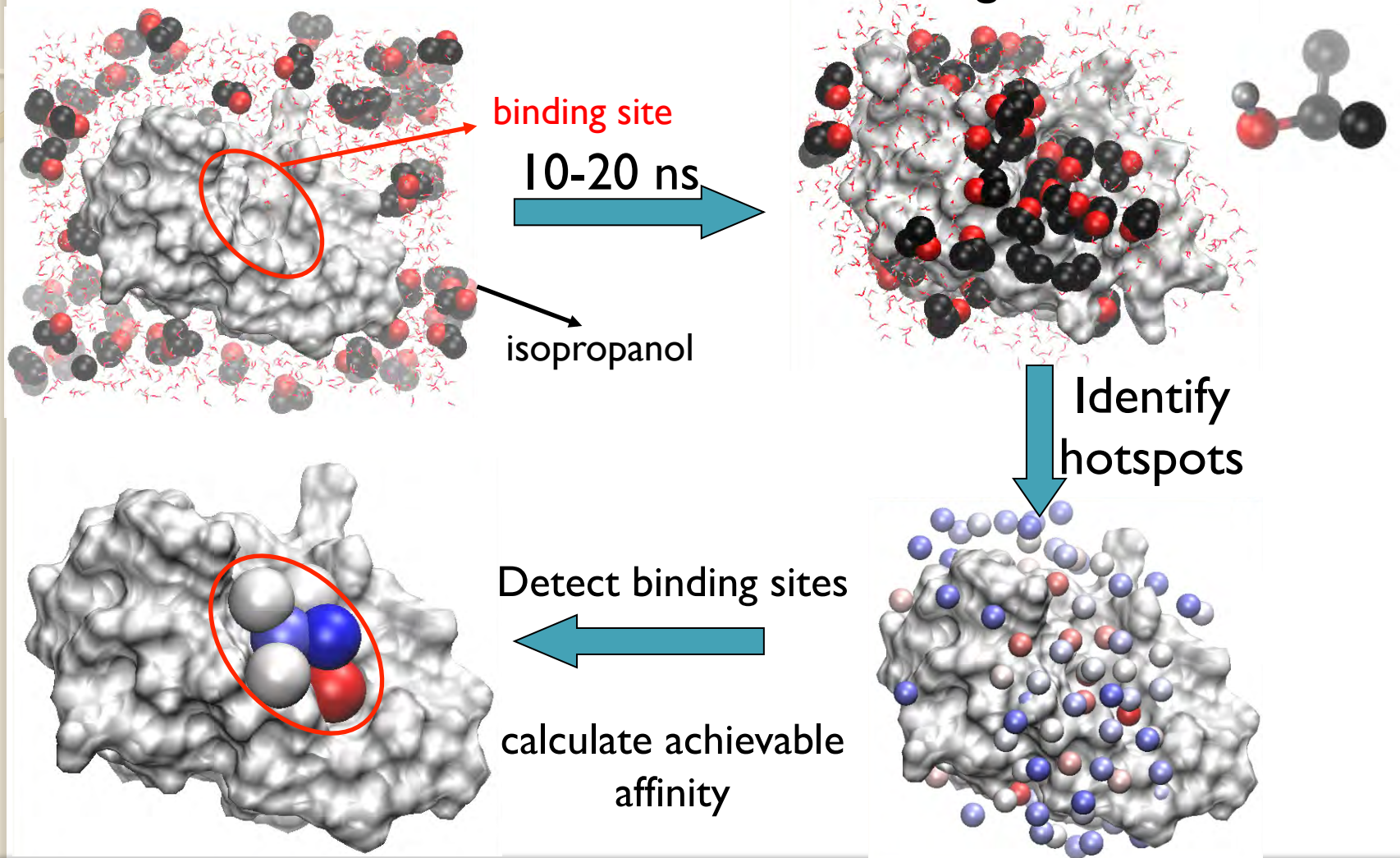


# Cytochrome c druggability



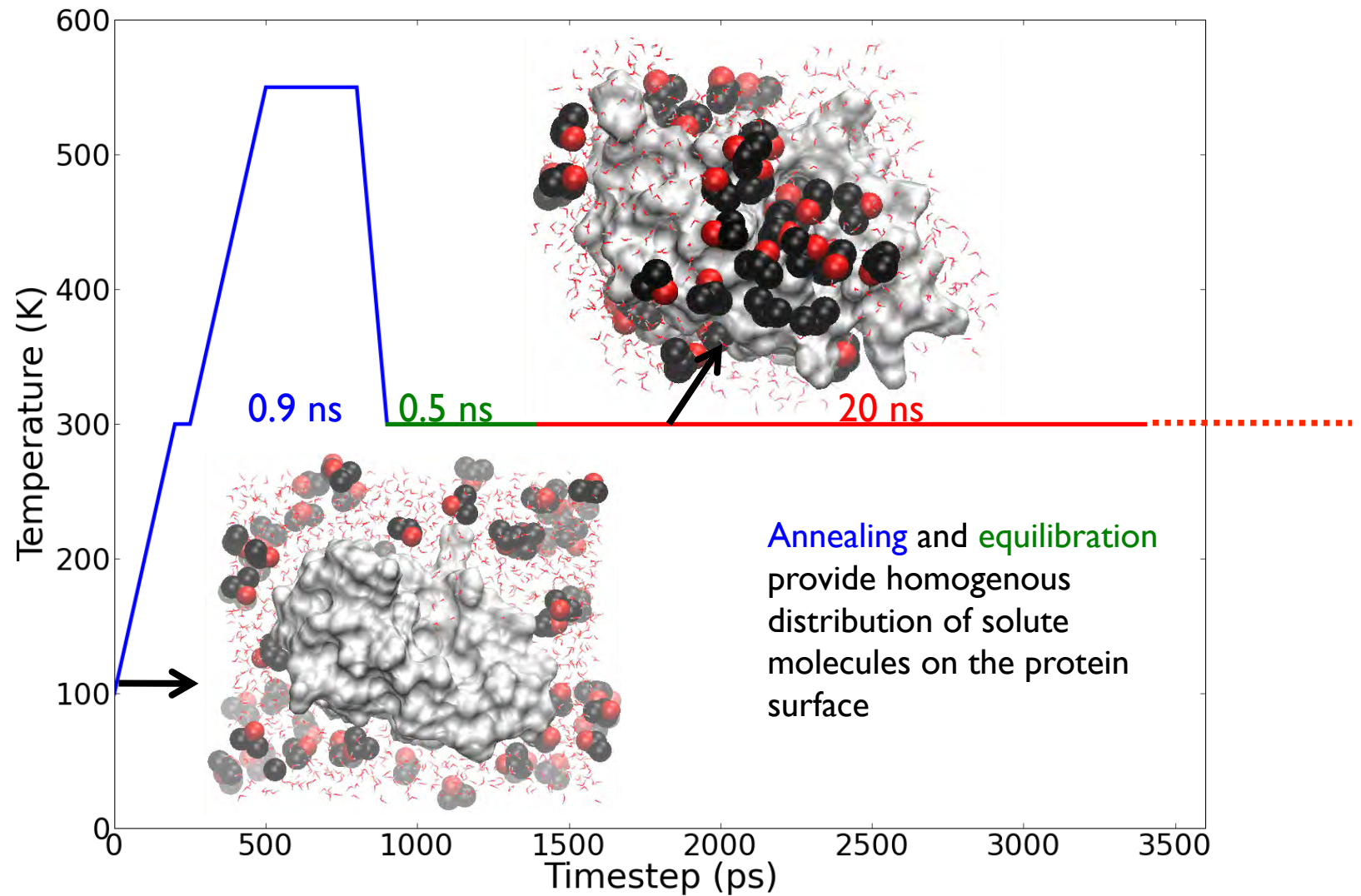
# Methodology Overview

From MD simulations to achievable drug affinities





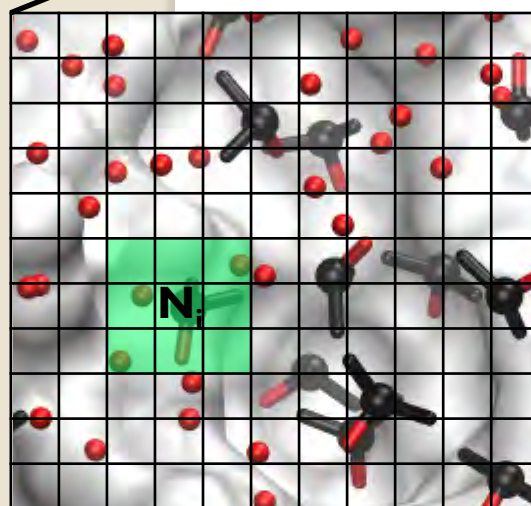
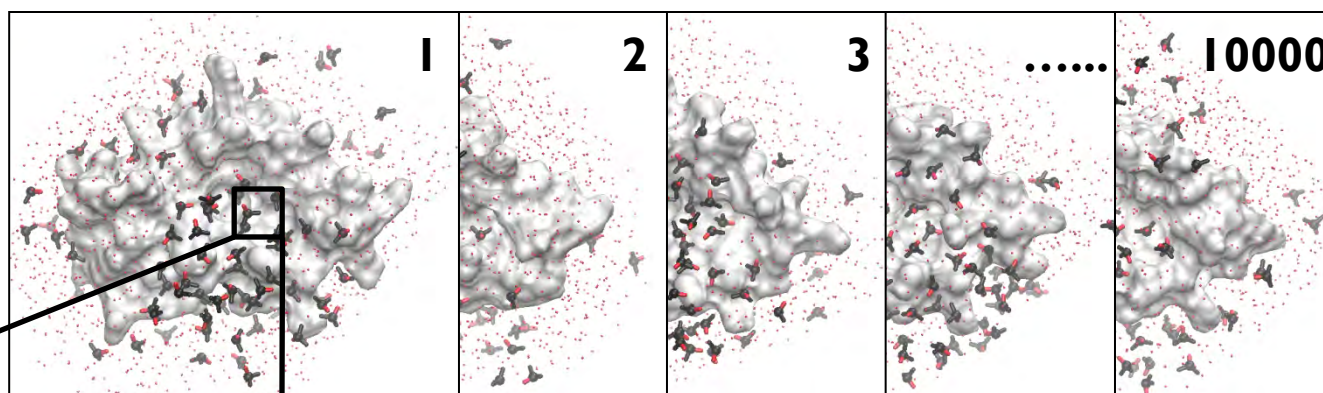
# Annealing, Equilibration, Simulation



NAMD2 with CHARMM force field was used for simulations.

# Free Energy of Binding for Isopropanol

Assuming that MD sampling converged to a **Boltzmann ensemble**

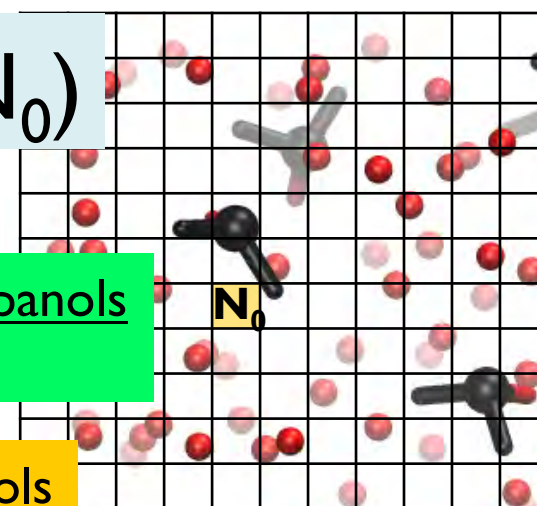


0.5 Å (not to scale)

$$\Delta G_i = -RT \ln(N_i/N_0)$$

$N_i$  = observed number of isopropanols  
(# of frames) \* (# of cubes)

$N_0$  = total number of isopropanols  
total number of frames



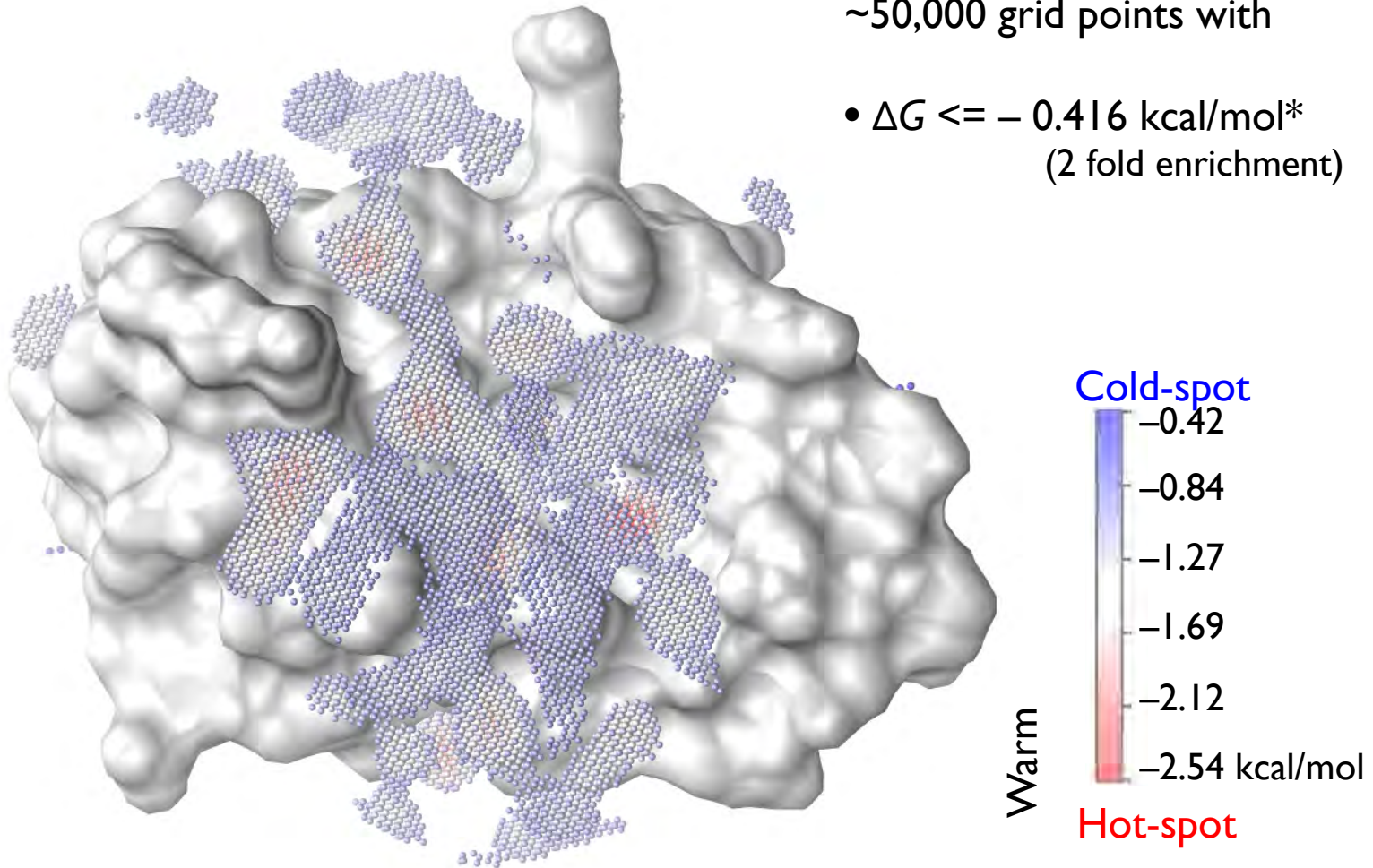
$N_i$  corresponds to the central highlighted grid element;  
number of cubes is introduced if multiple cubes are occupied by a single isopropanol

# Isopropanol Binding Spots

$\Delta G$  grid is mapped onto the protein structure

~50,000 grid points with

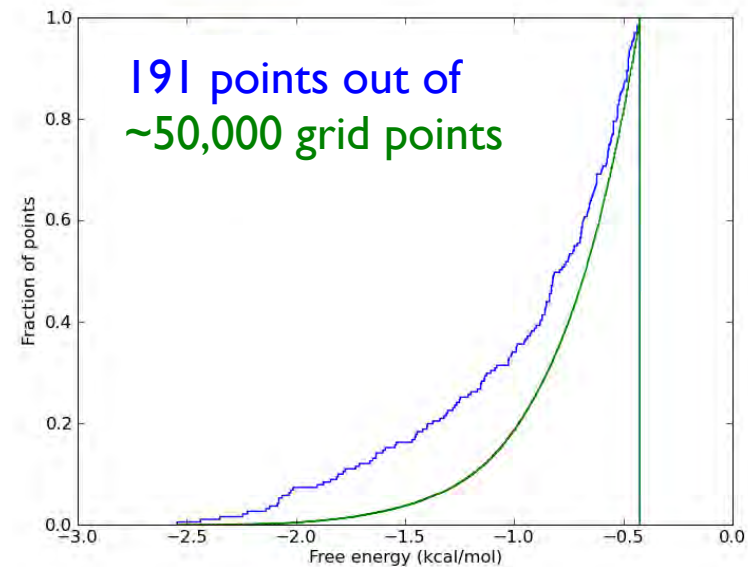
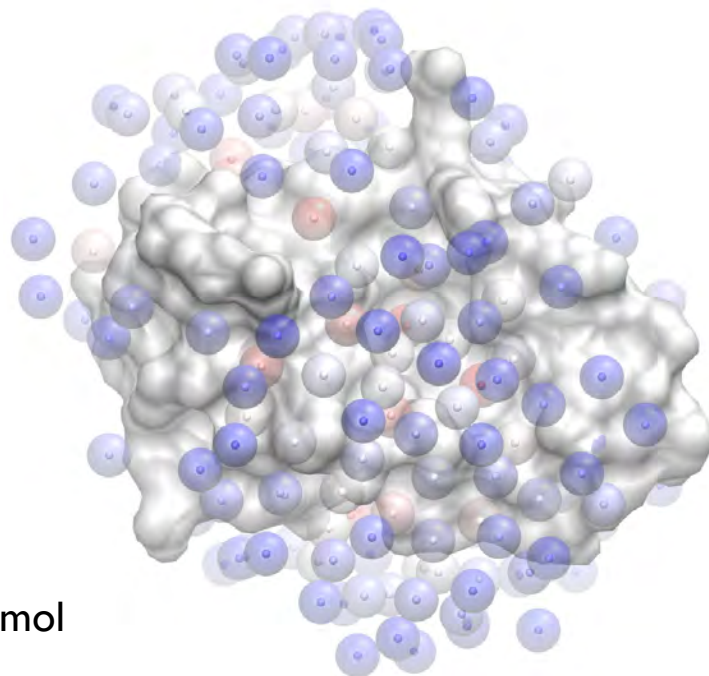
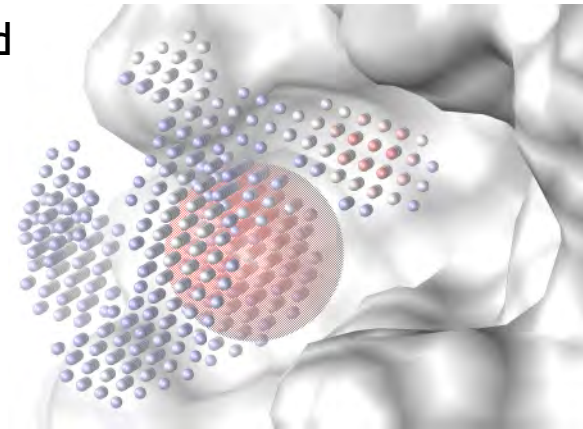
- $\Delta G \leq -0.416$  kcal/mol\*  
(2 fold enrichment)





# Selecting Isopropanol Binding Spots

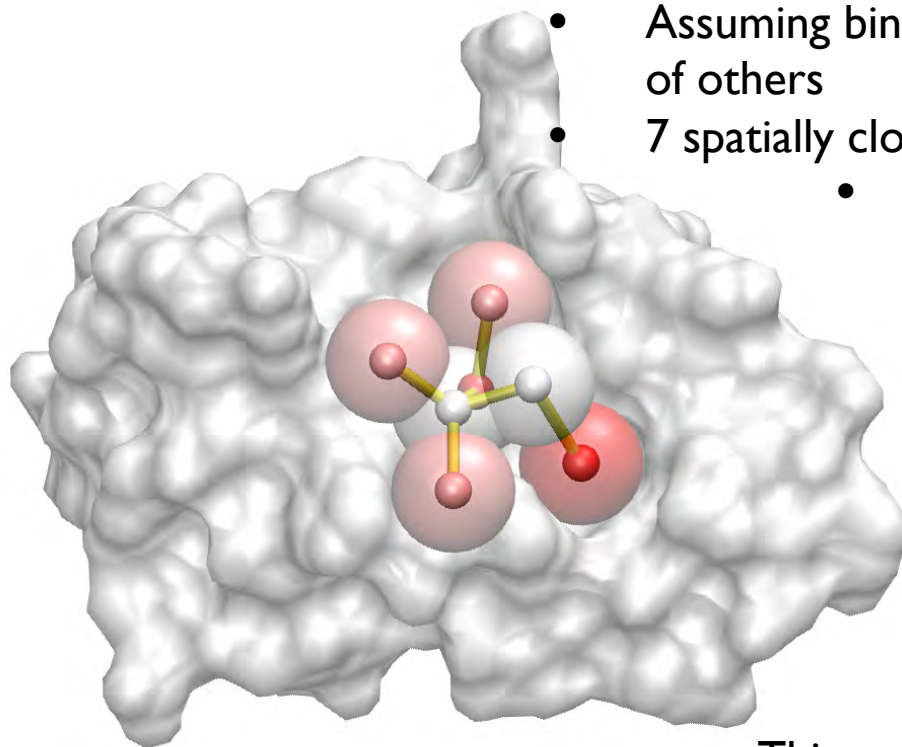
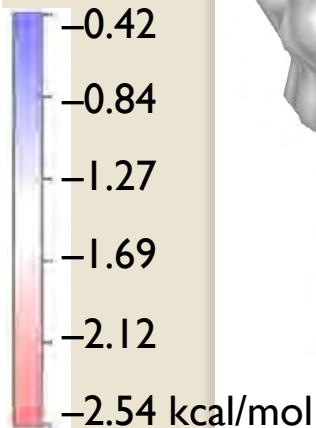
1. Grid element with lowest  $\Delta G$  value is selected
2. Other elements within **4 Å** are removed (elements inside the red sphere  $\rightarrow$ )
3. 1 and 2 are repeated until no more points are left to remove





# Affinity of a Drug-size Molecule

A heuristic approach for calculating achievable free energy of binding

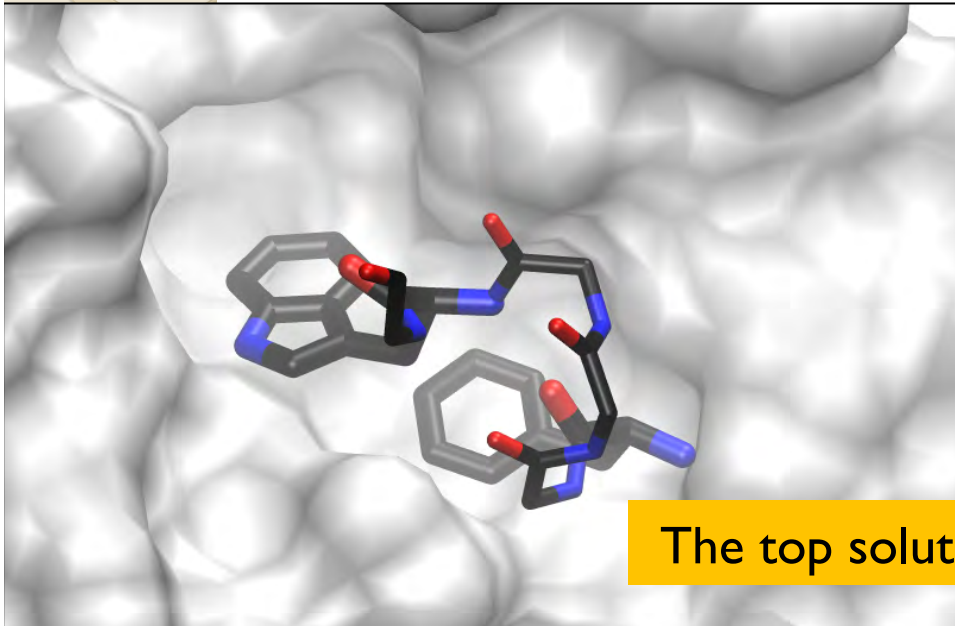


- Assuming binding of an isopropanol is independent of others
- 7 spatially close binding spots are selected
  - The sum of  $\Delta G_{binding}$  of individual points is considered as a binding free energy estimate that is achievable by a drug-like molecule

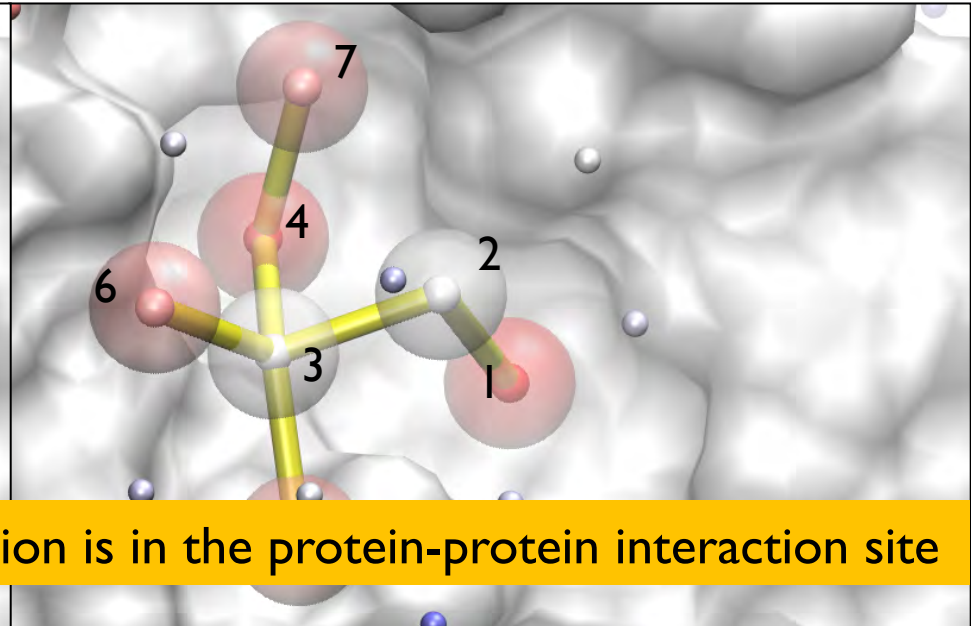
This way, the highest affinity we can observe is 5 fM ( $10^{-15}$ ).

# MDM2: p53 binding site

p53 peptide key interactions (X-ray)



Highest affinity solution (7 points)



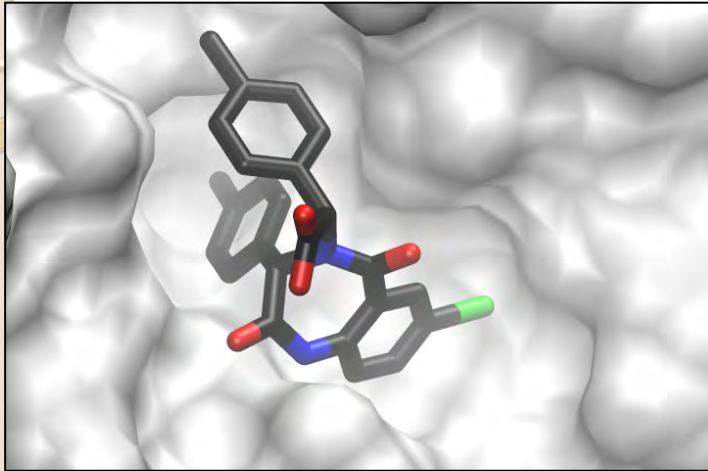
The top solution is in the protein-protein interaction site

Numbers indicate the order that hot spots were merged by the growing algorithm

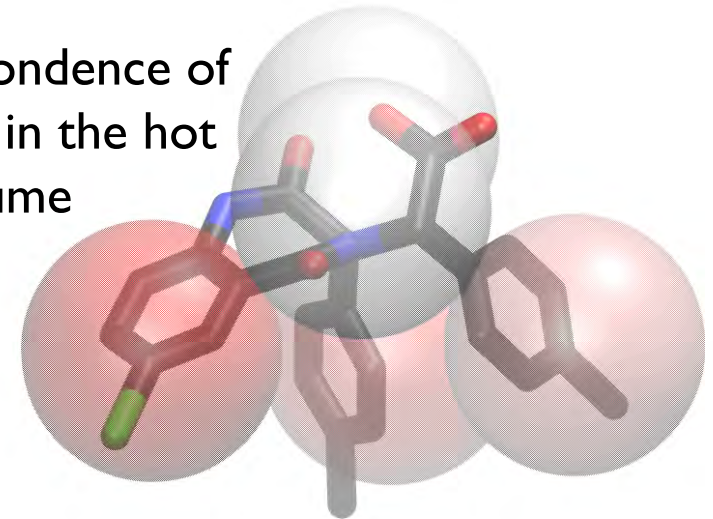
Predicted binding affinity range : **0.05-0.3 nM**  
Predicted max. affinity by Seco et al. : **0.02 nM**

# MDM2: p53 binding site

An inhibitor that disrupts p53 binding

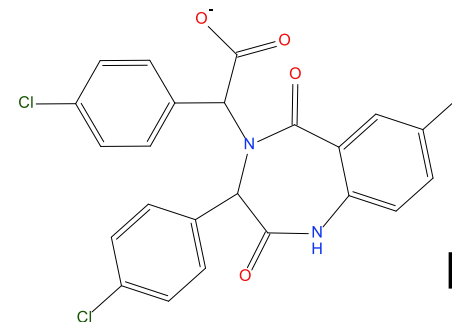
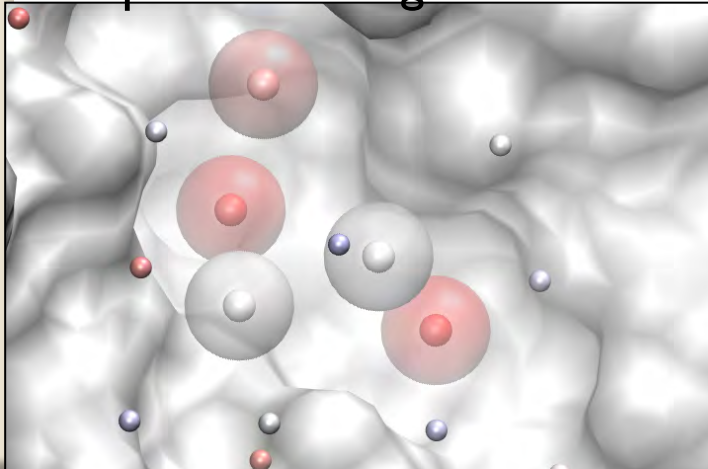


Correspondence of inhibitor in the hot spot volume



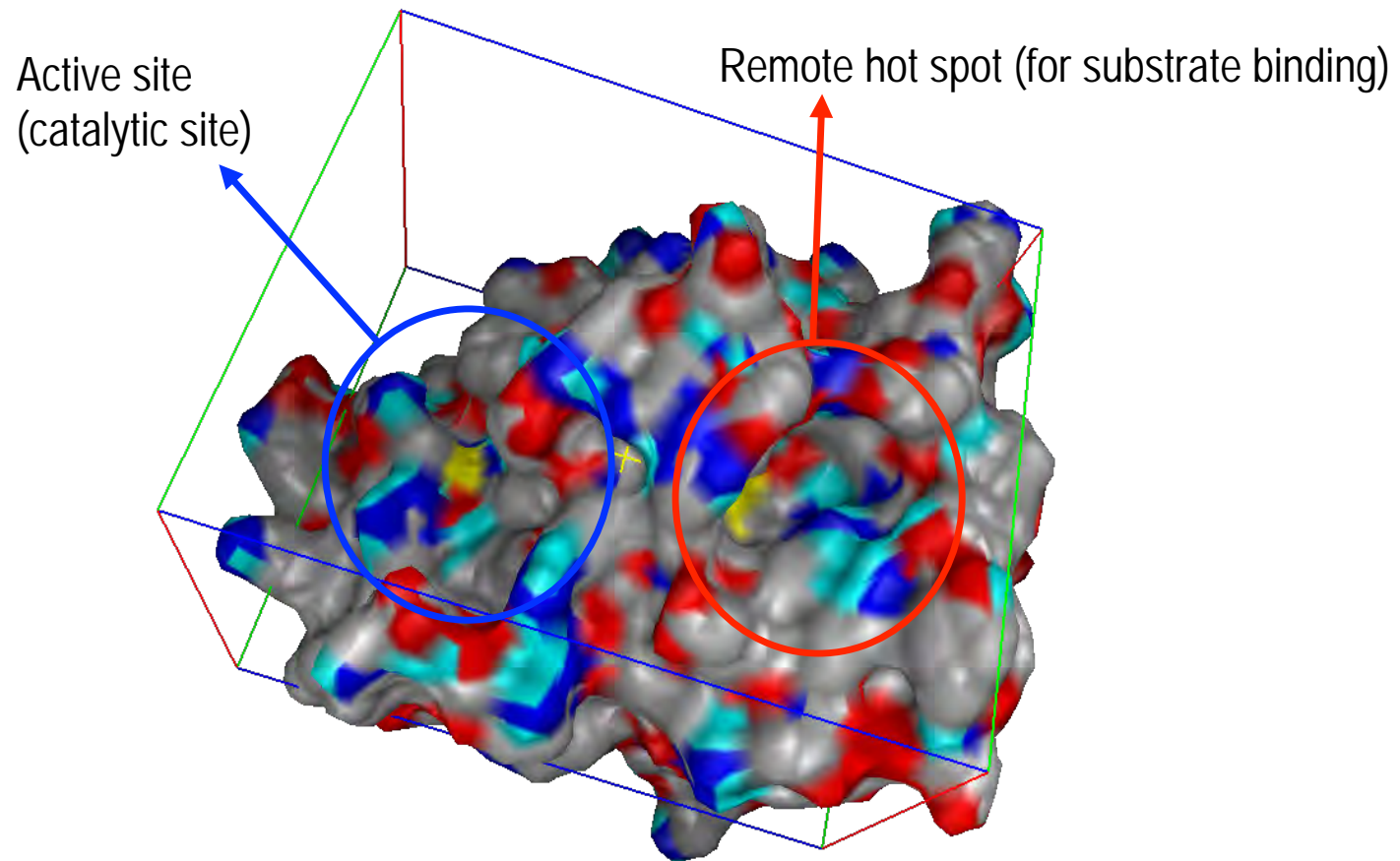
Predicted  $K_d$ : **47 nM**  
Known  $K_d$  : **80 nM**

Hot spots matching this inhibitor



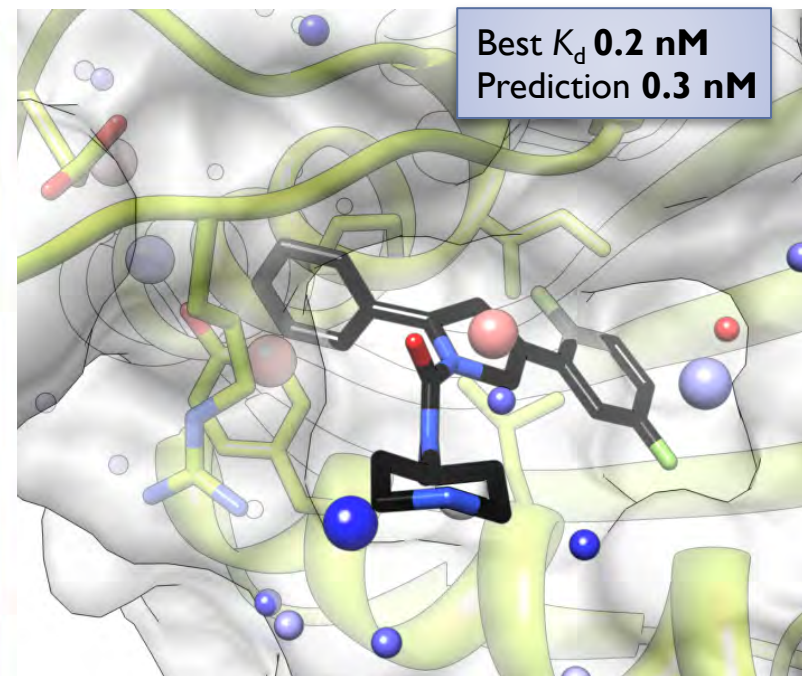
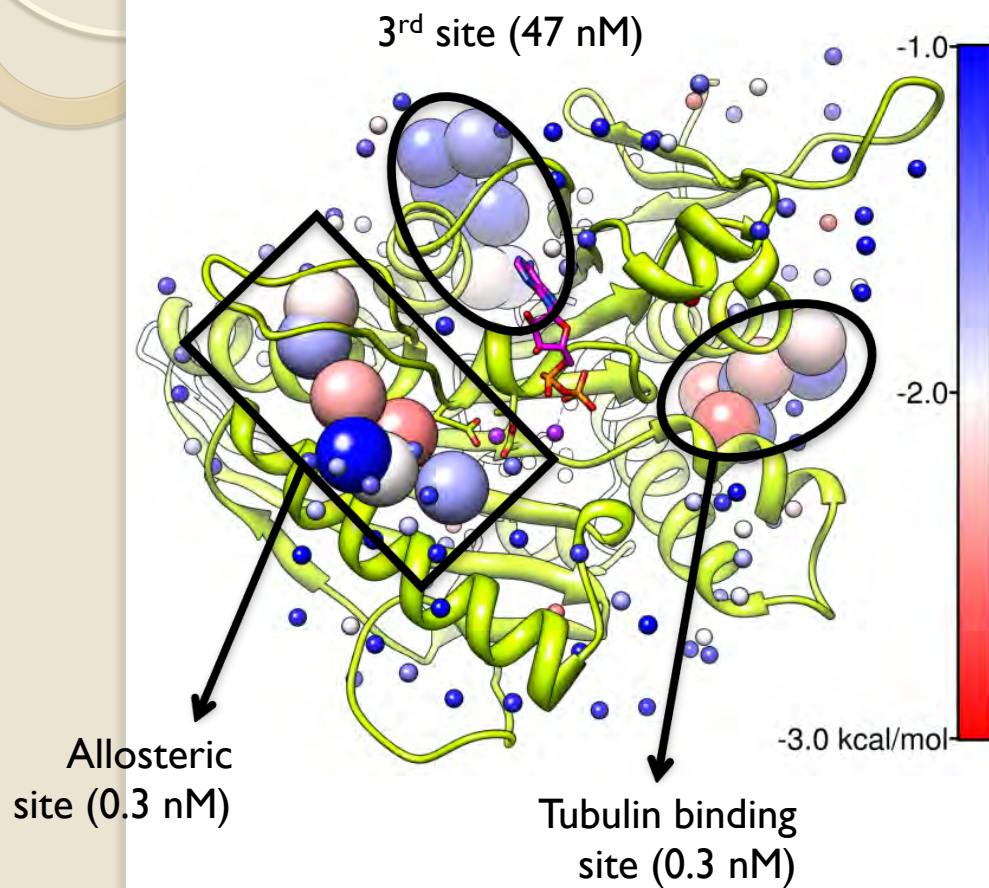
HAC = 32  
MW = 580

# Proteins may have multiple target sites



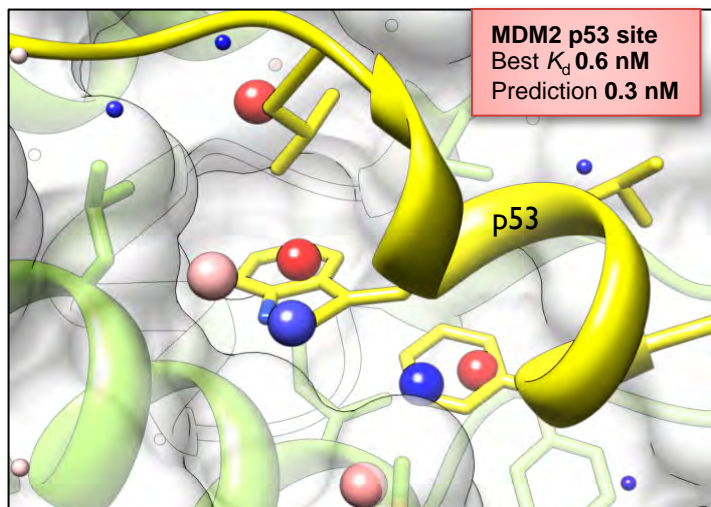


# eg5 Druggable Sites

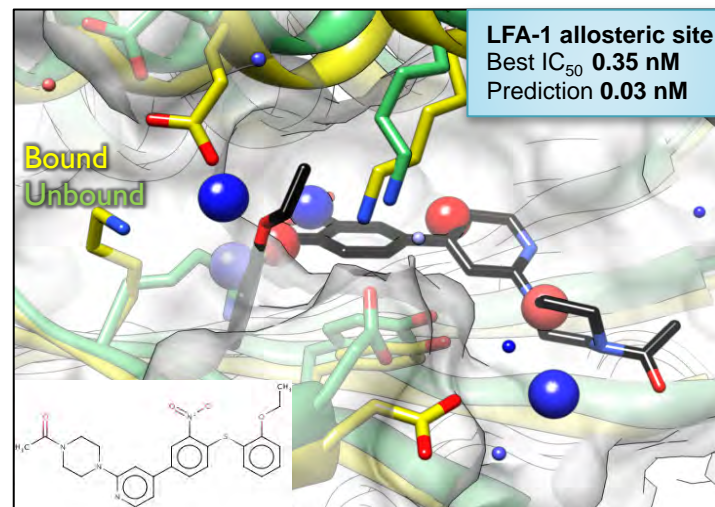


*Bioorg. Med. Chem. Lett.* **2007**, *17*, 5677-5682

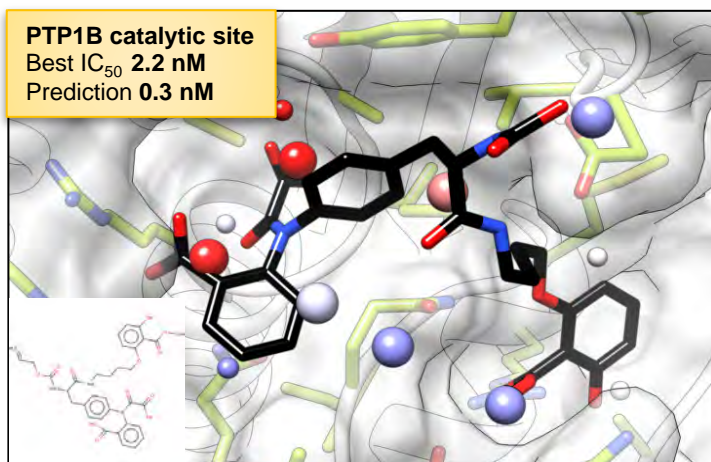
# Assessment of druggable allosteric sites



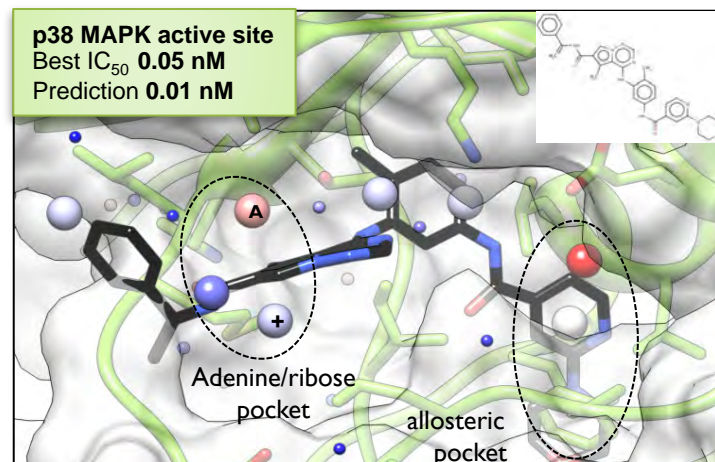
*J Med. Chem.* **2009**, 52, 7970-7973



*Biochemistry* **2004**, 43, 2394-2404



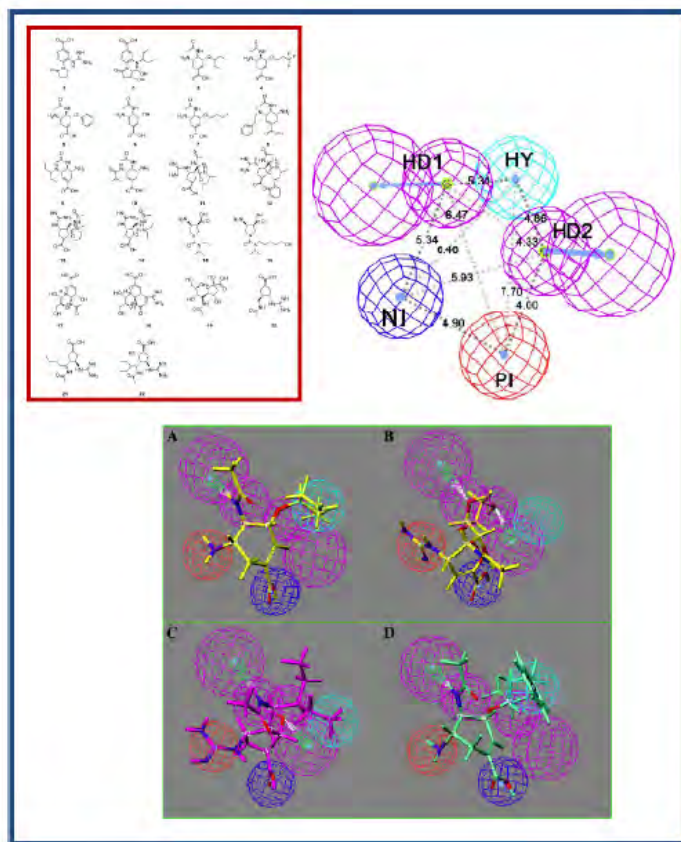
*Bioorg. Med. Chem. Lett.* **2003**, 13, 3947-3950



*J Med. Chem.* **2010**, 53, 2973-2985



# Probe distributions are used for building pharmacophore models



Zhang et al (2006) Bioorganic & Medicinal Chemistry Letters 16, 3009

## Pharmacophore Model: PM

Starting point: a series of hits

Method: clustering, identifying commonalities, assigning weights

Used for screening

- approved drugs

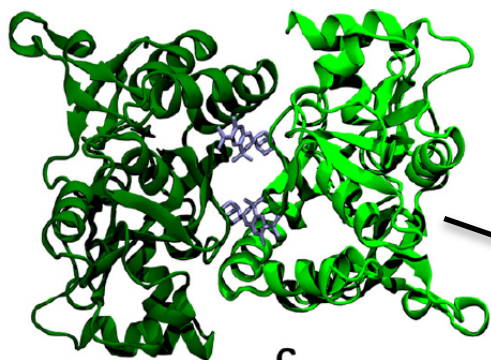
- libraries of small compounds

To identify repurposable or new drugs



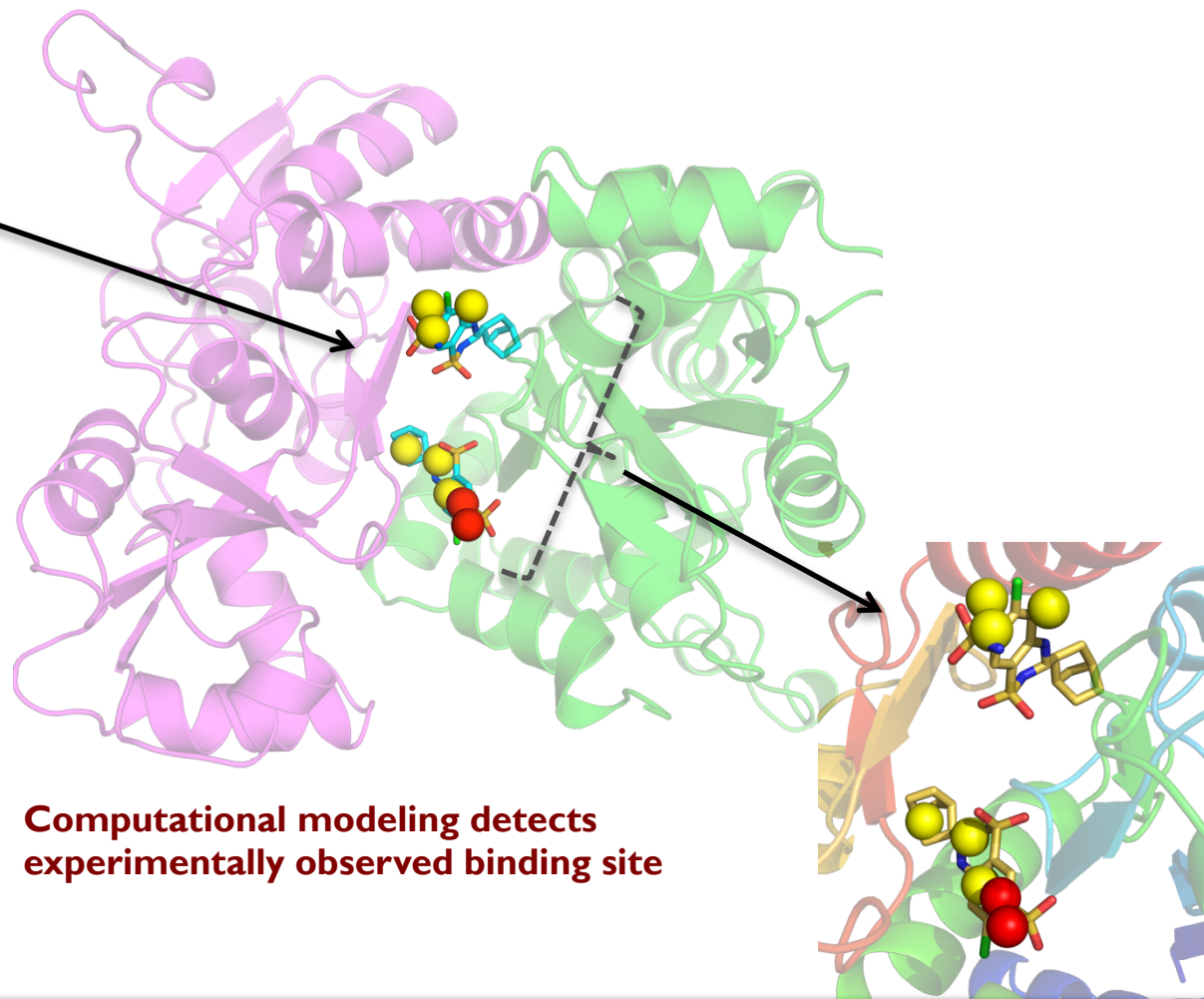
## 2

## Probes capture allosteric modulator site of AMPAR LBD Dimer



### Experimental Results

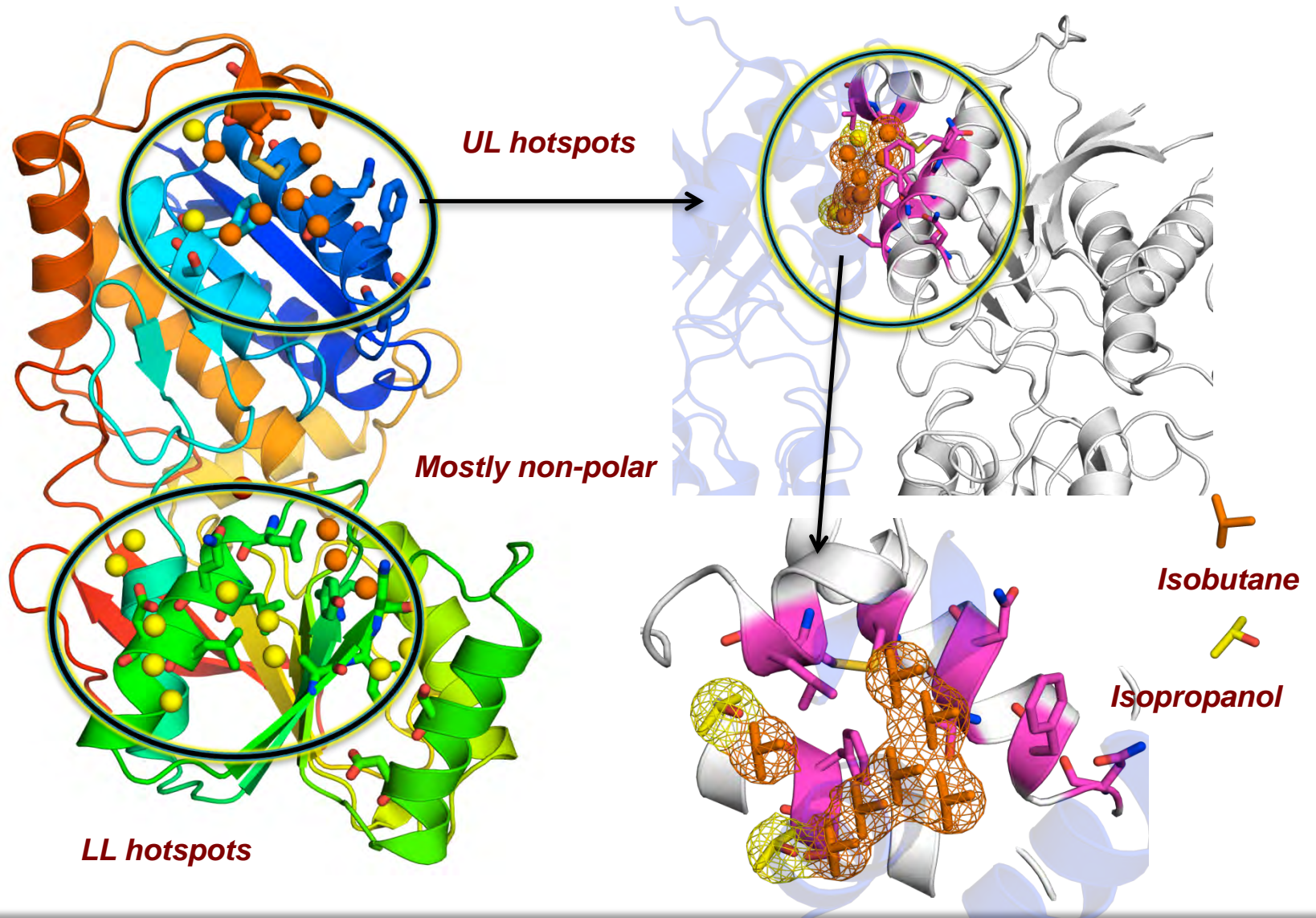
*Pohlsgarrd et al (2011).*  
*Neuropharmacology. 60,135-150.*



**Computational modeling detects  
experimentally observed binding site**

2

## Interfacial regions captured in AMPAR NTD

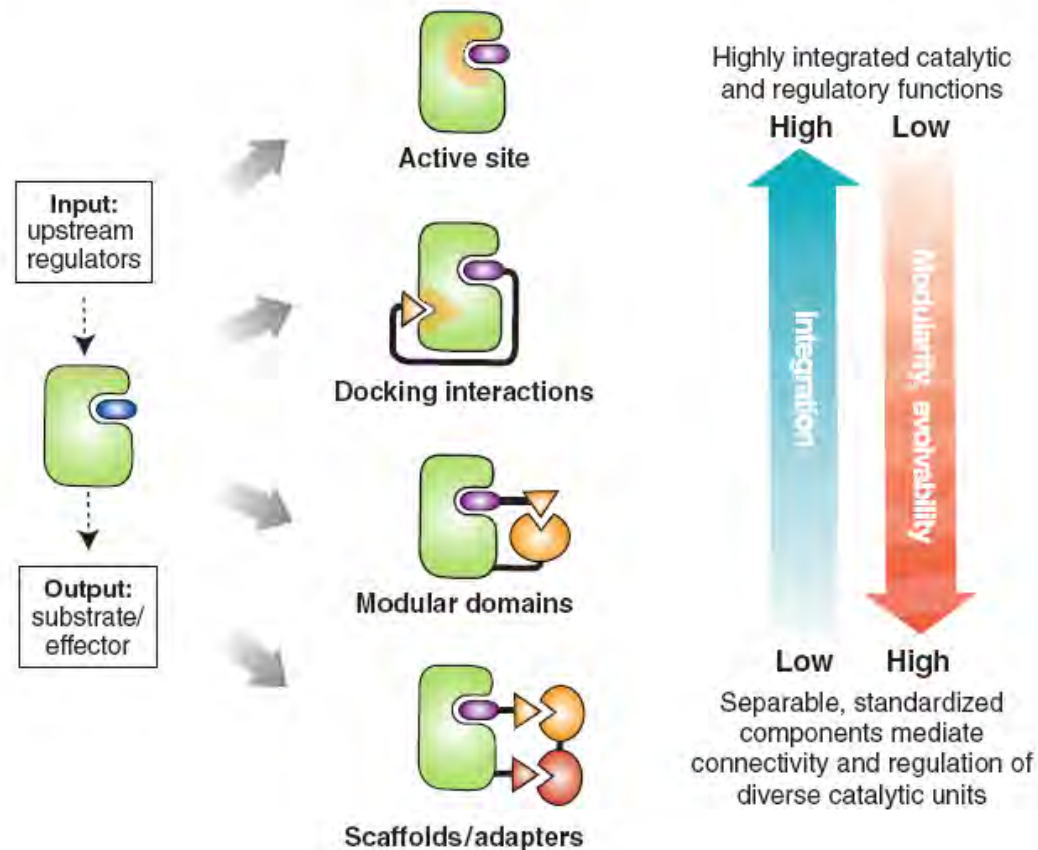




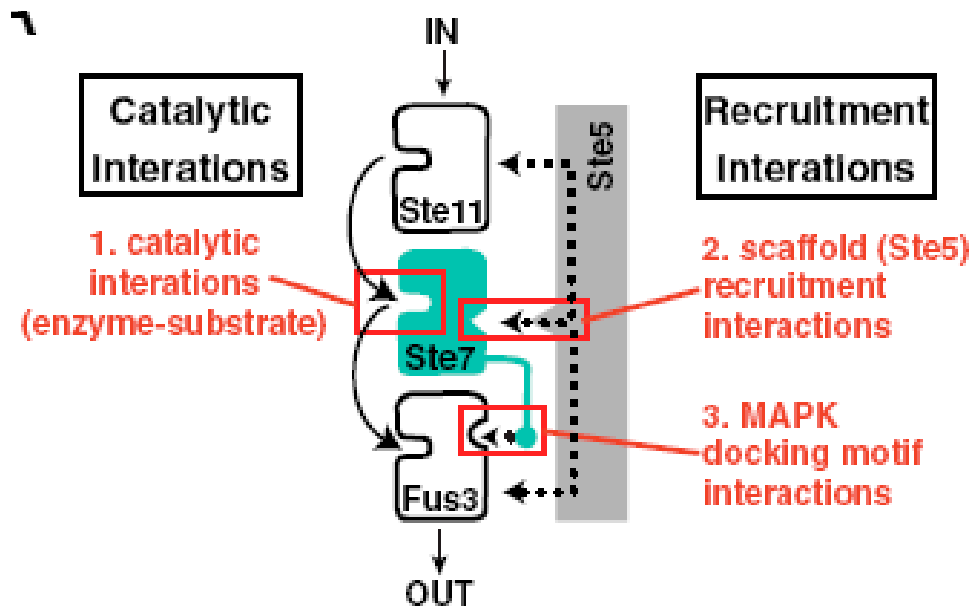
# SUMMARY

- 1 • Structure-encoded **flexibility** of drug targets and significance in drug discovery and design
- 2 • **Druggability** assessment: a first step before selecting a target
- 3 • Modularity and **promiscuity** of proteins and **quantitative systems pharmacology** methods

# Diversity & complexity of phenotypes arise from combinations of proteins & modular domains

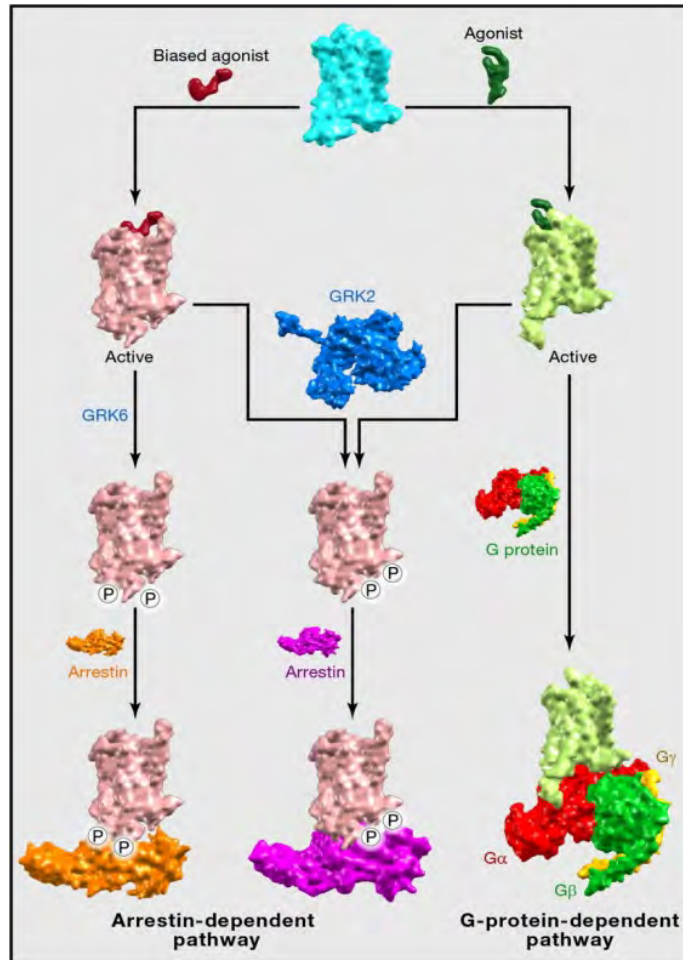


## Significance of targeting a specific site, not only a target protein





# Allostery Can Diversify Cellular Signaling Pathways through a Single Receptor



GPCRs use **conformational selection** to shape signaling.

Two (different) conformations of GPCR bind two (different) agonists, which branch into two pathways

# Protein Promiscuity

Many proteins are involved in multiple pathways.

Depending on the targeted **surface** region, or on the accessible **structural change/dynamics**

the interactions with different (or multiple) upstream or downstream partners/substrates may be affected,

which in turn would impact different (or multiple) pathways, and may result in various phenotypes



# Assessment of druggable allosteric sites

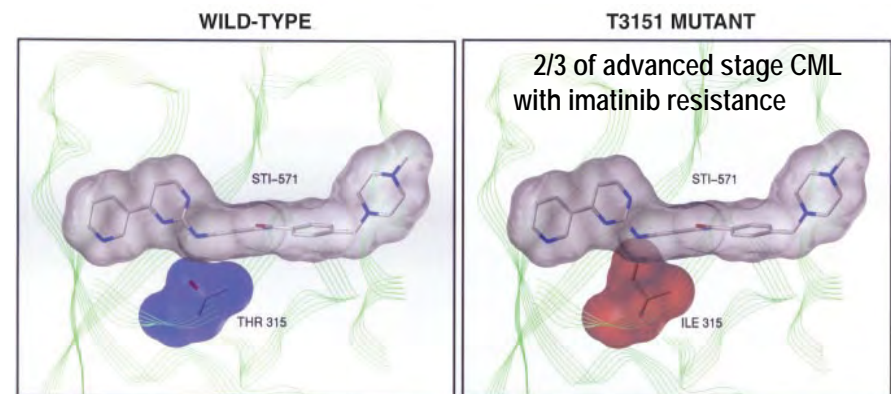
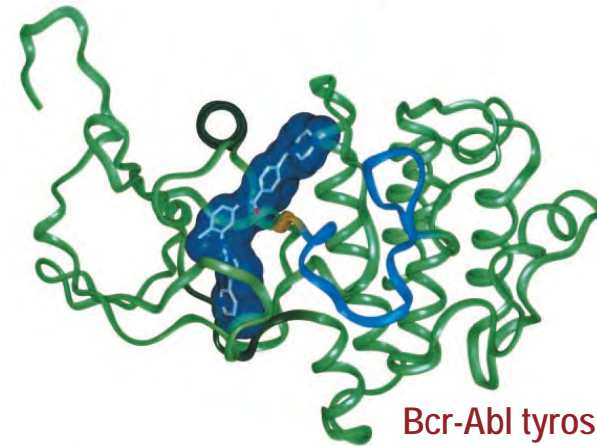
## Imatinib (Gleevec)

**TIME**  
MAY 25, 2003

THERE IS NEW **AMMUNITION**  
IN THE WAR AGAINST  
**CANCER.**  
**THESE ARE THE BULLETS.**

Revolutionary new pills like **GLEEVEC** combat cancer by targeting only the diseased cells. Is this the breakthrough we've been waiting for?

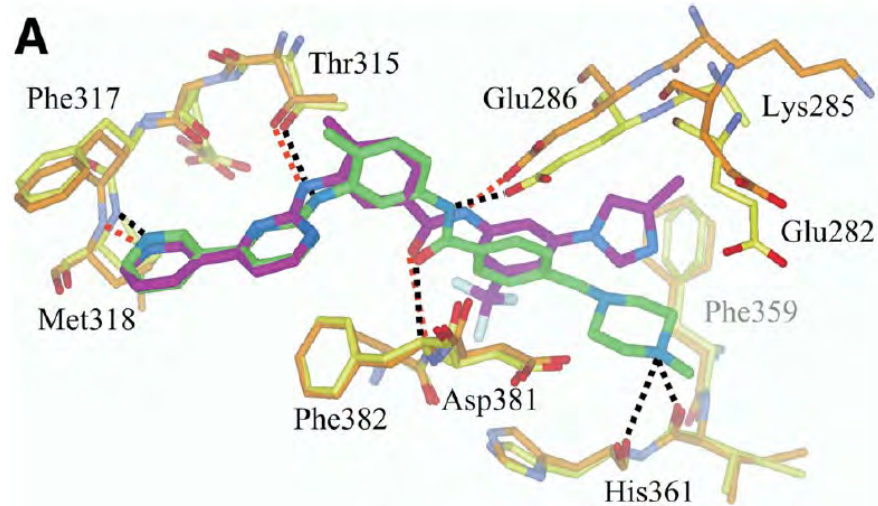
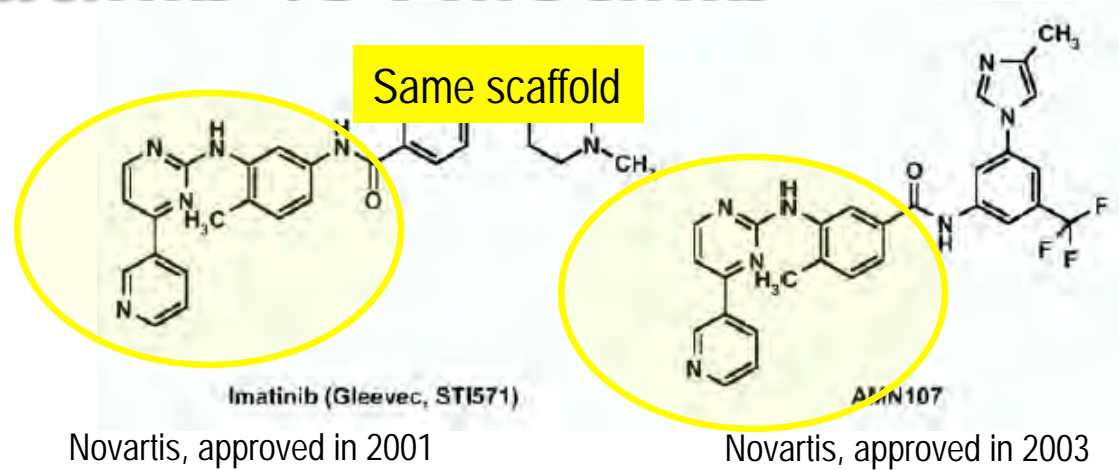
Imatinib was developed for chronic myelogenous leukemia (CML), but was also used for gastrointestinal stromal tumors (GISTs) and some other diseases.



$IC_{50} \sim 200 \text{ nM}$

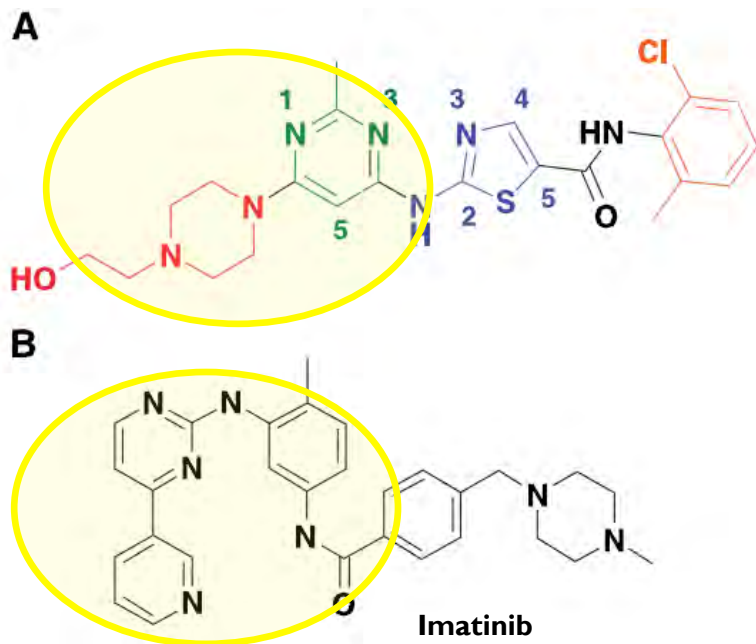
$IC_{50} > 10,000 \text{ nM}$

# Imatinib vs Nilotinib

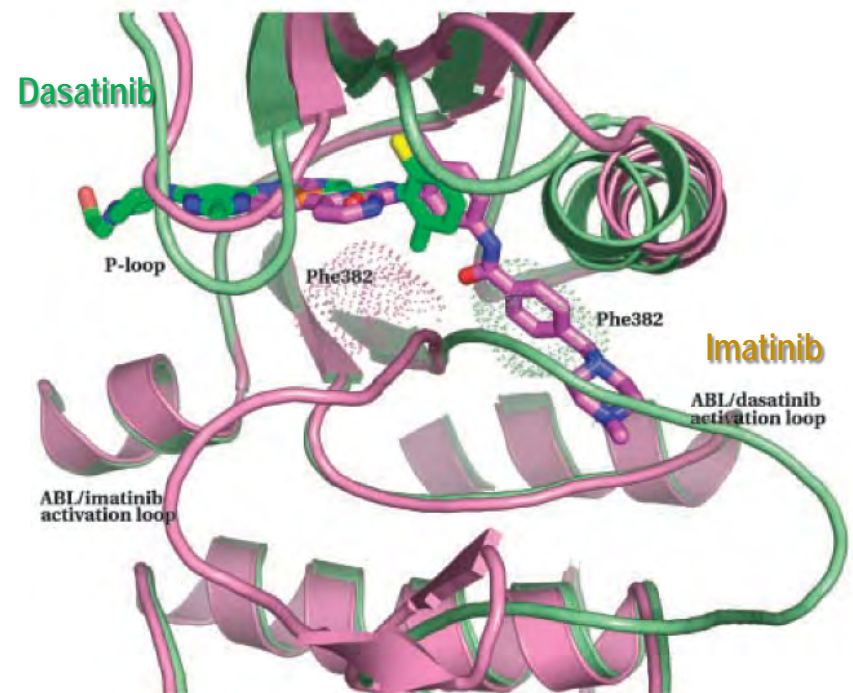


# Dasatinib addresses imatinib resistance mutations, **but** fails with mutant T315I

Dasatinib  
Bristol Myers Squibb, approved in 2011



Scaffold hopping via pharmacophore modeling



Cancer Res. 2006 , 66: 5790-7.



## ARTICLES

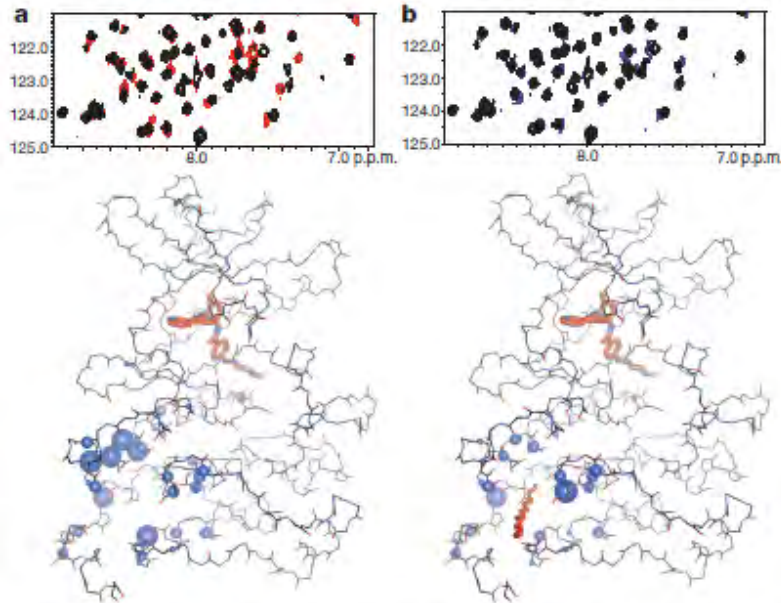
# Targeting Bcr–Abl by combining allosteric with ATP-binding-site inhibitors

Jianming Zhang<sup>1\*</sup>, Francisco J. Adrián<sup>2\*</sup>, Wolfgang Jahnke<sup>3</sup>, Sandra W. Cowan-Jacob<sup>3</sup>, Allen G. Li<sup>2</sup>, Roxana E. Iacob<sup>4</sup>, Taobo Sim<sup>1,5</sup>, John Powers<sup>6</sup>, Christine Dierks<sup>2</sup>, Fangxian Sun<sup>2</sup>, Gui-Rong Guo<sup>2</sup>, Qiang Ding<sup>2</sup>, Barun Okram<sup>7</sup>, Yongmun Choi<sup>1</sup>, Amy Wojciechowski<sup>1</sup>, Xianming Deng<sup>1</sup>, Guoxun Liu<sup>2</sup>, Gabriele Fendrich<sup>3</sup>, André Strauss<sup>3</sup>, Navratna Vajpai<sup>8</sup>, Stephan Grzesiek<sup>8</sup>, Tove Tuntland<sup>2</sup>, Yi Liu<sup>2</sup>, Badry Bursulaya<sup>2</sup>, Mohammad Azam<sup>6</sup>, Paul W. Manley<sup>3</sup>, John R. Engen<sup>4</sup>, George Q. Daley<sup>6</sup>, Markus Warmuth<sup>9</sup> & Nathanael S. Gray<sup>1</sup>

**GNF-2 binds to the myristate-binding site of Abl, leads to changes in the structural dynamics of the protein, and thus inhibits allosteric interactions!**

## 5

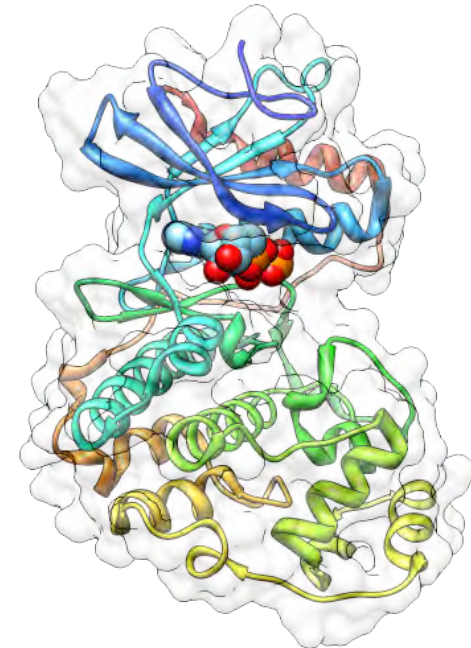
## Polypharmacological strategy: Inhibition of allosteric interaction site in addition to catalytic site



Evidence for GNF-2 binding to the myristate pocket of Abl. HSQC spectrum of Abl/imatinib with (red) and without (black) GNF-2 (top) shows chemical shift changes induced by ligand binding. Mapping of chemical shift changes to structure (PDB 10PK8) identifies the myristate pocket as the GNF-2 binding site. **b**, Same as **a** except myristic acid used instead of GNF-2.

Simultaneously targeting of

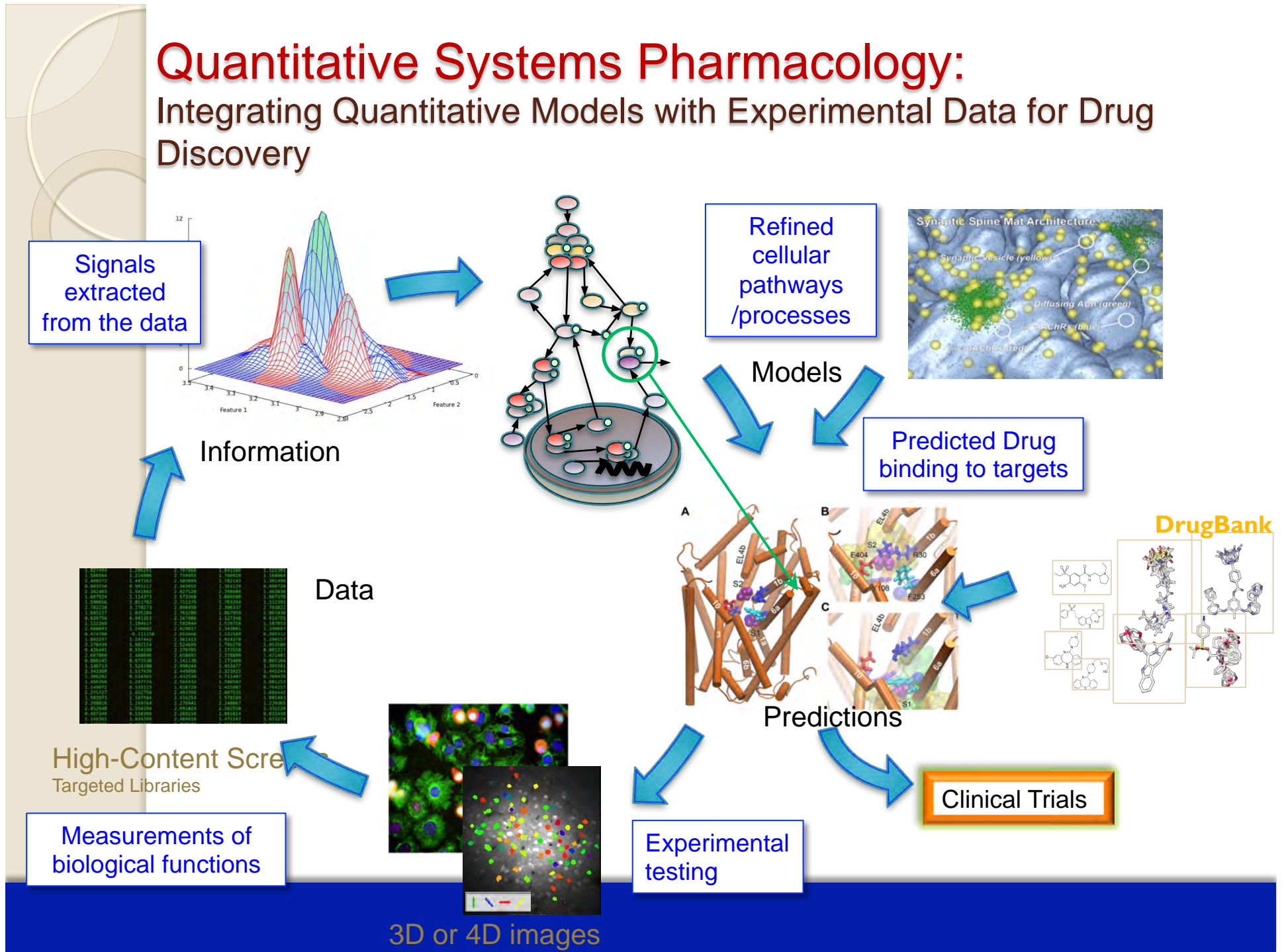
- the ATP binding site (by **Gleevec**)
- the myristate pocket (by **GNF-2**)



Khateb et al. [BMC Cancer](#). 2012 Overcoming Bcr-Abl T315I mutation by combination of GNF-2 and ATP competitors in an Abl-independent mechanism.

# Quantitative Systems Pharmacology:

## Integrating Quantitative Models with Experimental Data for Drug Discovery





# A Role for Computational Biology

## Box 2 A role for computational biology?

Predicting protein promiscuity is a problem of daunting complexity for bioinformaticians. Indeed, earlier work has shown that bioinformatics methods need improving to reliably uncover promiscuous reactions<sup>117</sup>, and our own *in silico* work in protein function prediction<sup>118,119</sup> has curbed our optimism. Even so, we do not doubt that certain areas of bioinformatics research will be important for progress in this field. Some of these avenues have already been pursued by computational biologists, but referencing individual studies is outside the scope of this review.

**Data analysis.** There is a great deal of data on protein promiscuity to be found in function-related databases. Collating information on promiscuous proteins would be a necessary first step, and existing enzyme, pathway or ontology databases can provide a lot of information on proteins with multiple EC numbers, reactions or substrates, or function categories for genes. Clues as to moonlighting might also be found from expression data. Unexpected expression patterns that do not correlate with our knowledge of existing networks are often indicative of a moonlighting function for a protein. Such data would be complemented by data on alternative splicing of a single gene, which will give hints to any additional roles in the cell.

**Sequence-based methods.** Large protein families with many relatives may indicate a trend toward promiscuity. Is there a correlation between number of orthologs and number of paralogs and how could it be explained?

**Structure-based methods.** Analyzing binding site characteristics could reveal those that make proteins more amenable to promiscuity.

**Docking profiles.** Probing the binding site with panels of selected ligands or other proteins can assess how restrictive the site is toward different types of molecules.

**Flexibility.** *In silico* studies of the flexibility of proteins can reveal how this may contribute to recognizing multiple partners.

**Redundancy in pathways.** The evidence of redundancy in metabolic and regulatory networks should be examined carefully, as it may also contain evidence for protein functional promiscuity.

**Calculation of promiscuity indices.** This could be based on *in silico* or experimental data and could help rank proteins and their partners according to their interaction promiscuity.

**Mapping of small-molecule space to protein space.** This would reveal any preferences of protein families for sets of chemical groups and possibly allow the engineering of mutants capable of binding small molecules from neighboring parts of the chemical space.

These are only some possible directions that could be explored to improve our chances of successfully exploiting promiscuity. Experimental verification of any rules learned and predictions made will be indispensable.