May 19-23, 2014

Hands-on Workshop on Computational Biophysics

by

The Theoretical and Computational Biophysics Group (TCBG)

and

The National Center for Multiscale Modeling of Biological Systems (MMBioS)

Workshop Program

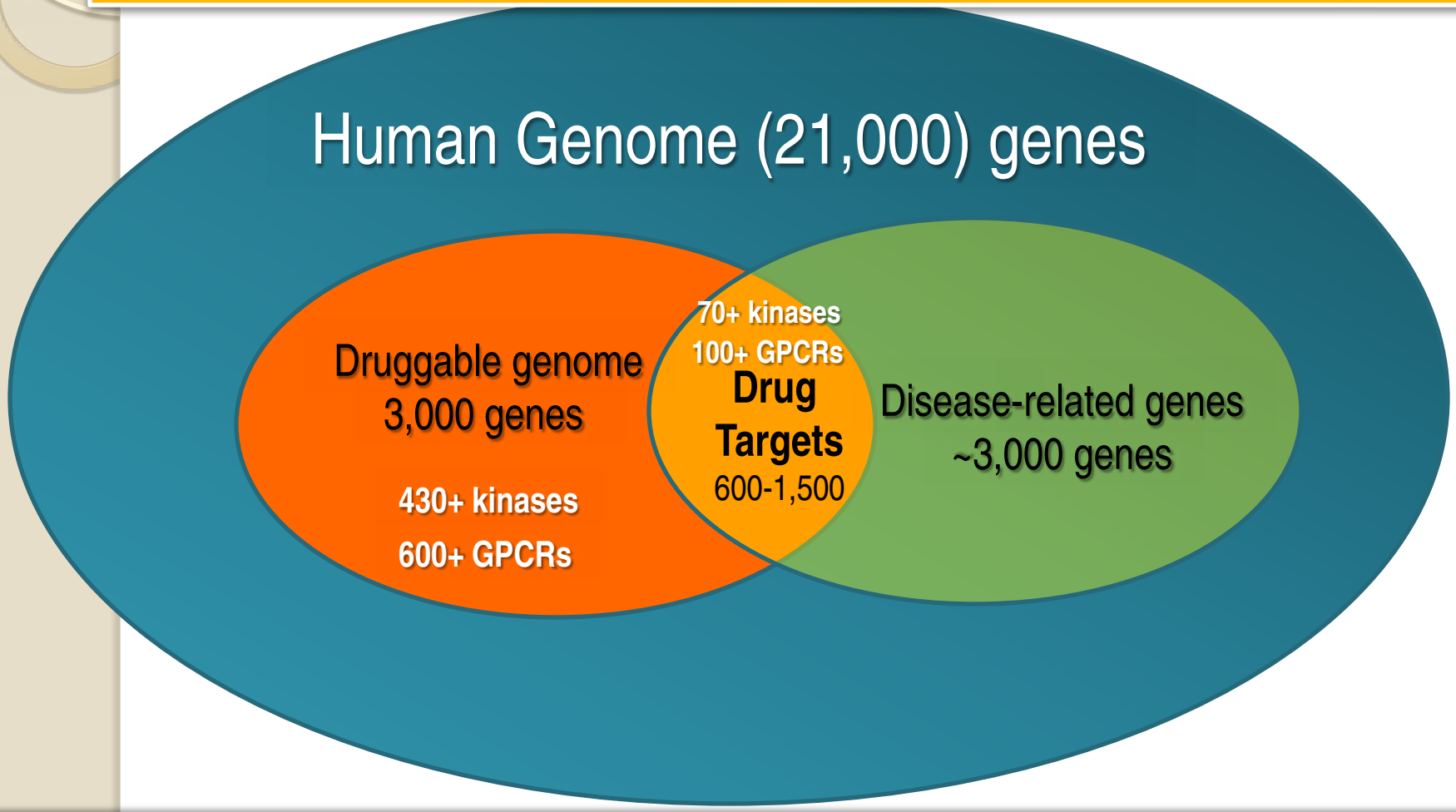
Thu, May 22: Collective Dynamics of Proteins Using Elastic Network Models - *Iv*

Bahar, Tim Lezon and Chakra Chennubhotla

Fri, May 23: Druggability Simulations, and Analyzing Sequence Patterns and Structural Dynamics - *Ivet Bahar , Indira Shrivastava, Chakra Chennubhotla,*

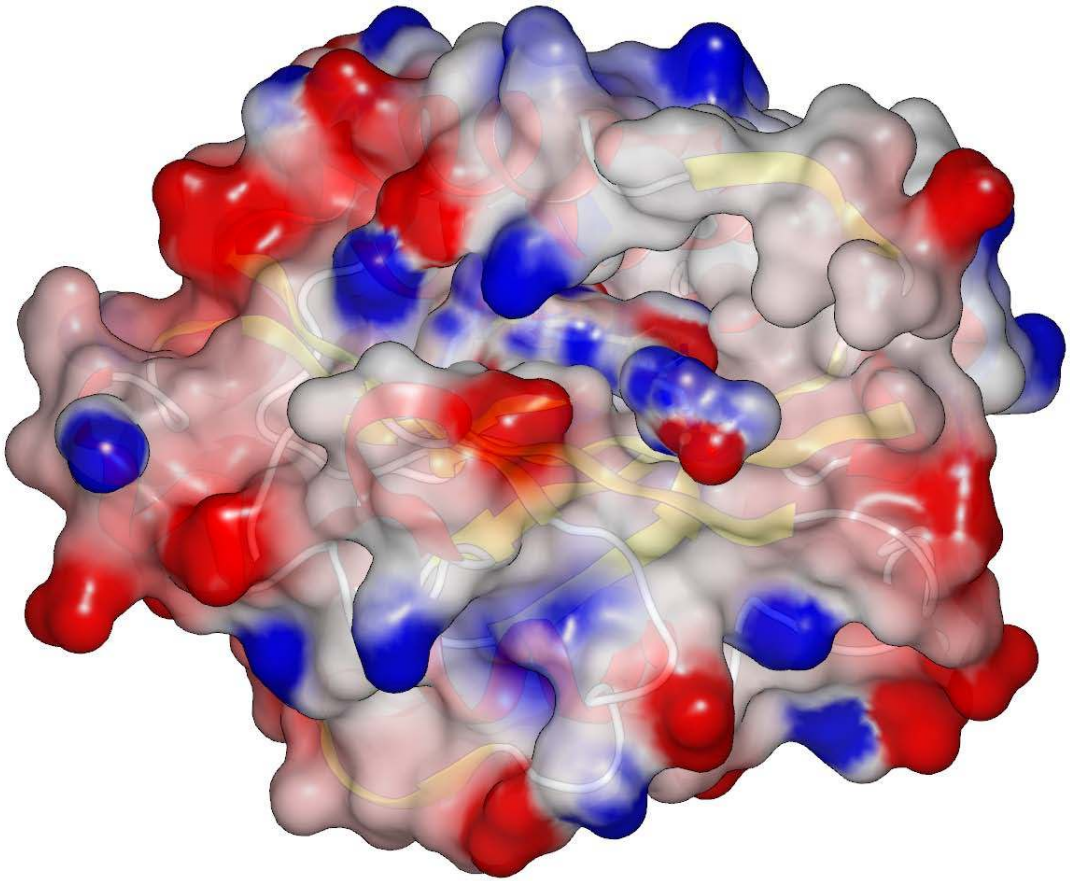
Druggable Genome

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



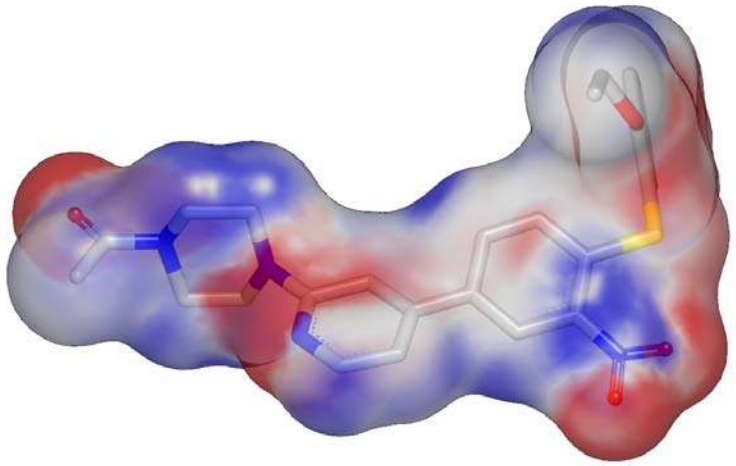
Hopkins and Groom, *Nat Reviews Drug Disc*, 2002

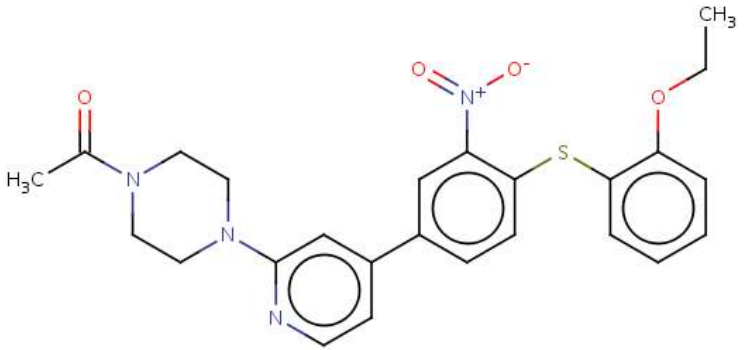
Druggable or not?



Active site druggability:

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

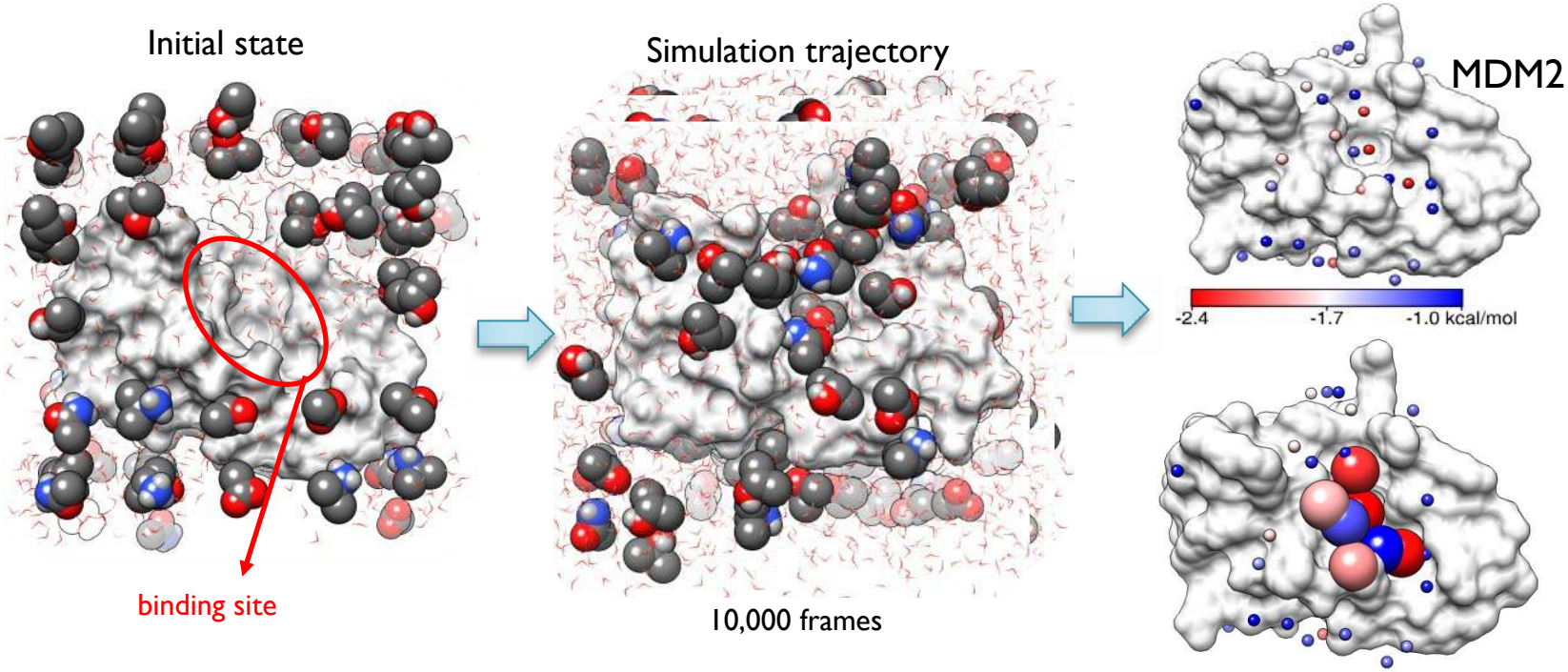


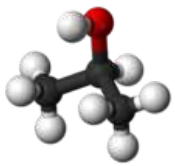


Lfa1 - a leukocyte glycoprotein that promotes intercellular adhesion and binds intercellular adhesion molecule 1

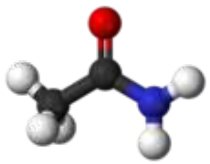
2

Druggability Simulations

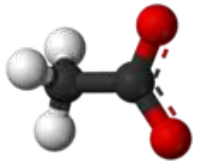




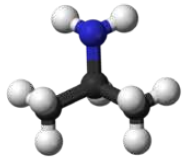
Isopropanol
(observed in 57% of drugs)



Acetamide
(21%)

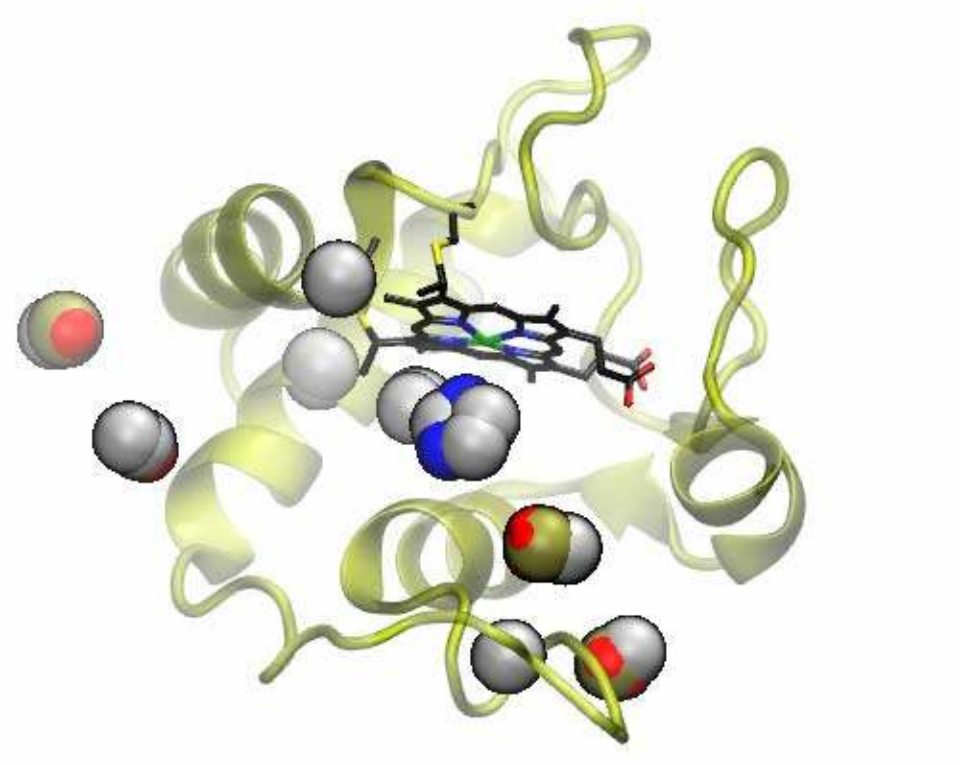


Acetate (-1)
(21%)



Isopropylamine (+1)
(25%)

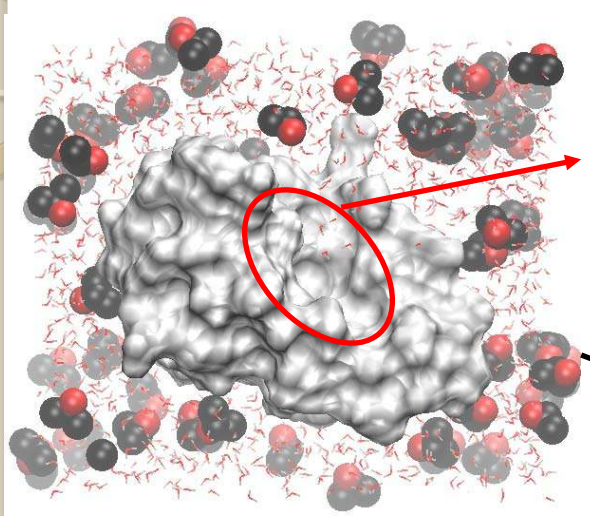
Cytochrome c druggability



Bakan & Bahar, *J Chem Theory Comput*. 8:2435-2447, 2012

Methodology Overview

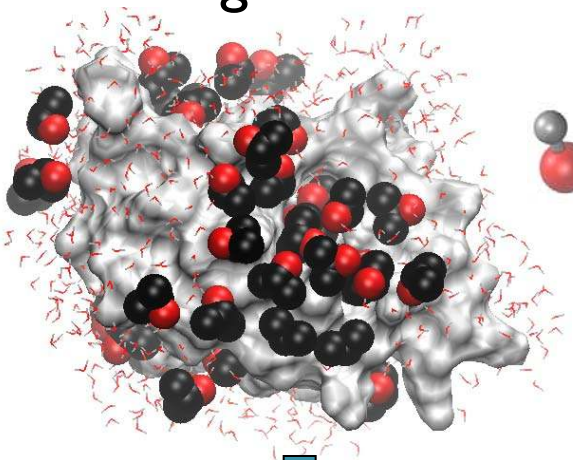
From MD simulations to achievable drug affinities

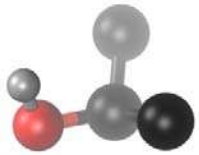


binding site

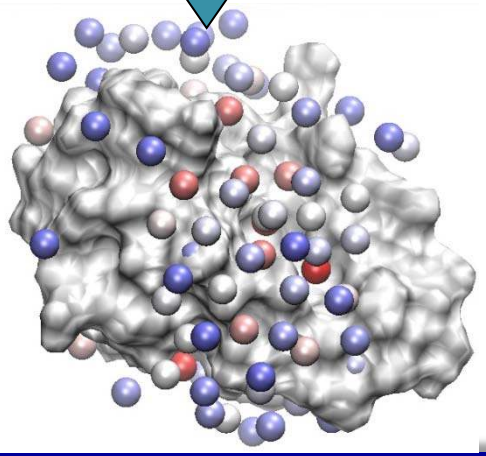
10-20 ns

isopropanol



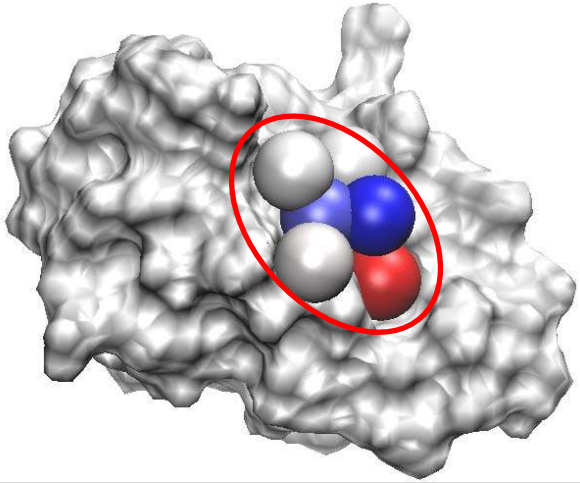


Identify hotspots

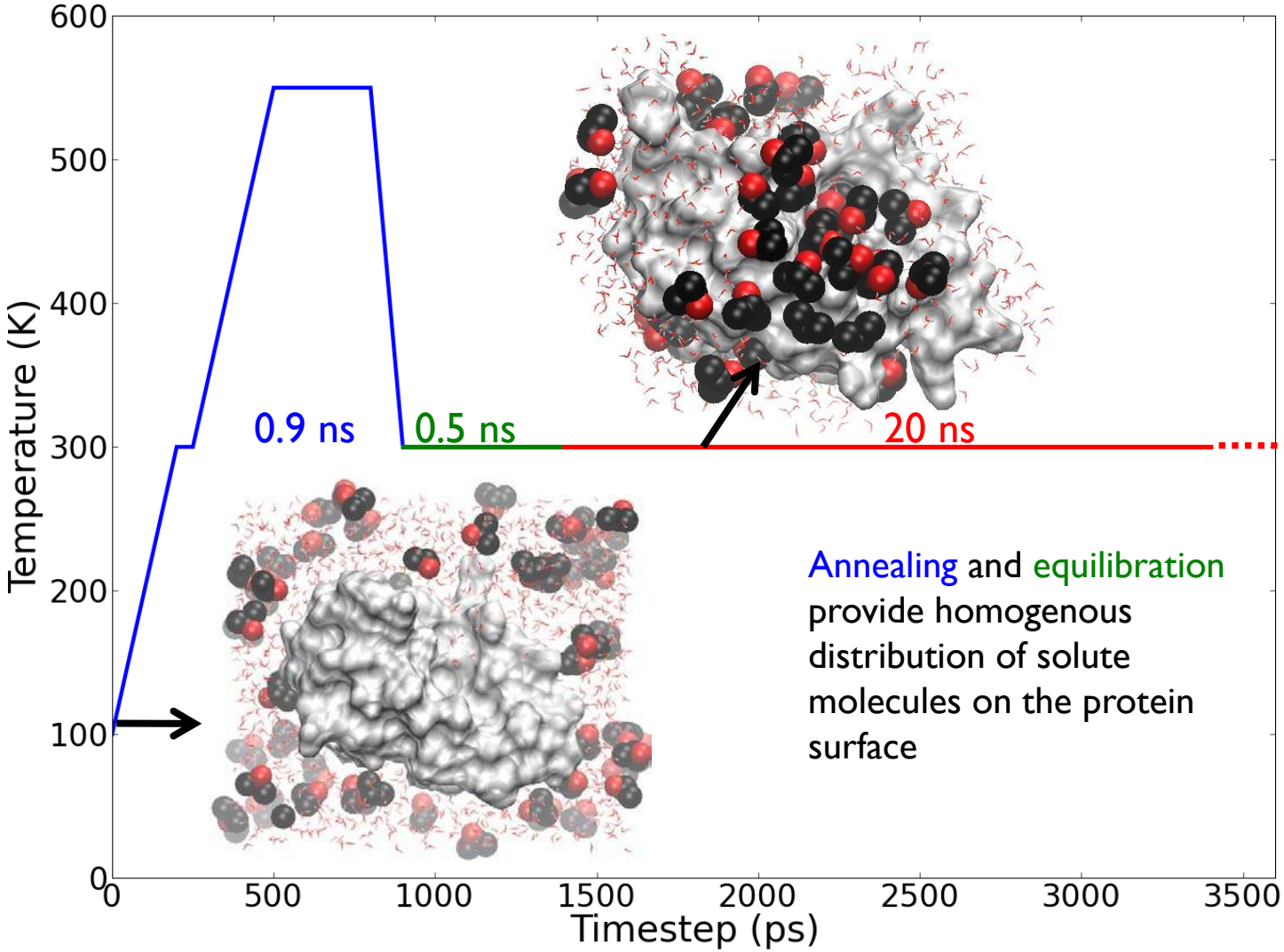


Detect binding sites

calculate achievable affinity



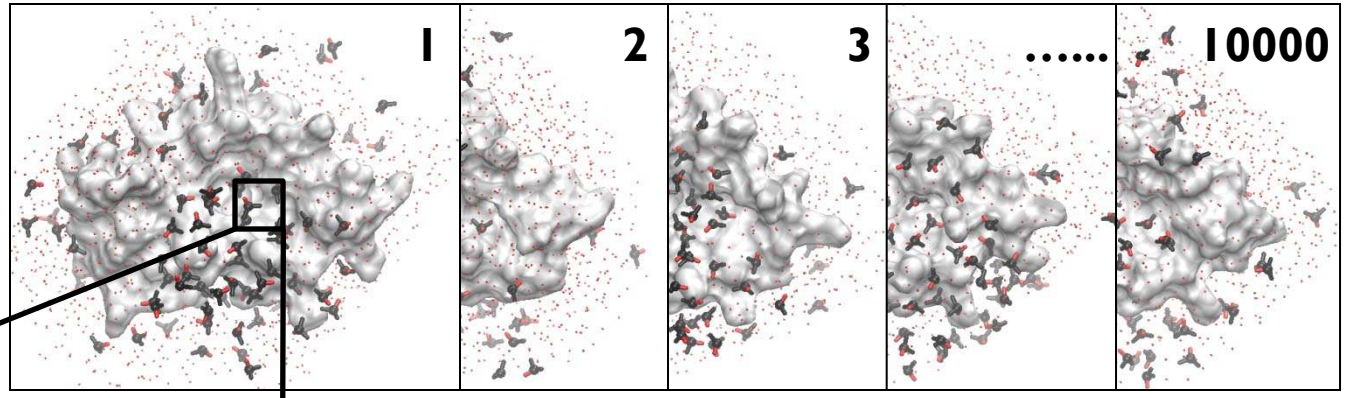
Annealing, Equilibration, Simulation

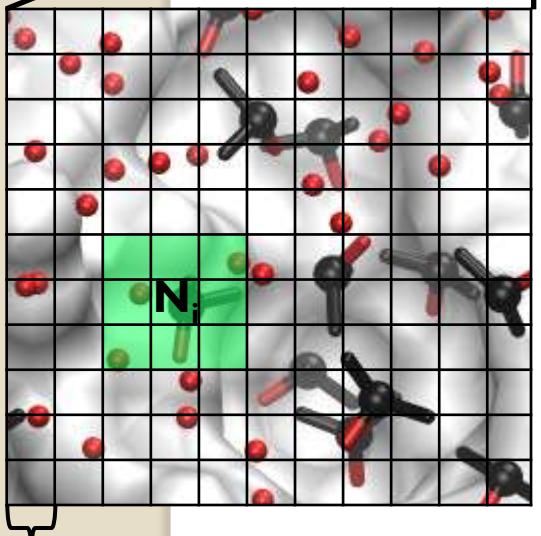


NAMD2 with CHARMM force field was used for simulations.

Free Energy of Binding for Isopropanol

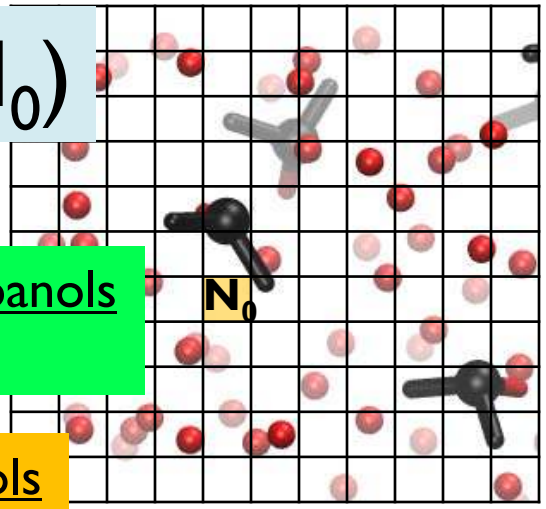
Assuming that MD sampling converged to a **Boltzmann ensemble**





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

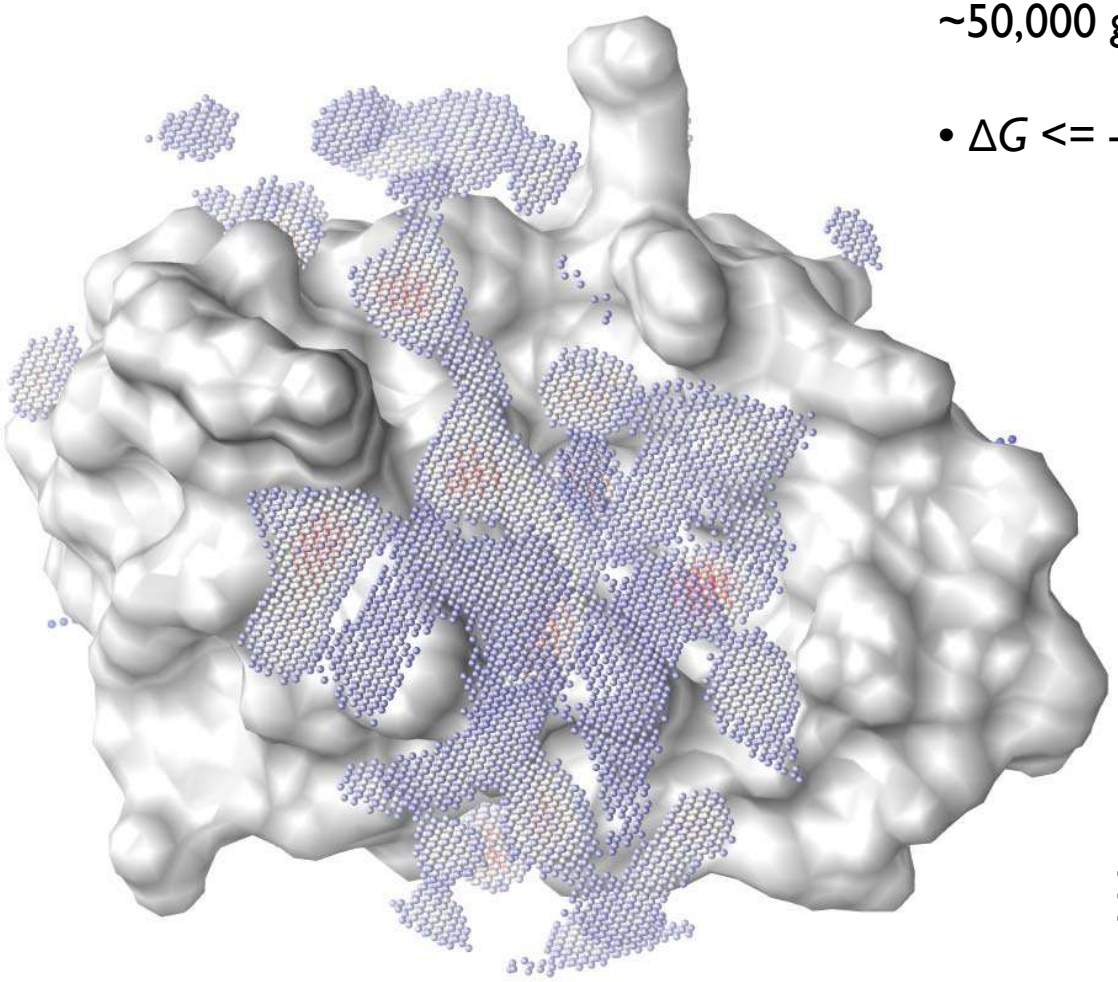
N_i corresponds to the central highlighted grid element;
number of cubes is introduced if multiple cubes are occupied by a single isopropanol

Isopropanol Binding Spots

ΔG grid is mapped onto the protein structure

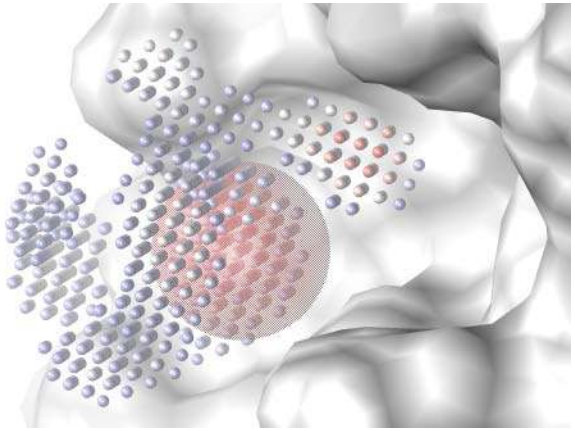
~50,000 grid points with

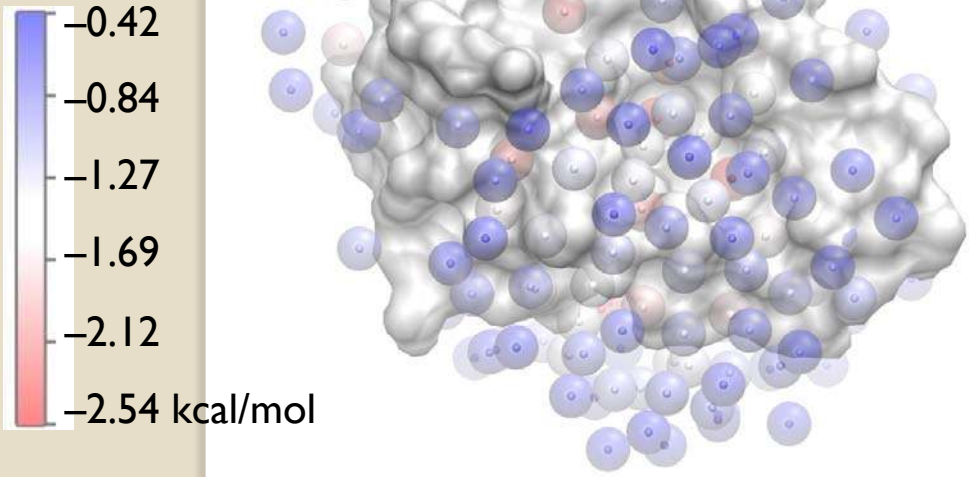
- ΔG <= – 0.416 kcal/mol*
  (2 fold enrichment)

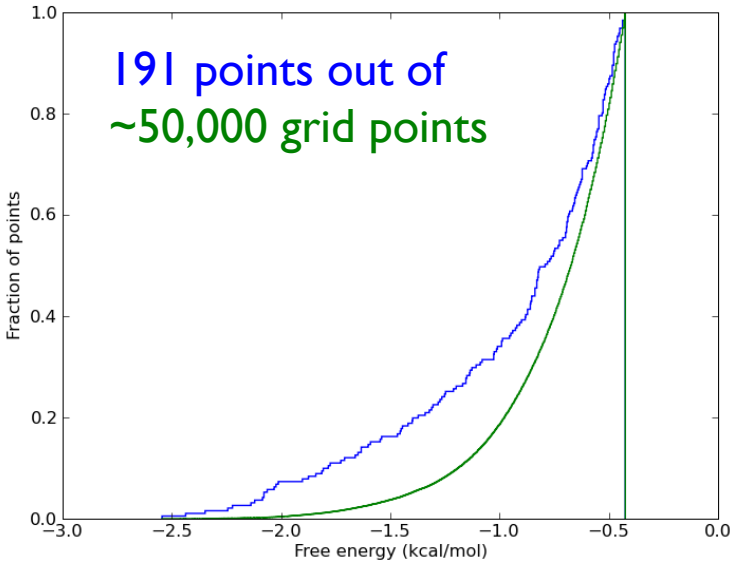


Selecting Isopropanol Binding Spots

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

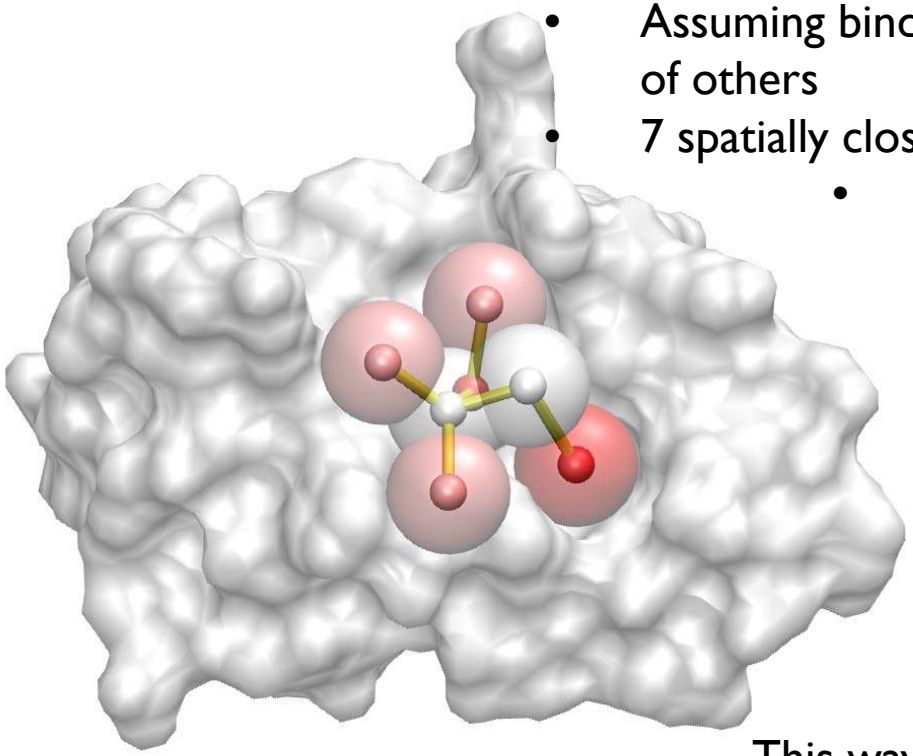






Affinity of a Drug-size Molecule

A heuristic approach for calculating achievable free energy of binding



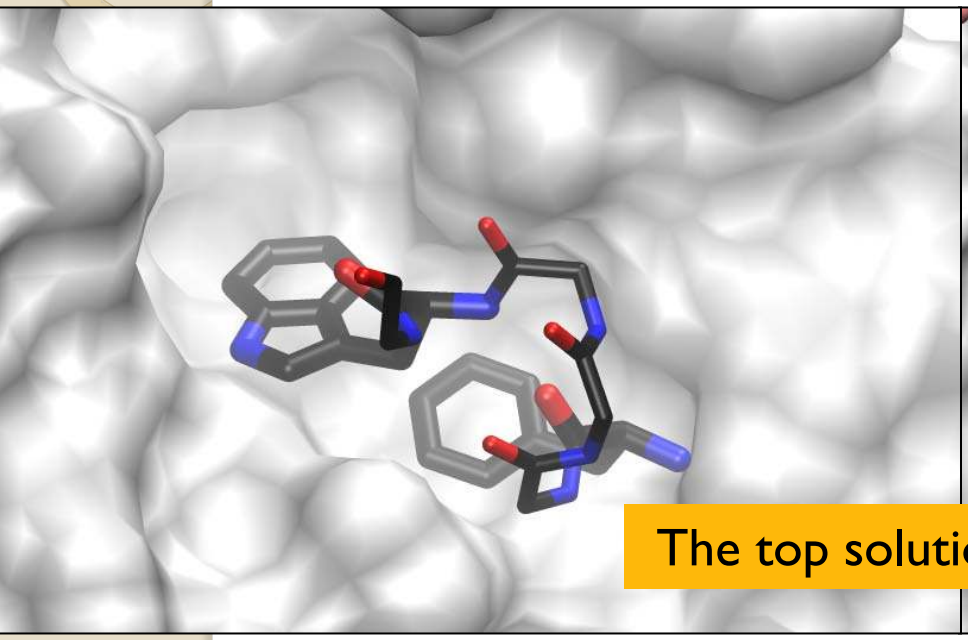
–0.42
–0.84
–1.27
–1.69
–2.12
–2.54 kcal/mol

- 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

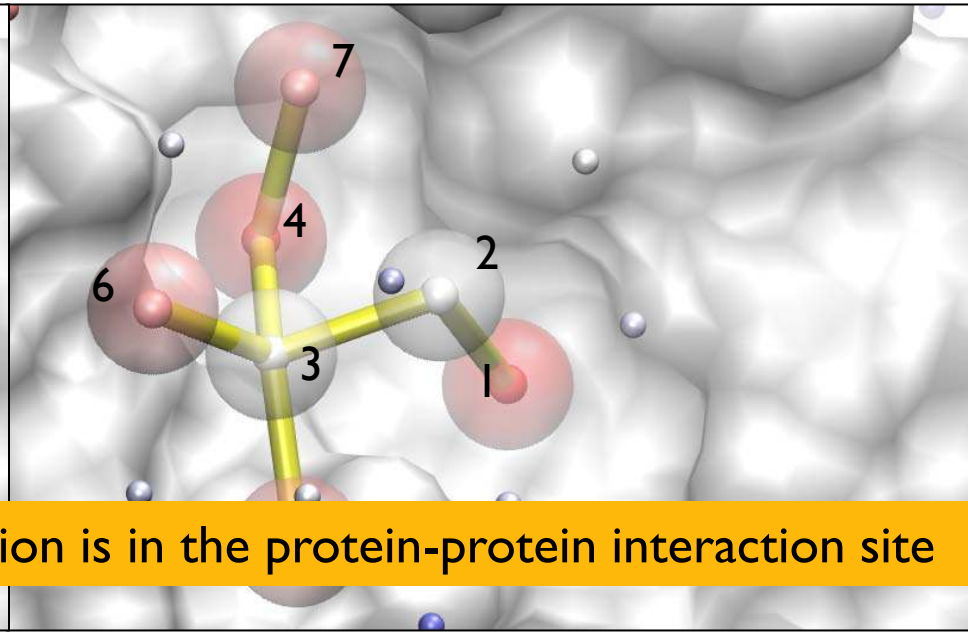
This way, the highest affinity we can observe is 5 fM (10^{-15}).

MDM2: p53 binding site

p53 peptide key interactions (X-ray)



Highest affinity solution (7 points)



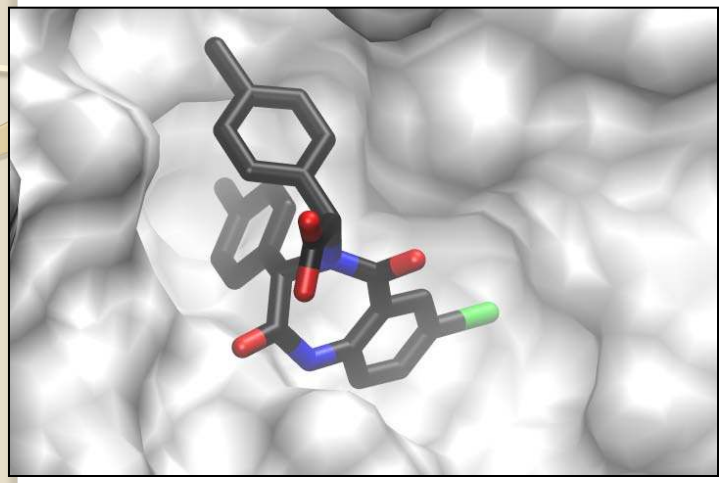
The top solution is in the protein-protein interaction site

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

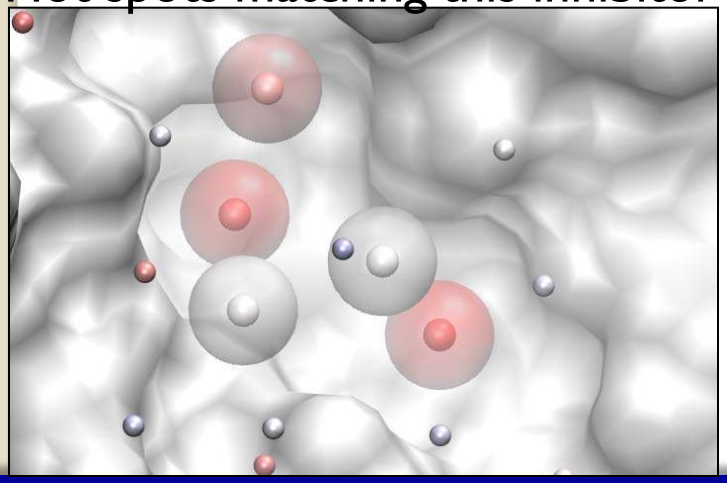
Predicted binding affinity range : **0.05-0.3 nM**
Predicted max. affinity by Seco et al. : **0.02 nM**

MDM2: p53 binding site

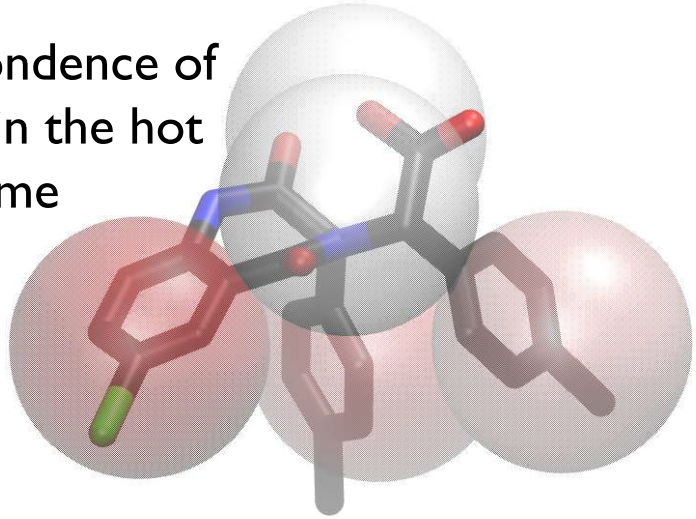
An inhibitor that disrupts p53 binding



Hot spots matching this inhibitor

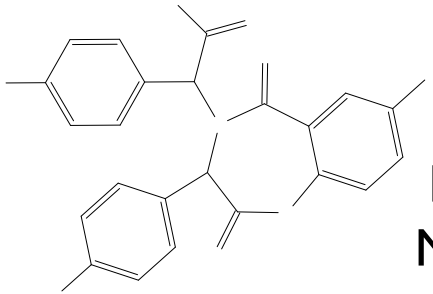


Correspondence of inhibitor in the hot spot volume



Predicted K_d: **47 nM**

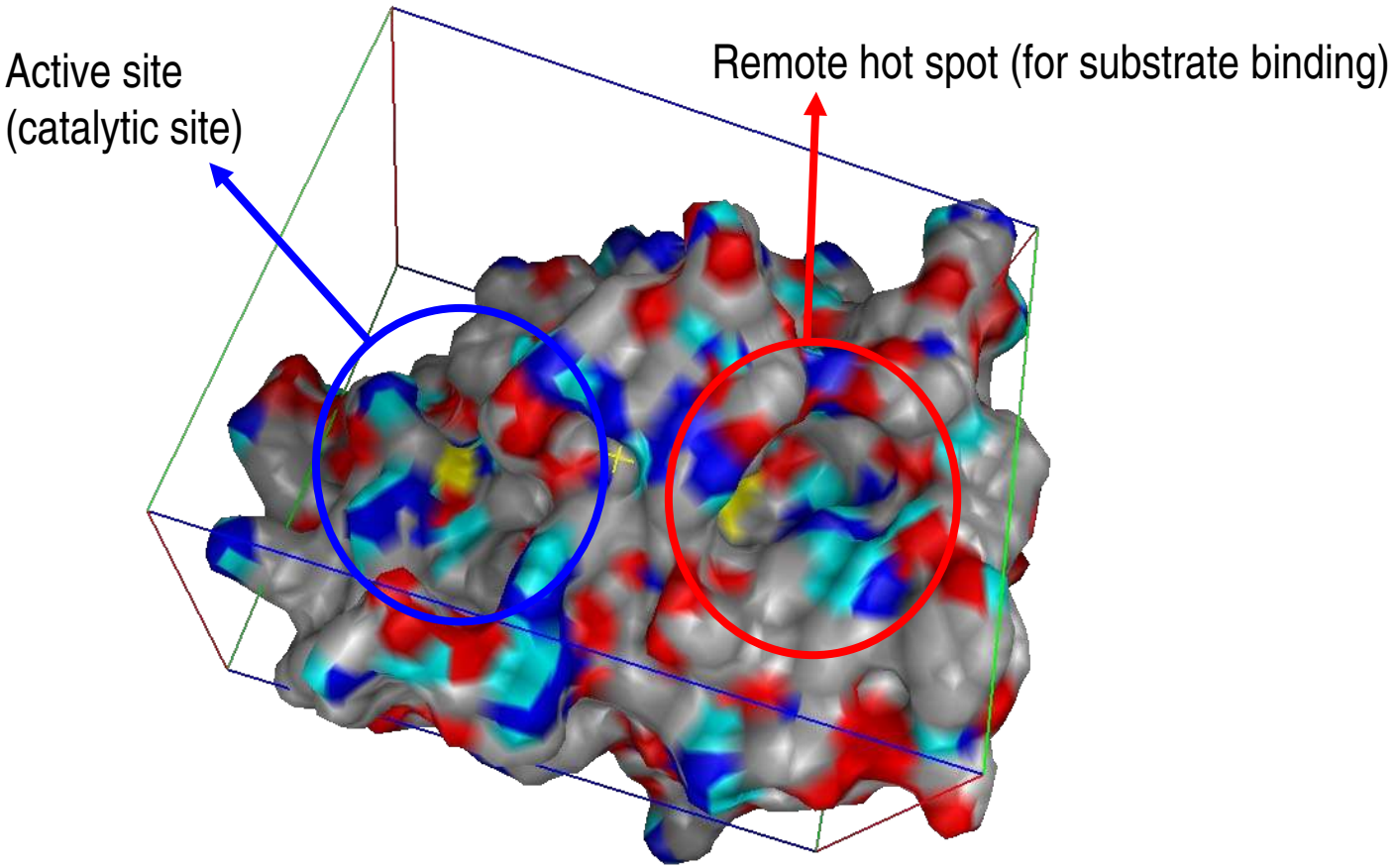
Known K_d : **80 nM**



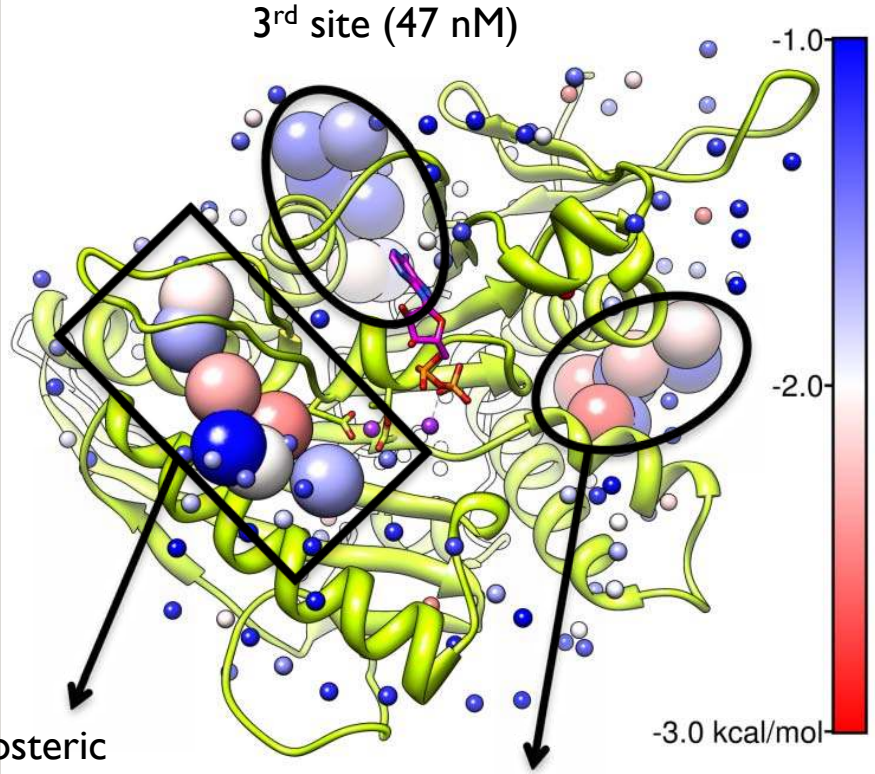
HAC = 32

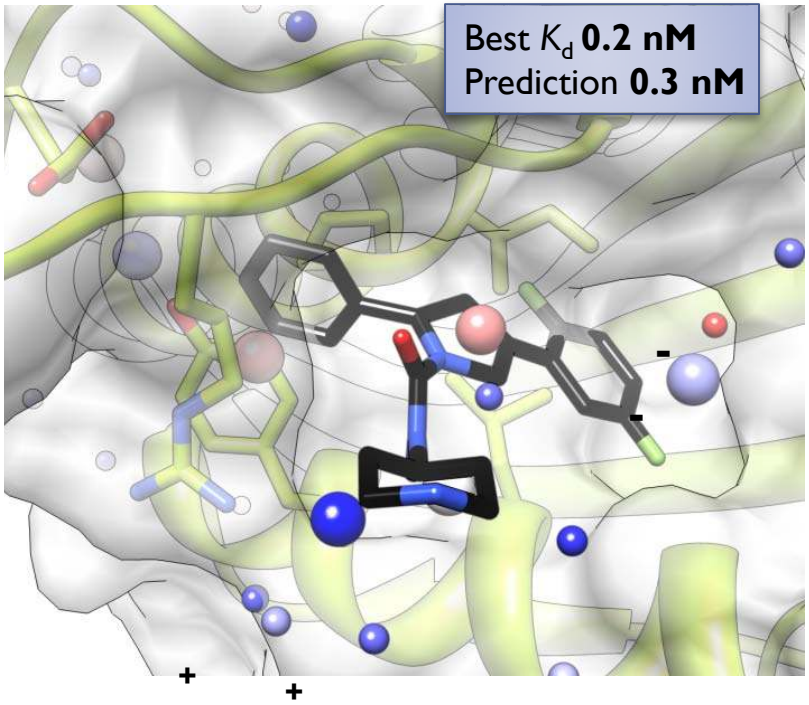
MW = 580

Proteins may have multiple target sites



eg5 Druggable Sites

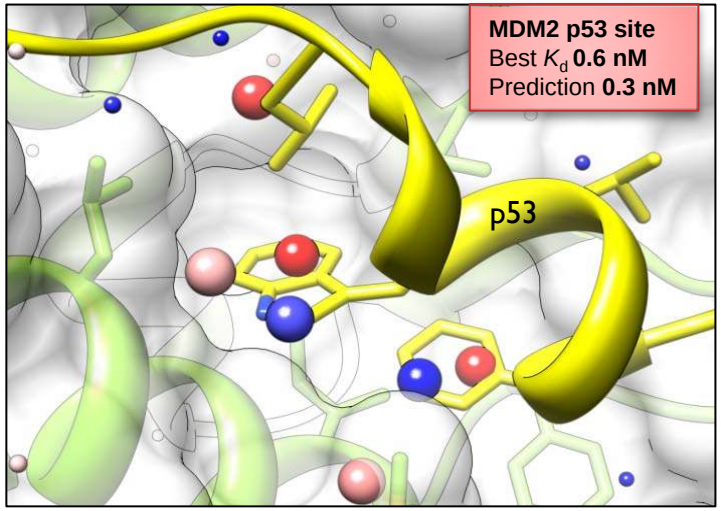




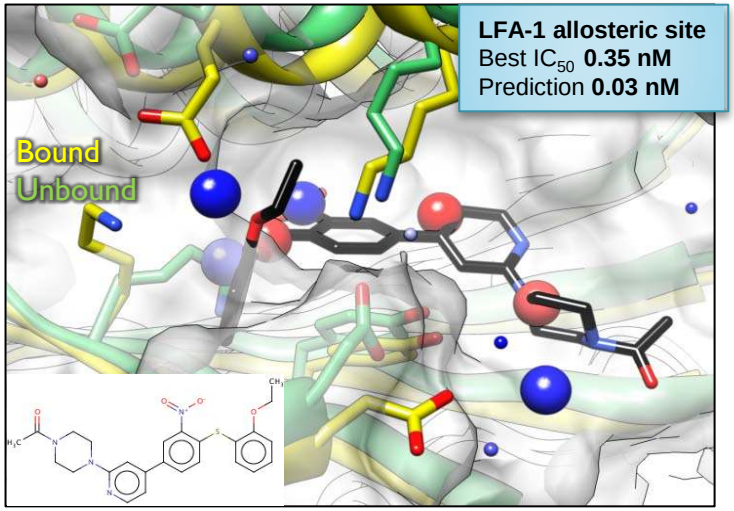
Bioorg. Med. Chem. Lett. **2007,** *17*, 5677-5682

Bakan & Bahar, *J Chem Theory Comput.* 8:2435-2447, 2012

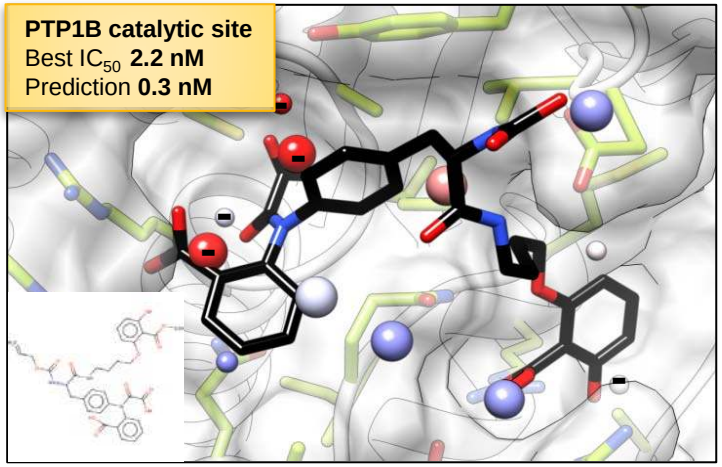
2 Assessment of druggable allosteric sites



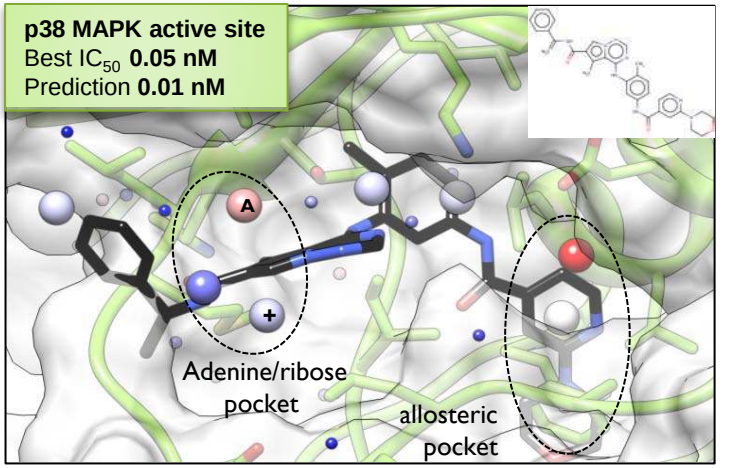
J Med. Chem. **2009,** *52*, 7970-7973



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

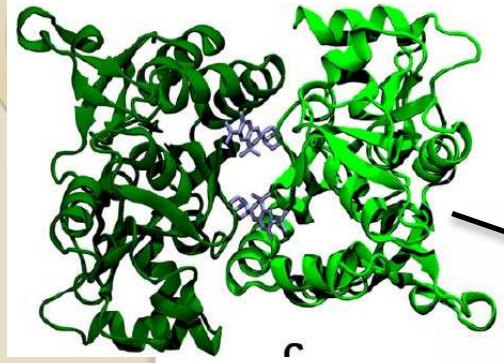


Bioorg. Med. Chem. Lett. **2003,** *13*, 3947-3950



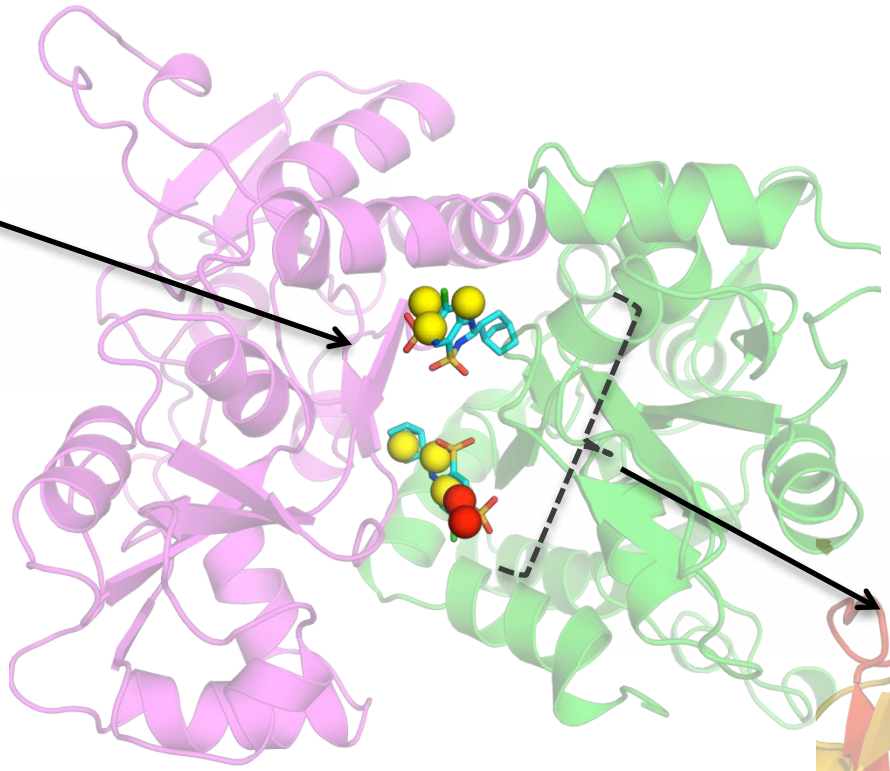
J Med. Chem. **2010,** *53*, 2973-2985

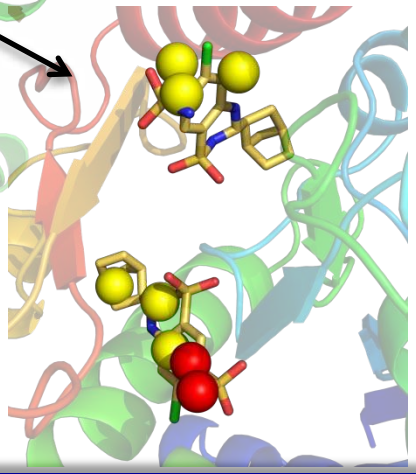
2 Probes capture allosteric modulator site of AMPAR LBD Dimer



Experimental Results

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

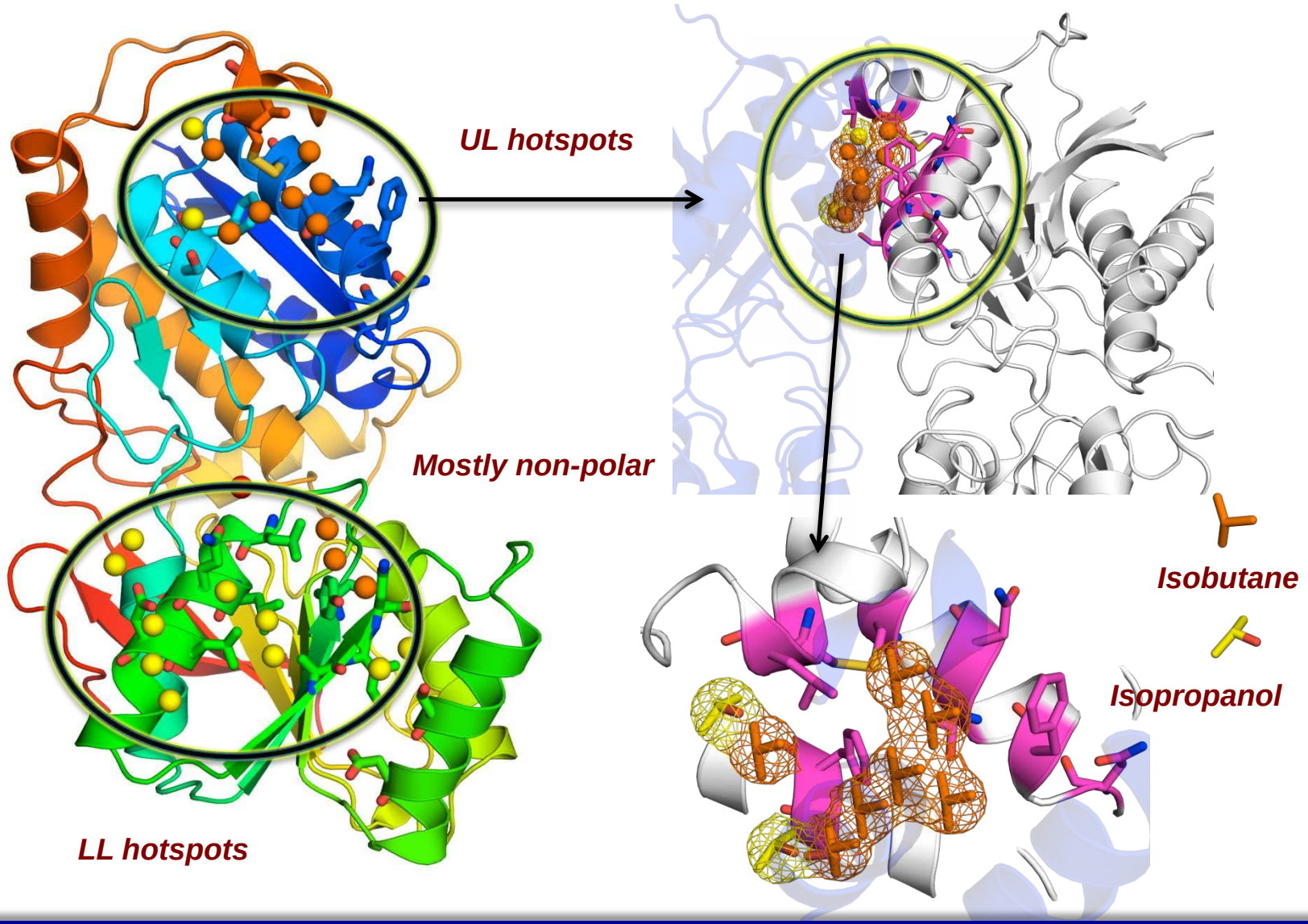




Computational modeling detects experimentally observed binding site

Dutta, Greger, Bahar, manuscript in preparation.

2 Interfacial regions captured in AMPAR NTD

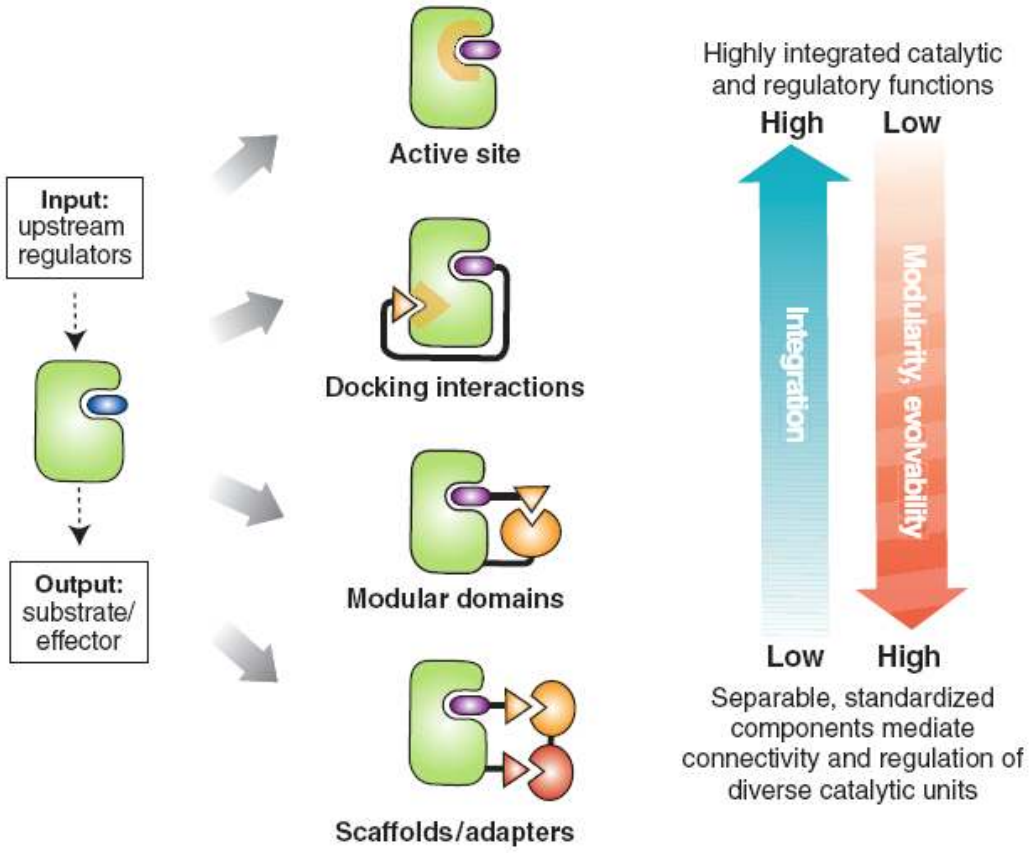


Dutta, Greger, Bahar, manuscript in preparation.

SUMMARY

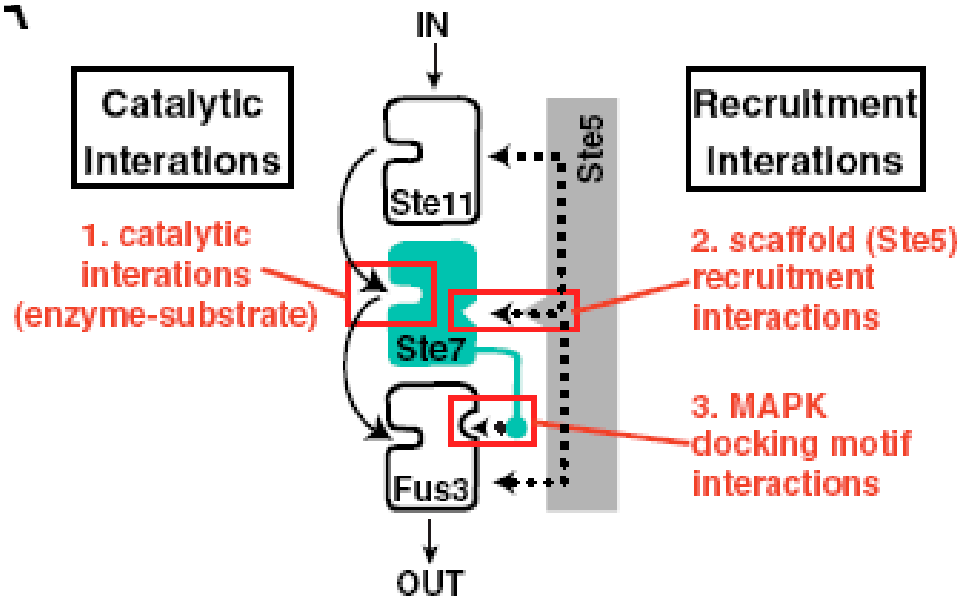
1. Structure-encoded **flexibility** of drug targets and significance in drug discovery and design
2. **Druggability** assessment: a first step before selecting a target
3. Modularity and **promiscuity** of proteins and **quantitative systems pharmacology** methods

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

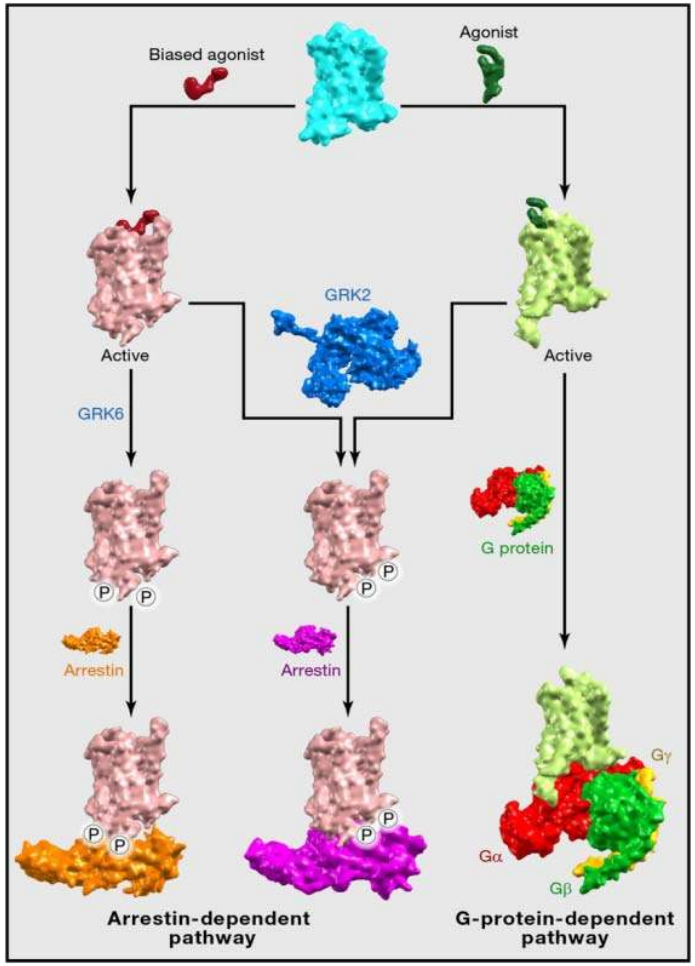


Bhattacharyya et al. *Annual Rev Biochem* 2006

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**

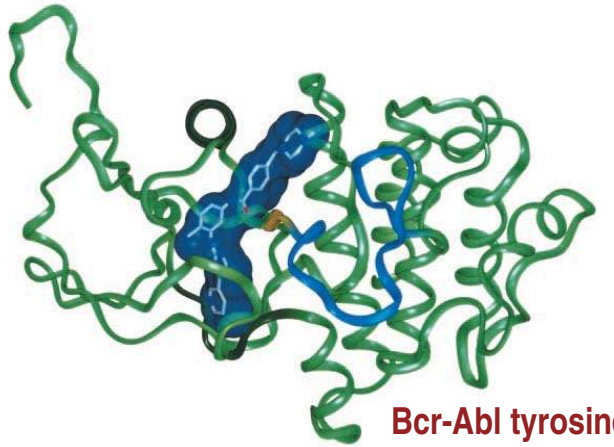
the interactions with different (or multiple) upstream or downstream partners/substrates may be affected,

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

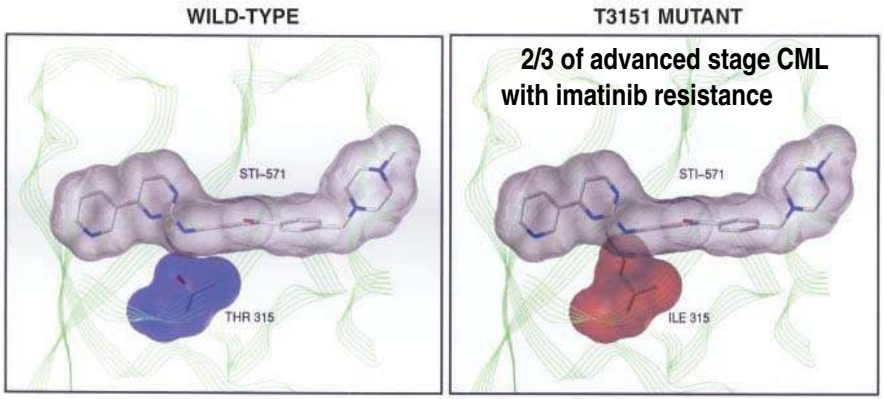
Assessment of druggable allosteric sites

Imatinib (Gleevec)

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



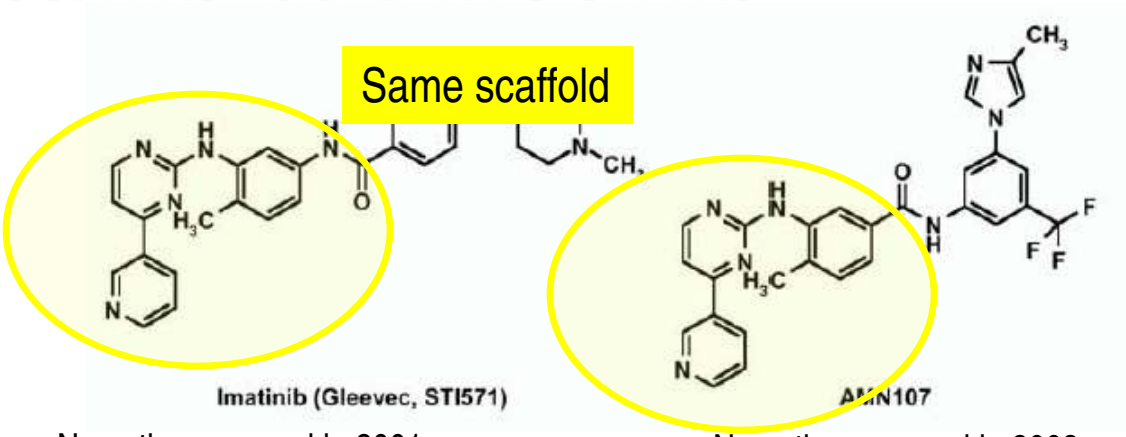
Bcr-Abl tyrosine kinase



IC_{50} ~200 nM

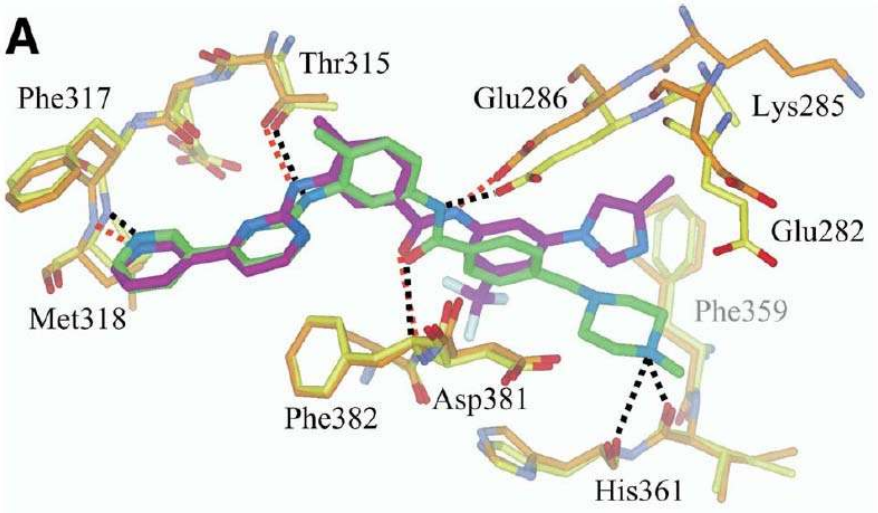
IC_{50} > 10,000 nM

Imatinib vs Nilotinib



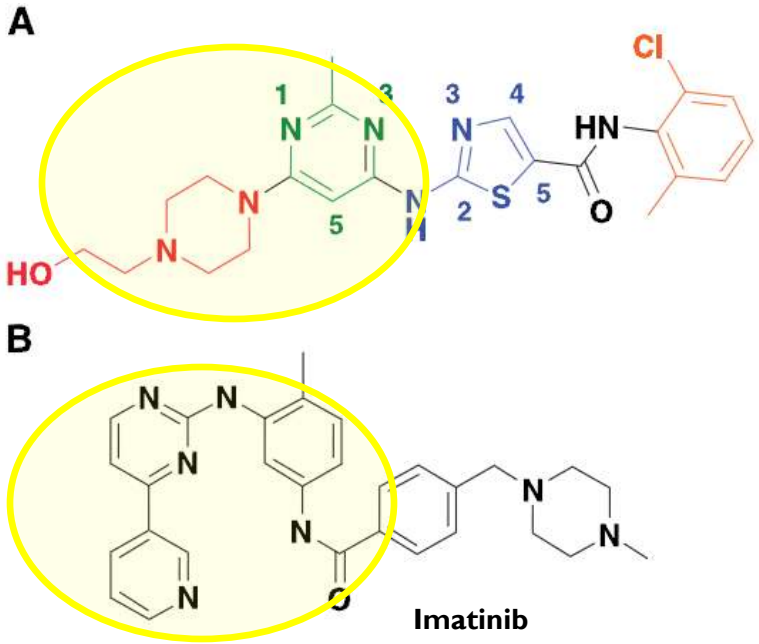
Novartis, approved in 2001

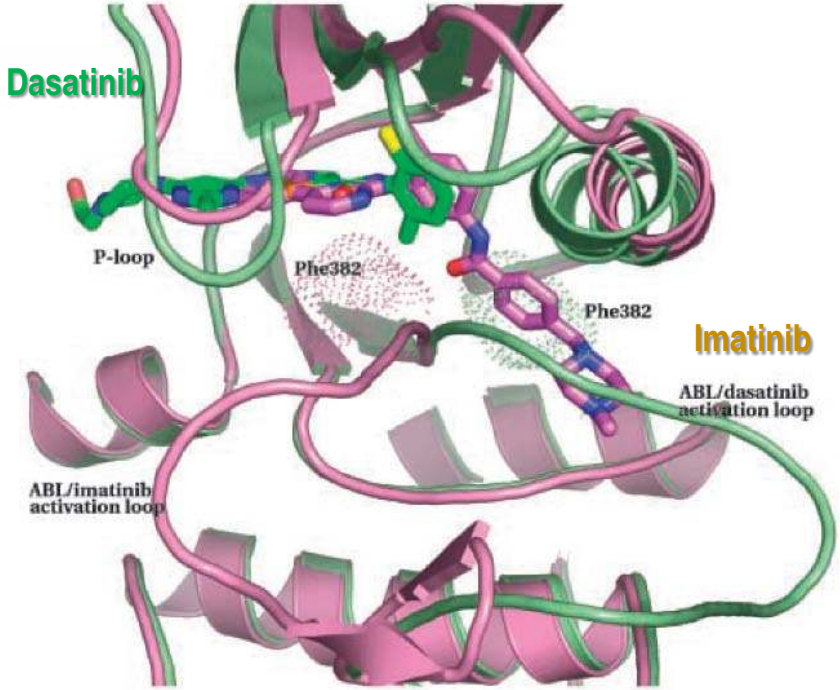
Novartis, approved in 2003



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.

5

Vol 463|28 January 2010|doi:10.1038/nature08675 nature

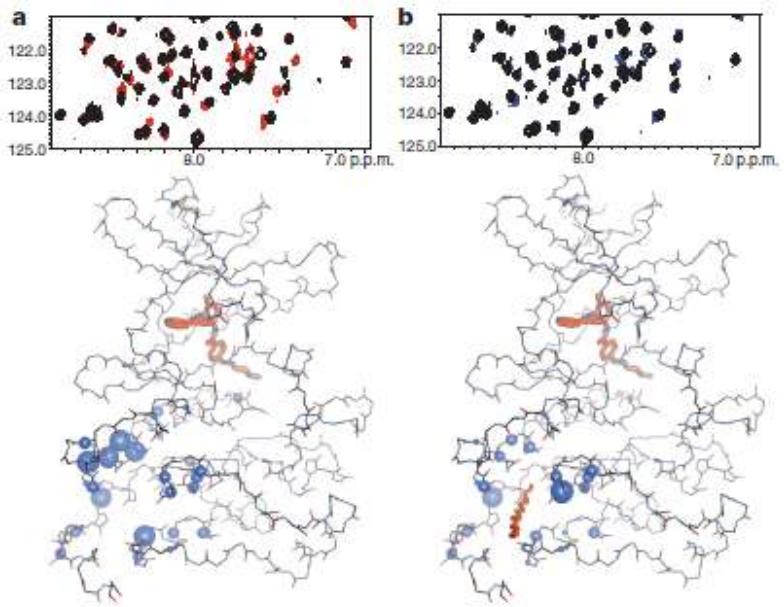
ARTICLES

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

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

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!

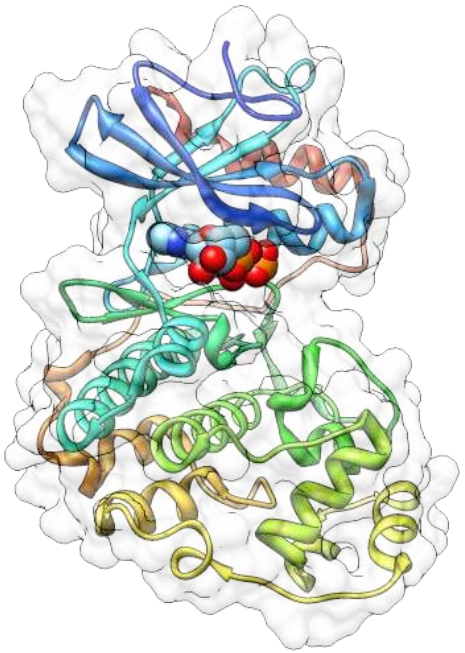
Polypharmacological strategy: Inhibition of allosteric interaction site in addition to catalytic site



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 1OPK8) 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)

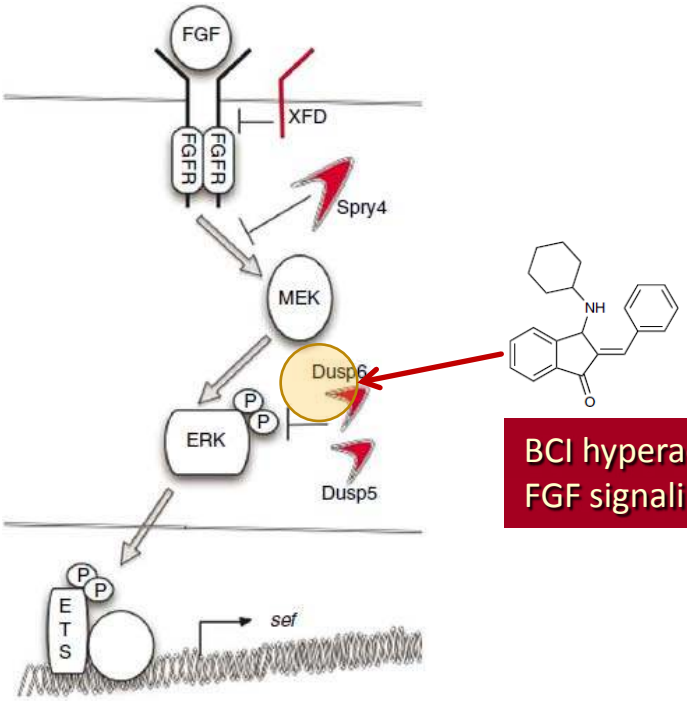


Khateb et al. BMC Cancer. 2012 Overcoming Bcr-Abl T315I mutation by combination of GNF-2 and ATP competitors in an Abl-independent mechanism.

nature chemical biology

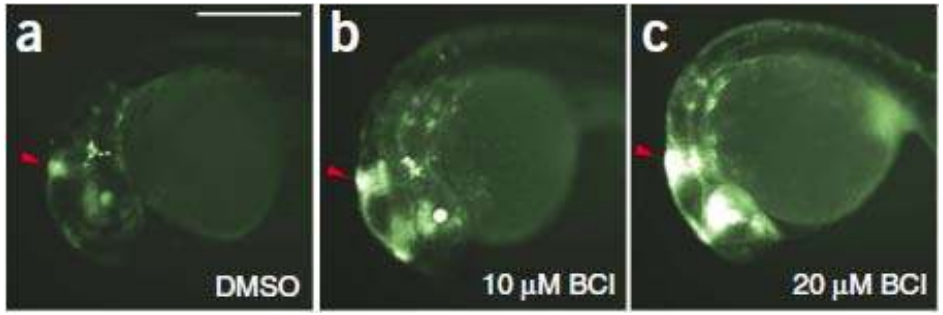
Zebrafish chemical screening reveals an inhibitor of Dusp6 that expands cardiac cell lineages

Molina G,* Vogt A,* **Bakan A,*** et al. **Nat Chem Biol, 2009,** 9, 680-7.



BCI hyperactivates FGF signaling

Fibroblast growth factor binding activates the MAPK pathway, leading to cell proliferation, organ development. Dusp6 serves as an attenuator/regulator by inhibiting ERK



Experiments with transgenic zebrafish embryo showed that FGF signaling is enhanced in the presence of BCI

nature chemical biology

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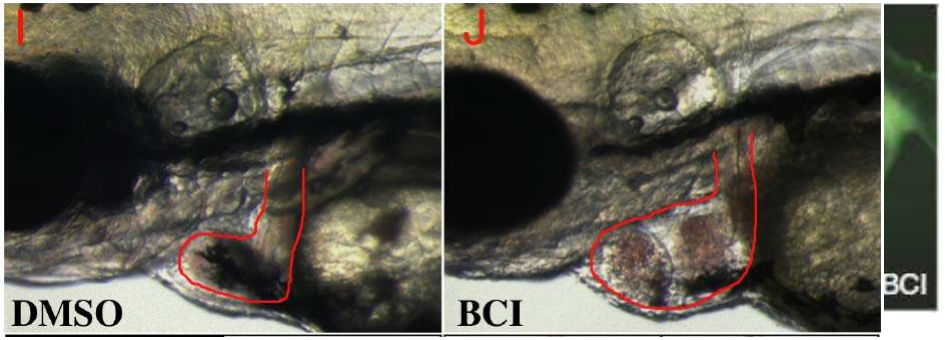
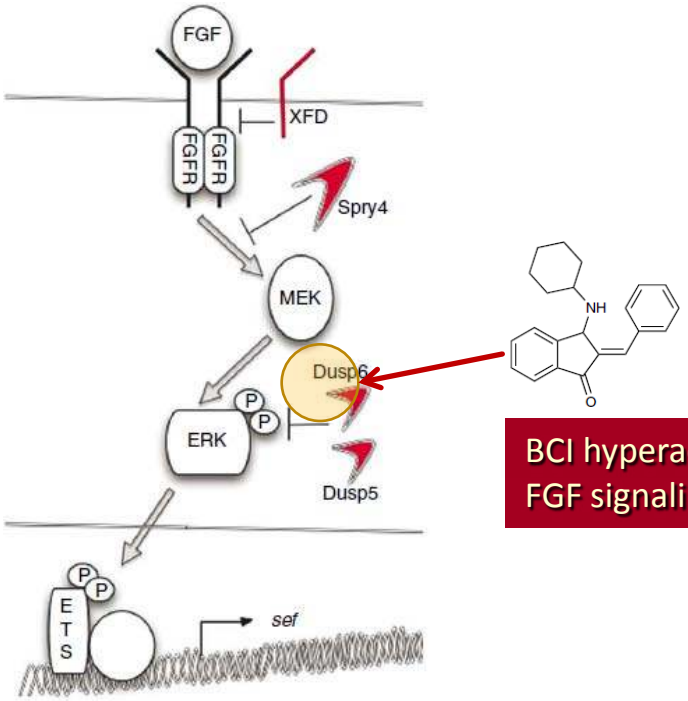
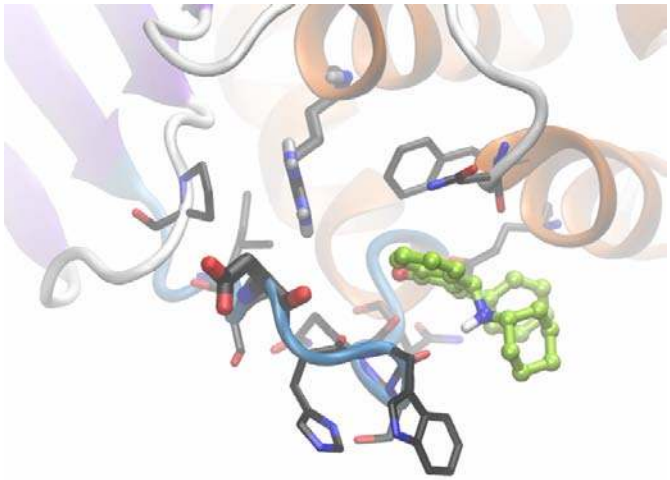


Zebrafish embryos treated with BCI have enlarged hearts!



BCI hyperactivates FGF signaling

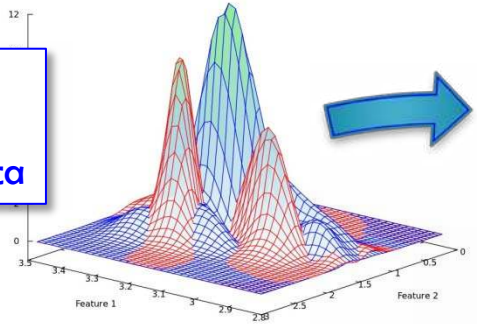


Fibroblast growth factor binding activates the MAPK pathway, leading to cell proliferation, organ development. Dusp6 serves as an attenuator/regulator by inhibiting ERK

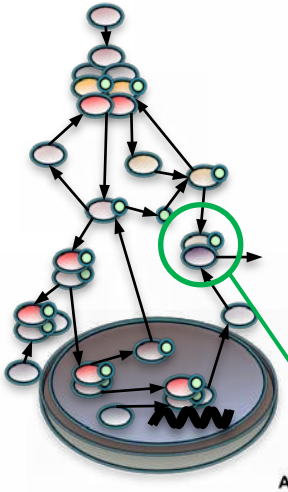
Quantitative Systems Pharmacology:

Integrating Quantitative Models with Experimental Data for Drug Discovery

Signals extracted from the data

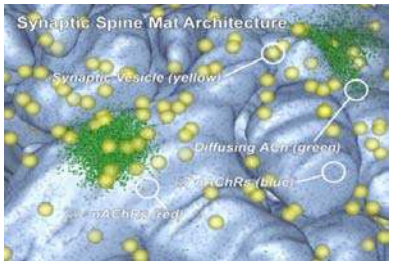


Information

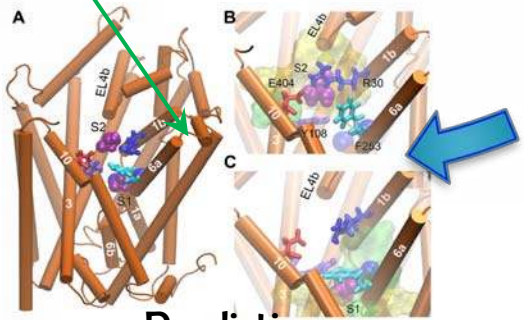


Refined cellular pathways /processes

Models

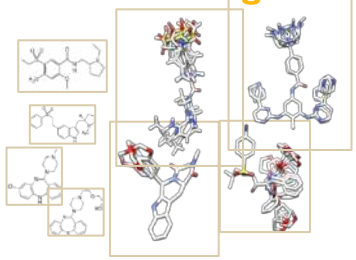


Predicted Drug binding to targets

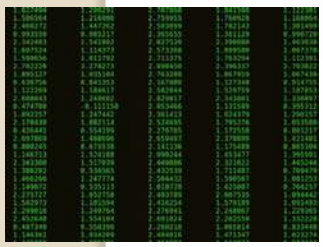


Predictions

DrugBank



Clinical Trials

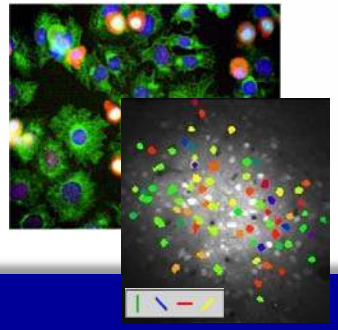


Data

High-Content Scree

Targeted Libraries

Measurements of biological functions



3D or 4D images

Experimental testing