Recent Advances in Small Molecule and Biologics Design using Schrödinger Suites

Annette Höglund, Jianxin Duan

Helsingfors

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Many people worked on these projects

Protein FEP:

- Teng Lin
- **Thomas Steinbrecher**
- Robert Abel
- Lingle Wang
- Byungchan Kim
- Chongkai Zhu
- Woody Sherman
- **Fiona McRobb**
- **Jeffrey Sanders**

CovDock:

- **Dora Toledo Warshaviak**
- Kenneth Borrelli
- Kai Zhu

Enzyme Engineering:

- **Sarah Sirin**
- David Pearlman

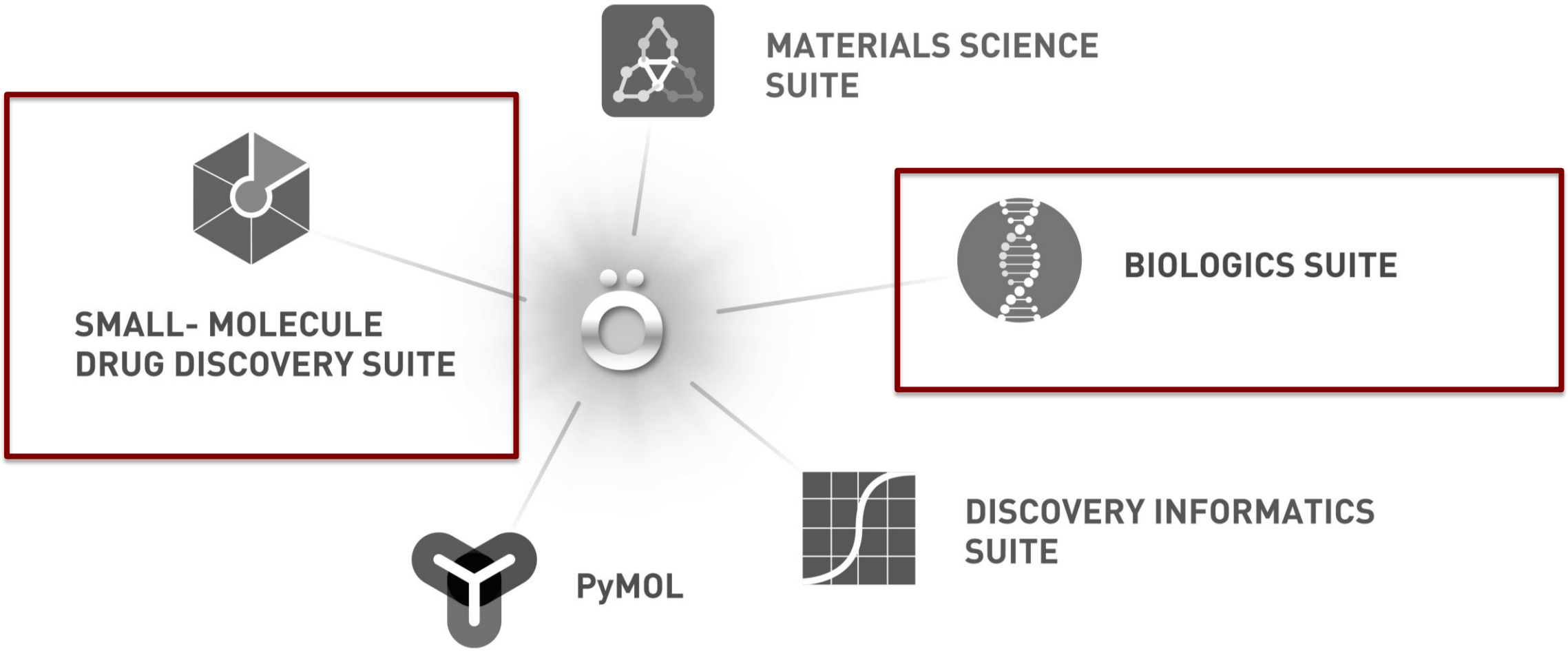
3D QSAR:

- **Daniel Cappel**
- Steve Dixon
- Jianxin Duan

WaterMap:

- **Daniel Cappel**

Our Five Main Software Suites



Agenda

Recent Advances in Small Molecule Design

1. Screening for covalently bound inhibitors
2. Using water energetics to guide the optimization of Platelet Derived Growth Factorβ inhibitors
3. Improving alignments for 3D ligand-based design

Recent Advances in Biologics Design

4. Computational approaches for enzyme design
5. Predicting protein-protein binding affinity using free energy perturbation

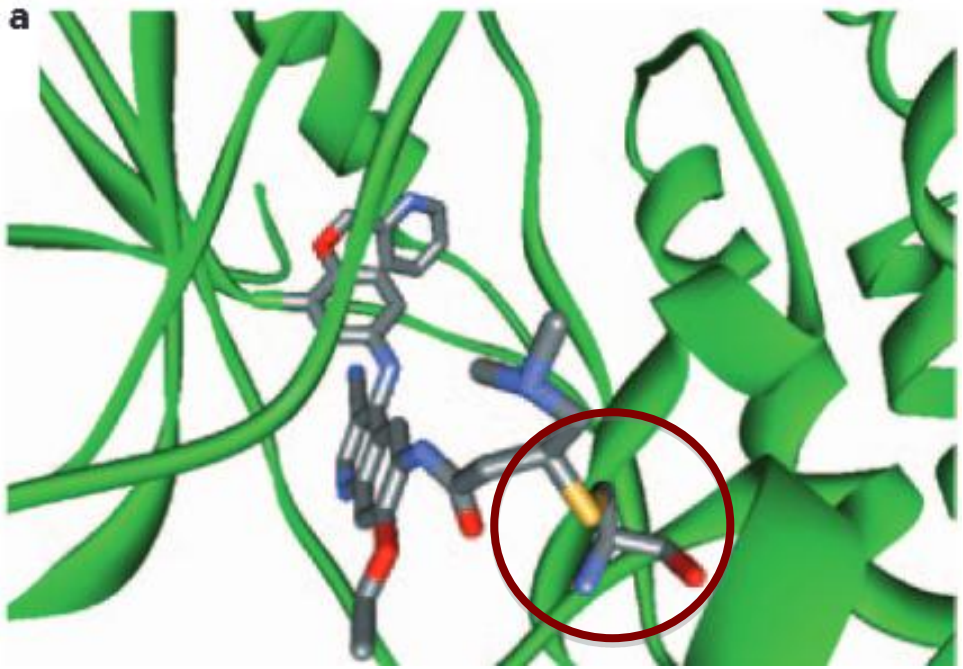
Part 1: Recent Advances in Small Molecule Design

Case Study # 1: Screening for Covalently-bound Inhibitors

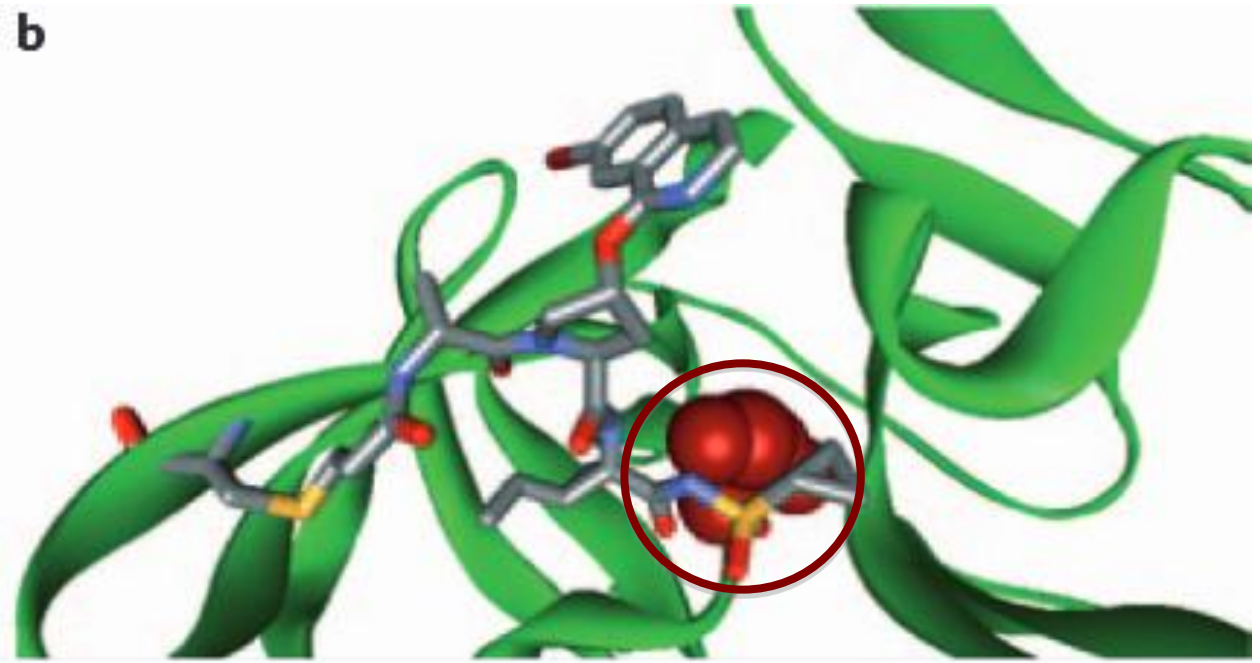
Toledo Warshaviak, D. et al. A Structure-Based Virtual Screening Approach for Discovery of Covalently Bound Ligands. *J Chem Inf Model*. 2014. 54(7):1941-50

Tools used: Glide, Prime, CovDock

Mechanism of Covalent Inhibition



EFGR protein bound **Neratinib** (2JIV)

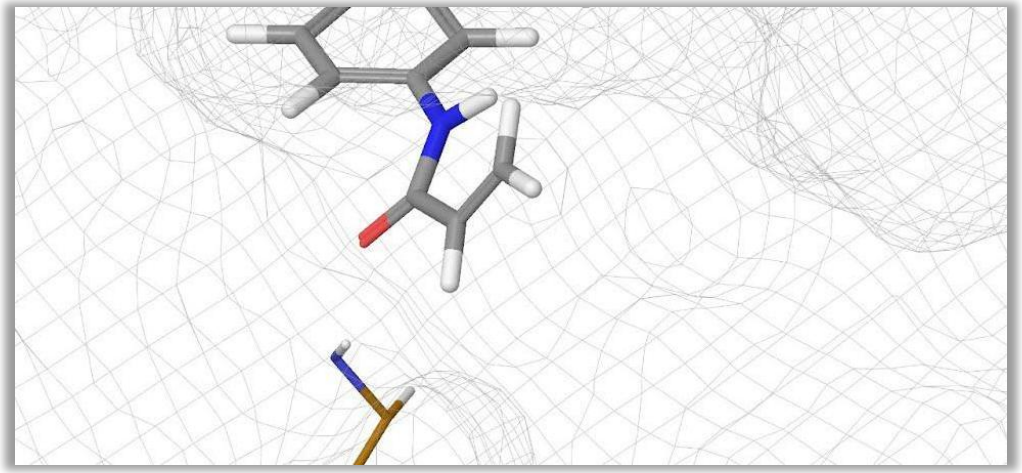


Hep C Virus protease bound peptide-like ligand (3OPY)

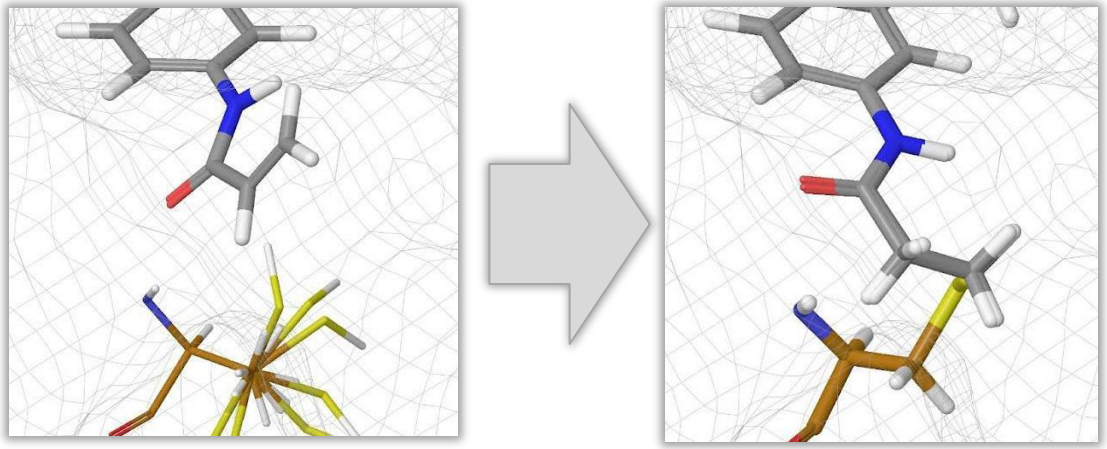
- A covalent bond is formed between the target and the inhibitor
- The inhibition can be either reversible or irreversible

CovDock Key Steps

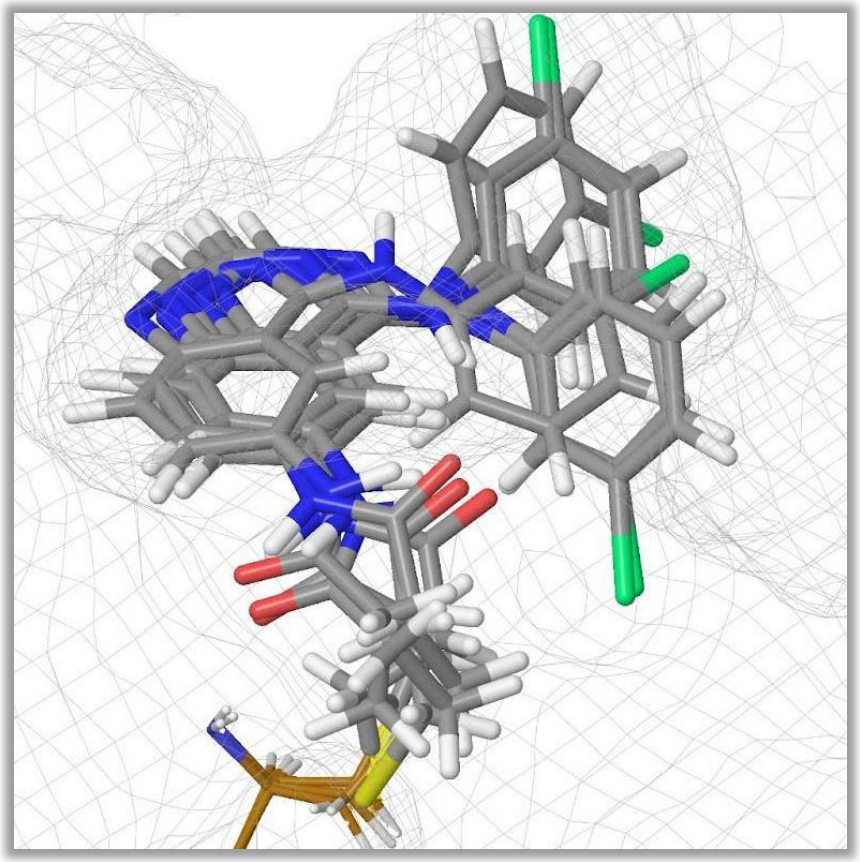
1. Initial docking (Glide)



1. Residue sampling (Prime)



1. Ligand refinement (Prime)



1. Final scoring (Glide)

CovDock Performs Very Well on Pose Prediction

- Results from 76 Ouyang et al. complexes
  - 13 Michael Addition; 63 acetylation beta-lactam
- Additional comparison made with AutoDock and GOLD
- RMSD is measured between the docked pose and the reference crystal structure

Pose	Schrödinger CovDock-LO	CovalentDock*	Autodock*	GOLD*
Top scoring pose	1.8	3.4	3.5	4.0
Best of 10 lowest energy poses	1.4	1.9	2.5	3.4

*Ouyang, X. et al. (2012) Journal of Computational Chemistry, 34(4), 326–336

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CovDock for Virtual Screening

- Limited VS applications/tools for covalent docking currently exist and process is not well automated
  - Across tools: limited auto preparation of ligands and protein, manual definition of reactive atoms and reaction type
- CovDock “pose prediction” mode takes about 1-3 hours/ligand per CPU. Need better speed to screen thousands of ligands efficiently
- The CovDock virtual screening protocol is tailored to address throughput needs, while retaining good pose-prediction

Karyopharm Therapeutics

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Virtual Screening Results

	Potency Range	Known Actives	Decoys	EF 1%	EF 10%	BEDROC (a= 20)
HCV NS3 Protease	2-4300 nM	25	1562	52	7	0.70
Cathepsin K	0.13 – 460 nM	21	1562	9	8	0.48
EGFR	0.5 – 1 uM	34	5000	46	8	0.65
XPO1	25nM – 5 uM	21	5000	33	7	0.52

- Retrospective study of four targets with covalent inhibitors
- Decoy libraries with matched physicochemical properties were generated

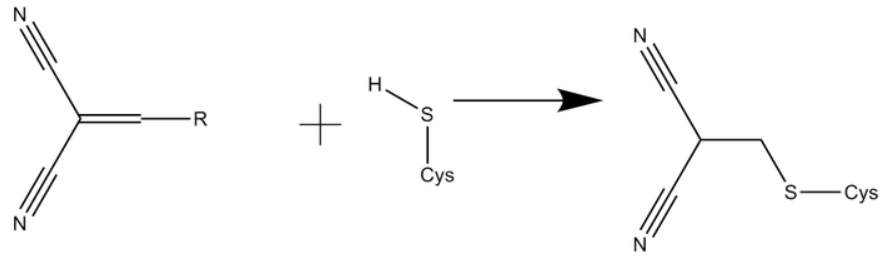
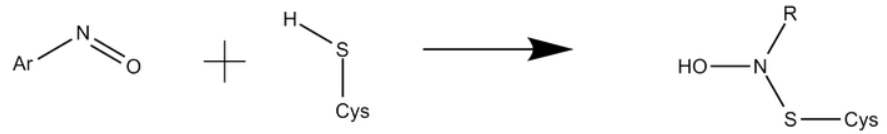
Applying hydrogen bonding constraints improves results

	With Filters		Without Filters	
	EF1%	EF10%	EF1%	EF10%
HCV NS3 Protease	52	7.2	16	2.7
Cathepsin K	9.4	8.1	4.7	1.4
EGFR	46	7.7	38	6.2
XPO1	33	6.7	33	6.7

Covalent Docking

- Reduce setup time by downloading pre-generated custom reaction inputs

www.schrodinger.com/CovDock/Covalent-Reactions-Repository

Index	Reaction	Ligand SMARTS	Receptor SMARTS	CDOCK File
1		[C,c]=[C,c]-[C,c]#[N,n]	[C]-[S,O;H1,-1]	Download
2		N=O	[C][S;H1,1]	Download

- Dock ligands with multiple reactions in a single experiment (command line only)
- Supports Glide positional, H-bond and torsional constraints (command line only)

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Case Study # 2: Using Water Energetics to Guide the Optimization of Platelet Derived Growth Factorβ inhibitors

Horbet, R. et al. Optimization of Potent DFG-in Inhibitors of Platelet Derived Growth Factorβ (PDGF-Rβ) Guided by Water Thermodynamics. *J Med Chem*. 2015. 58(1):170-182.

Tools used: Glide and WaterMap

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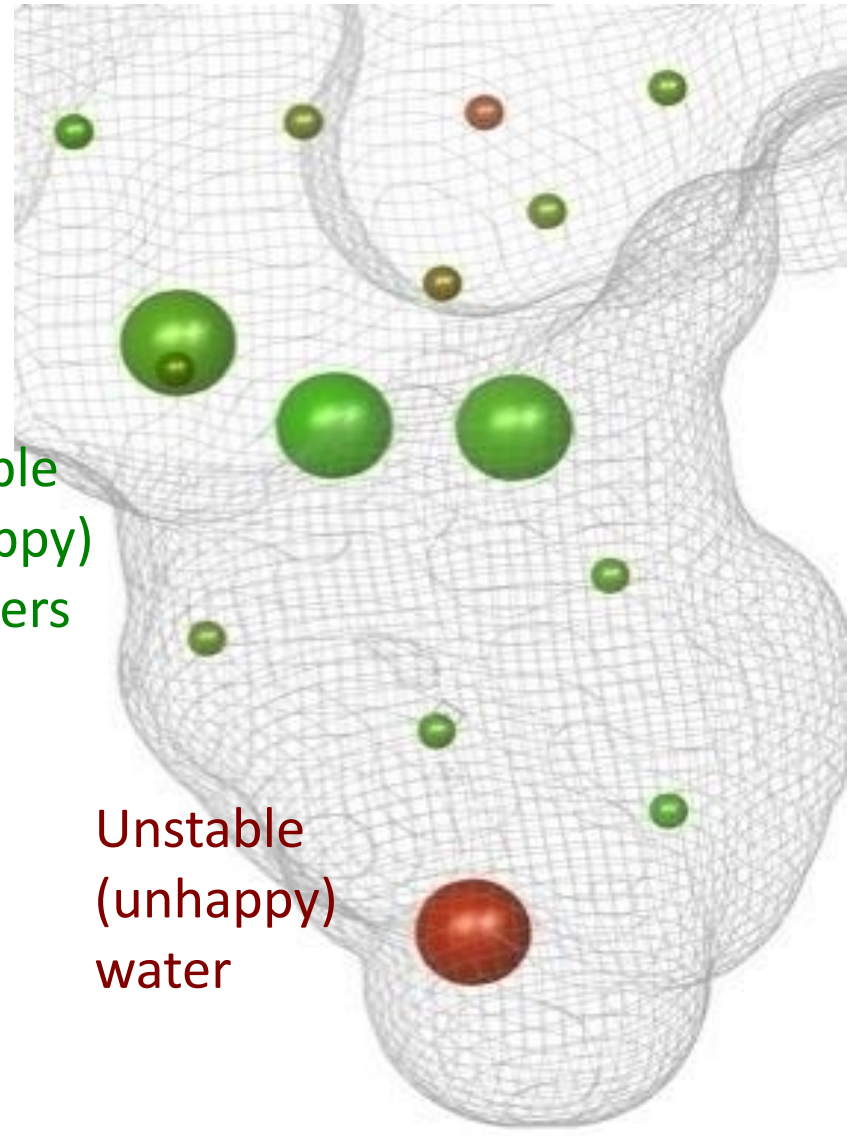
Why Is water important?

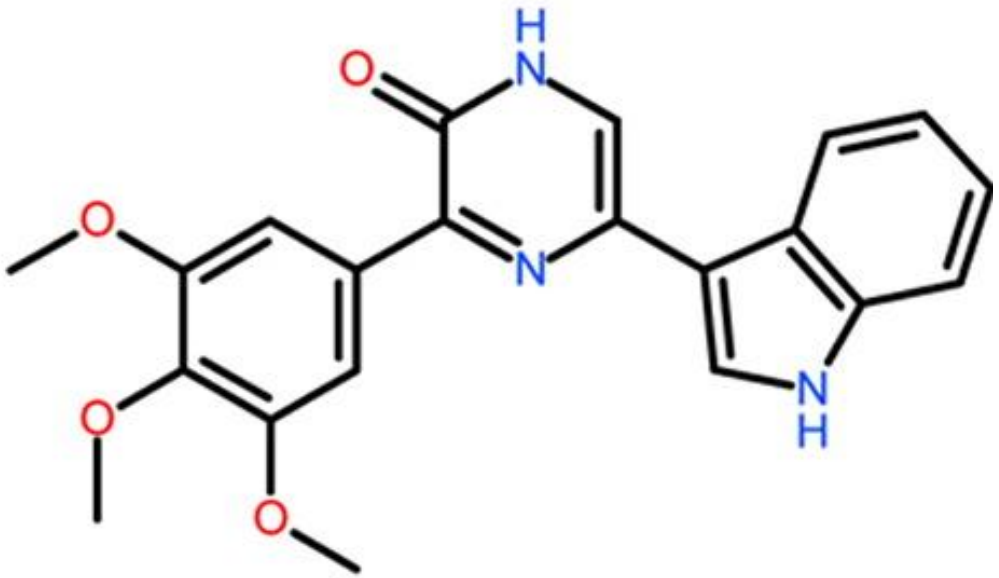
- Water is everywhere in biology
- Protein binding sites are mostly filled with water
- Water is a direct competitor in ligand and substrate binding
- Displacement of unhappy waters can lead to big potency gains
- But…water energetics cannot be determined from structure alone

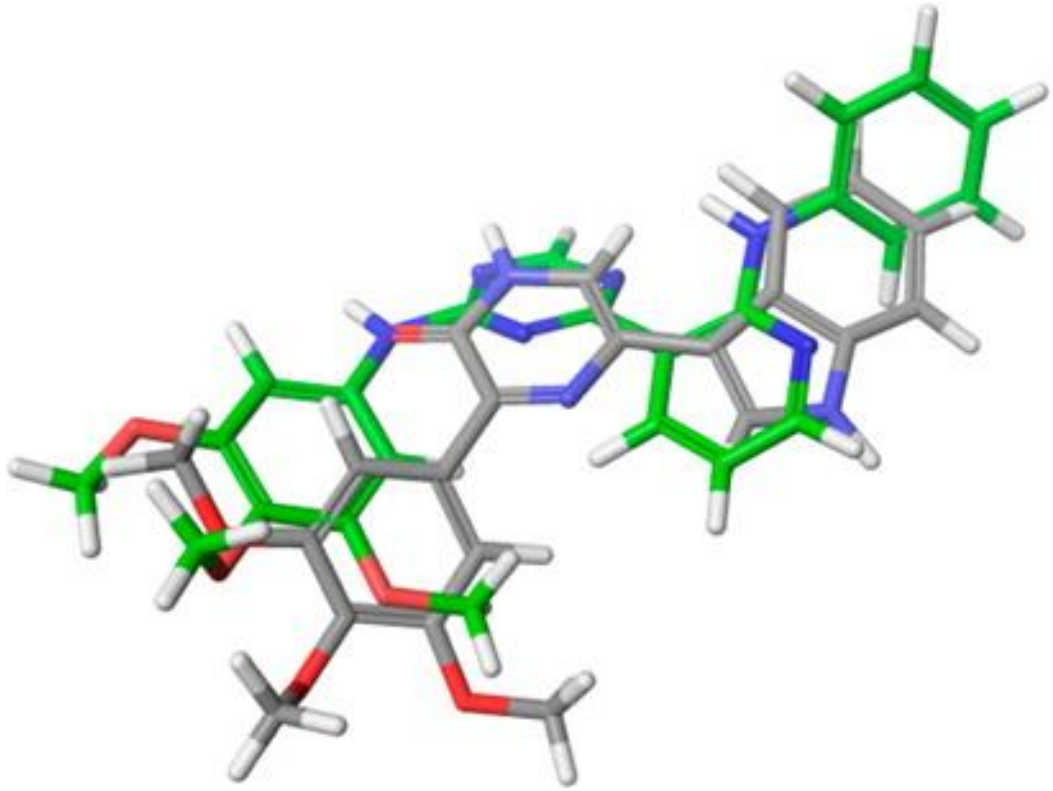
WaterMap visualization

- WaterMap computes the entropy and enthalpy of "hydration sites"
- These can be used to rationalize SAR, drive potency, and tune selectivity
  - Green = stable
  - Red = unstable
- Provides a "map", not a GPS



Optimization of a 3,5-Diaryl-pyrazin-2(1*H*)-one inhibitors of PDGF-Rβ

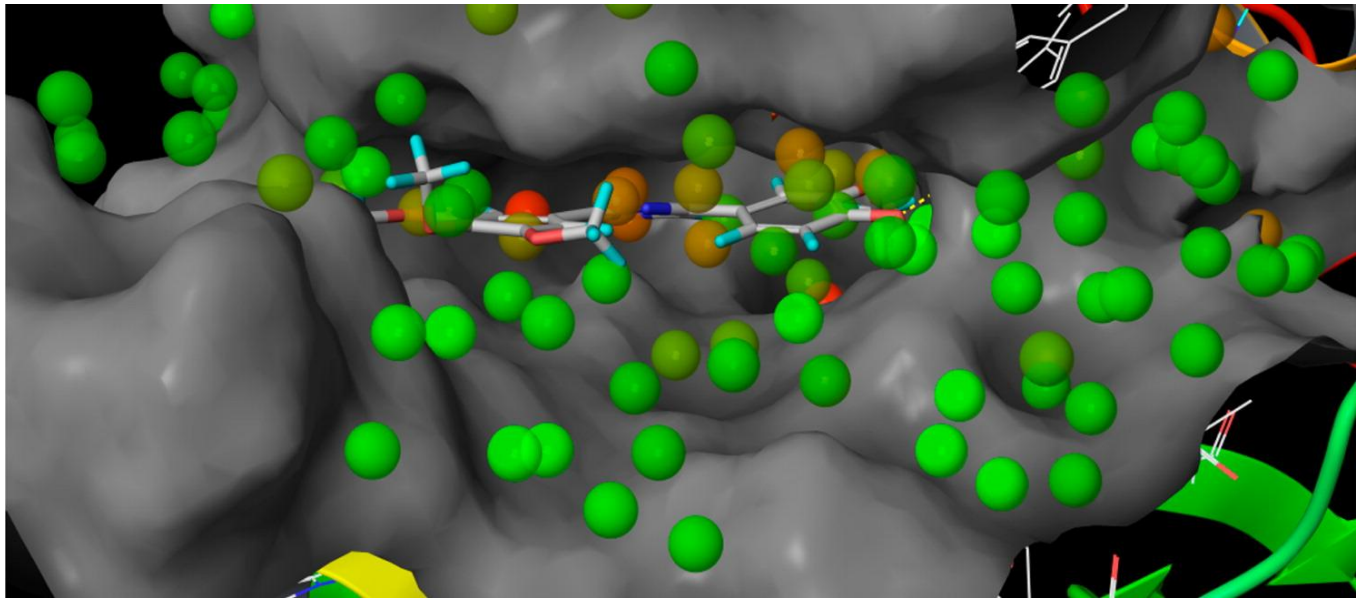




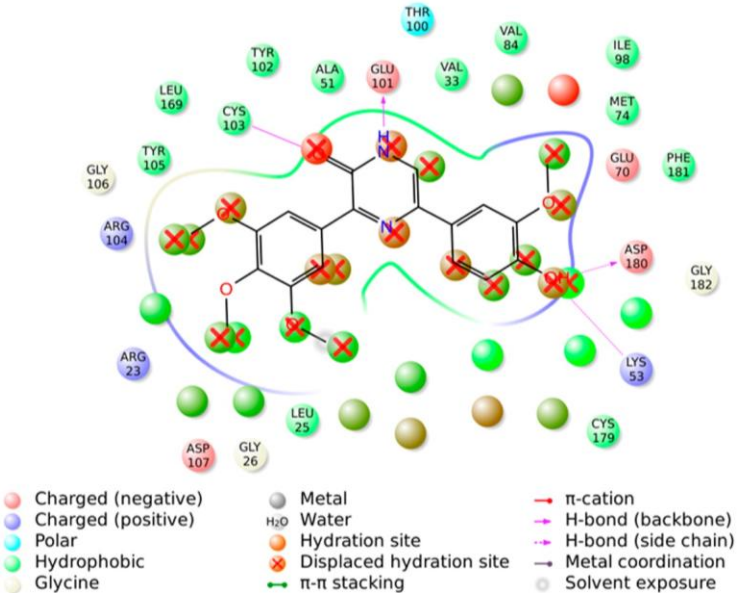
Starting compound, IC_{50} = 0.5uM,
Selective for the β isoform.

Docked pose overlaid with a VEGF-R2 DFG-in inhibitor

Using WaterMap, the authors predicted an unstable water to target for displacement, resulting in a potency boost



Docked pose of designed analog overlaid with WaterMap results



2D Ligand Interaction Diagram

- Modifications were introduced based on the WaterMap-derived hypothesis
- Resulted in a 10x increase in potency

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Case Study # 3: Improving Alignments for 3D Ligand-Based Design

Cappel C. et al. Exploring Conformational Search Protocols for Ligand-Based Virtual Screening and 3-D QSAR Modeling . *J Comput Aid Mol Des*. 2015. 29(2):165-182.

Tools used: ConfGen, MacroModel, Phase, Field-based QSAR

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The success of 3D LBDD methods is dependent on two things

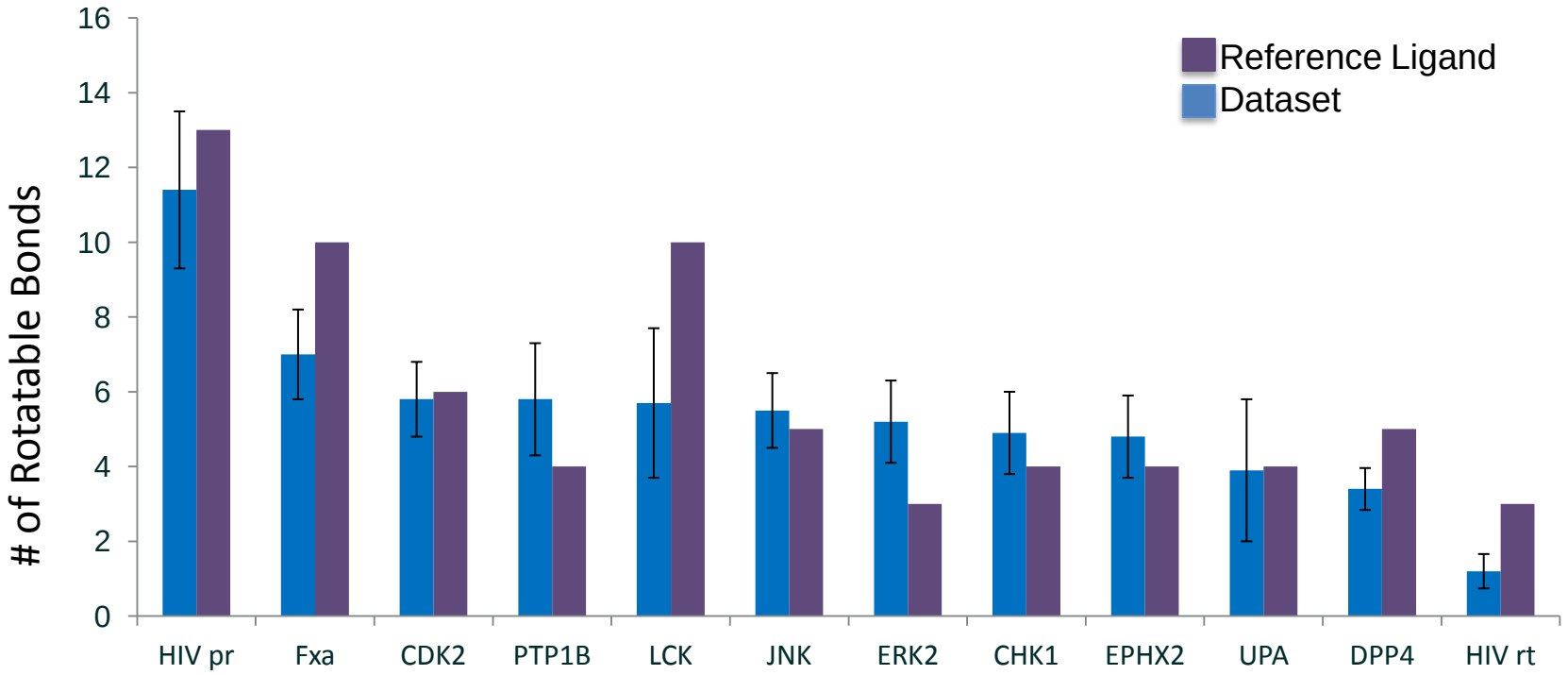
1. Adequate 3D conformational sampling of compounds in your screening library
   - Exhaustive sampling can be performed prior to initiating a screen, or may be during the screen to a lesser extent.

2. Accurate alignment of conformers to the 3D conformation of your reference (i.e. active) compound.

Study to assess the effects of alignment and conformational search algorithms

12 series of congeneric series from literature, criteria:

- Activity data from the same protocol from one publication
- Share common scaffold
- At least one co-crystallized ligand



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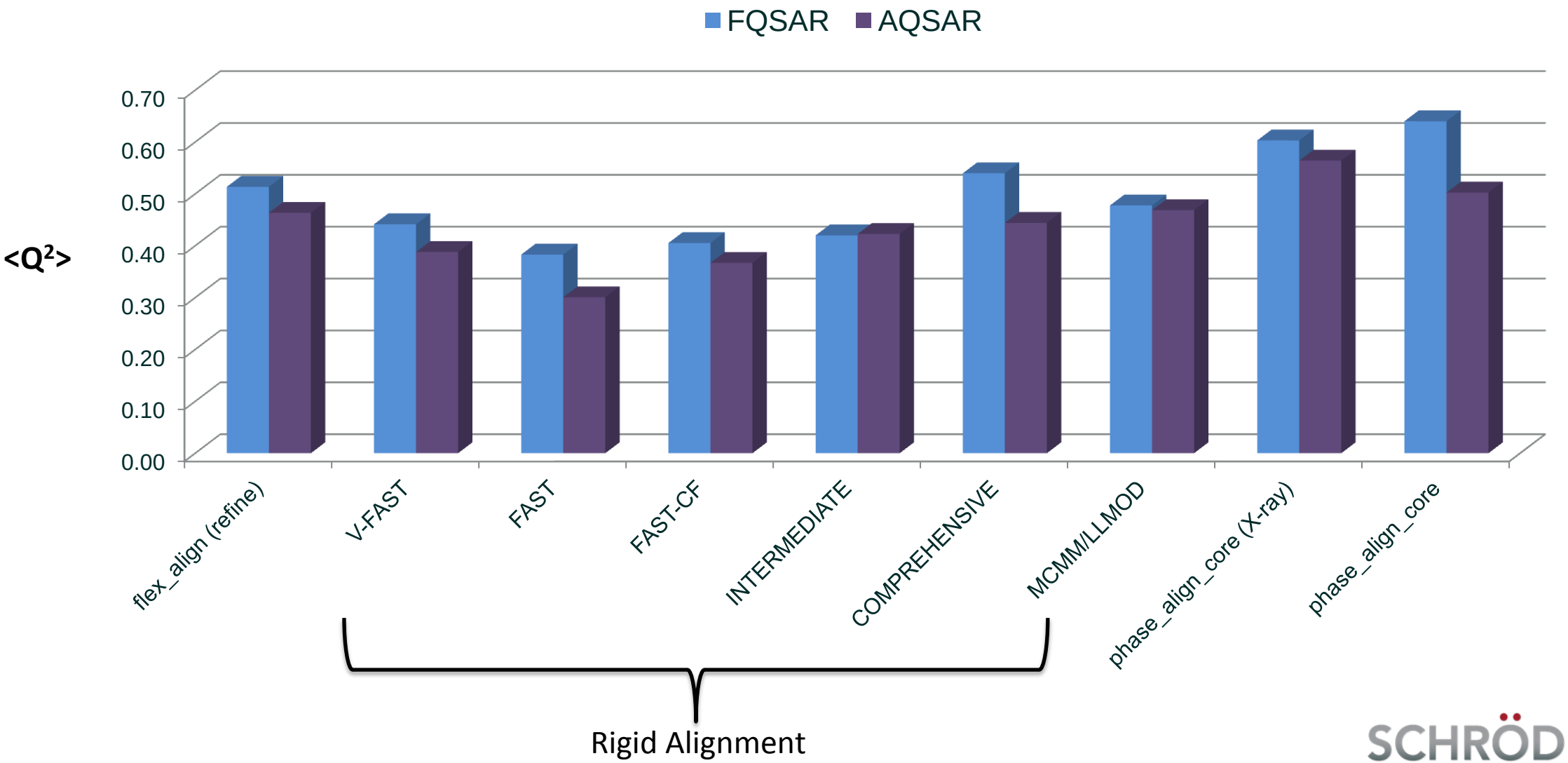
Phase_Align_Core outperformed the rest



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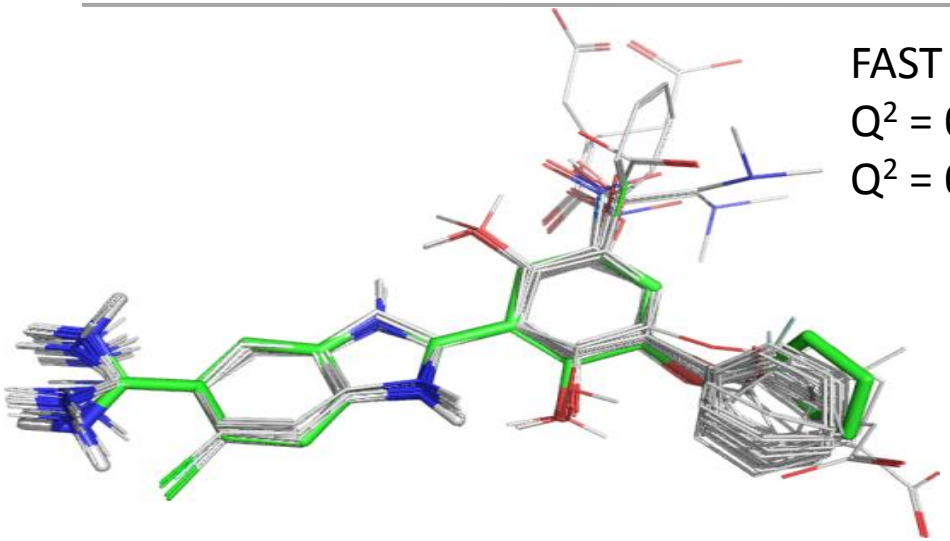
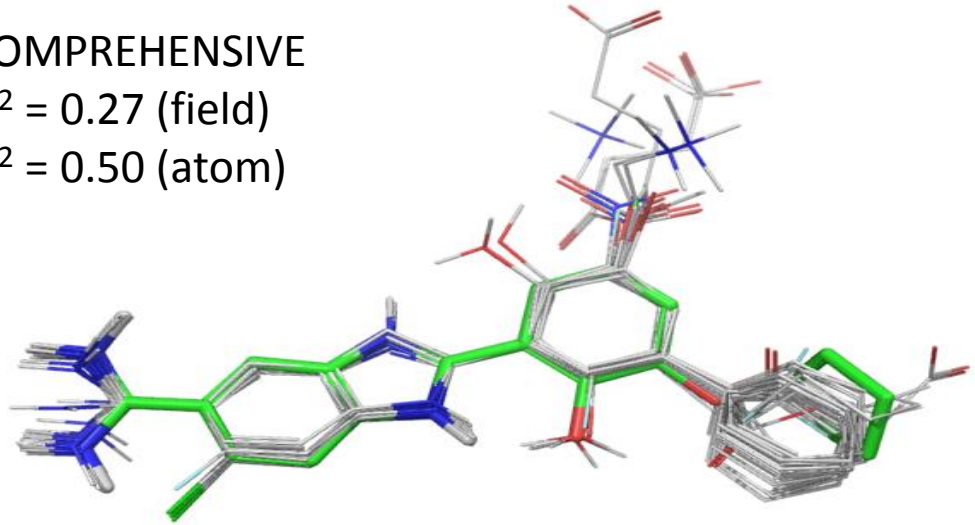
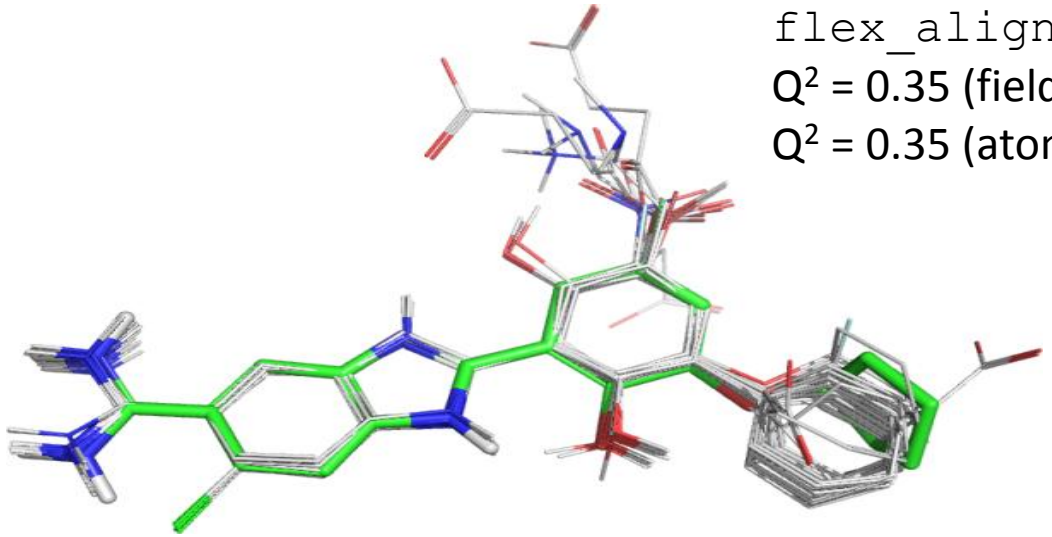
Example: UPA



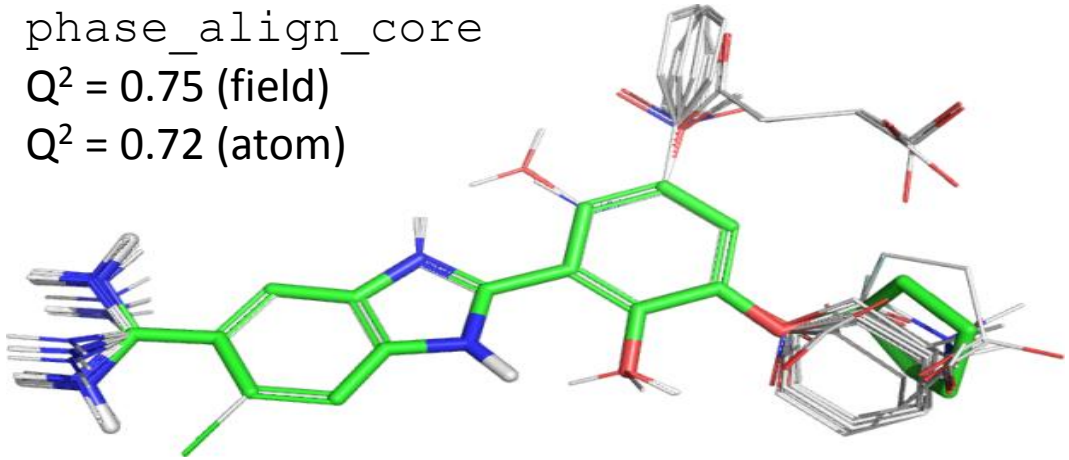
FAST
Q^2 = 0.14 (field)
Q^2 = 0.08 (atom)



COMPREHENSIVE
Q^2 = 0.27 (field)
Q^2 = 0.50 (atom)



`flex_align`
Q^2 = 0.35 (field)
Q^2 = 0.35 (atom)



`phase_align_core`
Q^2 = 0.75 (field)
Q^2 = 0.72 (atom)

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How phase_align_core works

Find maximum common scaffold (largest ring system) between alignment template and each ligand

Change coordinates of common scaffold to the ones of template

Constrain core and sample conformations of remaining part of the molecule

Take conformation with maximized shape overlap

Take home messages from this study

- QSAR predictions sensitive to conformational search protocols
  - Best predictions obtained when core of the molecule restrained to core of query
  - Amongst unconstrained search methods, the most thorough one performed best
- Manual refinement of alignment would likely produce better models
- Overall the best conformational search and alignment protocol is `phase_align_core`
- `phase_align_core` also generates good alignments for non-Xray alignment template

Part 2: Recent Advances in Biologics Design

Biologics Suite Features

- Protein-protein docking
- Antibody structure prediction from sequence
- Antibody humanization
- Fast homology model generation
- Accurate long loop predictions
- Residue scanning
- Affinity Maturation
- Cysteine scanning
- Crosslink design
- Peptide QSAR
- Aggregation hot spot ID

Case Study # 4: Computational Approaches for Enzyme Design

Sirin, S. et al. A Computational Approach to Enzyme Design: Predicting ω-aminotransferase activity using docking and MM-GBSA rescoring . *J Chem Inf Model*. 2014. 15(8):2334-2346.

Tools used: Glide, MM-GBSA, Desmond, Residue Scanning, KNIME

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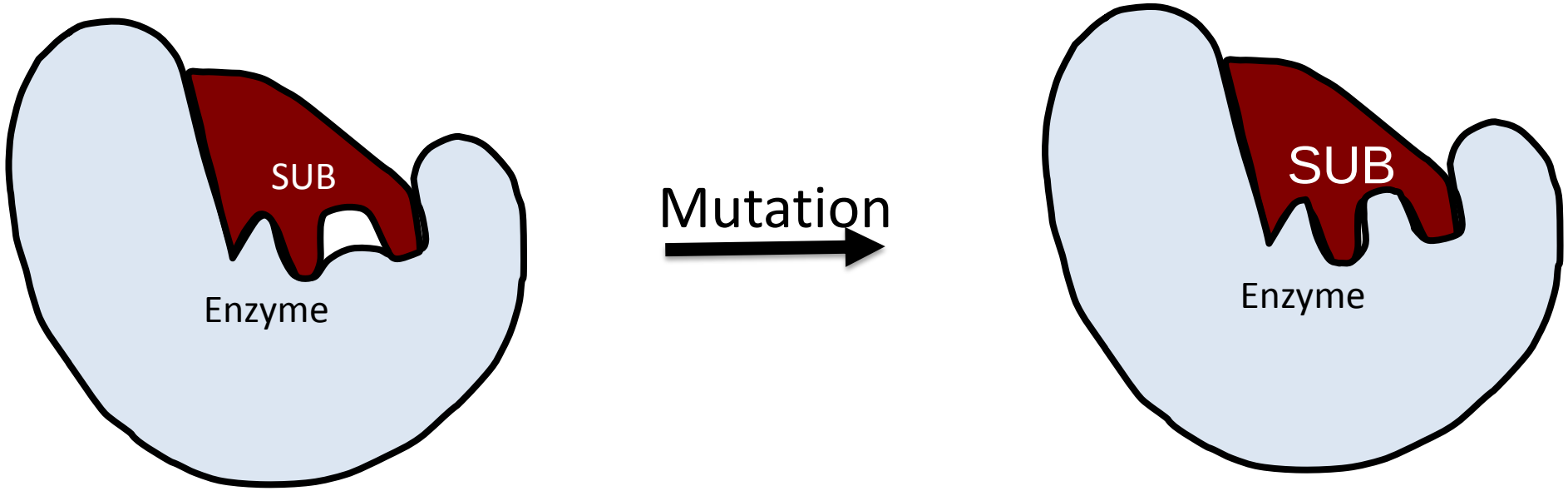
Enzyme Engineering Seeks to optimize binding and/or turnover



Applications:

- Biocatalysis
- Biosensors
- Food and detergent additives

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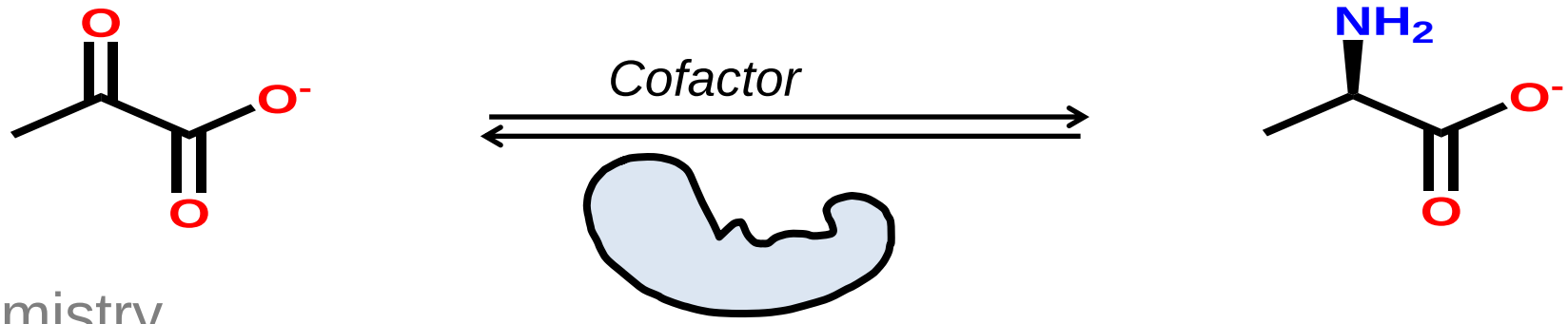
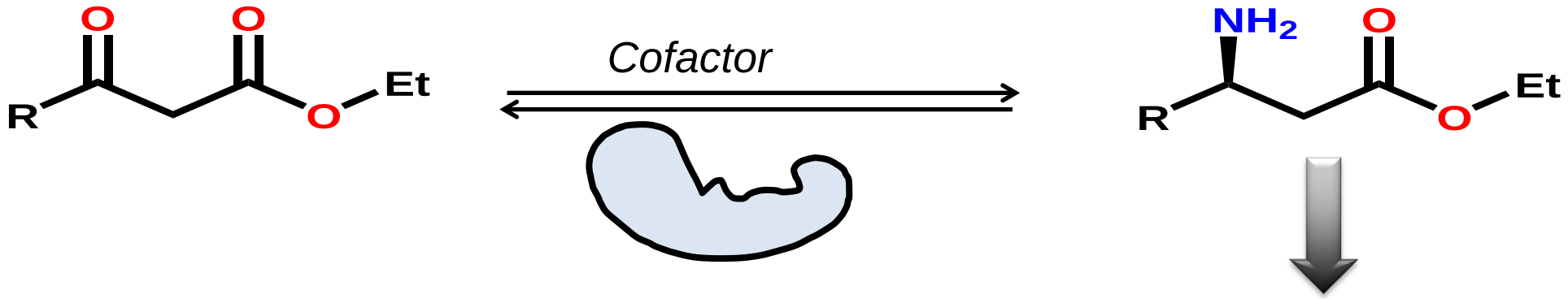
ω-Aminotransferase example

Wild-type ω-aminotransferase chemistry

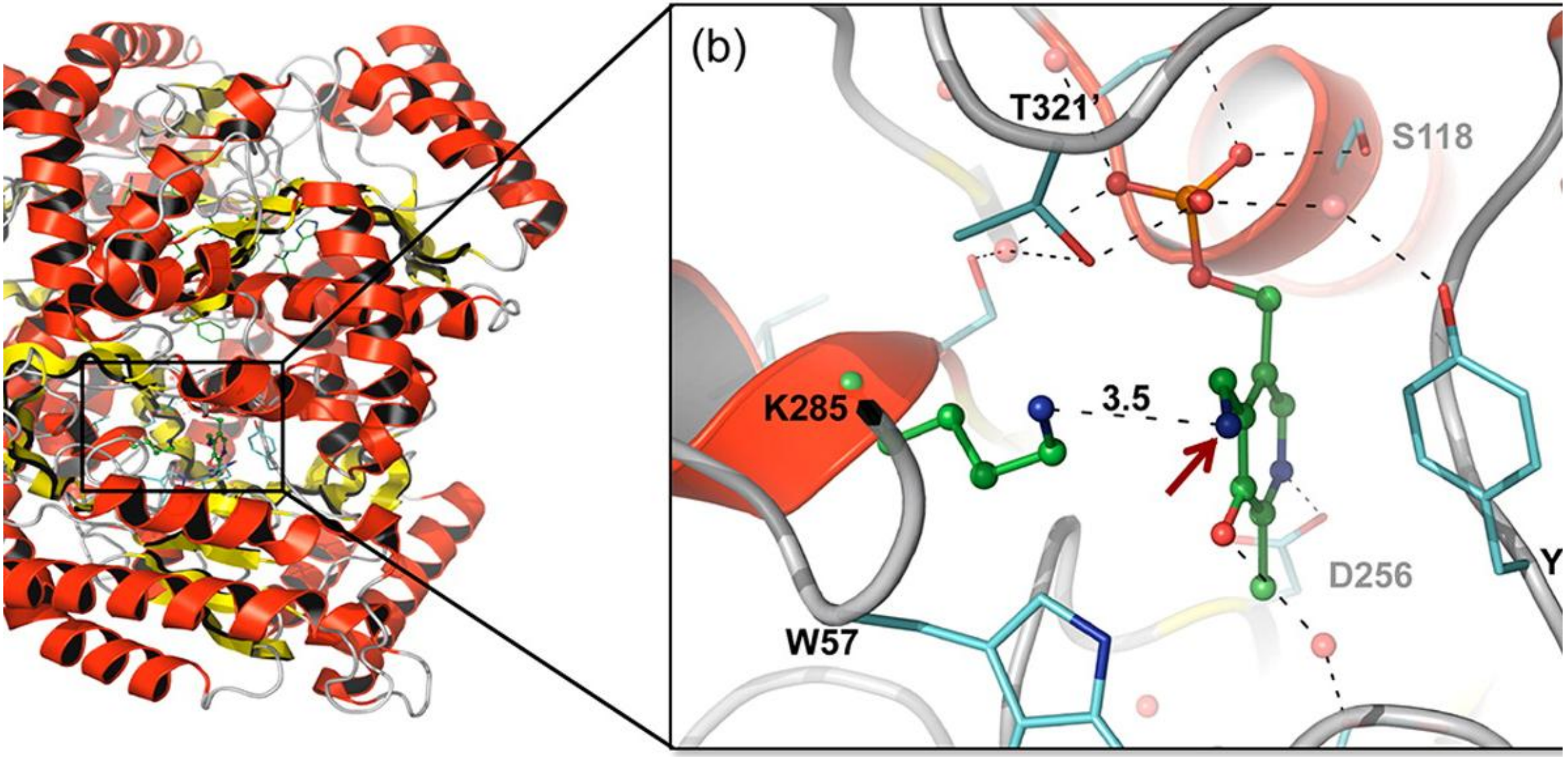


Desired chemistry



Imagabalin

Structure of wild type ω-Aminotransferase



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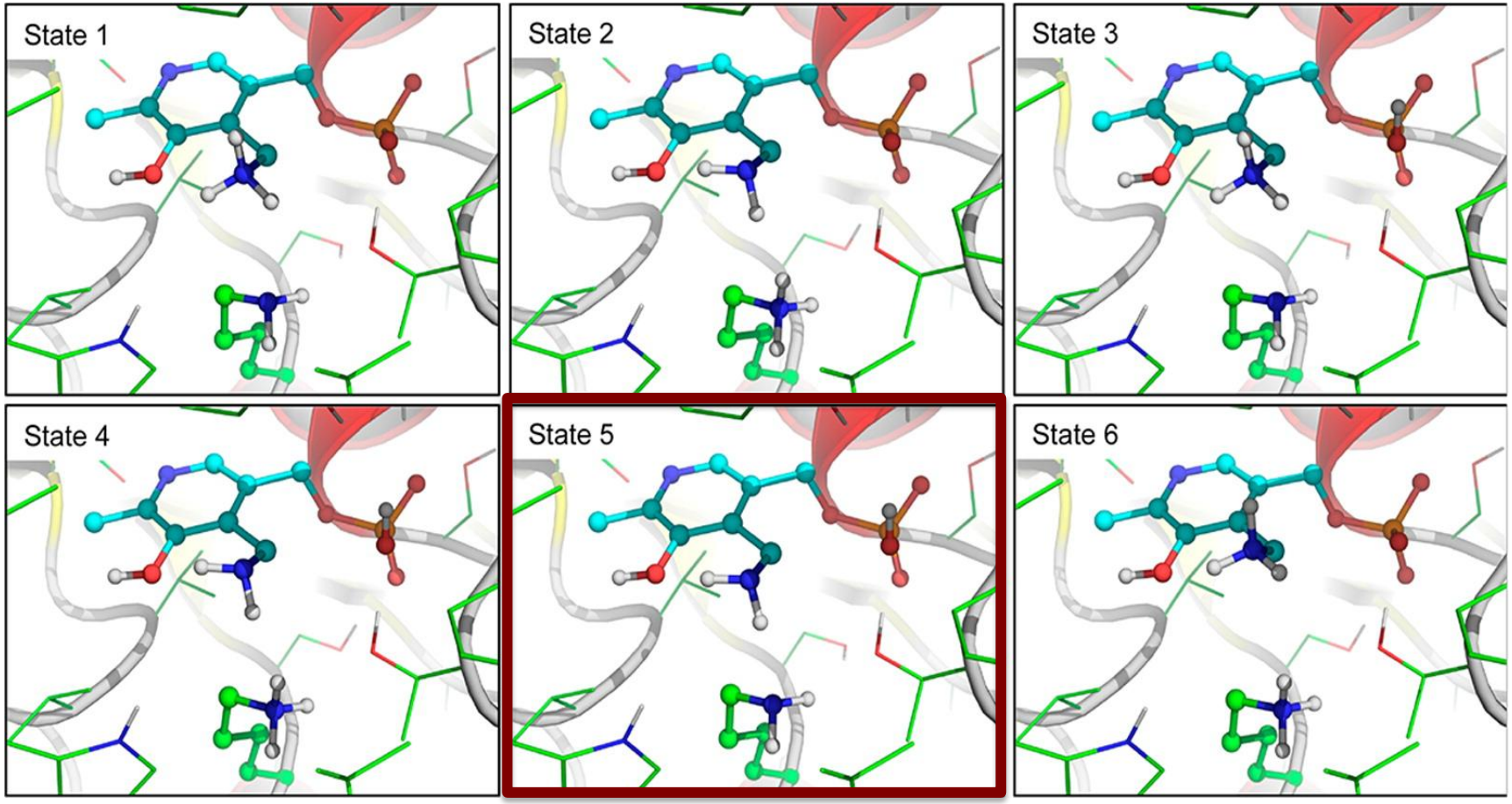
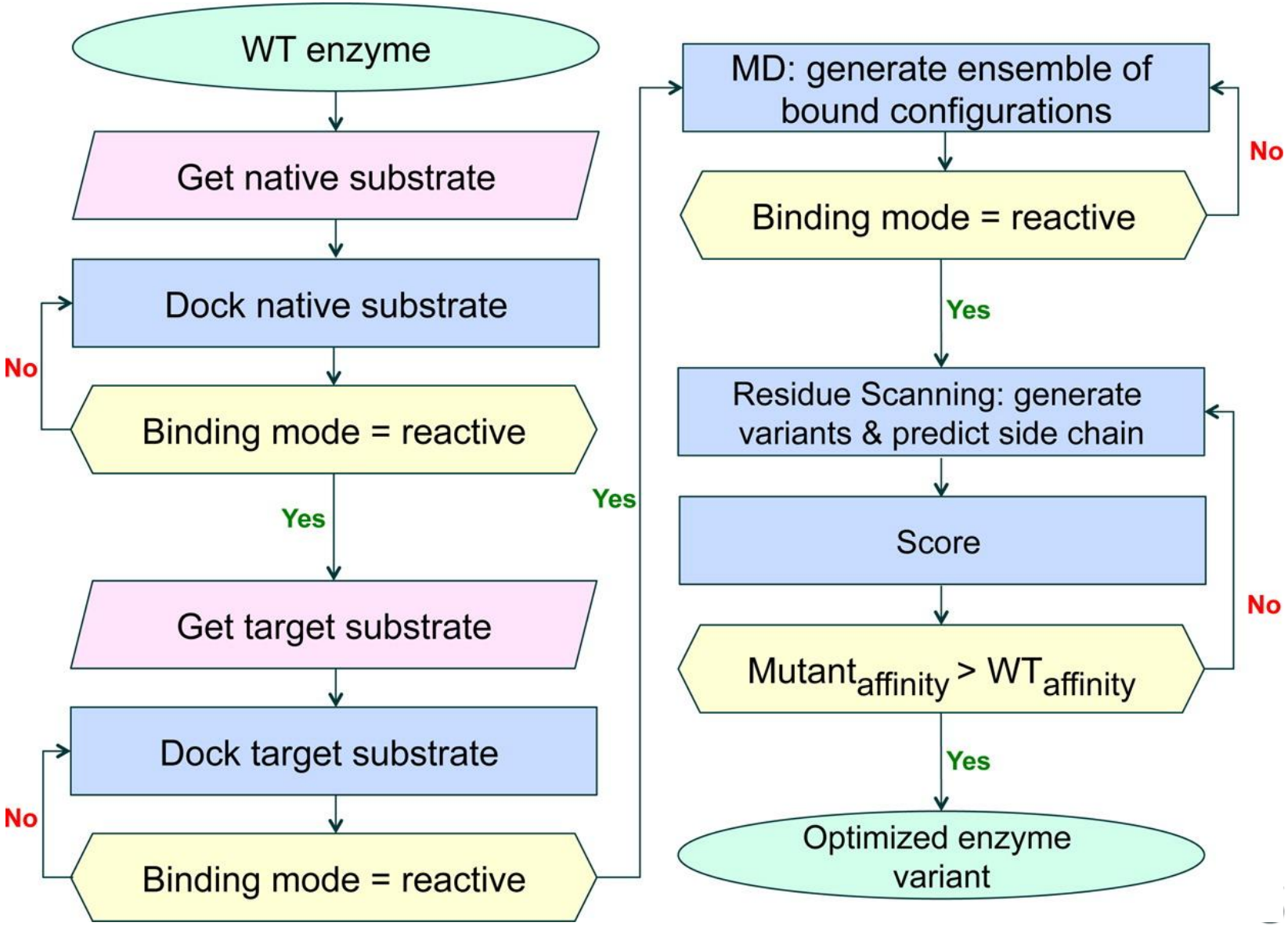
Protonation states were examined to identify an optimal starting structure



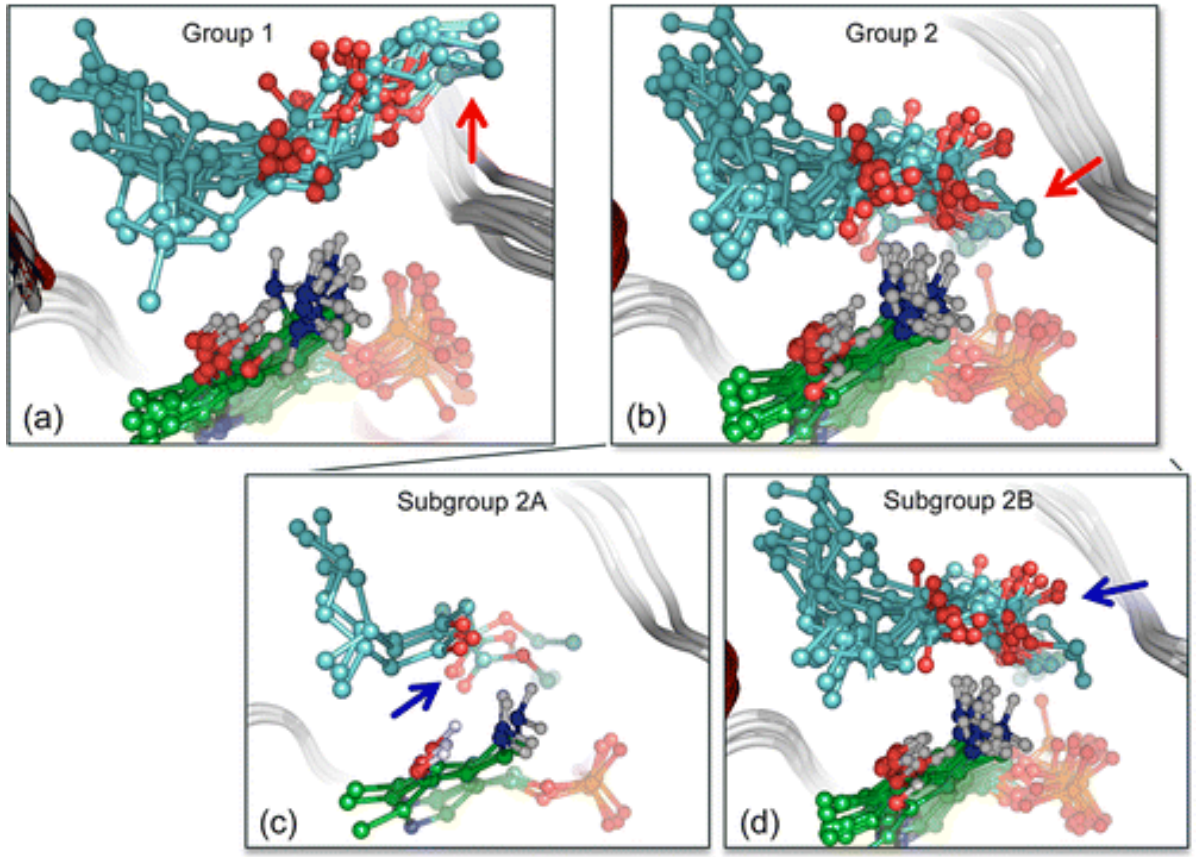
PROPKA was used to determine protonation states of active site residues and substrate

MD analysis revealed that State 5 is the most stable reactive conformation

Computational workflow

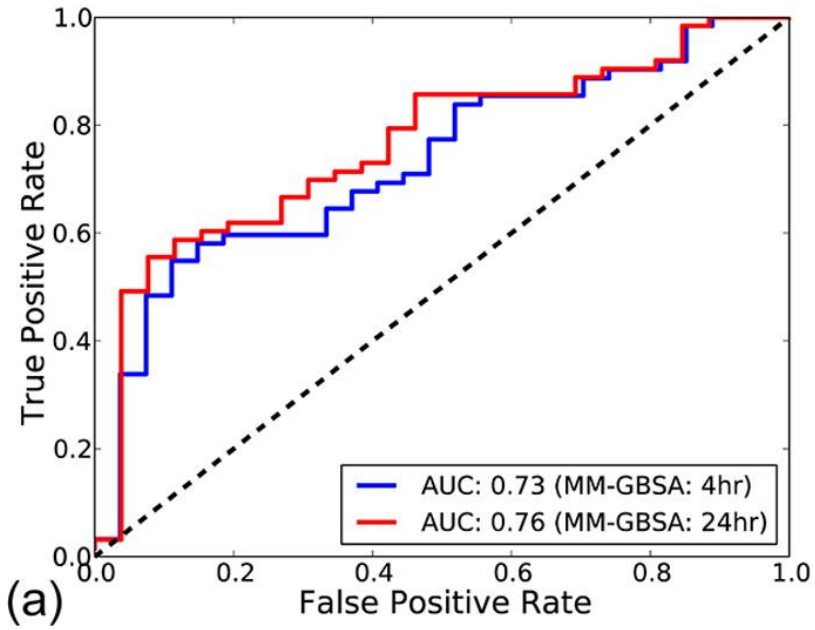


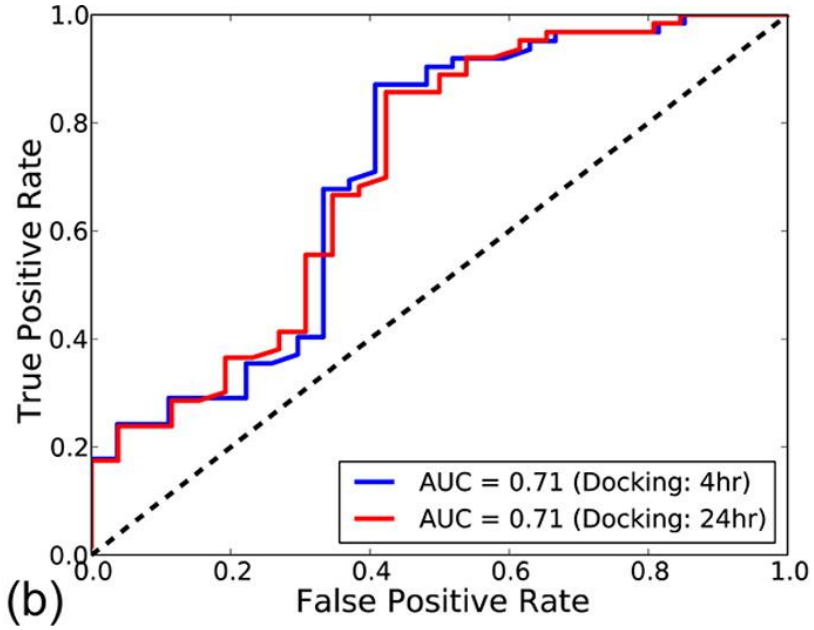
Prediction of target substrate binding was carried out by induced fit docking and metadynamics

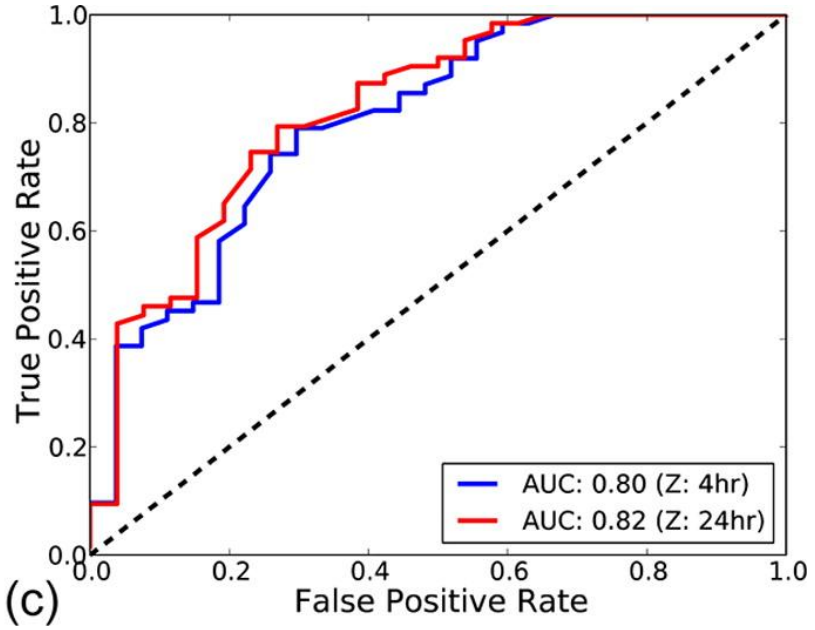


Clustering of 30ns metadynamics simulation

Retrospective analysis of predictions: ROC







- 89 variants were analyzed, with activities measured experimentally at 4 and 24 hours.
- 27 variants were inactive, while 62 had some activity toward the target substrate

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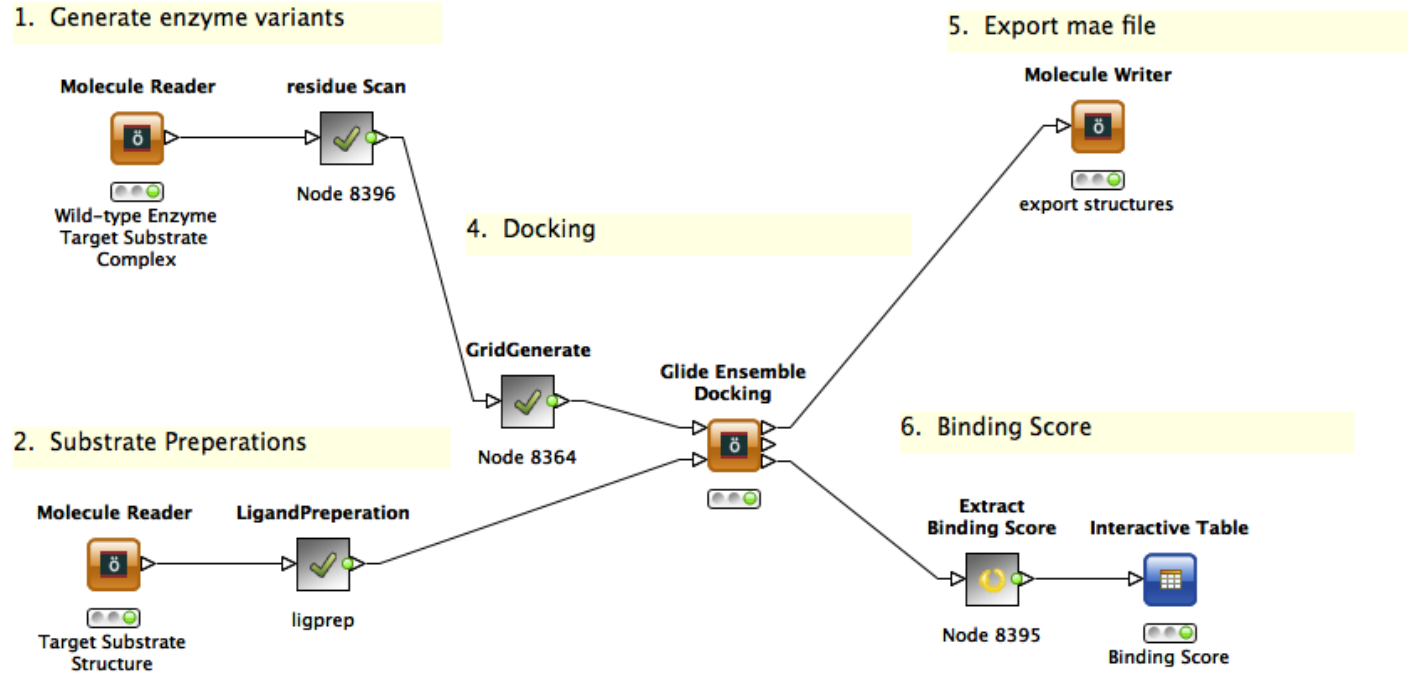
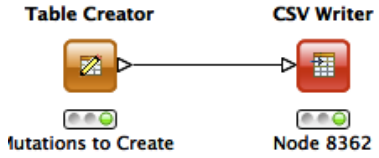
Automated KNIME workflow – available for use





Enzyme -- Substrate Binding Activity:

[Requires: Glide, Bioluminate]

Need to Specify:
Section 1: path for starting enzyme-substrate complex (mae file)
Section 2: path for substrate (mae file)
Section 3: list of mutations to generate the enzyme variant of interest
Section 5: path for mae output

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Case Study # 5: Predicting Protein-Protein Binding Affinity Using Free Energy Pertubation

Unpublished data

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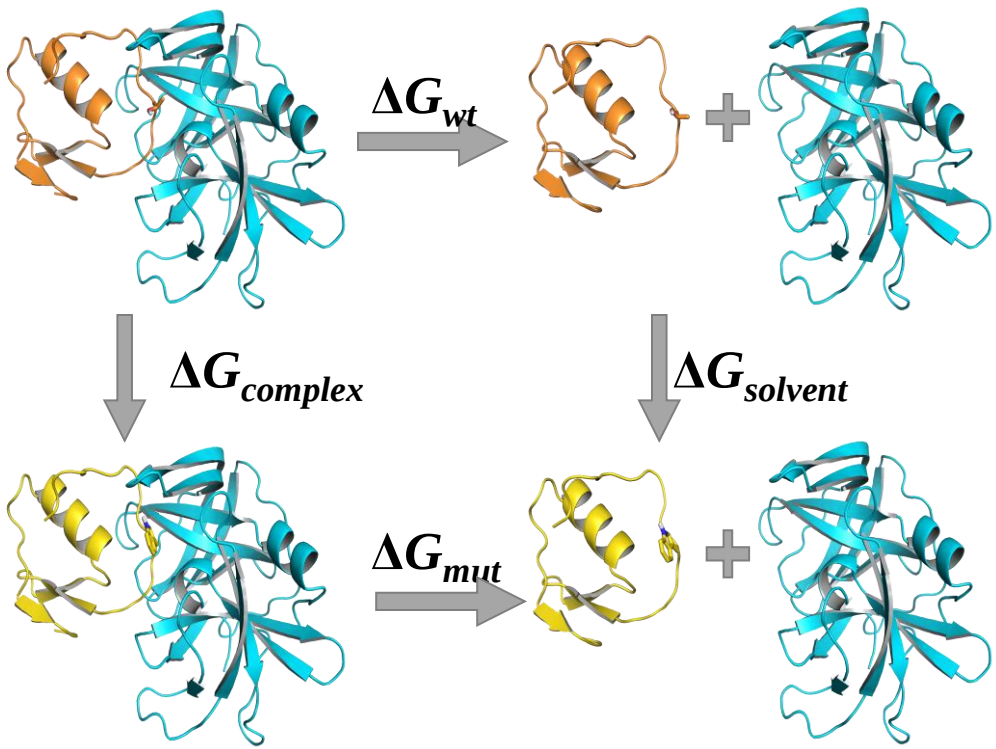
Free energy perturbation is the most accurate free energy calculation

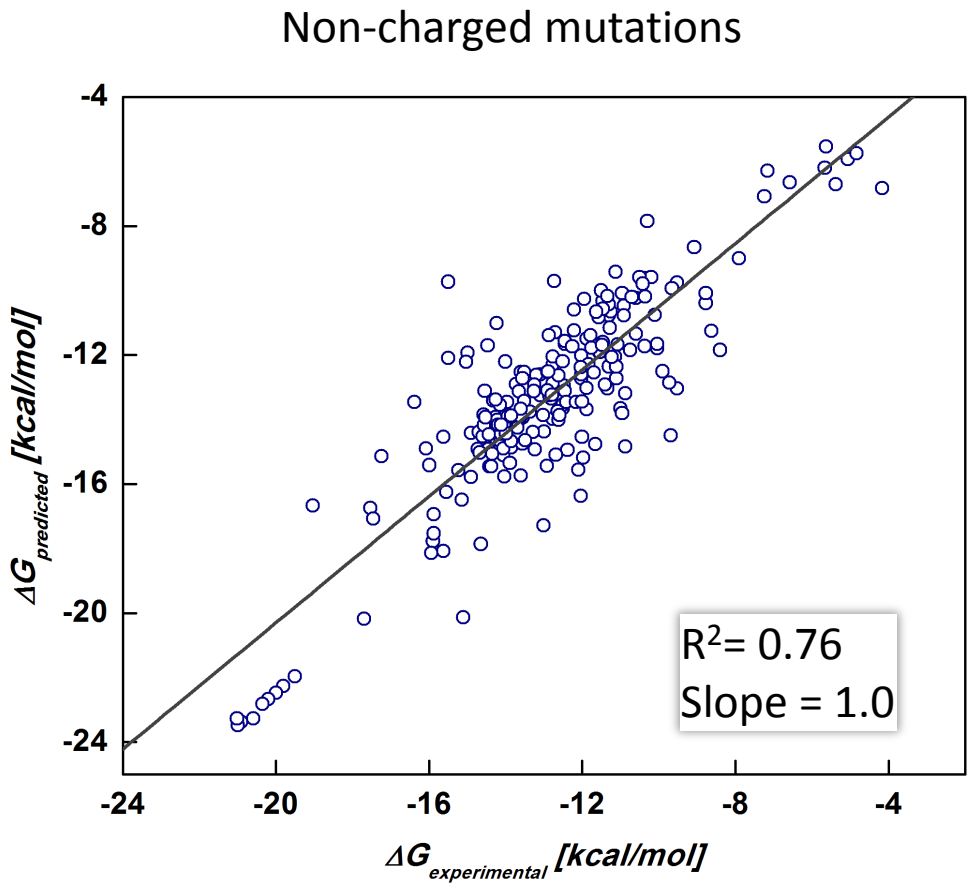


$$\Delta\Delta G = \Delta G_{wt} - \Delta G_{mut} = \Delta G_{complex} - \Delta G_{solvent}$$

To calculate rigorous ΔΔG values, alchemical intermediates are employed. These intermediates allow gradual perturbation of the wild-type protein to the mutant protein.

Overview of Study

System	PDB ID	No. mutants
CAL-PDZ/ical36 peptide	4E34	5
TEM-1 Beta-Lactamase/BLIP	1JTG	16
Barnase/Barnstar	1BRS	27
SC Serine Protease/OMTKY3	1R0R	137
SG Protease B/OMTKY3	3SGB	140
SG Protease B	1SGP	13
Ribonuclease inhibitor/angiogenin	1A4Y	18
Ras/RalGDS	1LFD	19
TPR scaffold protein/CTR Hsp90	3FWV	6



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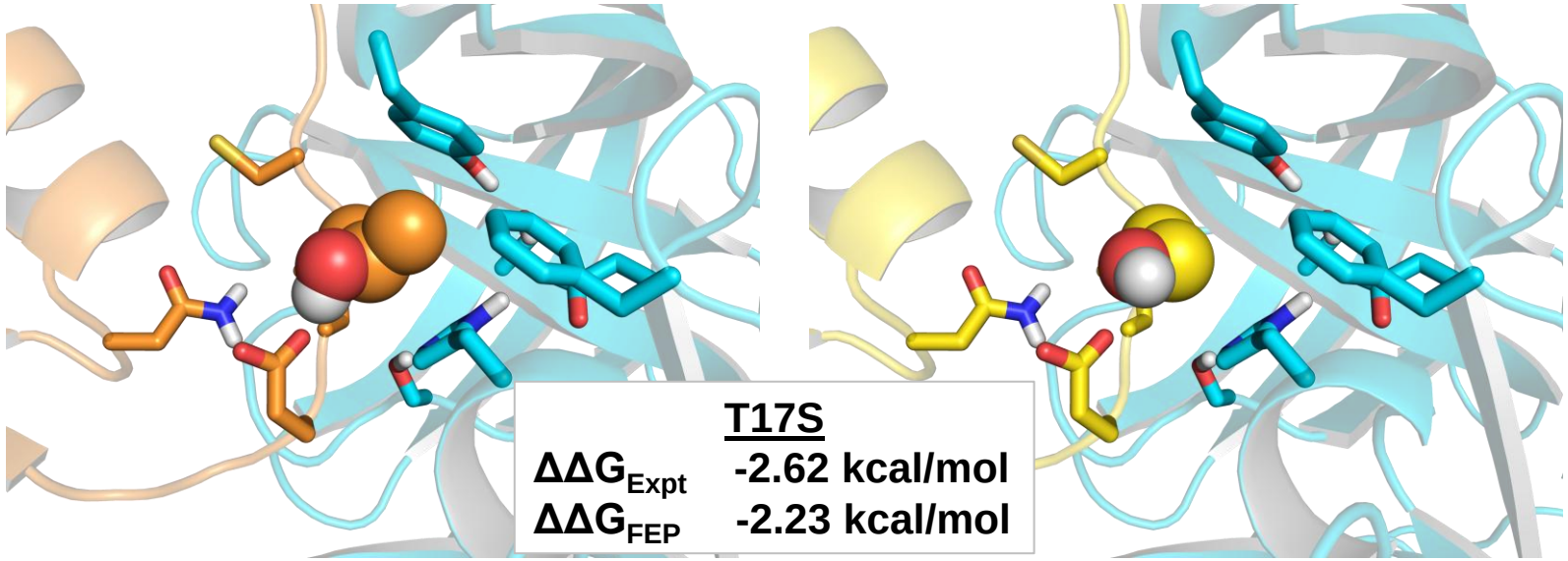
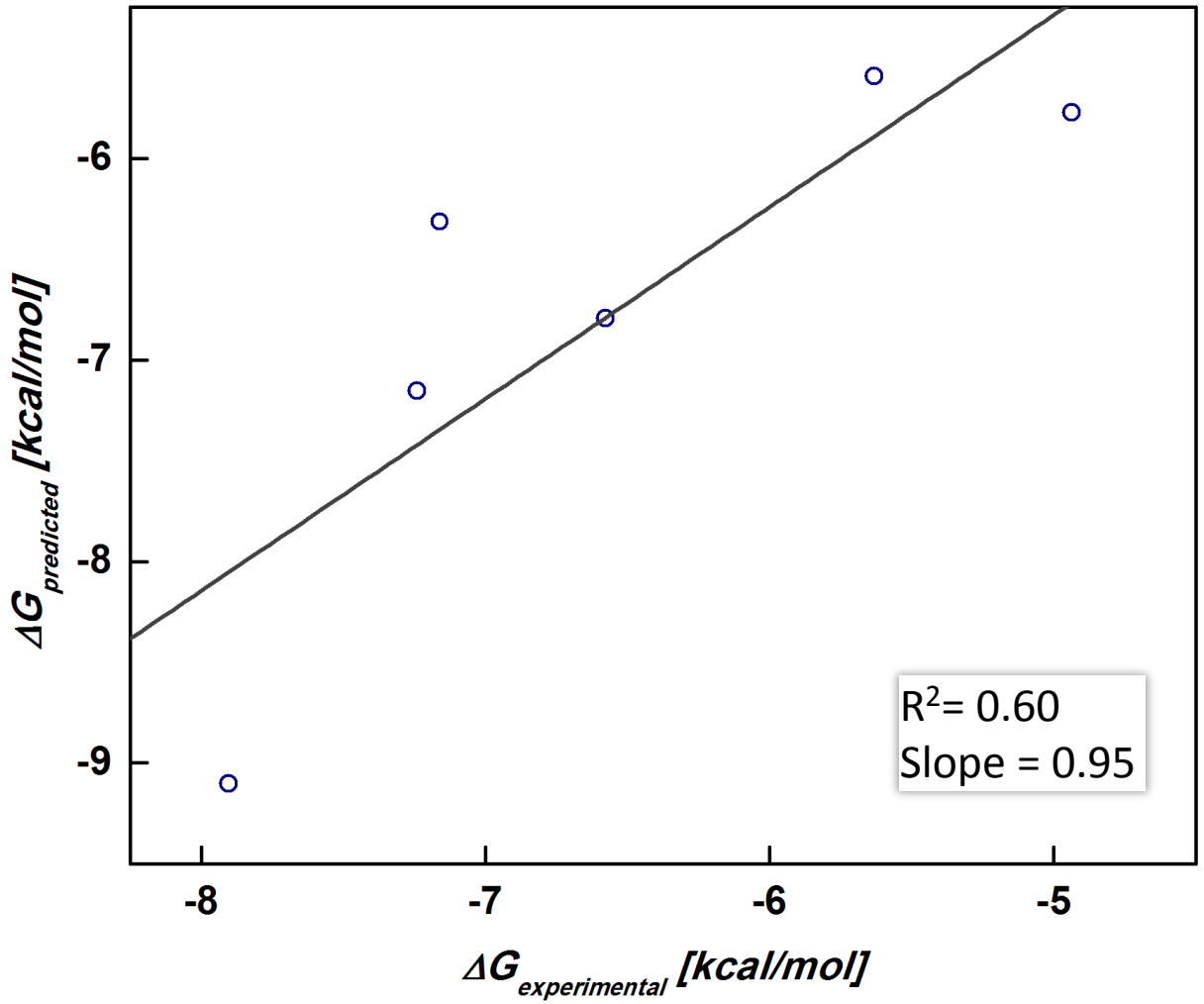
Example 1: SG Protease B/Inhibitor (3SGB)

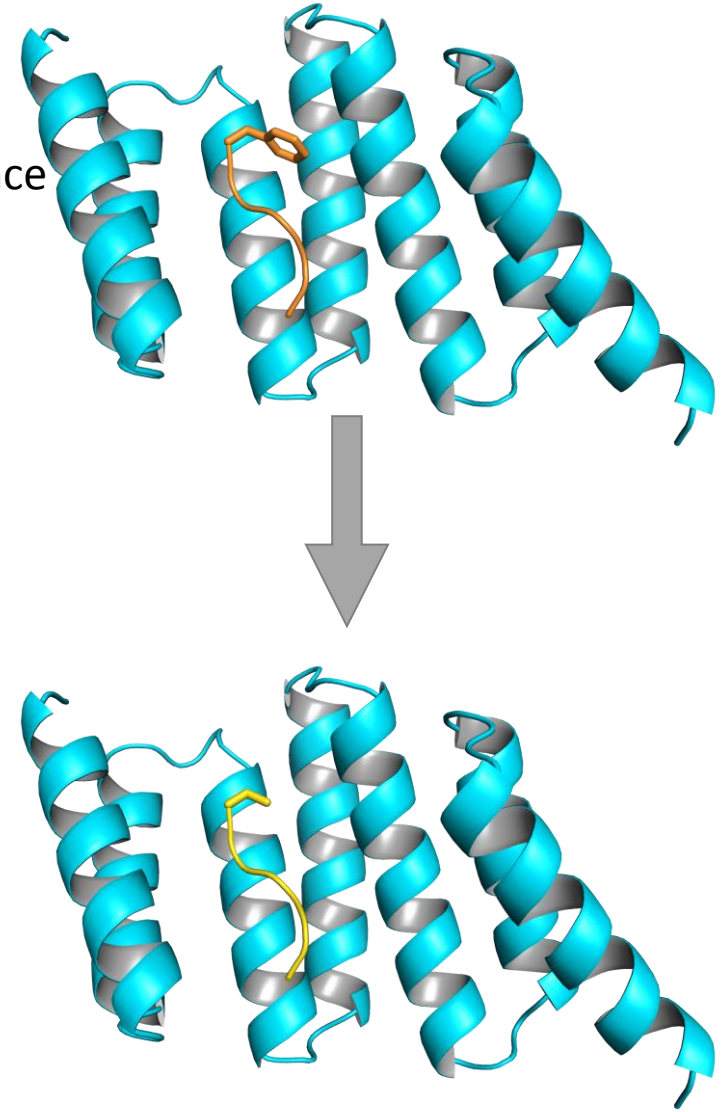
Mutation type	R^2	Slope	MUE	RMSE
Non polar	0.48	0.81	1.04	1.43
Aromatic	0.63	0.67	1.01	1.21
Polar	0.76	1.02	0.73	0.98
Charged	0.46	1.46	2.17	2.81



Example 2: TPR Scaffold Protein/CTR Hsp90 (3FWV)



Peptide Sequence
MEEV**F**



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Thank you!

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