New Developments in RosettaLigand

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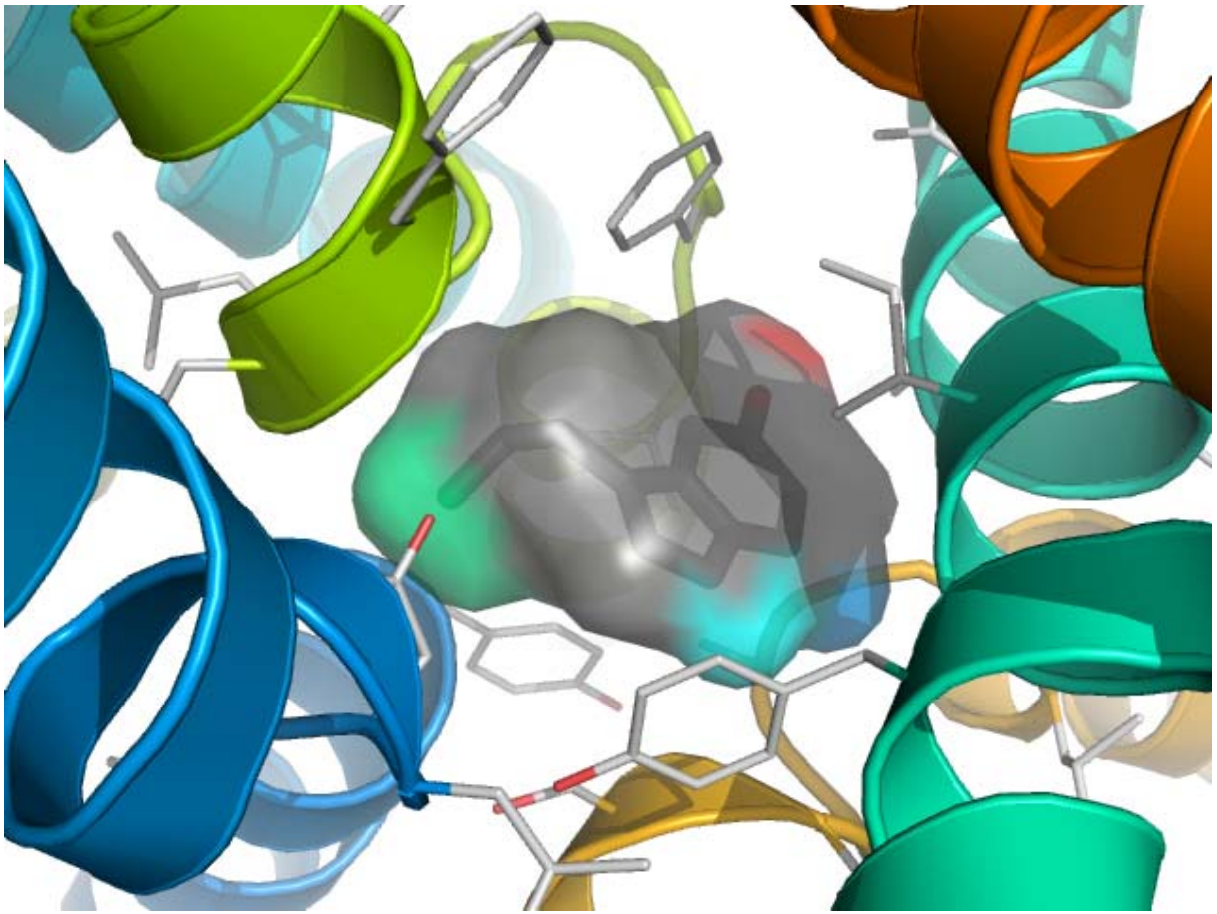
QSAR Model Validates 5-HT Binding Mode to Serotonin Transporter



- hSERT is a Neurotransmitter :: Sodium Symporter (NSS) with twelve TM domains
- A homolog $LeuT_{Aa}$ was crystallized with 22% sequence identity to hSERT, increases to ~45% in substrate binding site
- RosettaLigand docking into homology model with full Ligand and Protein Flexibility

Kaufmann, K. W.; et al. "Structural determinants of species-selective substrate recognition in human and Drosophila serotonin transporters revealed through computational docking studies" *Proteins* ***2009, 74, 630-42.***

Outline

- Ligand-Guided Virtual High-Throughput Screening Identifies Allosteric Modulaters of Metabotropic Glutamate Receptors
- BCL::PharmMap: Comprehensive, Rapid, and Robust Pharmacophore Mapping using QSAR Models
- RosettaLigand: 80% Success Rate for Docking into Comparative Models with full Ligand and Protein Flexibility
- RosettaLigand Algorithms for Ligand Ranking and Fragment-Based Drug Design
- QSAR-Derived Pharmacophore Maps Discriminate incorrect Poses in Ligand Docking into Comparative Models

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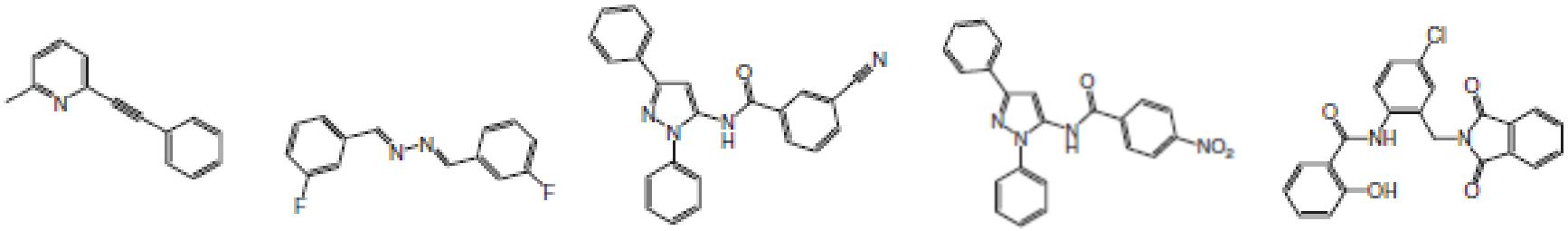
Treatment Strategies for CNS Disorders through Modulation of $mGluR_5$

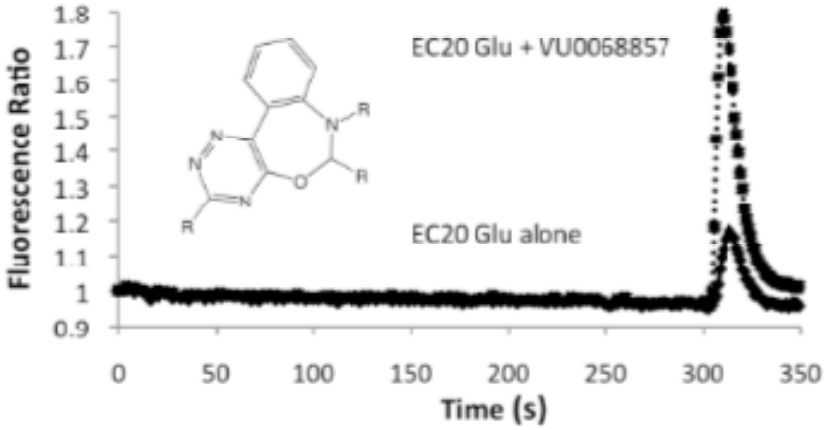


- Allosteric positive modulation (activation) of $mGluR_5$ may ameliorate the symptoms of schizophrenia.

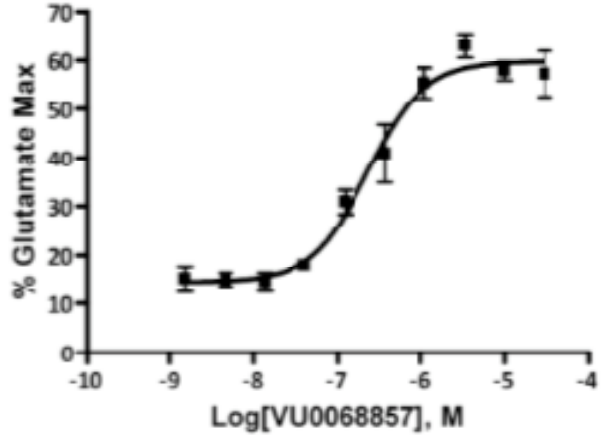
- Allosteric negative modulation of $mGluR_5$ offers a potential treatment strategy of fragile X syndrome symptoms, a CNS disorder associated with autism spectrum disorders (ASD).

High-Throughput Screen yields 1387 PAMs and 345 NAMs of $mGluR_5$

a)



b)

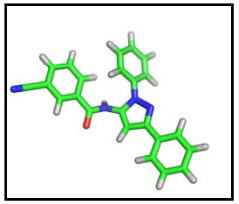


- 150,000 compounds were tested for allosteric modulation of $mGluR_5$ measuring receptor-induced intracellular release of calcium. 1,387 (0.94%) compounds were verified as PAMs of $mGluR_5$. 345 (0.23%) compounds were verified as NAMs of $mGluR_5$.

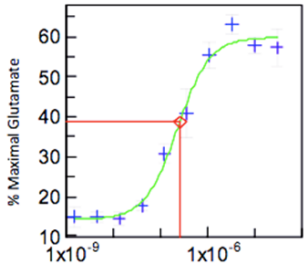
Niswender, C. M.; Johnson, K. A.; Luo, Q.; Ayala, J. E.; Kim, C.; Conn, P. J.; Weaver, C. D. *Mol Pharmacol* ***2008, 73, 1213-24.***

Relate Chemical Structure and Biological Activity

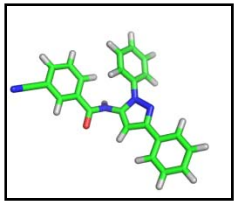
Chemical Structure



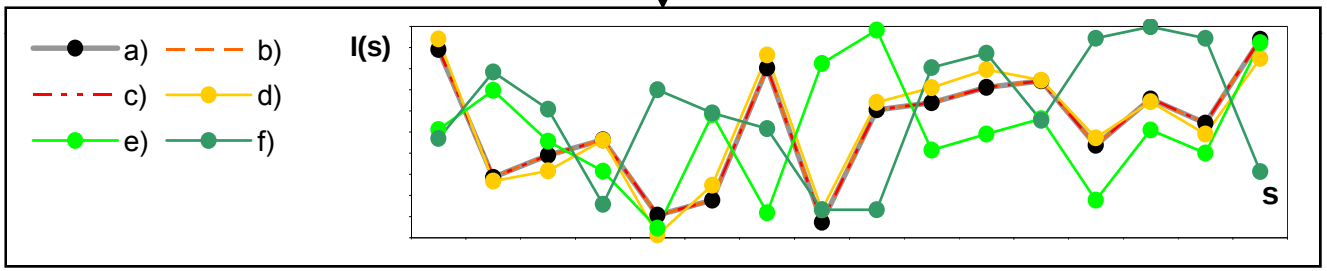
Biological Activity

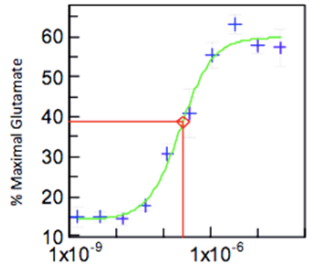


Transformation-Invariant, Problem-Optimized Numerical Description



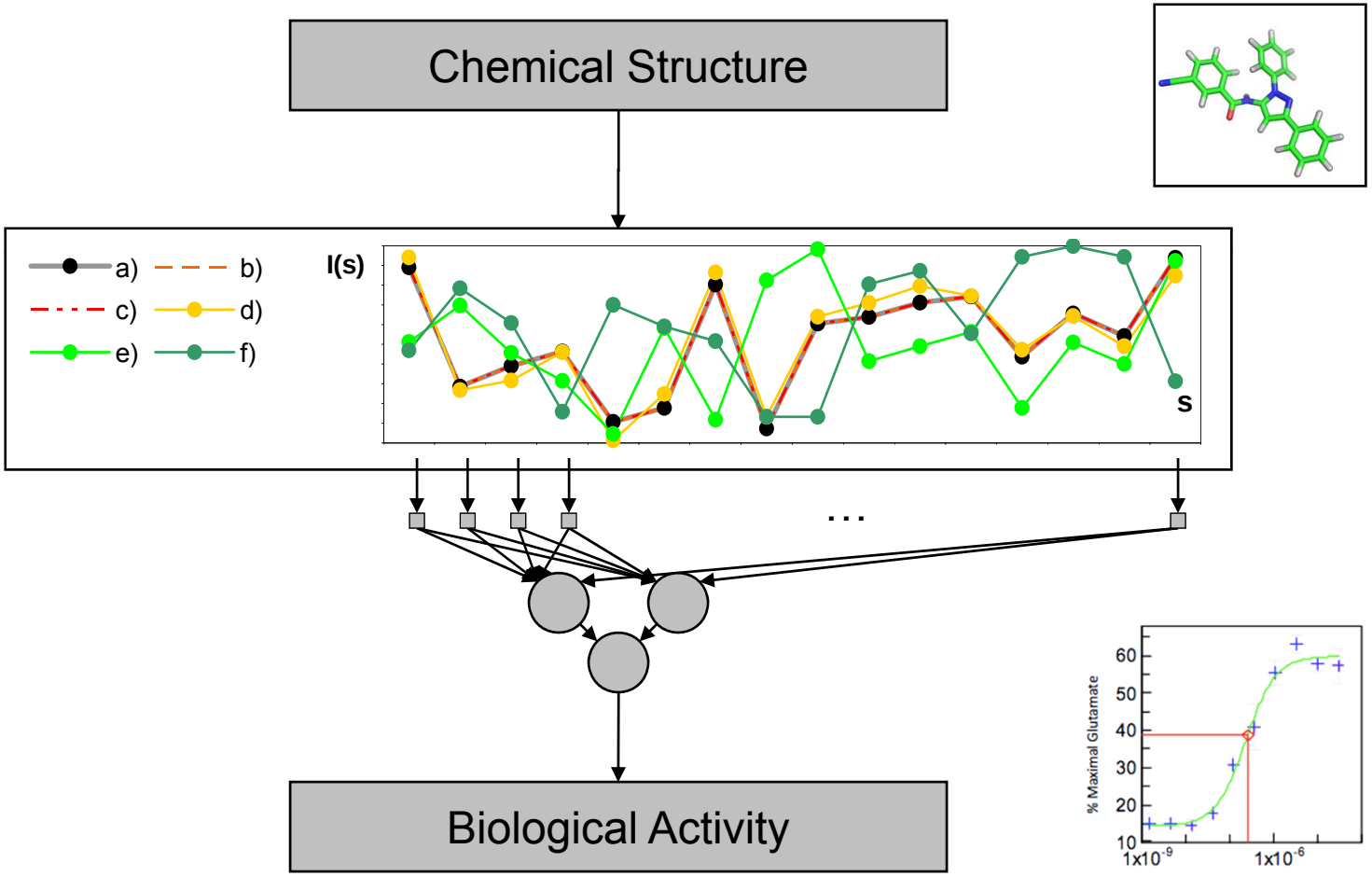
Chemical Structure



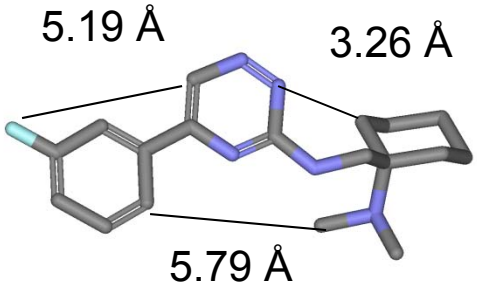


Biological Activity

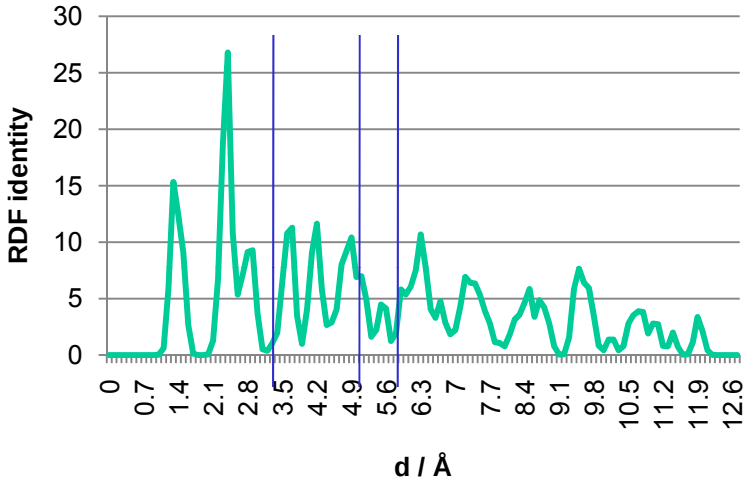
Machine Learning Calculates Activity from Numerical Description



Radial Distribution Functions describe 3D shape …



$$g(d) = \sum_{\substack{atom\\pairs\\i,j}} e^{-B(d-d_{ij})^2}$$



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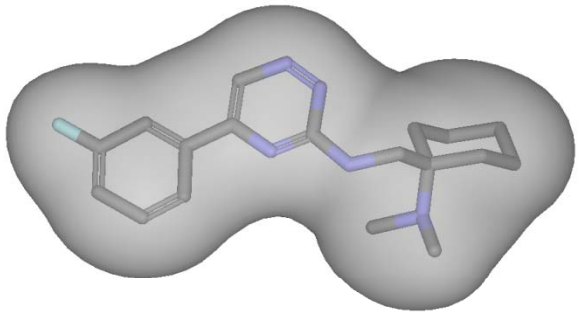
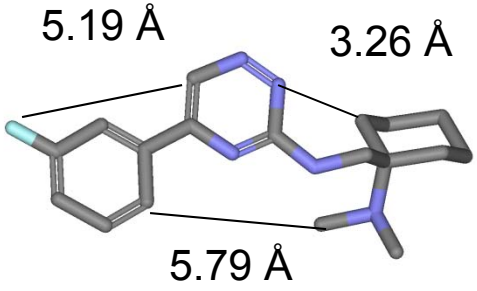


where:

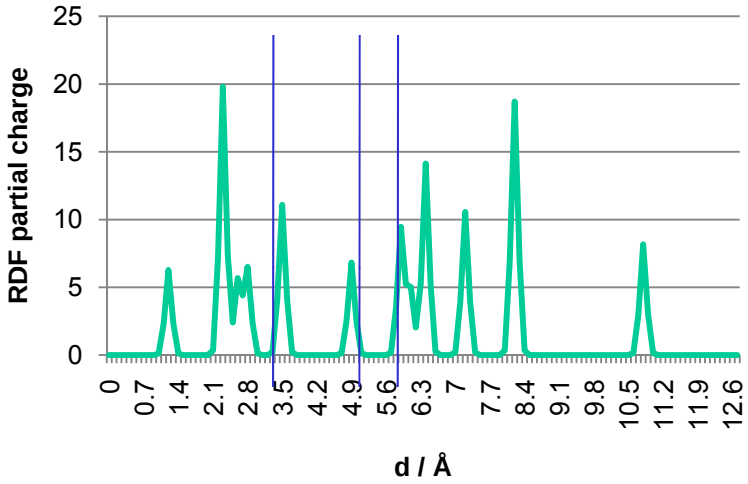
d_{ij} – distance between two atoms

B – temperature factor, here 100

… but can also Encode Chemical Properties such as Polarizability



$$g(d) = \sum_{\substack{atom\\pairs\\i,j}} A_i A_j \, e^{-B(d-d_{ij})^2}$$



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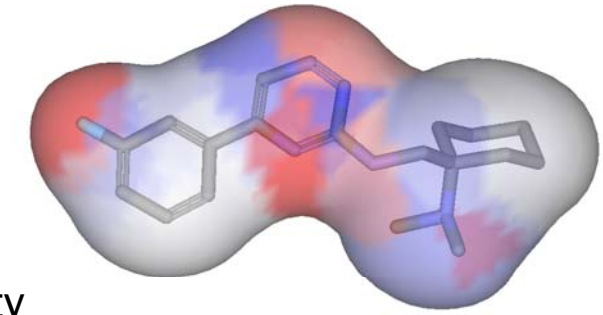
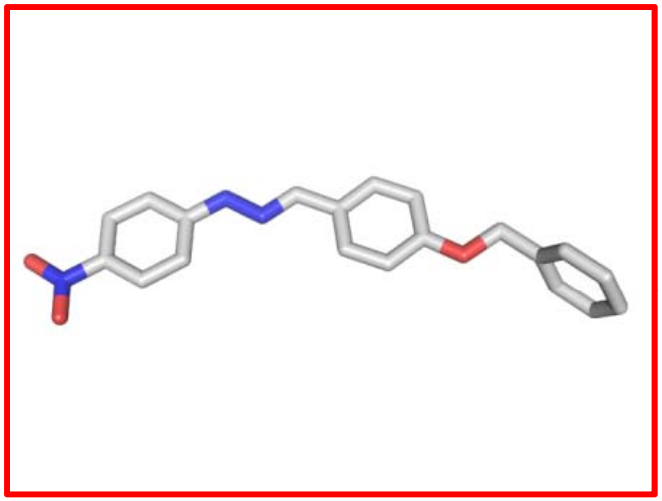
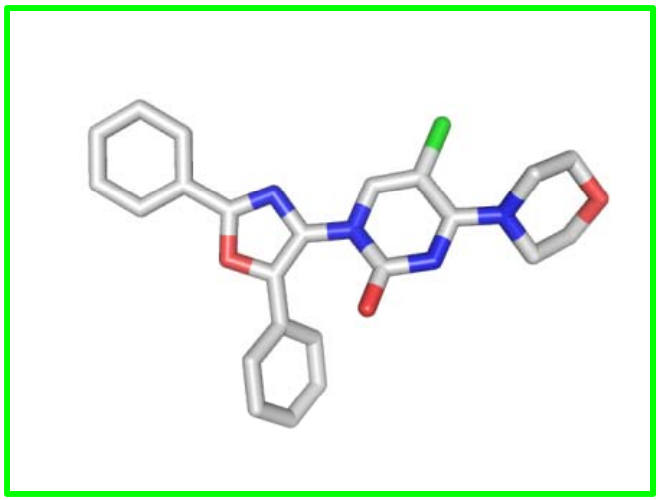


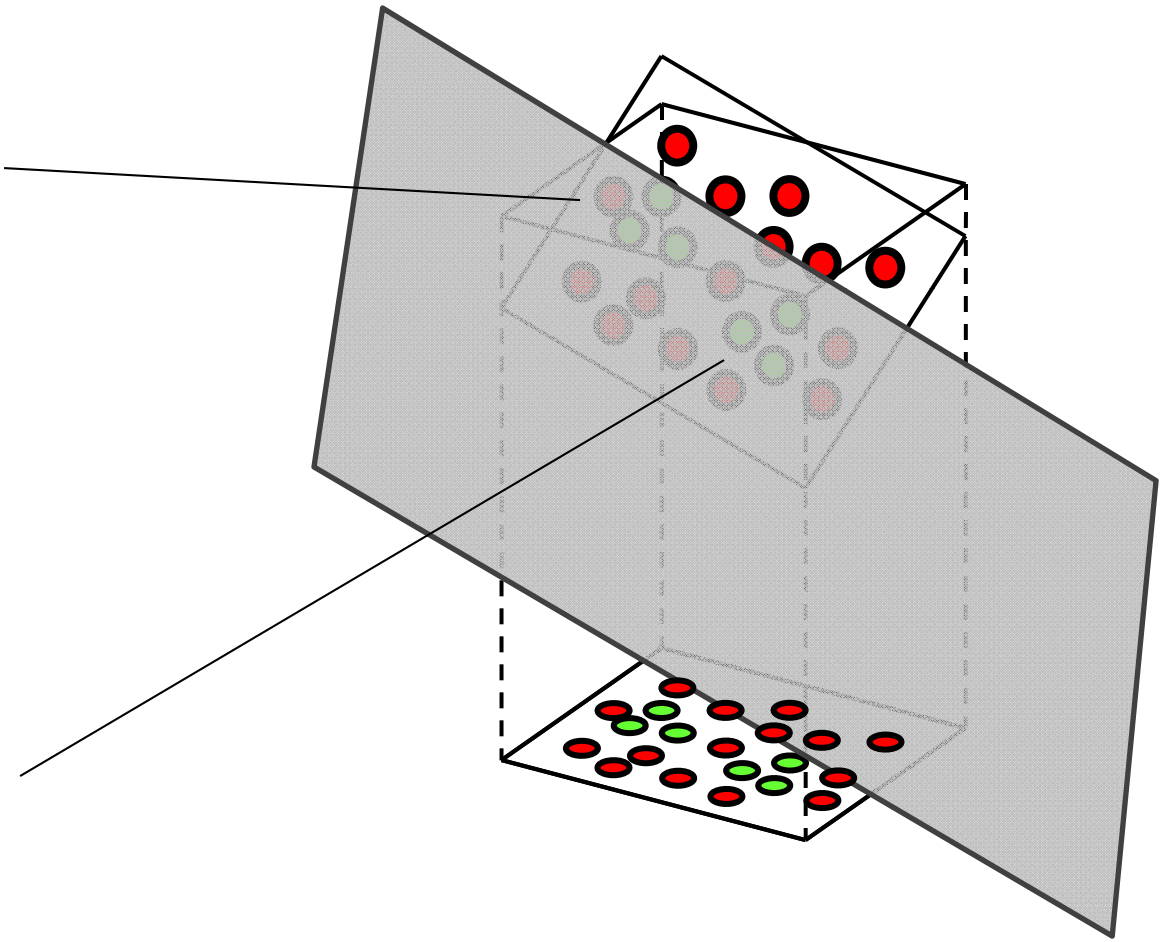
where:

- d_{ij} – distance between two atoms
- A_i , A_j – atom properties, here lone pair electro negativity
- B – temperature factor, here 100

Mapping Descriptor Space into Hyperspace







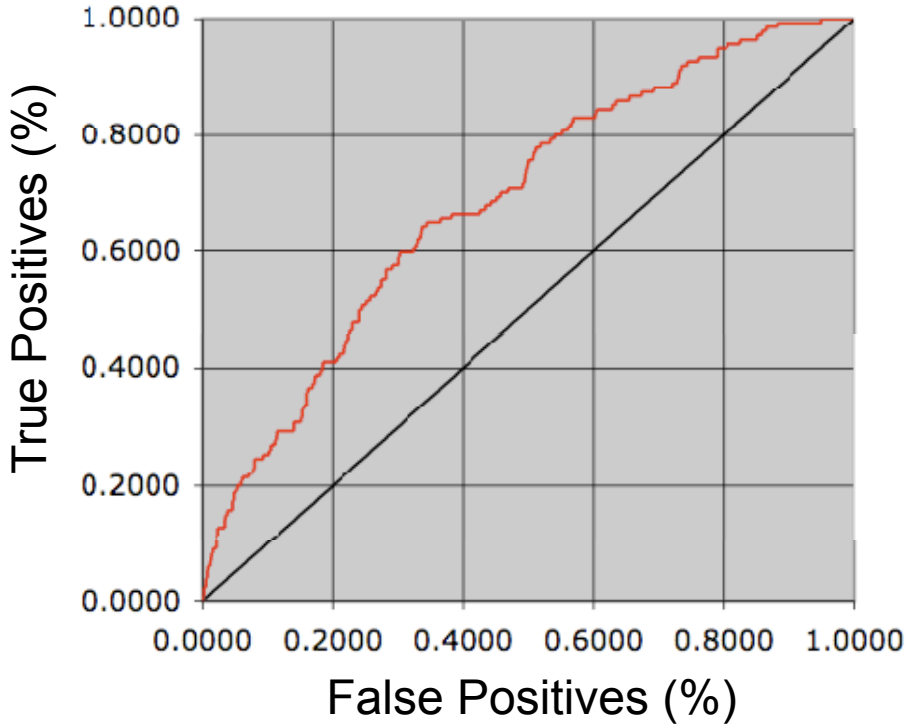
Optimizing the set of chemical descriptors for the given target

Number descriptors

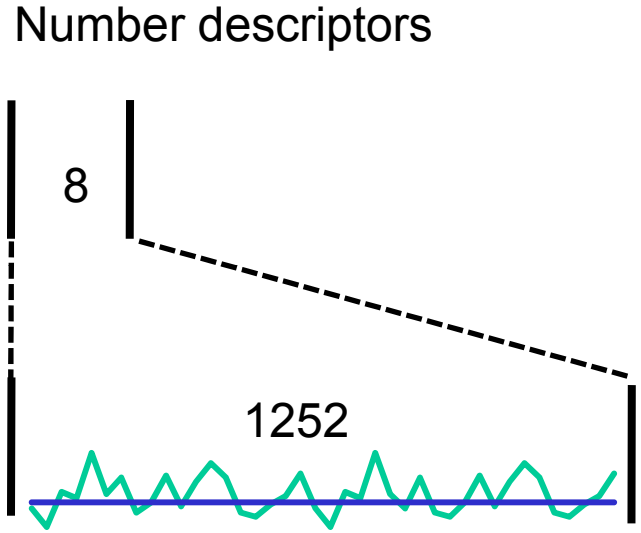
8

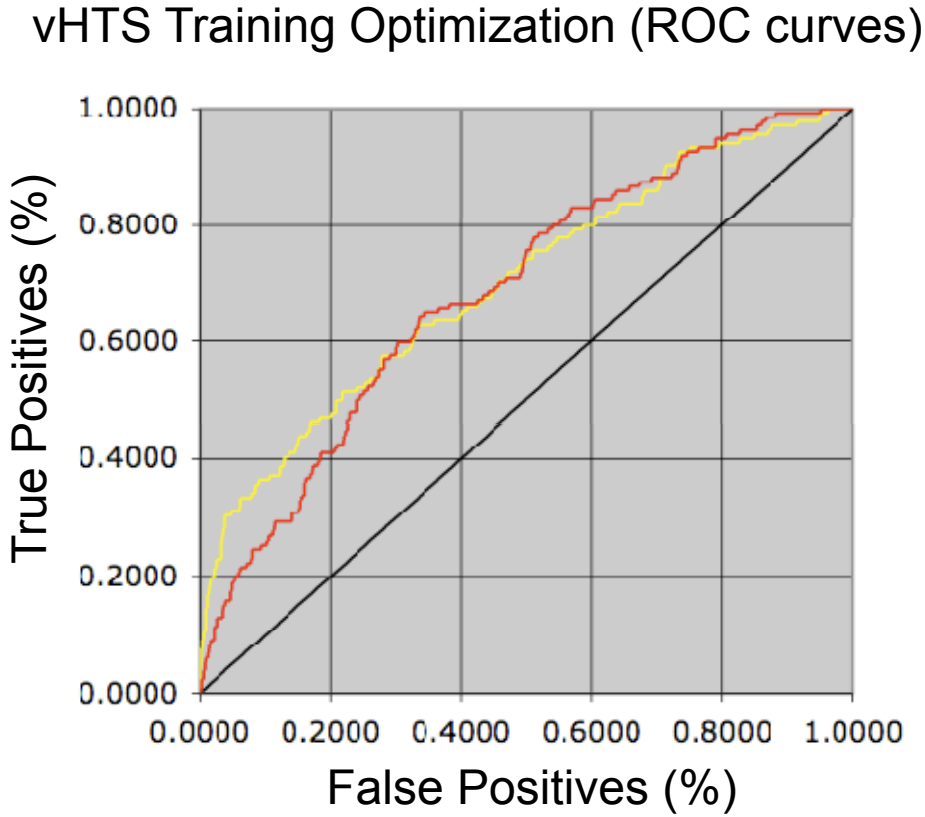
- Molecular Weight
- Number H bond donors
- Number H bond acceptors
- XlogP
- Polar surface area
- Mean molecular polarizability
- Molecular dipole moment
- Aqueous solubility

vHTS Training Optimization (ROC curves)



Optimizing the set of chemical descriptors for the given target





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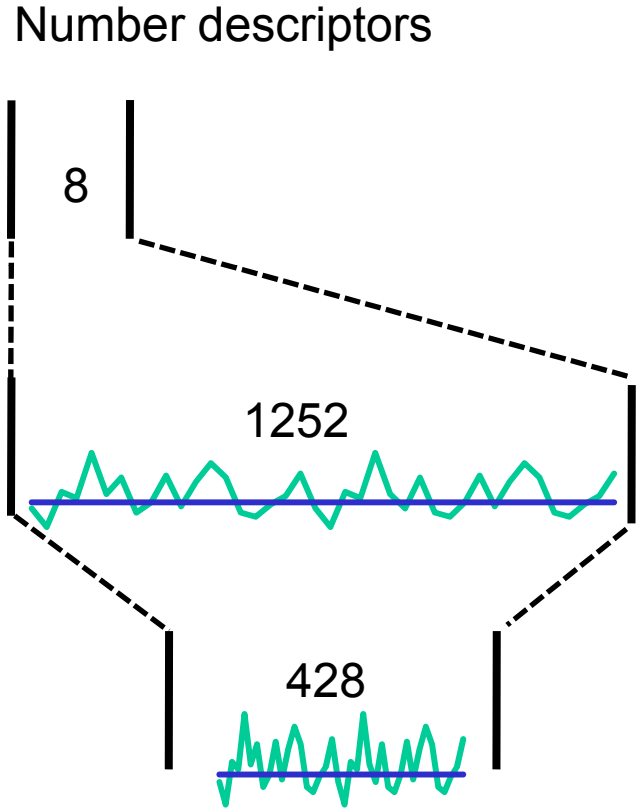

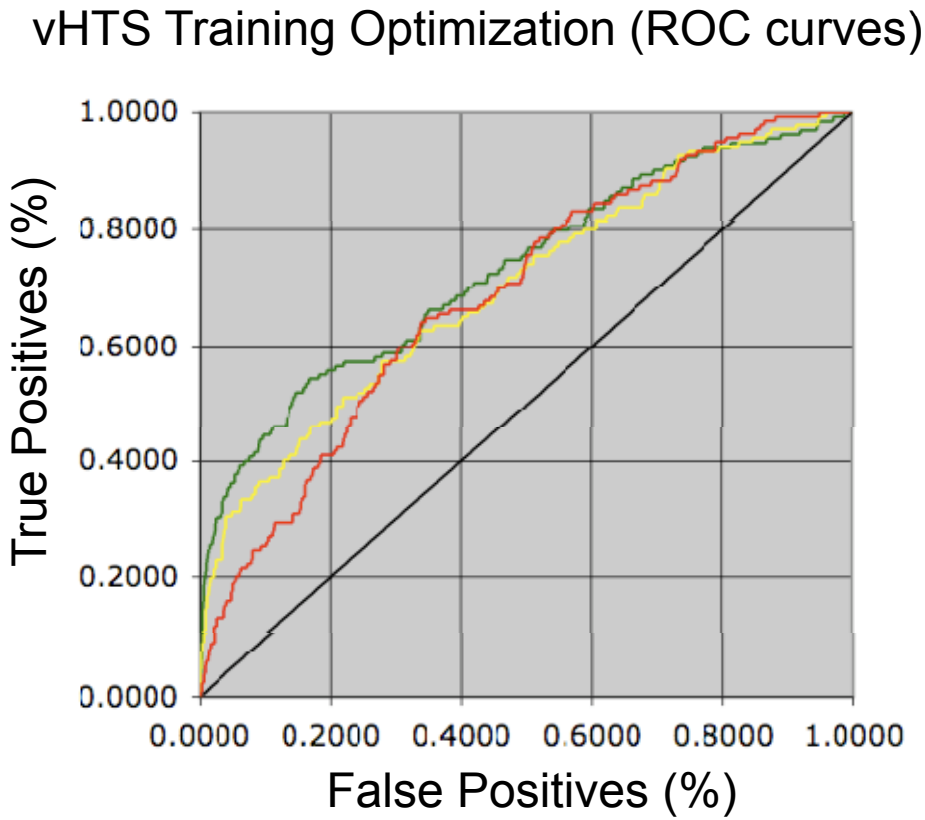

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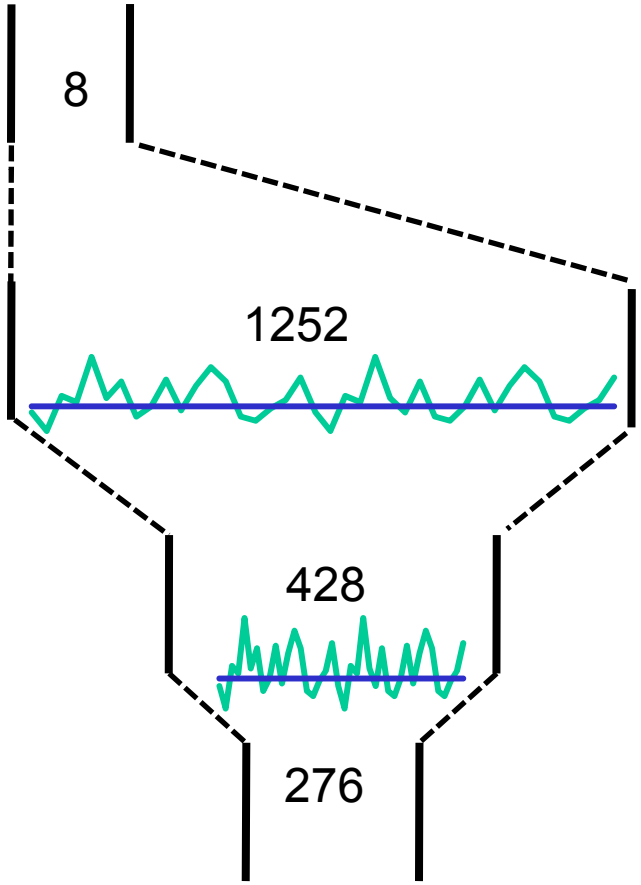
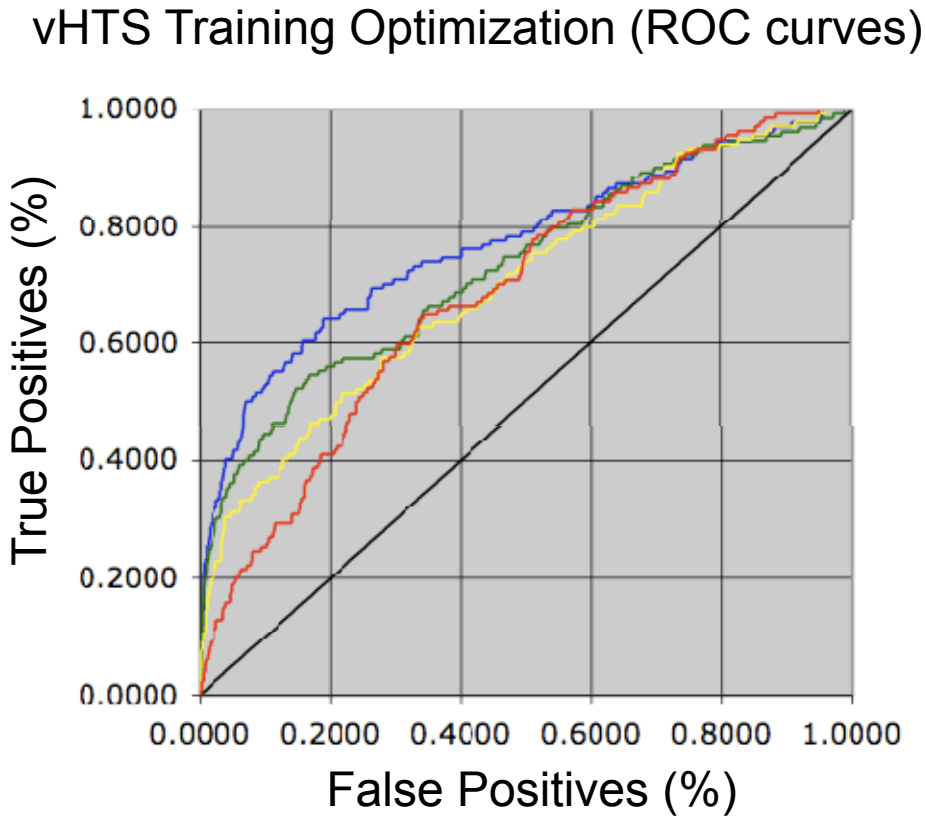
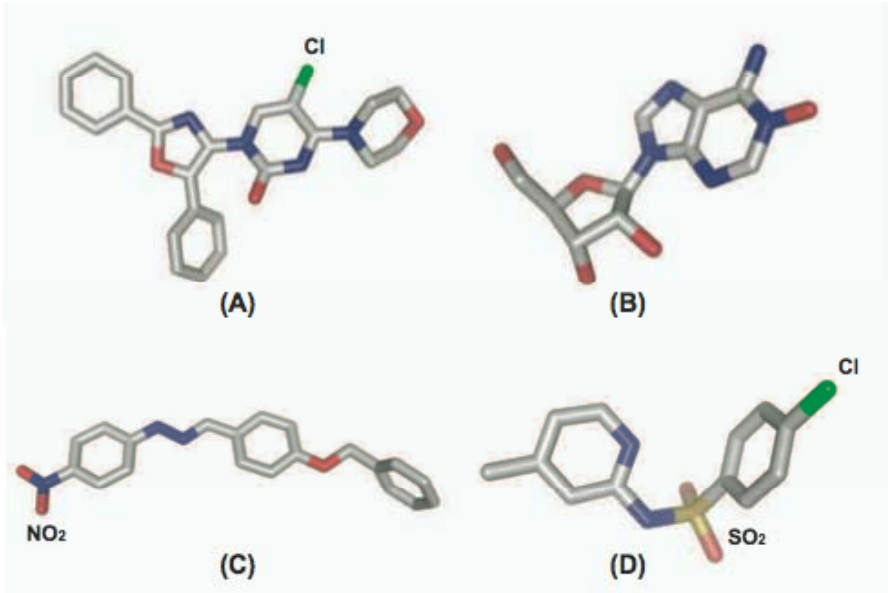
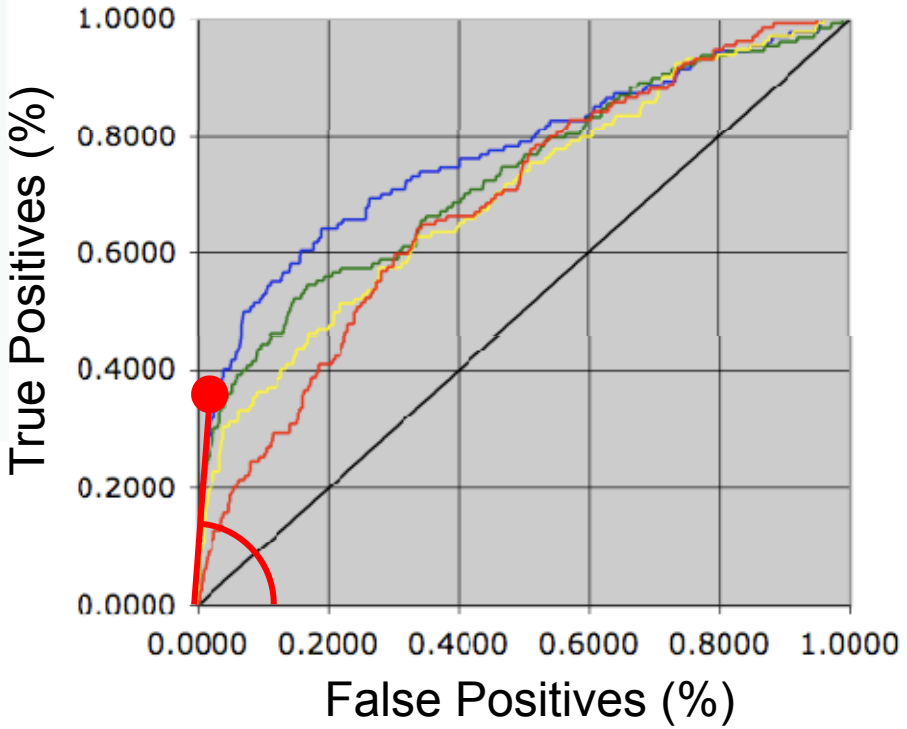




Virtual Screen for Highly Active Compounds and Novel Leads



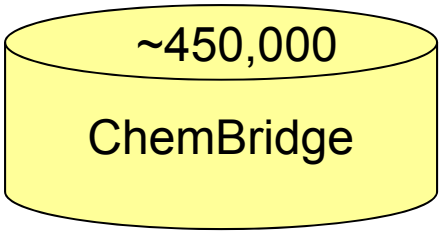
vHTS Training Optimization (ROC curves)



- A) True positive
- B) False negative
- C) False positive
- D) True negative

- Enrichment of Active Compounds by 43x

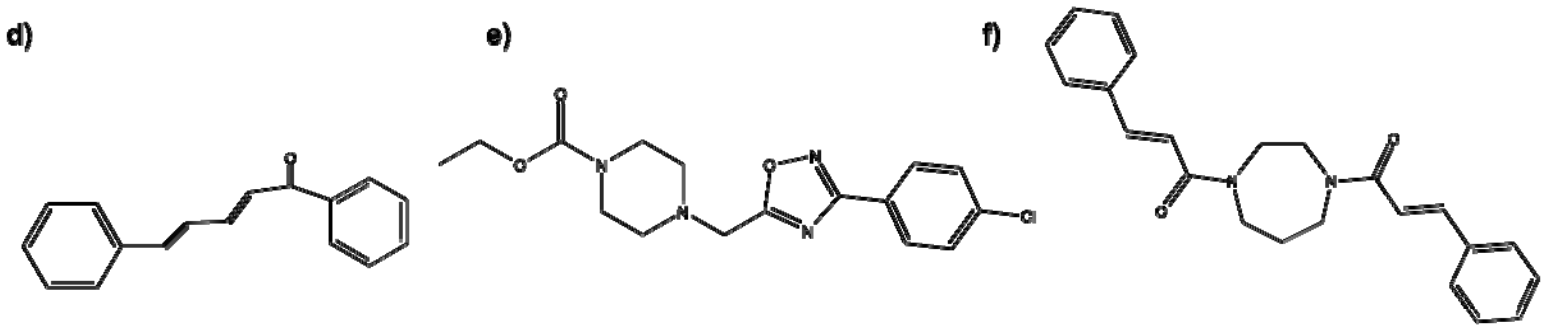
Experimental Results $mGluR_5$ Positive Allosteric Modulators



Mueller, R.; et al. "Identification of Metabotropic Glutamate Receptor Subtype 5 Potentiators Using Virtual High-Throughput Screening" *ACS Chem. Neurosci* ***2010, 1, 288-305.***

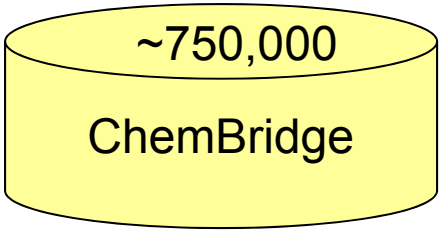
824 Compounds predicted with EC_{50} < 1µM by QSAR model

232 Compounds (28.1%) were confirmed as $mGlur_5$ PAMs
Enrichment = 28.1% / 0.96% = 30



Non-trivial scaffold modifications with mGluR5 PAM activity

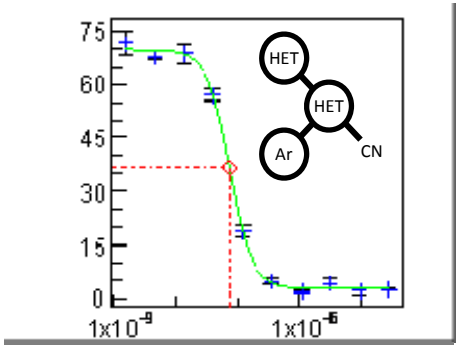
Experimental Results $mGluR_5$ Negative Allosteric Modulators



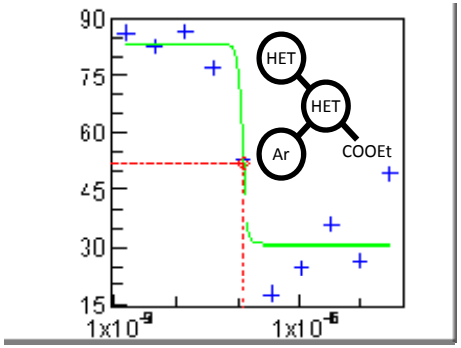
749 Compounds with novel Scaffolds predicted
with EC_{50} < 10μM by QSAR model

12 Compounds (1.6%) were confirmed as $mGlur_5$ NAMs
Enrichment = 1.6% / 0.23% = 7

VU0240790-4
EC_{50} = 75 nM



VU0360620-1
EC_{50} = 124 nM



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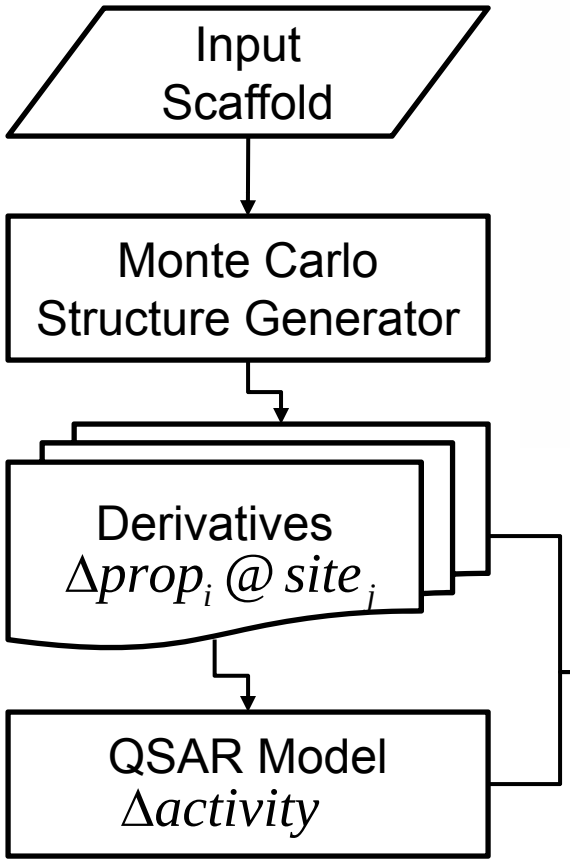
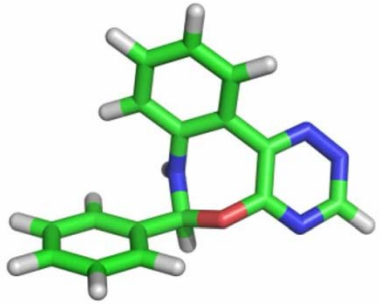
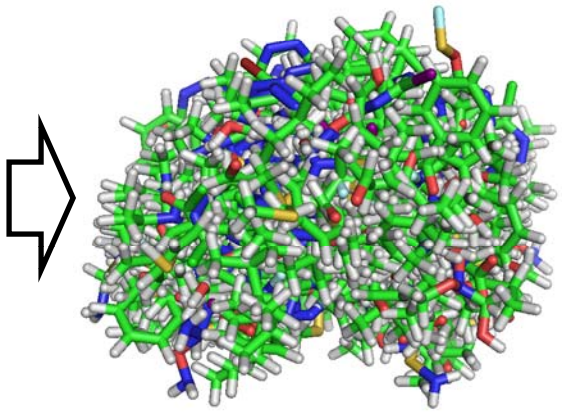
BCL::PHARMMAP – Computes Partial Derivatives of Property vs. Structure

- The Algorithm

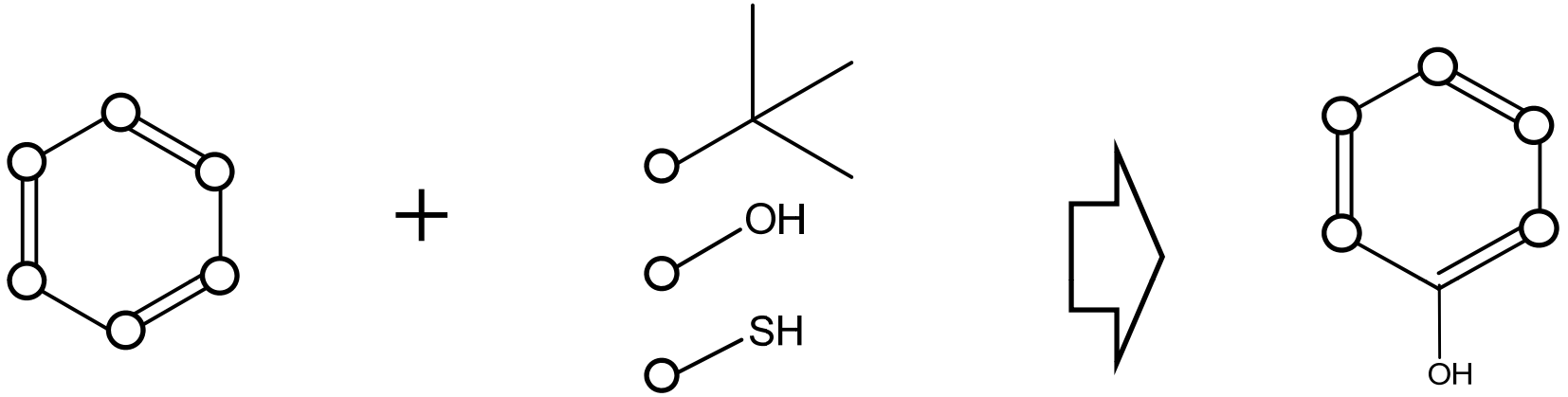






$$sensitivity\left(prop_i @ site_j\right) = \left(\frac{\partial activity}{\partial prop_i @ site_j}\right)_{prop_{k\neq i} @ site_{l\neq j}} \approx \left\langle \frac{\Delta activity}{\Delta prop_i @ site_j} \right\rangle$$

BCL::PHARMMAP – Generation of Chemical Derivatives

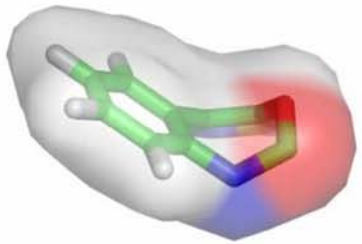


scaffold substituents derivatives

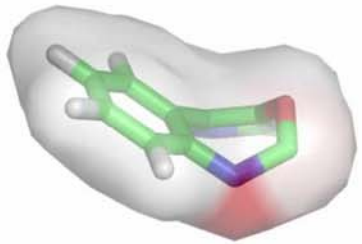
-X				-X-	÷X÷	=X-
-OH	$-C(CH_3)_3$	$-NH_2$	-H	$-CH_2-$	÷CH÷	=CH-
-COOH	$-NH(CH_3)$	$-CH_3$	-F	-NH-	÷N÷	=N-
-CHO	$-N(CH_3)_2$	-Ph	-Cl	-O-		
-SH	$-OCH_3$	$-CH_2CH_3$	-Br	-S-		
$-SCH_3$	$-CH=CH_2$	$-CH(CH_3)_2$	-I	-CO-		

The Benzoxazepine Scaffold

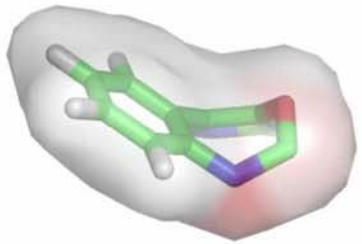
Hbond Donor



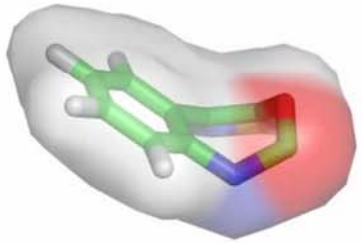
Hbond Acceptor



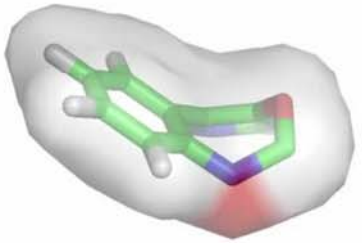
Polarizability



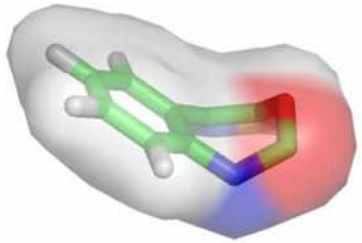
Steric Bulk



Positive Charge

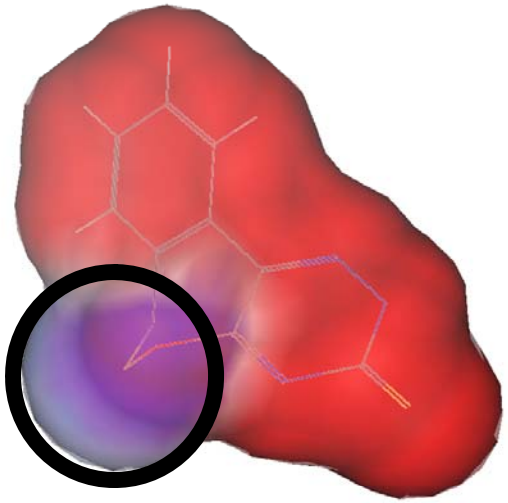


Negative Charge



■ increase ☐ neutral ■ decrease

BCL::PHARMMAP versus CoMFA



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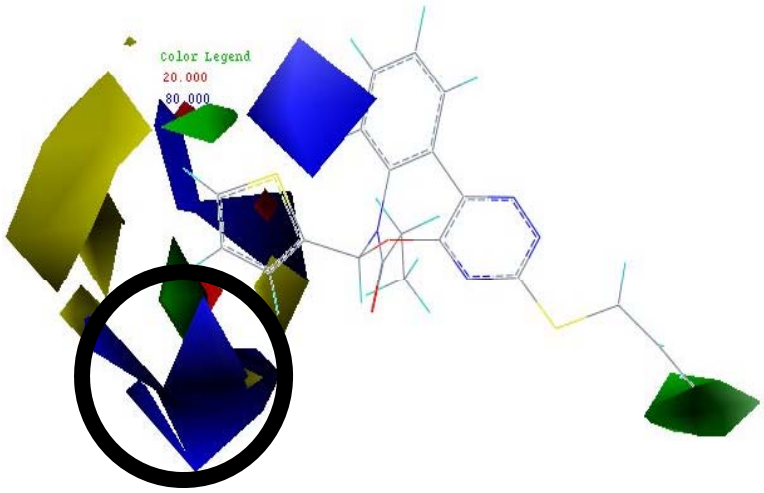
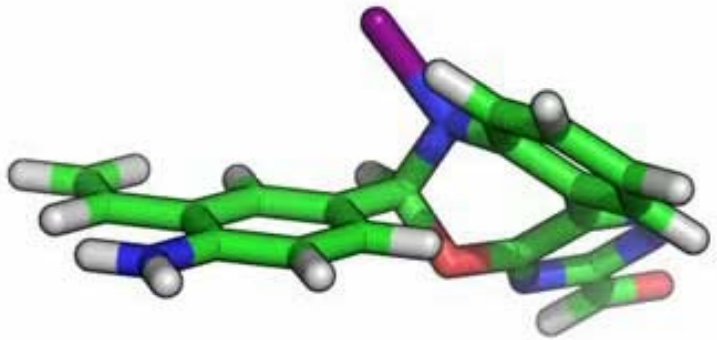


PHARMMAP		COMFA
150,000	# Compounds & biological activities used	118
s-m	Runtime	h-y
NO	Superimposition on common scaffold required?	YES
Charge Bulk Polarizability # H-bond D/A	Physicochemical properties considered	Charge Bulk

Prioritizing Compounds for Chemical Synthesis using 3D SAR



Efficiency: 7.0

EC50 [µM]: 1.33

Outline

- Ligand-Guided Virtual High-Throughput Screening Identifies Allosteric Modulaters of Metabotropic Glutamate Receptors
- BCL::PharmMap: Comprehensive, Rapid, and Robust Pharmacophore Mapping using QSAR Models
- RosettaLigand: 80% Success Rate for Docking into Comparative Models with full Ligand and Protein Flexibility
- RosettaLigand Algorithms for Ligand Ranking and Fragment-Based Drug Design
- QSAR-Derived Pharmacophore Maps Discriminate incorrect Poses in Ligand Docking into Comparative Models

RosettaLigand: Docking with Full Ligand and Receptor Flexibility

Meiler, J.; Baker, D. "ROSETTALIGAND: Protein-small molecule docking with full side-chain flexibility" *Proteins* ***2006, 65, 538-548.***

Kaufmann, K.; Glab, K.; Mueller, R.; Meiler, J. "Small Molecule Rotamers Enable Simultaneous Optimization of Small Molecule and Protein Degrees of Freedom in ROSETTALIGAND Docking" In *German Conference on Bioinformatics; Beyer, A., Schroeder, M., Eds.: Dresden,* ***2008; pp 148-157.***

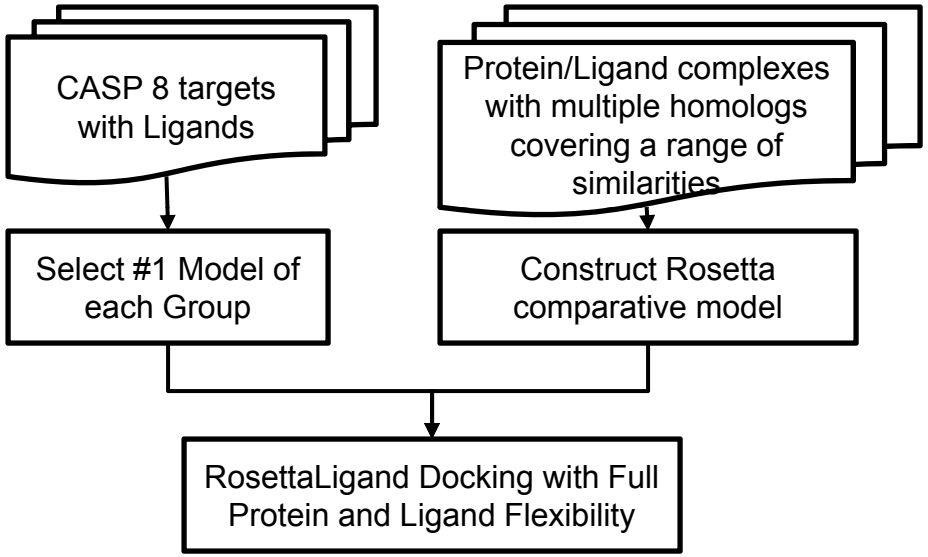
Davis, I. W.; Baker, D. "RosettaLigand docking with full ligand and receptor flexibility" *J Mol Biol* ***2009, 385, 381-92.***

Davis, I. W.; Raha, K.; Head, M. S.; Baker, D. "Blind docking of pharmaceutically relevant compounds using RosettaLigand" *Protein Sci* ***2009, 18, 1998-2002.***

Kaufmann, K. W.; Dawson, E. S.; Henry, L. K.; Field, J. R.; Blakely, R. D.; Meiler, J. "Structural determinants of species-selective substrate recognition in human and Drosophila serotonin transporters revealed through computational docking studies" *Proteins* ***2009, 74, 630-42.***

Docking to Comparative Models is Successful in 80% of Cases

- Flowchart



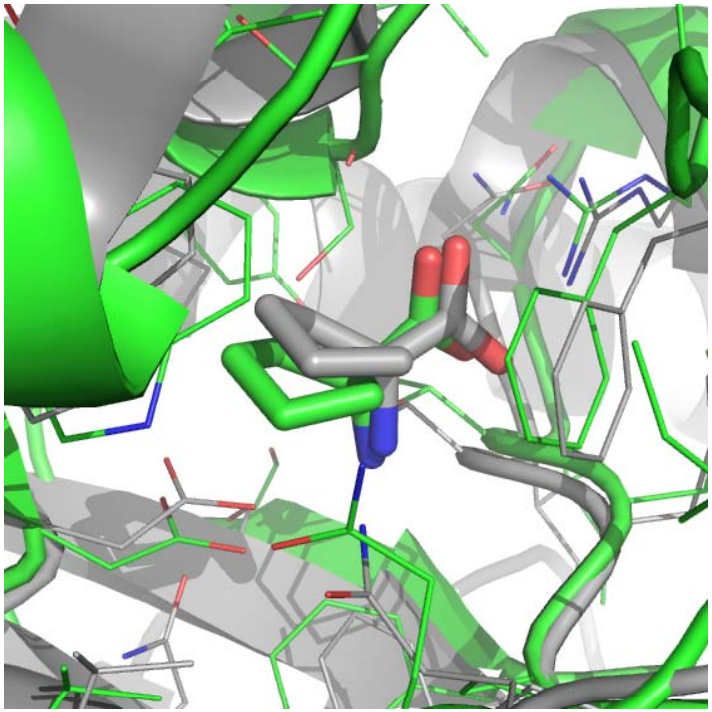
- Docking to Rosetta Comparative Models succeeds for at least one comparative model in 18 of 21 cases
- Docking to CASP models succeeds in 7 of 9 cases

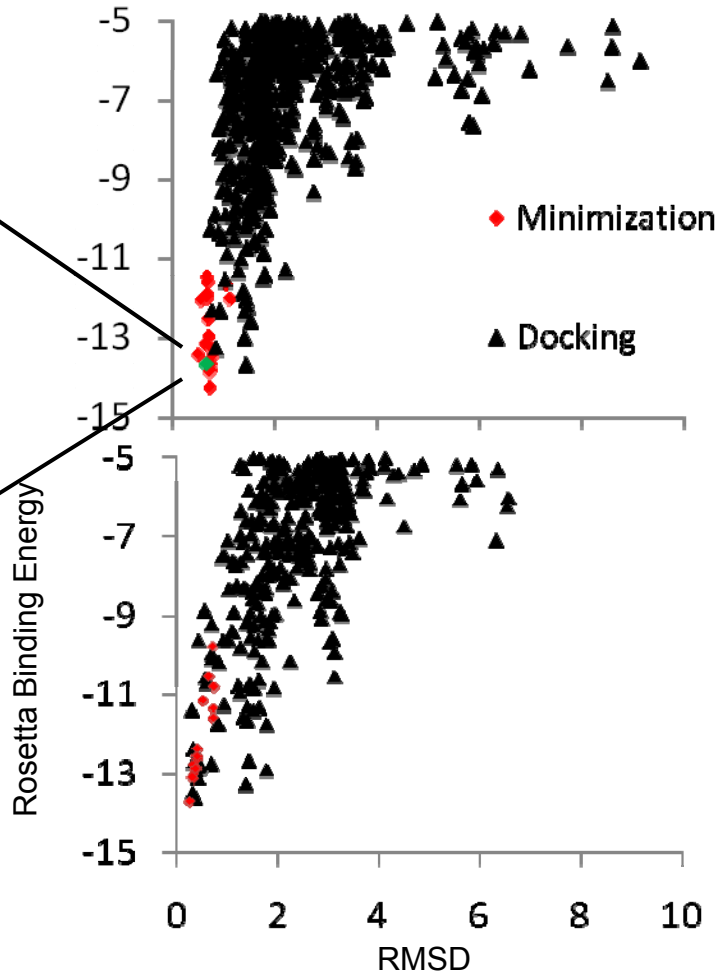
Rosetta Models	RMSD < 2.5 Å in top 10
2AYR	1/3
2FAI	2/3
2B1V	2/3
1FD0	2/4
1FCX	1/4
1FCZ	0/4
1Y1M	5/5
1PBQ	2/5
1PB9	4/5
2QWE	2/3
2QWD	2/3
2QWB	2/3
1TSY	0/3
1NJE	2/3
1NJA	1/3
1V48	2/3

Rosetta Models	RMSD < 2.5 Å in top 10
1B8O	1/3
1VFN	3/3
1SQA	0/2
1O3P	1/2
1F5K	1/2

CASP Models	
3D8B	0/(N/A)
3DLZ	1/(N/A)
3DAO	0/(N/A)
3DA1	1/(N/A)
3DKP	1/(N/A)
3DLS	1/(N/A)
3DLC	1/(N/A)
3DME	1/(N/A)
3DOU	1/(N/A)

Comparative Models Show Similar Binding Funnels to X-ray Structures

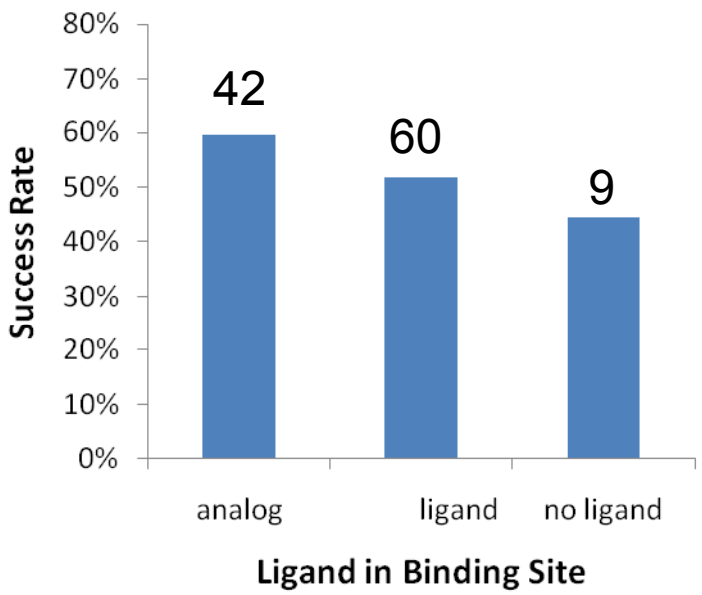


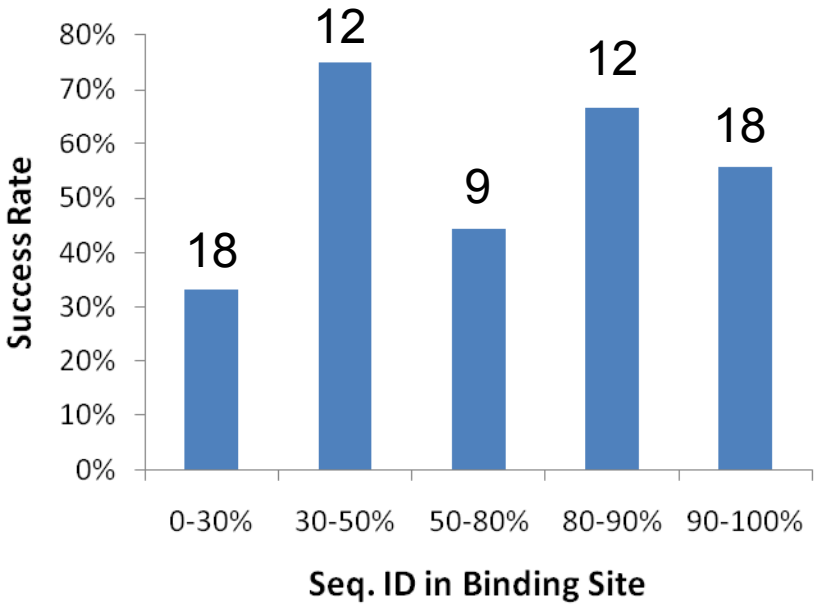


- Proline(1Y1M) in model (2RC7)
- 33% Seq ID. / 36% in Binding Site
- Ligand RMSD 0.67 Å
- Interface RMSD 1.63 Å

Success Rate is Largely Independent From Sequence Similarity

- Success Rate improves if ligand is bound in template
- Success rate is independent from sequence similarity





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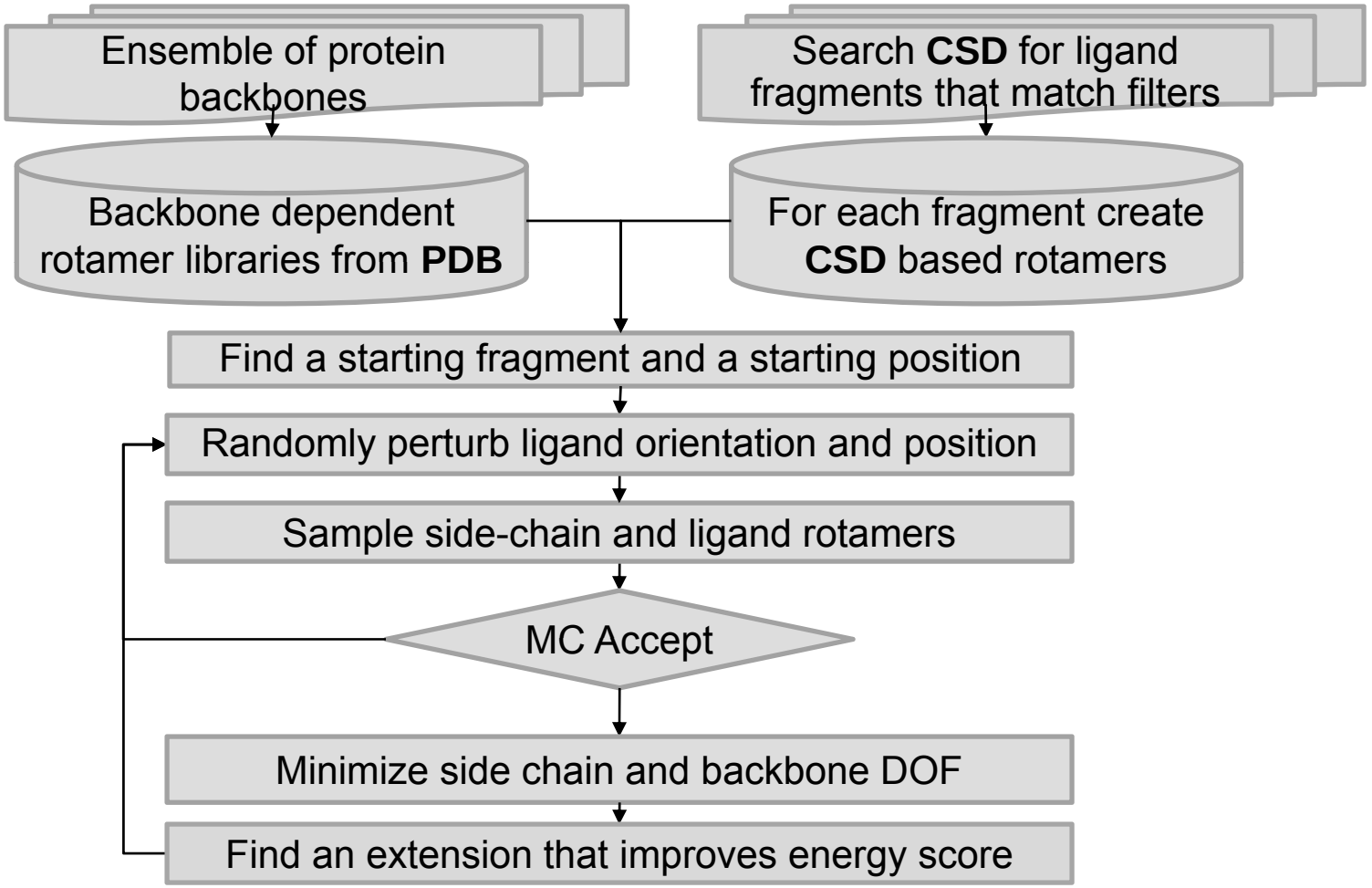
Computer-Aided Drug Design in Rosetta



Ligand Design using Rosetta Scripts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  <AddHydrogens name=add_h chain=X/>
compound movers
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    <Add mover name=grow/>
    <Add mover_name=translate/>
    <Add mover_name=rotate/>
    <Add mover_name=slide_together/>
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  <LoopOver name=grow_loop mover_name=low_res_dock filter_name=both/>
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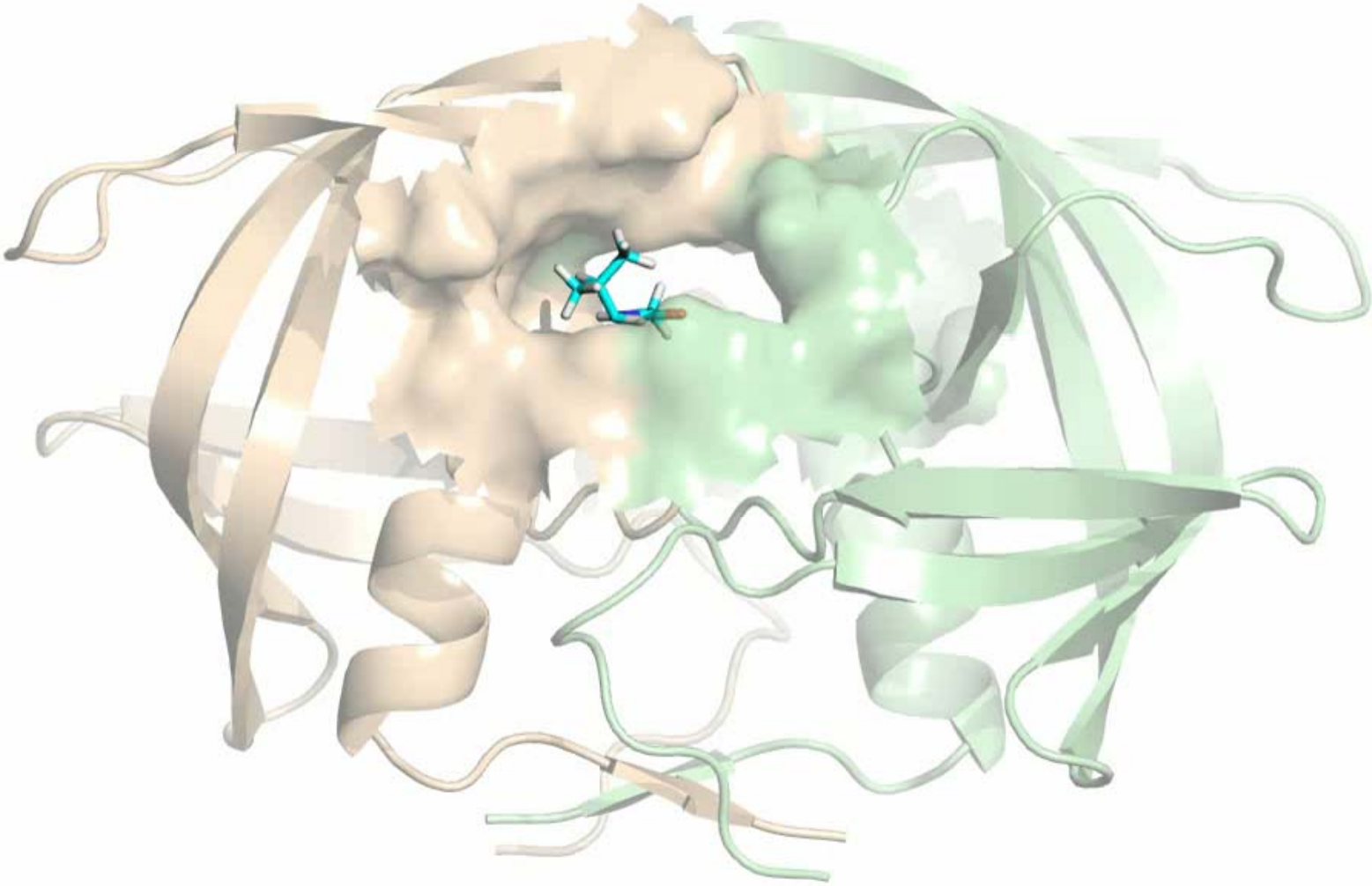

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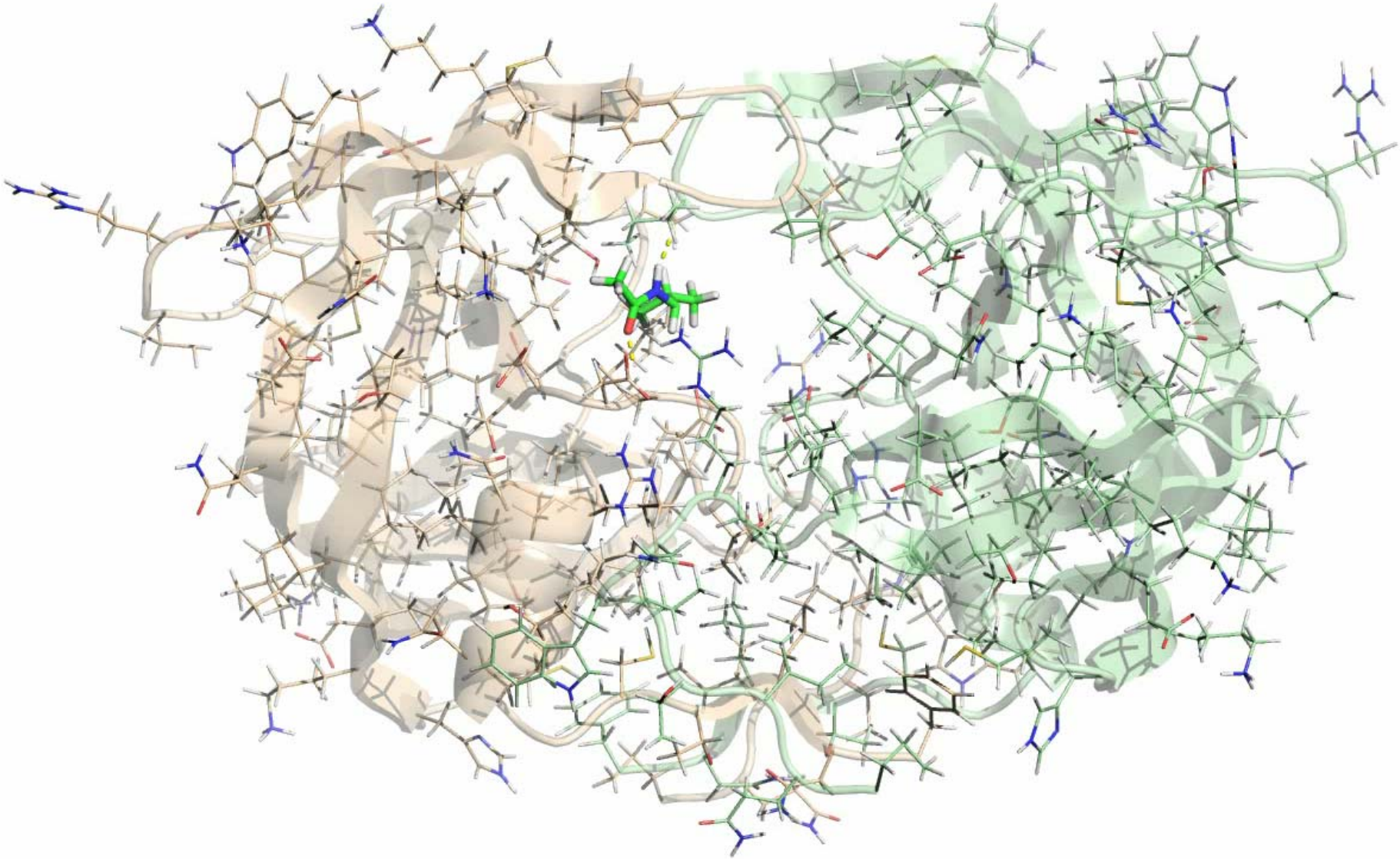

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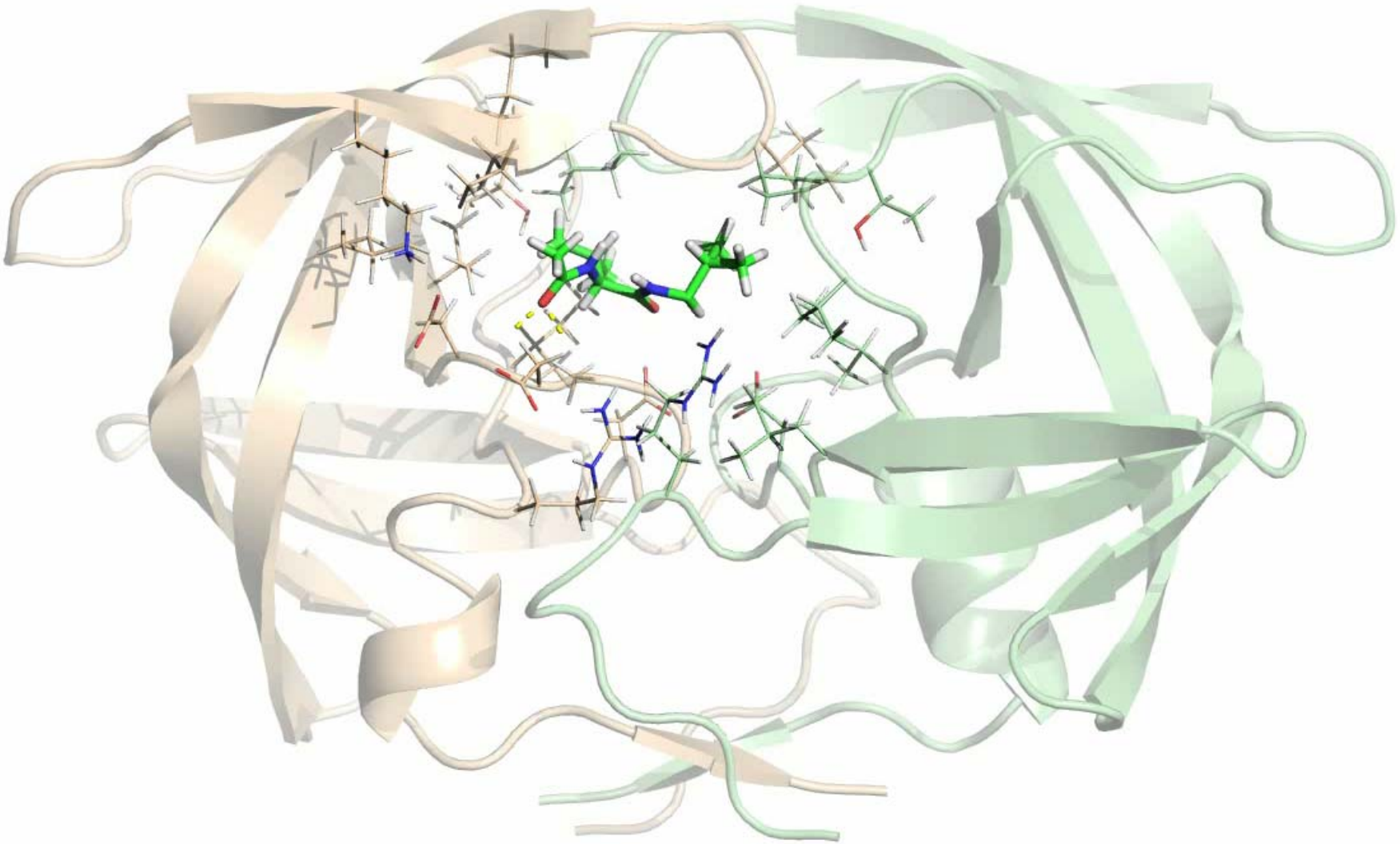
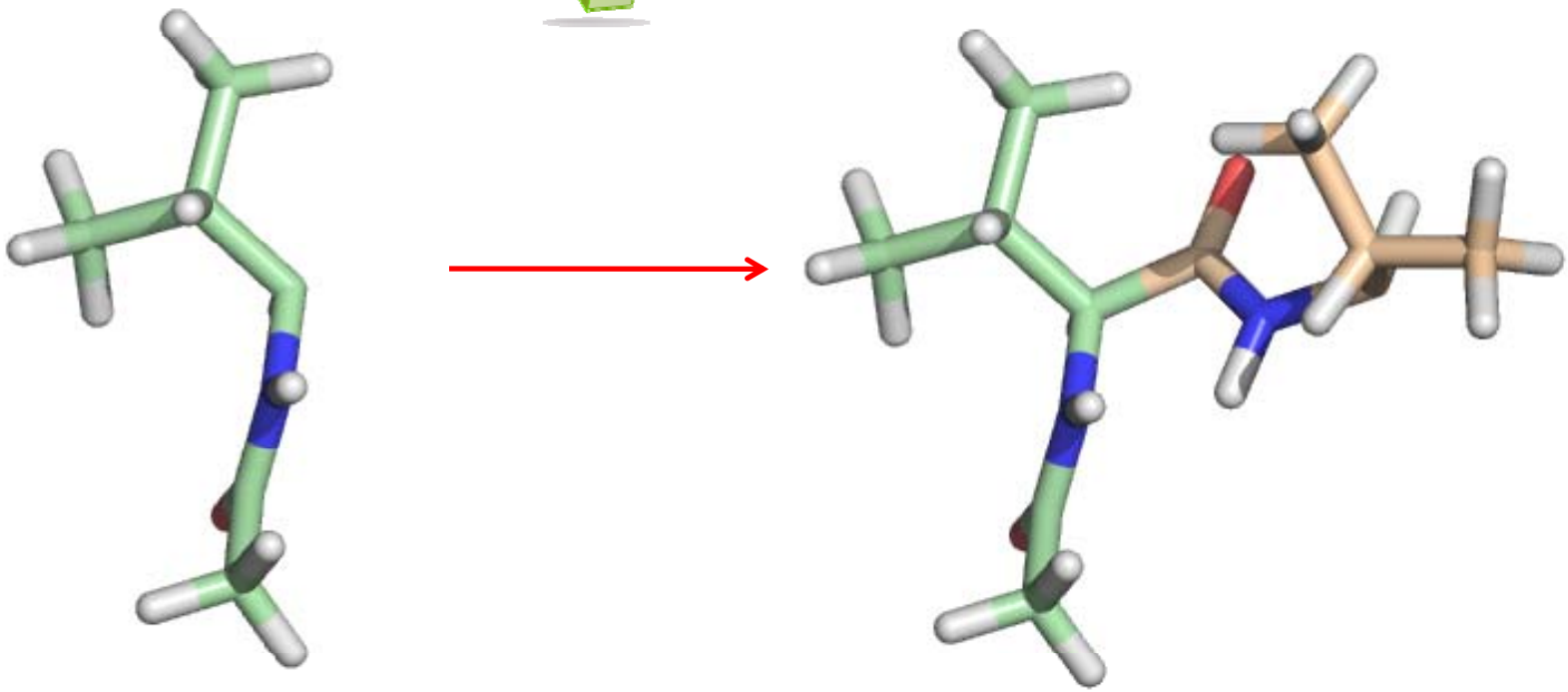


Fragment Extension 1 is Accepted as Predicted Binding Energy Increases

-7.20

-9.31



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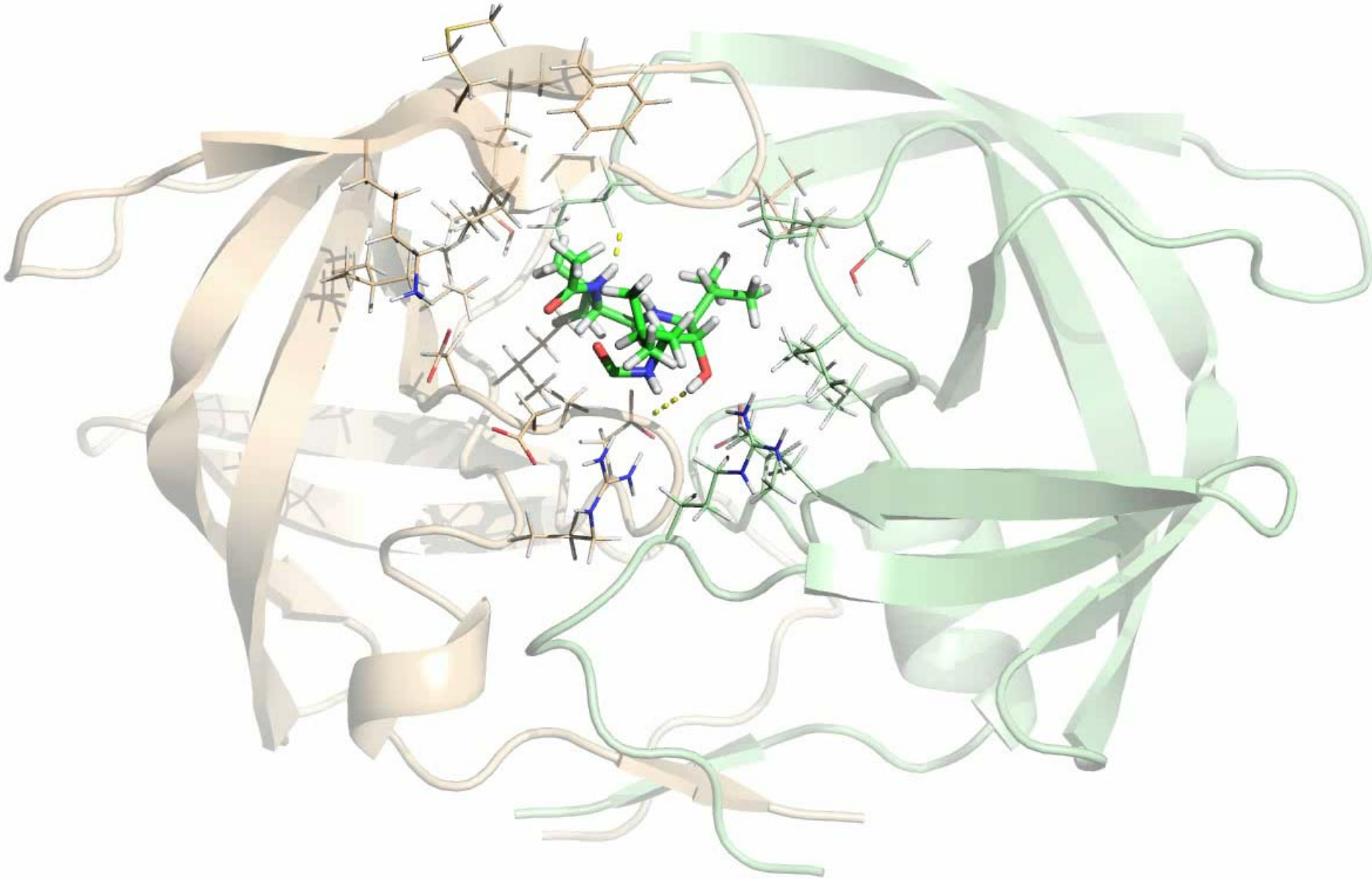
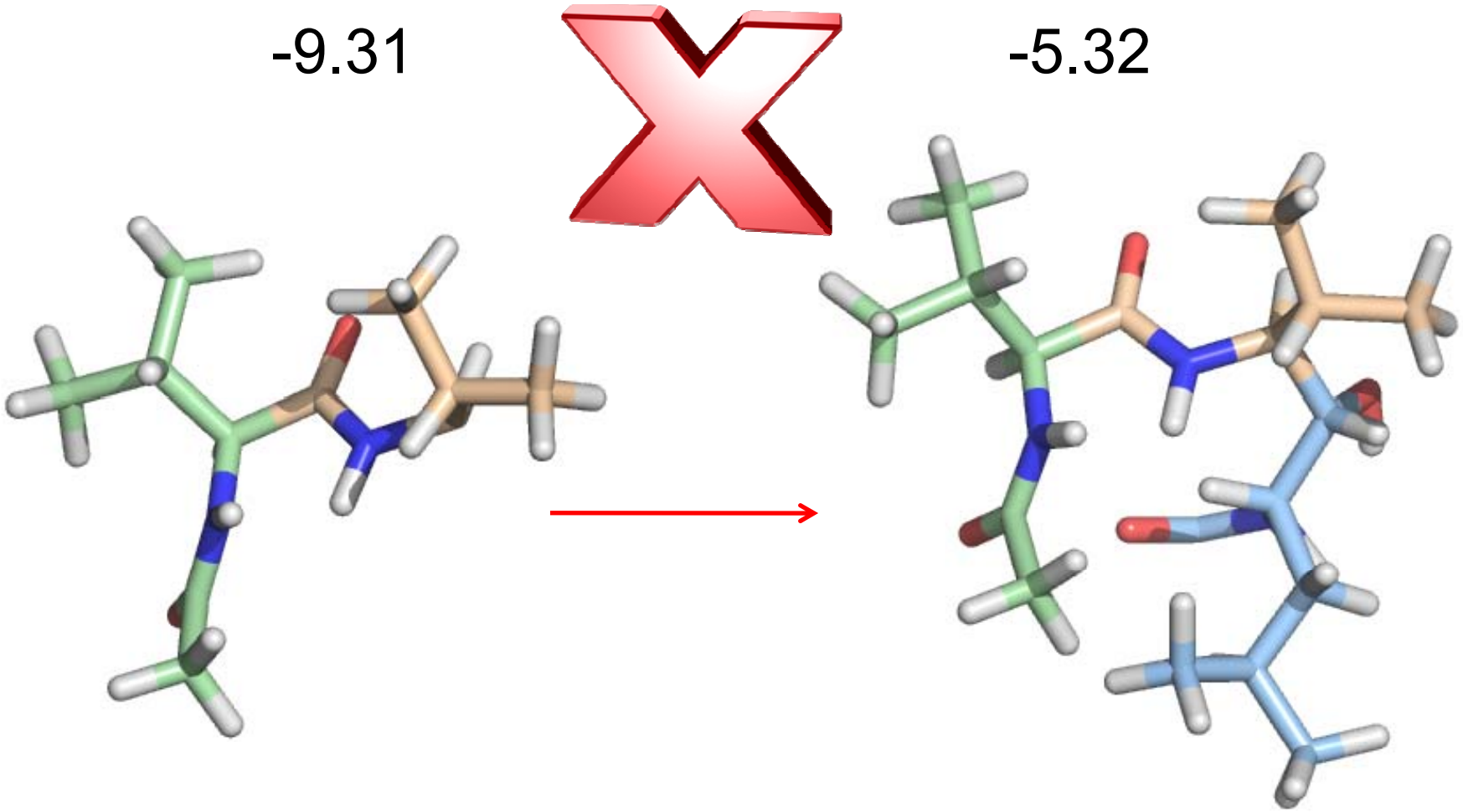


Fragment Extension 2 is Rejected as Predicted Binding Energy Decreases



www.rosettacommons.org

- Rosetta consists of multiple modules: protein folding, comparative modeling, ligand docking, protein design, antibody/antigen interactions, etc.
- Rosetta is developed in a consortium of twelve laboratories by around 50 developers
- Rosetta is free for academic us; user guide and tutorials are available
- PyRosetta is a python interface that allows integration with Pymol
- FoldIt is the better video game for you and your kids
- Rosetta@home uses your computer for our research

RosettaCon 2009, Leavenworth, WA, USA

Kaufmann, K. W.; et al. "Practically Useful: What the Rosetta Protein Modeling Suite Can Do for You" *Biochemistry* ***2010.***

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Amyloid β-Peptide-Binding Alcohol Dehydrogenase (ABAD) Inhibitors

- Inhibitor bound crystal structure (PDB ID:1U7T)
- High-throughput screening data (PubChem ID: 893)

J Mol Biol. 2004 Sep 17;342(3):943-52.

Crystal structure of human ABAD/HSD10 with a bound inhibitor: implications for design of Alzheimer's disease therapeutics.

Kissinger CR, Rejto PA, Pelletier LA, Thomson JA, Showalter RE, Abreo MA, Agree CS, Margosiak S, Meng JJ, Aust RM, Vanderpool D, Li B, Tempczyk-Russell A, Villafranca JE.

Pfizer-La Jolla, 10777 Science Center Dr., San Diego, CA 92121, USA.

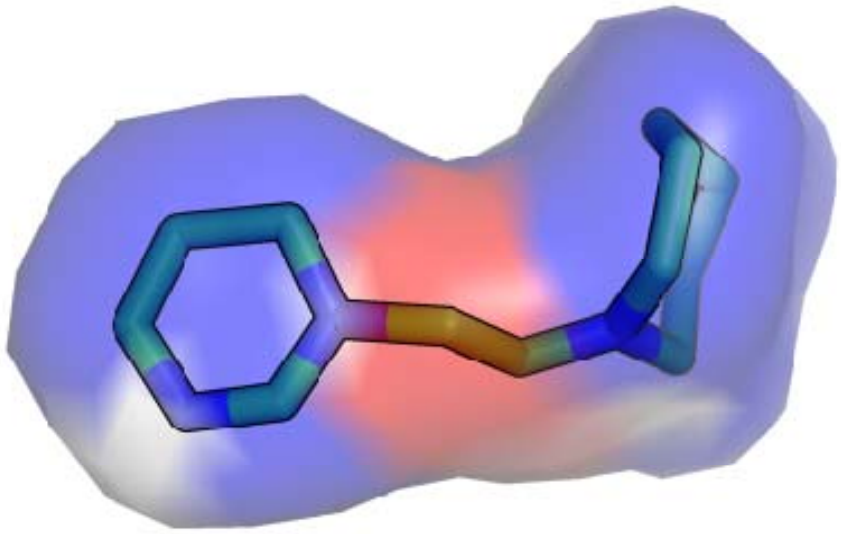
Abstract

The enzyme 17beta-hydroxysteroid dehydrogenase type 10 (HSD10), also known as amyloid beta-peptide-binding alcohol dehydrogenase (ABAD), has been implicated in the development of Alzheimer's disease. This protein, a member of the short-chain dehydrogenase/reductase family of enzymes, has been shown to bind beta-amyloid and to participate in beta-amyloid neurotoxicity. We have determined the crystal structure of human ABAD/HSD10 complexed with NAD(+) and an inhibitory small molecule. The inhibitor occupies the substrate-binding site and forms a covalent adduct with the NAD(+) cofactor. The crystal structure provides a basis for the design of potent, highly specific ABAD/HSD10 inhibitors with potential application in the treatment of Alzheimer's disease.

PMID: 15342248 [PubMed - indexed for MEDLINE]

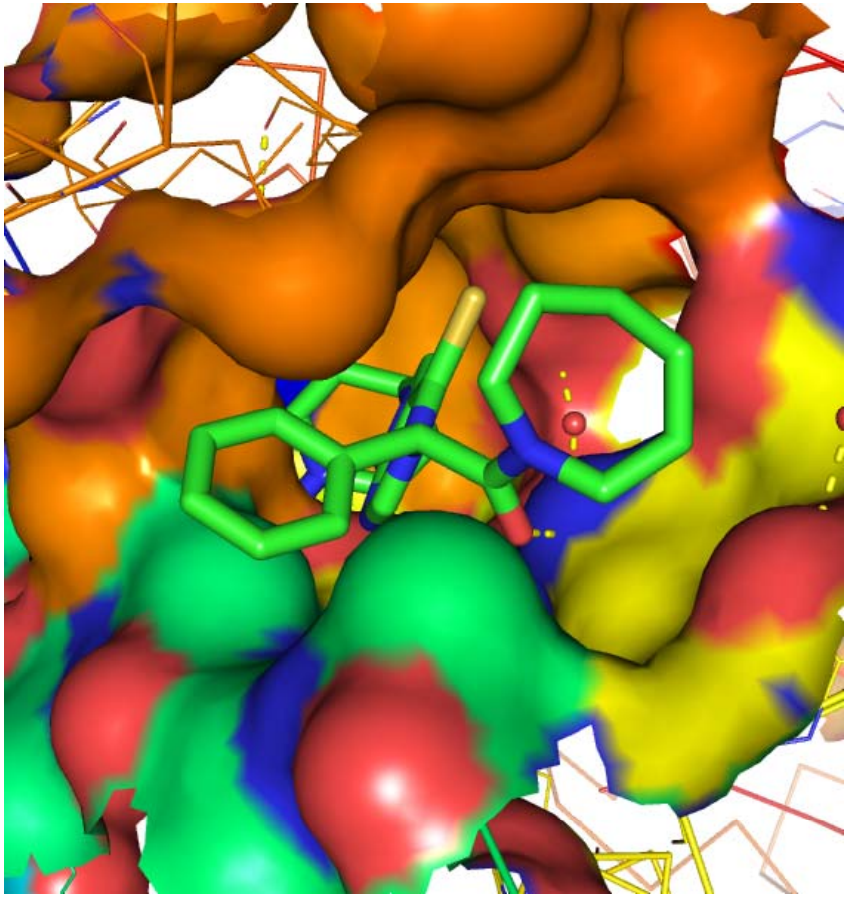
PharmMap Predictions Match Experimental Data

- PharmMap for steric bulk

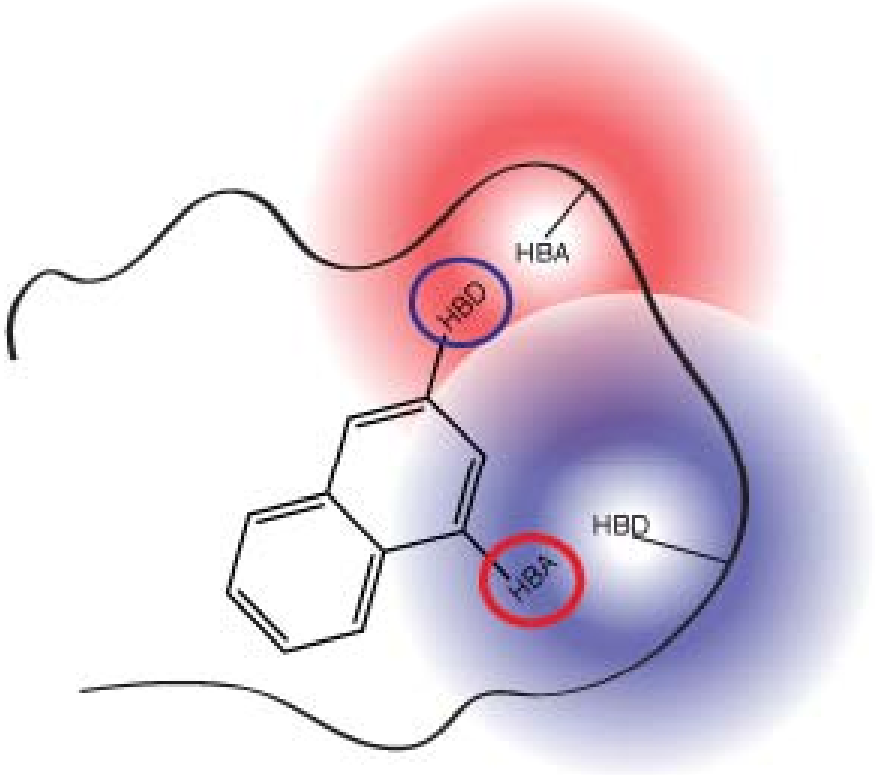


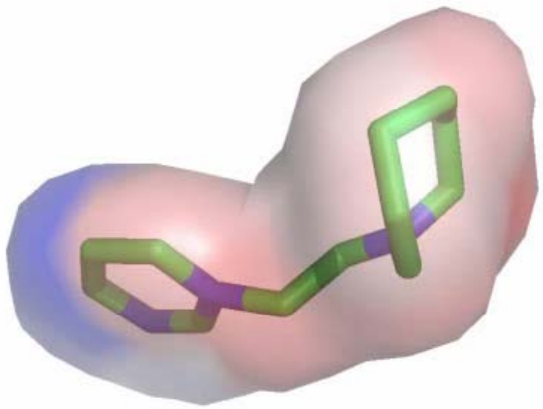
increase neutral decrease

- Co-crystallized Inhibitor



Pseudo-Potential Scores Agreement of Pharmacophore Map with Docking

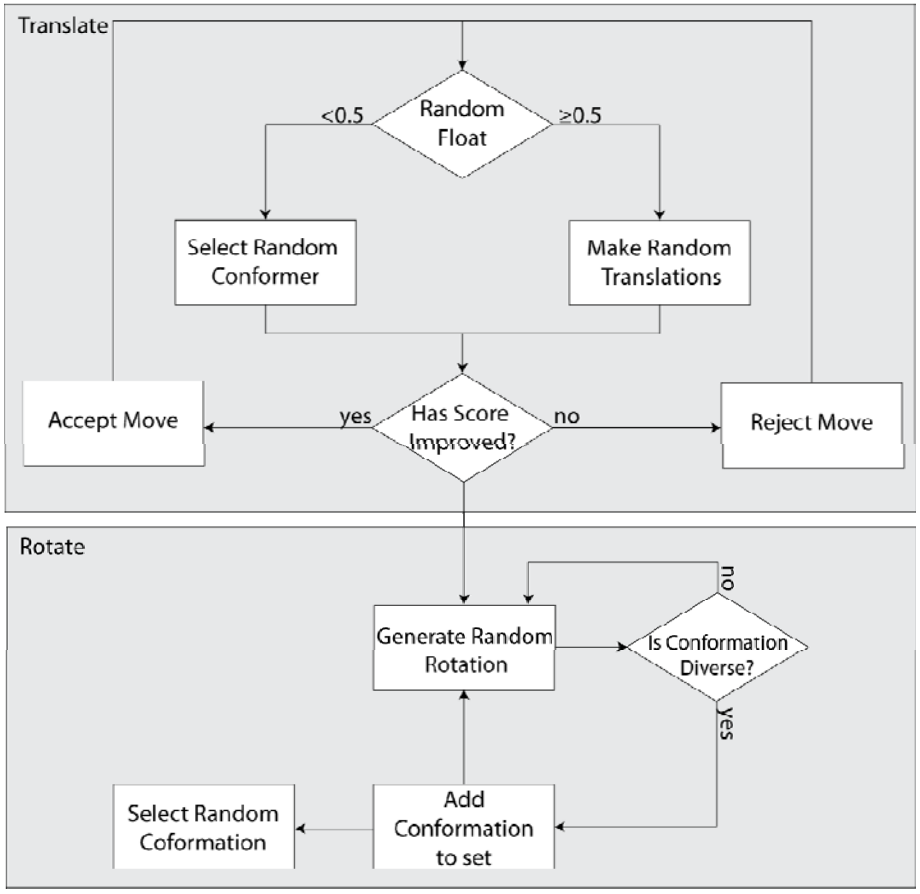




- Attractive interaction between sites in pharmacophore map that prefer addition of hBond acceptors (red) and hBond donor sites in the protein

Scoring Terms are Implemented in RosettaLigand Centroid Docking

- Additional grids store information about H-bond acceptors and H-bond donors, charge, and polarizability in the protein
- These grids are precalculated enabling rapid scoring needed for virtual screening
- The low-resolution sampling is enhanced to allow translations and rotations to improve original shape complementary score in addition to pharmmap scores



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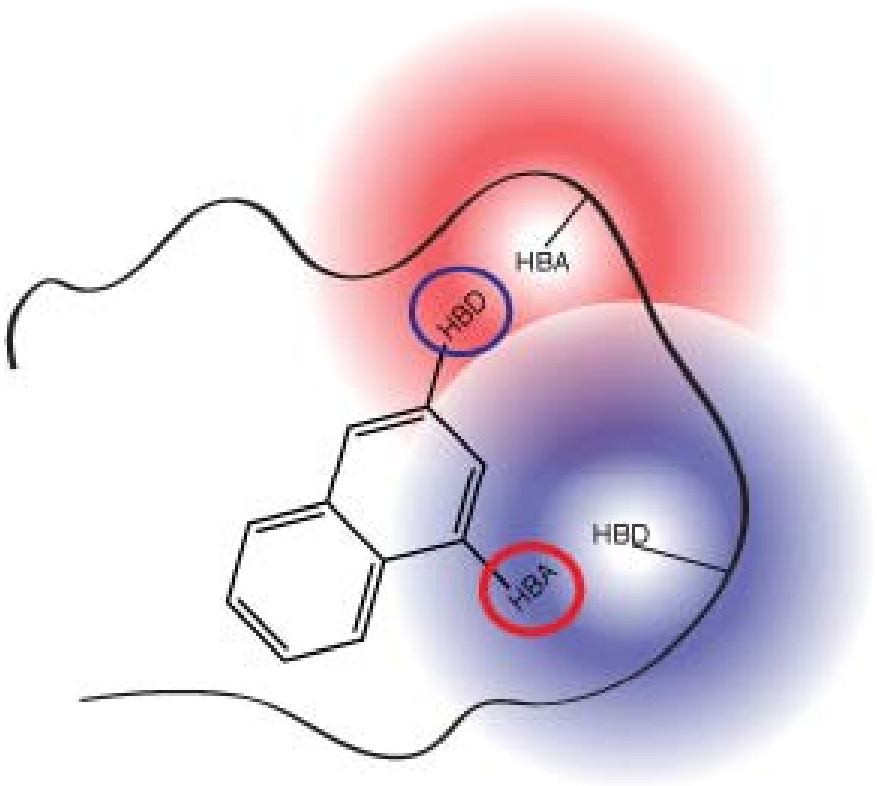

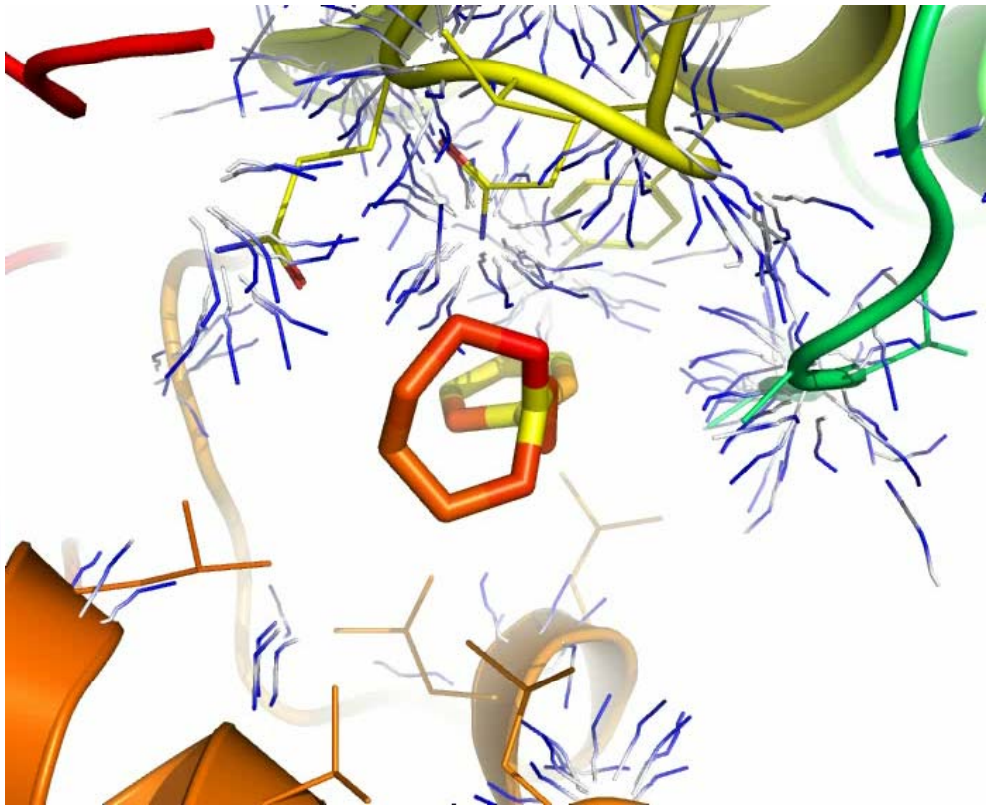

- Attractive interaction between sites in pharmacophore map that prefer addition of hBond acceptors (red) and protein hBond donor sites (blue)

Conclusion

- quantitative 3D SAR models enable virtual high-throughput screening of external substance databases to prioritize for acquisition. Novel chemotypes are detected.
- pharmacophore maps derived from 3D SAR models guide hit-to-lead optimization by prioritizing synthesis.
- 80% success rate for RosettaLigand docking into comparative models with full side chain and backbone flexibility.
- Coming next: RosettaLigand fragment-based drug design and pharmacophore maps as docking restraints.

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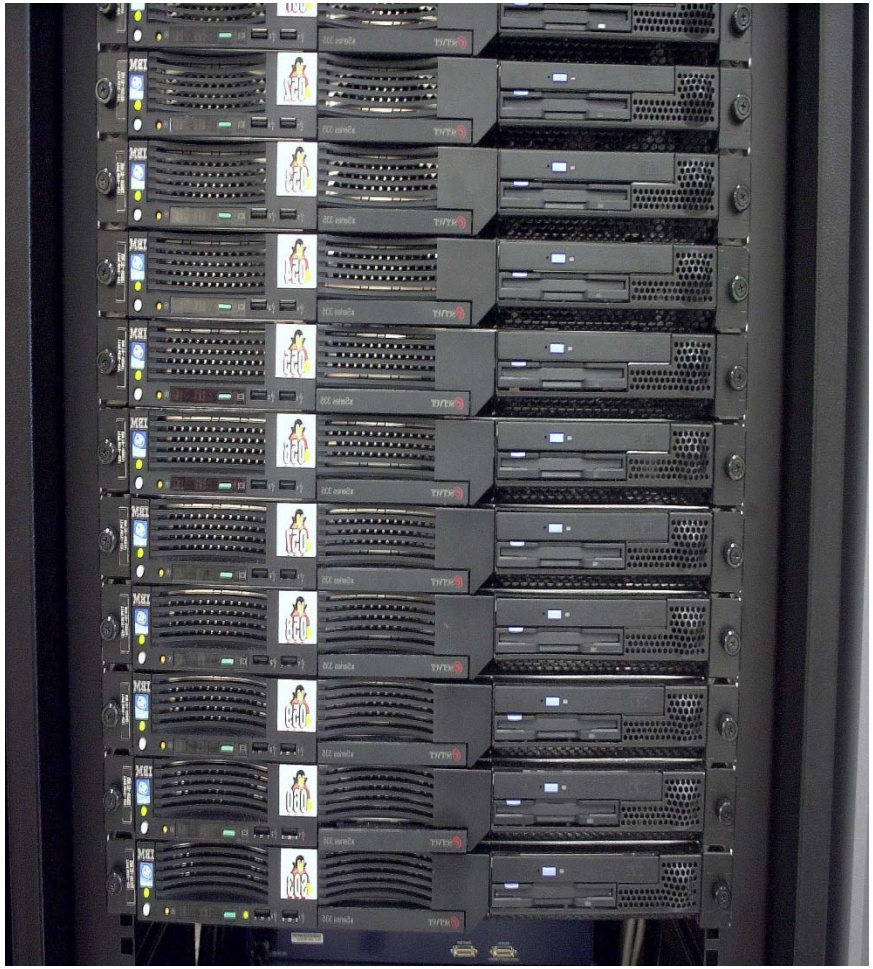

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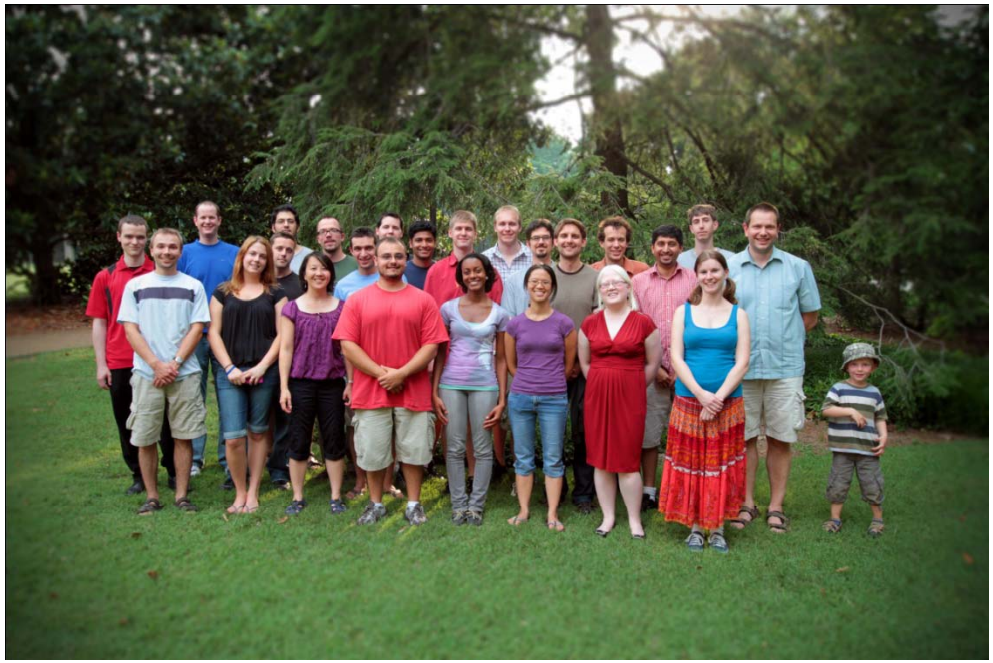

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