Beyond the Happy Water – recent advances in the quantification of contributions

Benjamin Tehan

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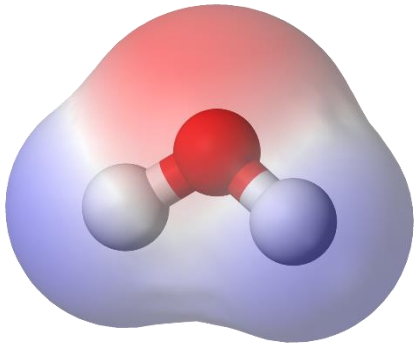
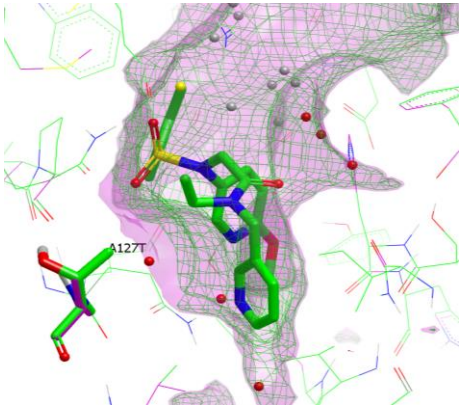
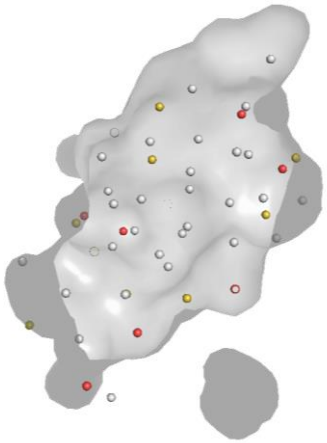
Water

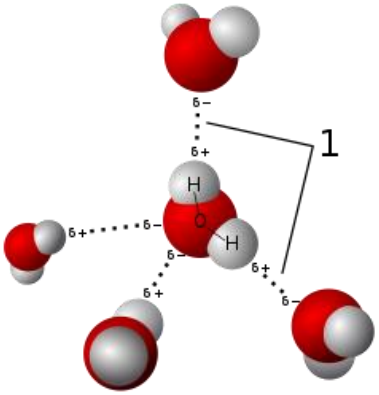




selectivity



SAR issues



quantifying

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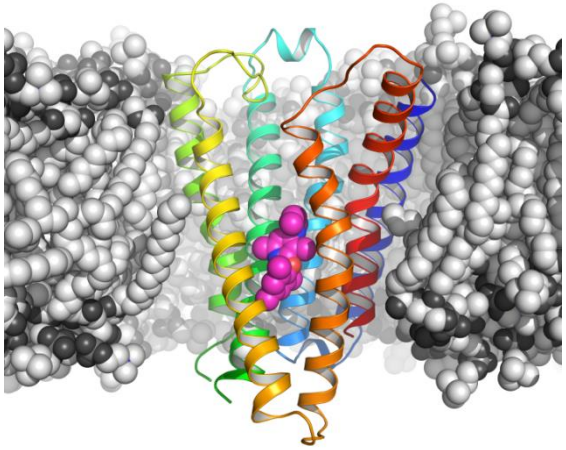
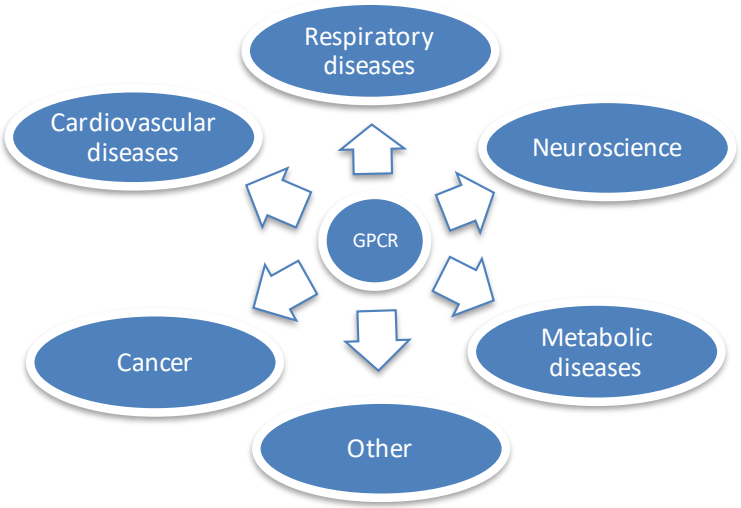
G Protein-Coupled Receptors

- Arguably the most important family of drug targets in industry
  - Source of approximately 30-40% approved drugs
  - 375 GPCRs in human genome (225 with known ligands, 150 orphan targets)
- Clinical validation and compelling biology across wide range of diseases





Respiratory diseases	Neuroscience	Cardiovascular diseases
Advair®	Zyprexa®	Opsumit®
Zyrtec®	Abilify®	Diovan®
Breo™ Ellipta™	Seroquel®	Benicar®
Anoro®	Suboxone®	Tracleer®
Seebri®		Zioptan™
Ultibro®	**Metabolic diseases**	Plavix®
Ventolin® HFA	Belviq®	
Singulair®	Byetta®	
Spiriva®	Myrbetriq®	**Cancer**
Tudorza® Pressair®	Signifor®	Erivedge®

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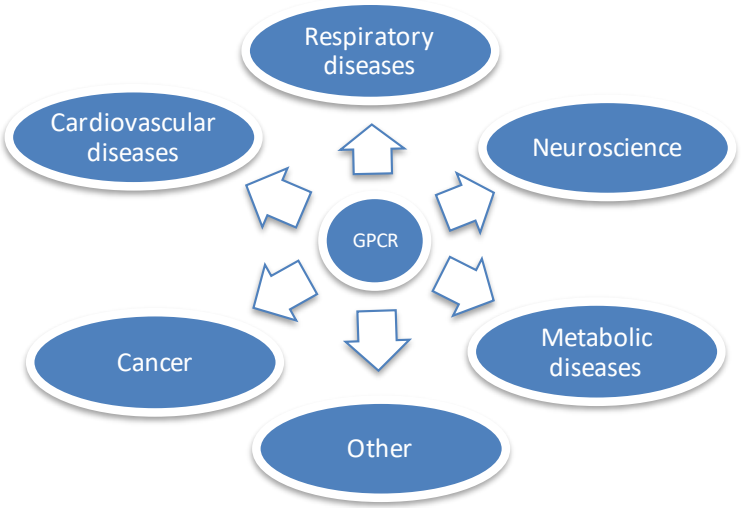
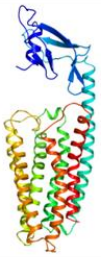
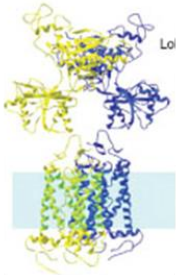


- Many therapeutically valuable yet challenging targets remain untapped

	Class A Rhodopsin	Class B Secretin	Class C Metabotropic
			
Liganded	187	22	15
Orphan	97	0	8
Drugged NCE	64	0	2
Drugged biologic	15	8	1

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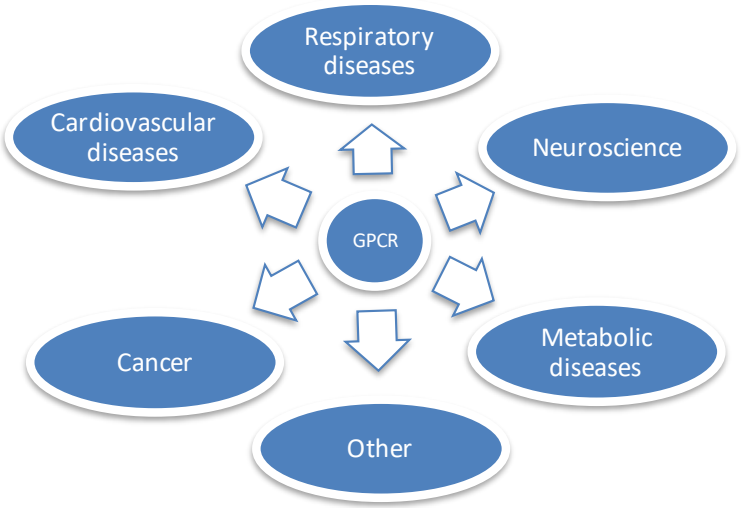

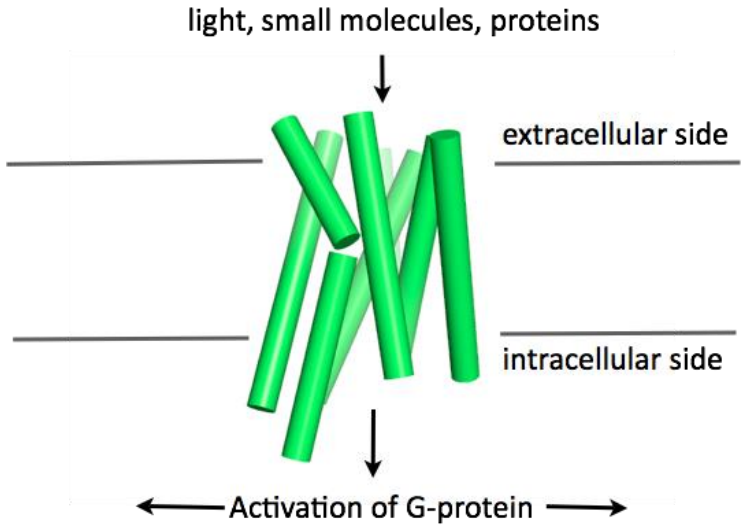

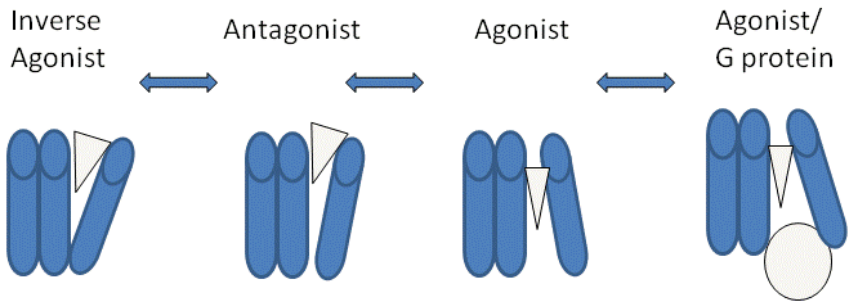

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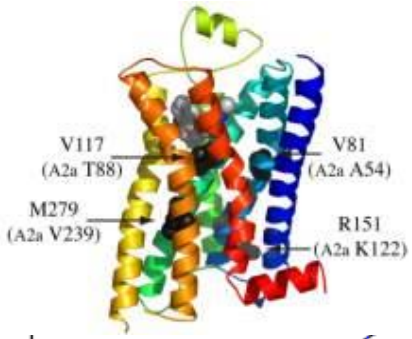
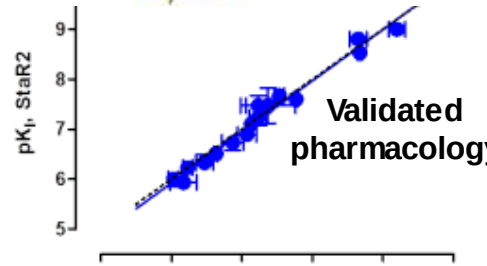
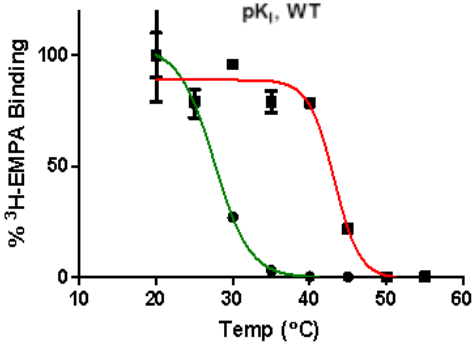
Heptares Technology Platform

Stabilised Receptor (StaR®)





Validated pharmacology

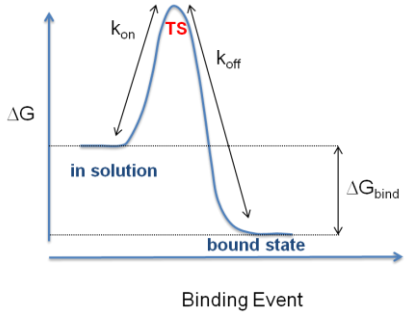


Increased stability

GPCR Structure Determination

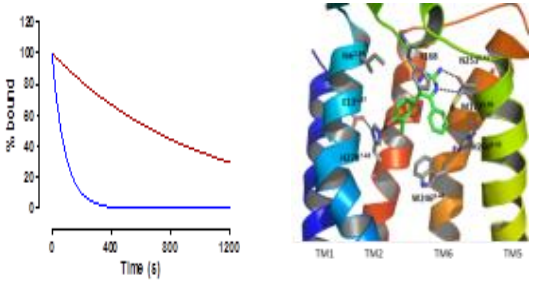
Landmark structure reveals new drug binding site in Family B GPCRs

GPCR Thermodynamics



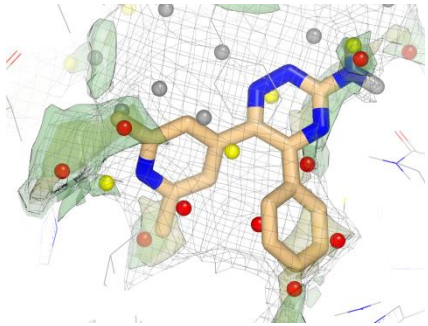
Understanding Entropy and Enthalpy

Controlling Receptor Kinetics



Drug kinetics by StaR® using SPR related to X-ray crystal structures

Computational Modelling



Computational modelling of protein, ligand and water interactions

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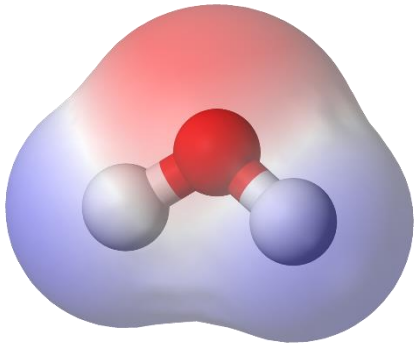
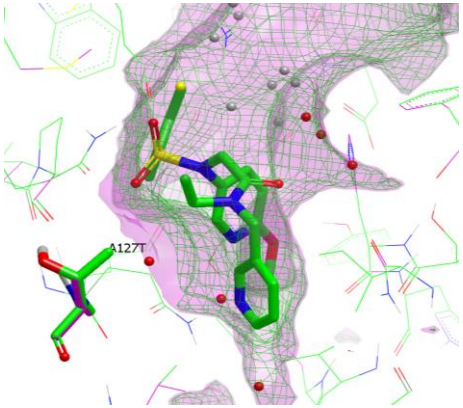
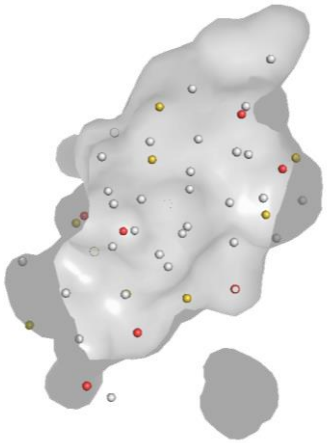
Water

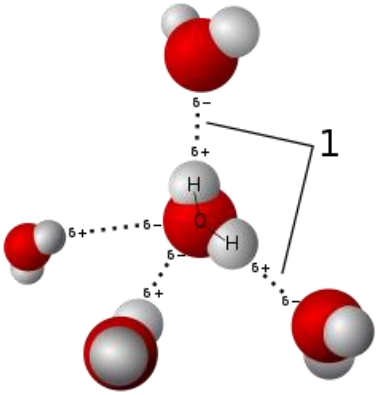




selectivity



SAR issues



quantifying

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The Role of Water

- Water molecules play an essential role in the structure and function of biological systems

  - Displacement of waters from a binding site is a key component of ligand binding, with significant binding energy, and thus potency, often from the entropic gain of the displacement

  - Stabilisation or Destabilisation of waters or water networks within a binding site can also enable control of activity, selectivity and even function of a receptor

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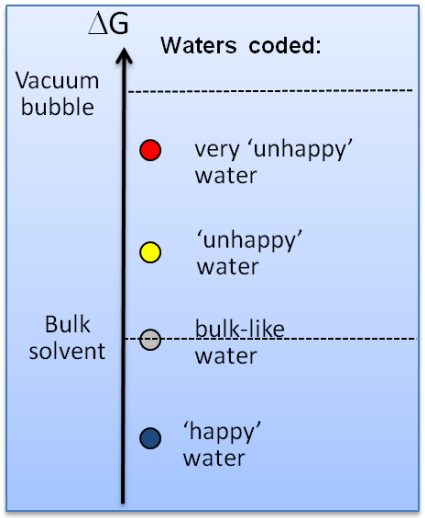
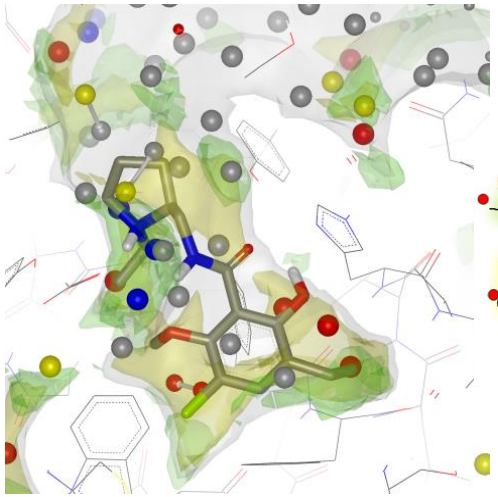
The Role of Water



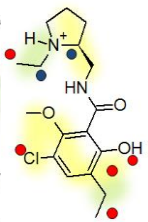
GRID C1= hot-spot (hydrophobic)

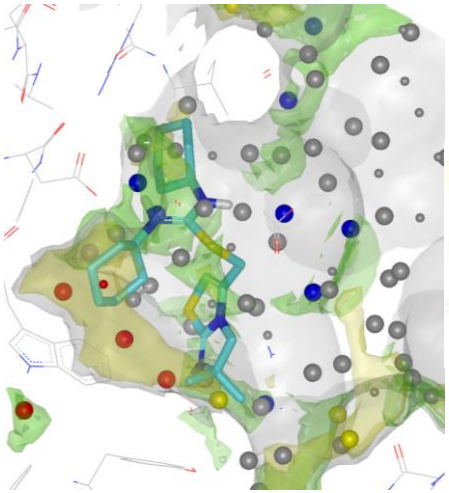
GRID OH2 hot-spot (hydrophilic)

GRID C3 pocket shape

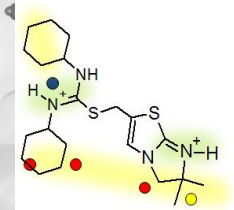


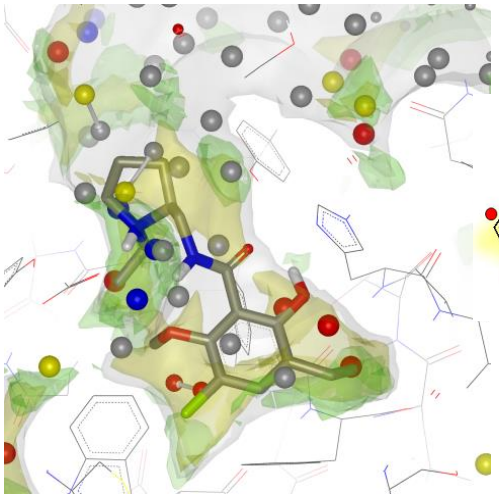
D_3





CXCR4





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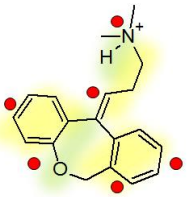

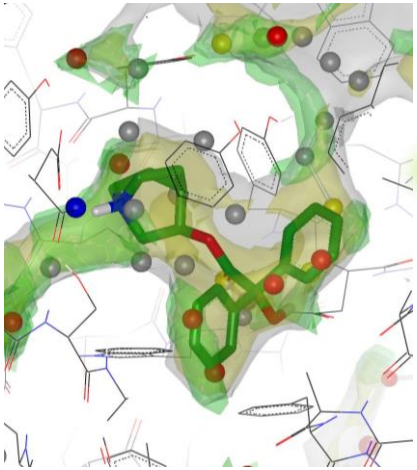

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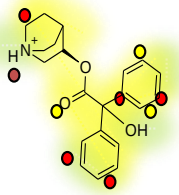

Marshall et al. TIPS *2012;33(5):249-60.*

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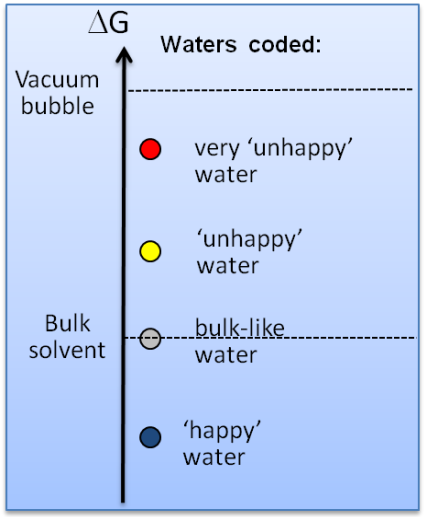
The Role of Water



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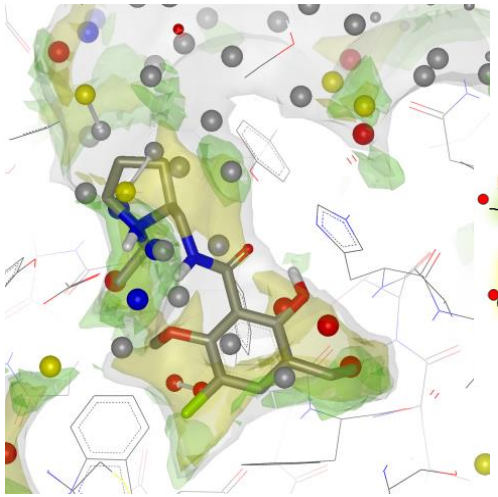
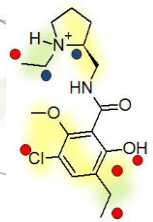
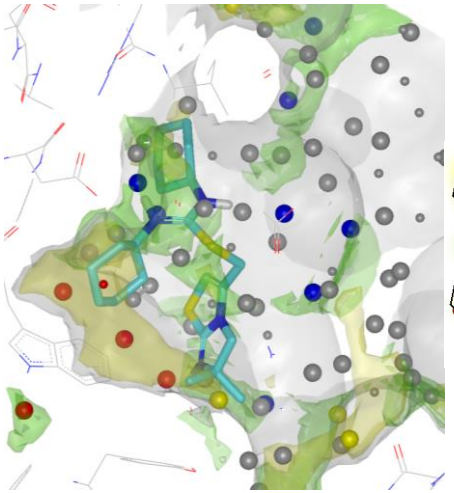
GRID C3 pocket shape



D_3





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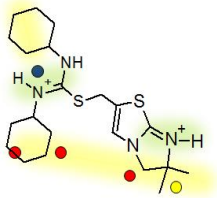

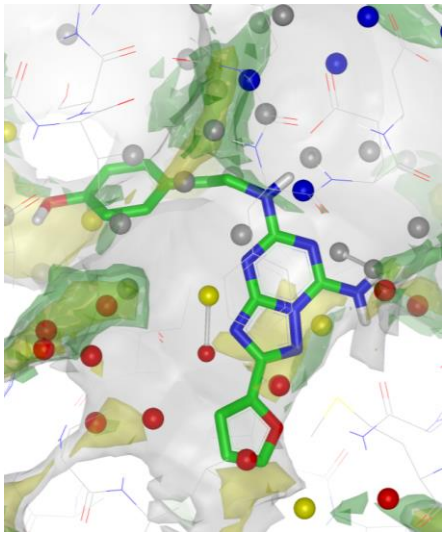

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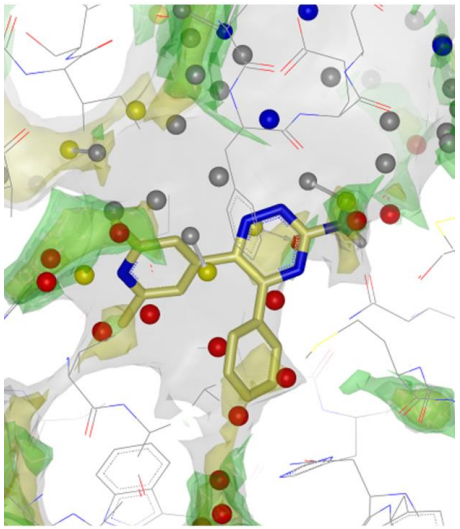

Congreve et al. JMC *2012; 55:1898-1903*

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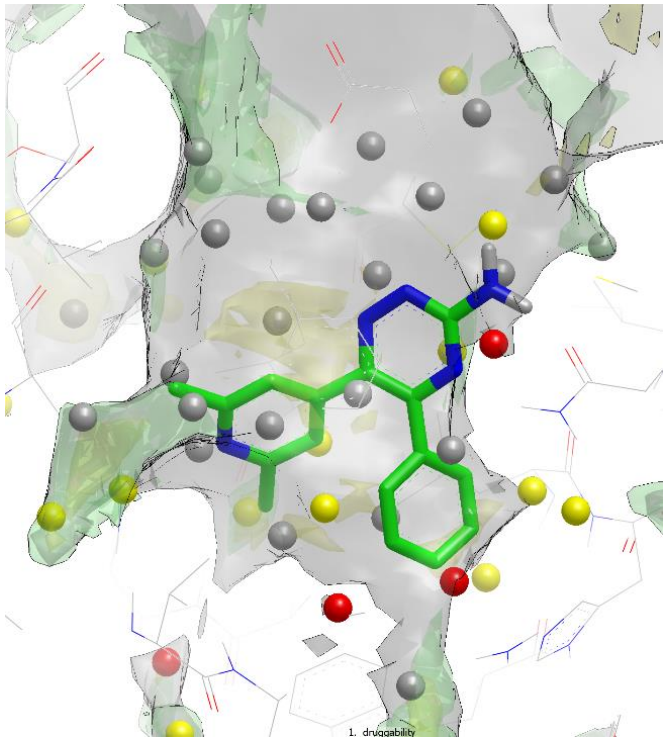
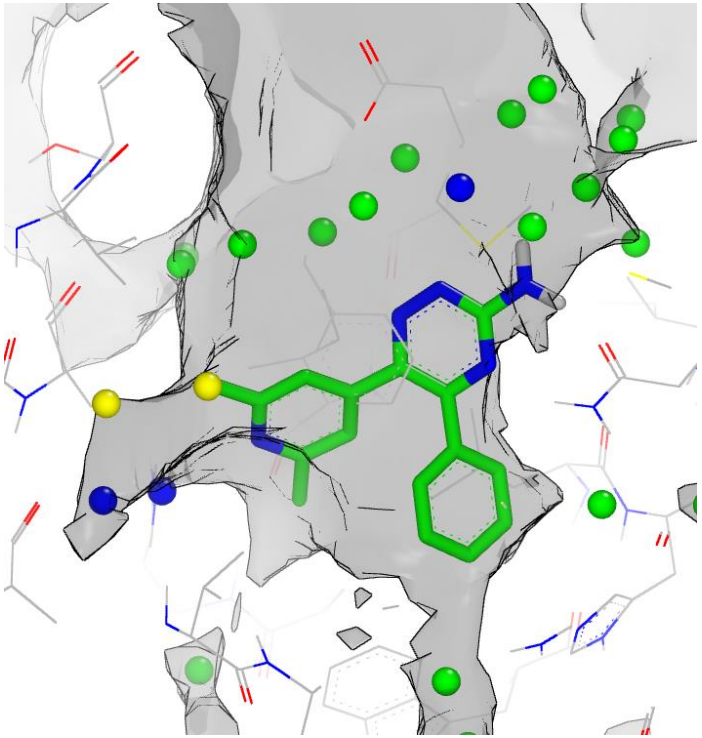
The Role of Water

- Pseudo-apo water network
  - (energetically coded: red & yellow unhappy)
- Triazine ligand complex
  - Perturbed water network
  - effect of pyridine





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Predicting Water Network Energetics

- WaterMap (Molecular Dynamics based, Schrödinger) & WaterFLAP (GRID based, Molecular Discovery) are complementary methods to predict water networks & estimate energy relative to bulk water

- WaterMap is a powerful physics-based method using molecular dynamics (MD) simulations with explicit waters, particularly useful in apo/open structures
  - GPU implementation allows calculation in < 1hour
  - GCMC issues appear to be resolved

- WaterFLAP is a rapid empirical approach using GRID energies of water, CRY lipophilic/hydrophobic probe and entropy estimation, particularly useful in ligand complexes with occluded regions with trapped waters
  - Waters placed in GRID water probe hotspots until no space remaining, can be used to seed MD (WaterMap, FEP+ etc)
  - NEW - rapid prediction of $\Delta\Delta G$ of waters not displaced by a ligand when docked (based on optimization of remaining waters from apo network)

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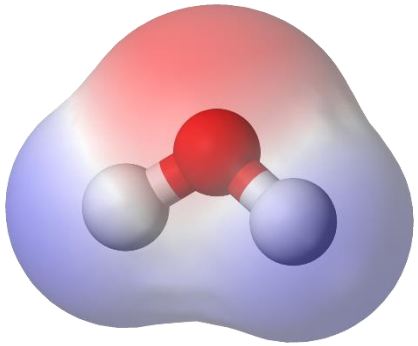
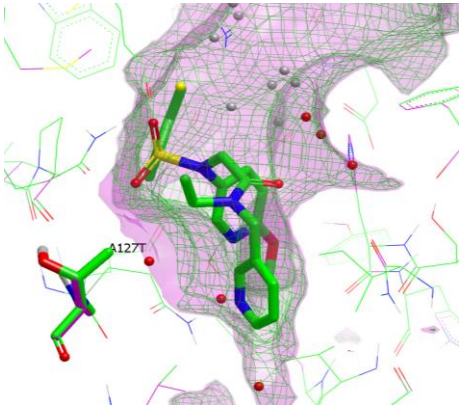
Water





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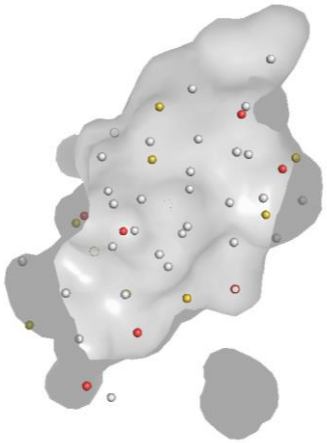
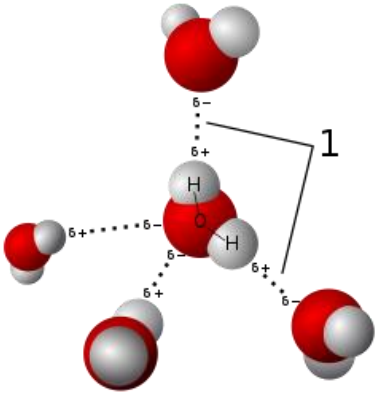


SAR issues



quantifying

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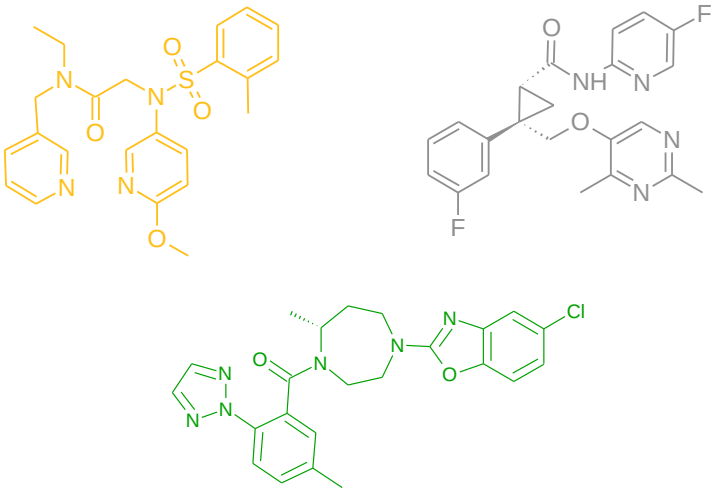
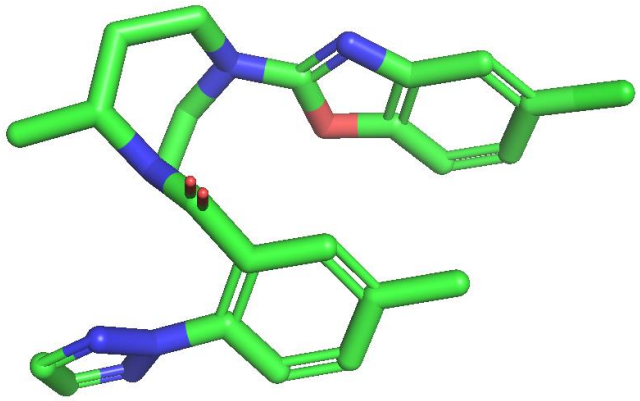
The Role of Water

- Water in Binding
  - Suvorexant – green
  - EMPA – orange
  - Lemborexant – grey



- Pharmacophores
  - Suvorexant crystal as reference



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The Role of Water

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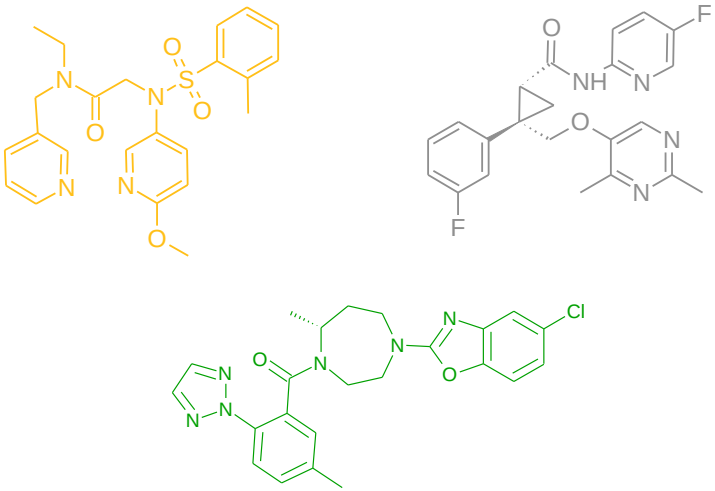
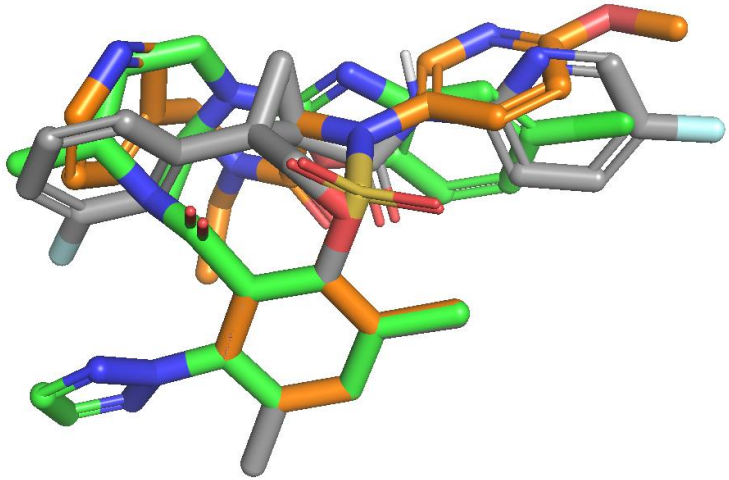


- Pharmacophores
  - Fuzzy pharmacophore definitions
  - No clear pharmacophore



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The Role of Water

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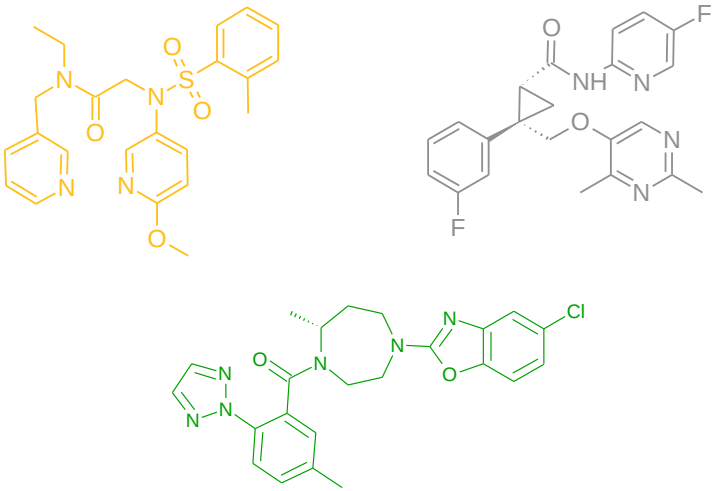
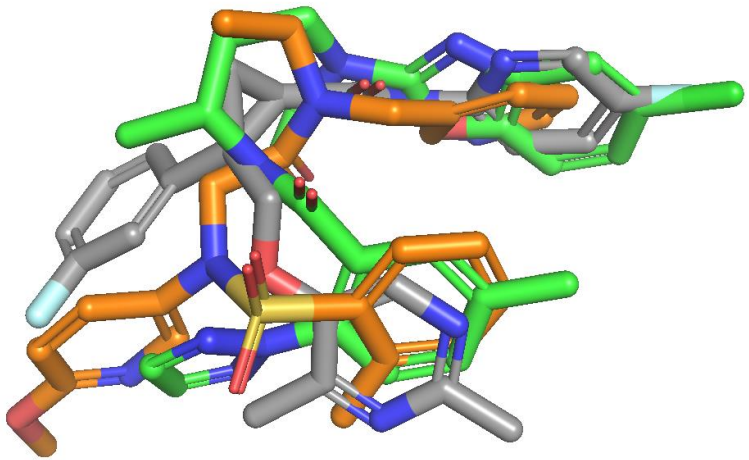


- Pharmacophores
  - Flexible alignment
  - Possible pharmacophore?



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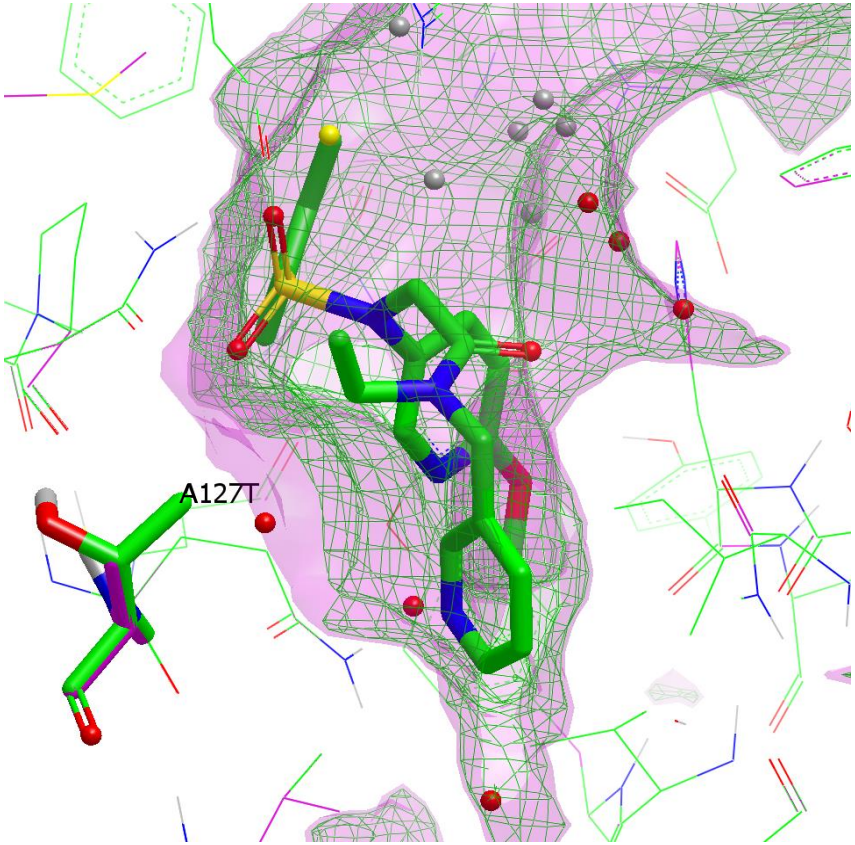
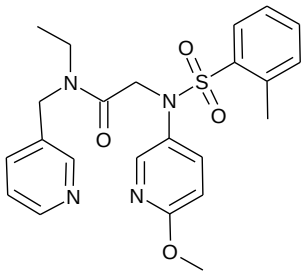
Selectivity

- Water in Selectivity



- EMPA selective for OX2 – not for steric reasons – fits in both sites (Thr → Ala)
- EMPA binding to OX1 traps a water - predicted by WaterFLAP / WaterMap to be high in energy



EMPA

OX1 pKi 6.1
OX2 pKi 8.9

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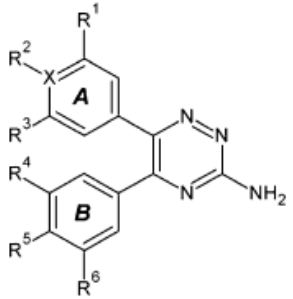
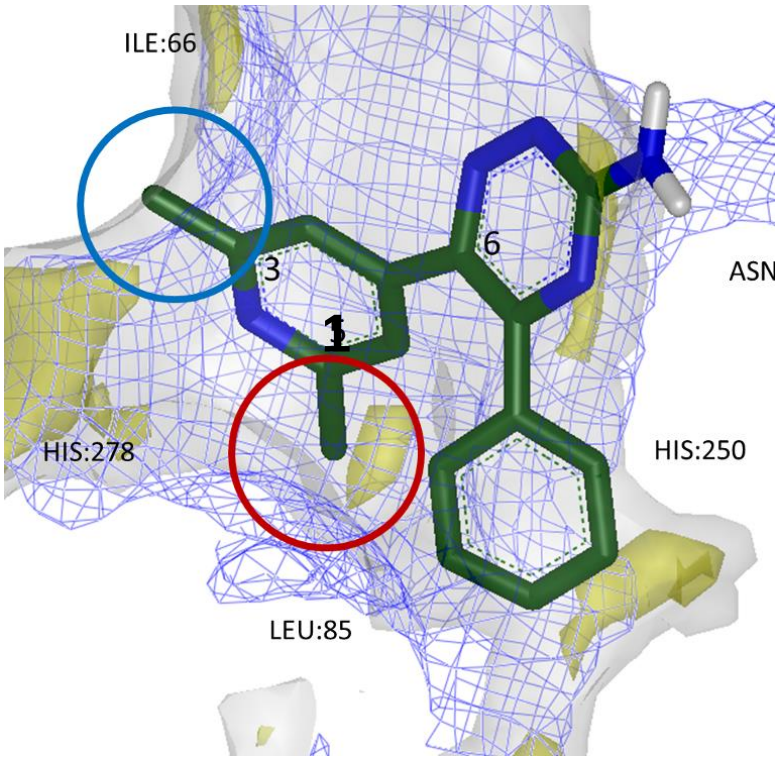
Selectivity

- Water in Selectivity
- Adenosine A_{2A} triazines



ID	formula	A_{2A} pK_i	A_1 pK_i
4a	X=C; R^{1-6}=H	6.93	6.56
4b	X=C; R^1=Cl; R^{2-6}=H	7.29	7.25
4c	X=C; R^1=R^3=Cl; $R^{2,4,5,6}$=H	8.40	7.36
4d	X=C; R^1=R^3=Me; $R^{2,4,5,6}$=H	7.67	6.71
4e	X=C; R^1=Cl; R^2=OH; $R^{3,4,5,6}$=H	8.85	9.79
4f	X=C; R^1=R^3=Me; R^2=OH; $R^{4,5,6}$=H	8.39	7.78
4g	X=N; R^1=R^3=Me; $R^{4,5,6}$=H	8.11	7.07
4h	X=N; R^1=R^3=Me; R^5=F; $R^{4,6}$=H	7.81	6.40
4i	X=N; R^1=R^3=Me; $R^{4,6}$=F; R^5=H	7.56	6.77
4j	X=N; R^1=R^3=Me; R^4=F; $R^{5,6}$=H	7.98	6.96
4k	X=N; R^1=Me; R^3=CF_3; $R^{4,5,6}$=H	8.46	7.50
4l	X=N; R^1=Me; R^3=CF_3; R^5=F; $R^{4,6}$=H	8.34	6.93

- In the beginning



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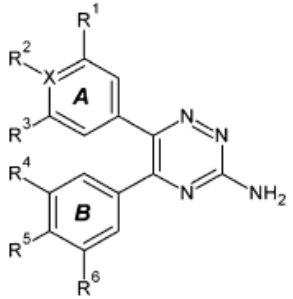
Selectivity

- Water in Selectivity
- Adenosine A_{2A} triazines



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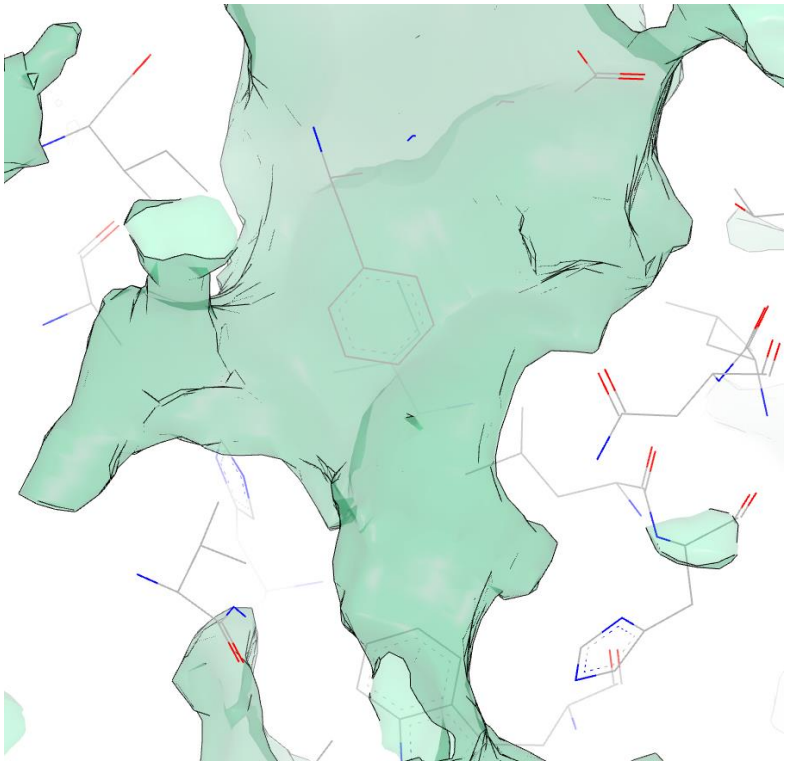
- A_1 crystal structure



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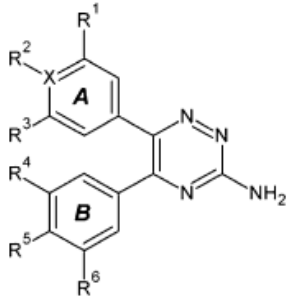
Selectivity

- Water in Selectivity
- Adenosine A_{2A} triazines



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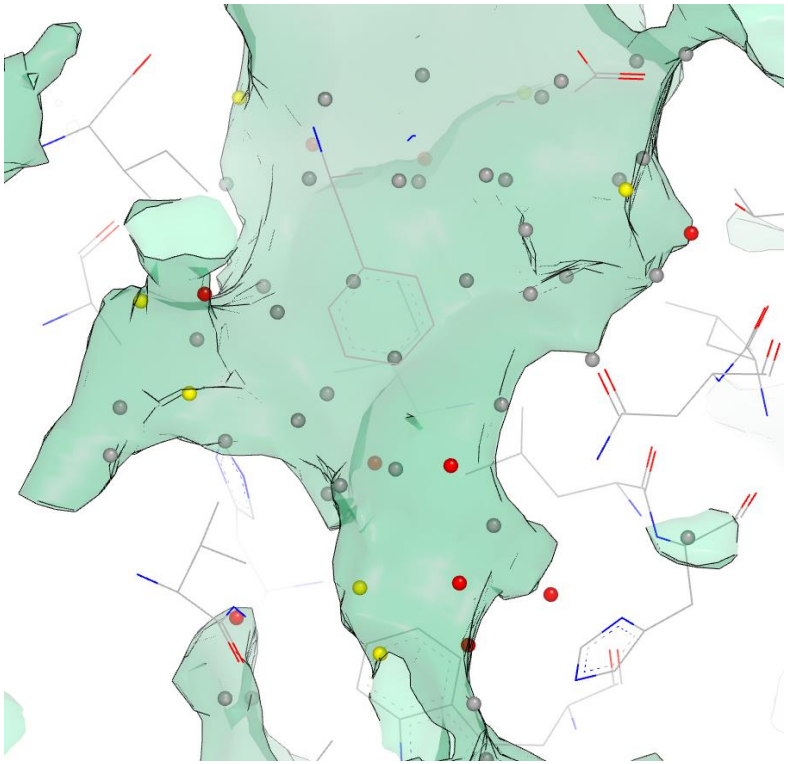
- A_1 with APO water network



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Selectivity

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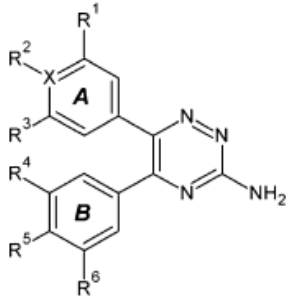
- Adenosine A_{2A} triazines



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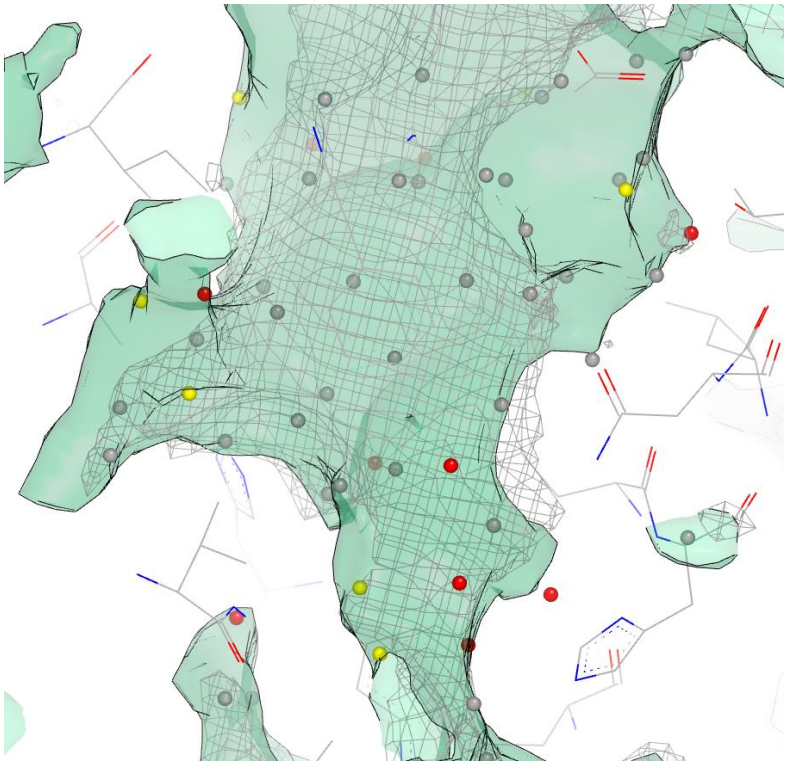
- A_1 compared to A_{2A}



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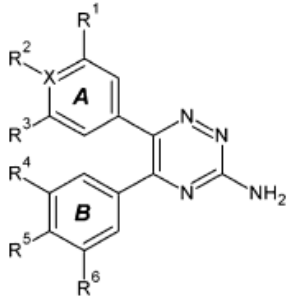

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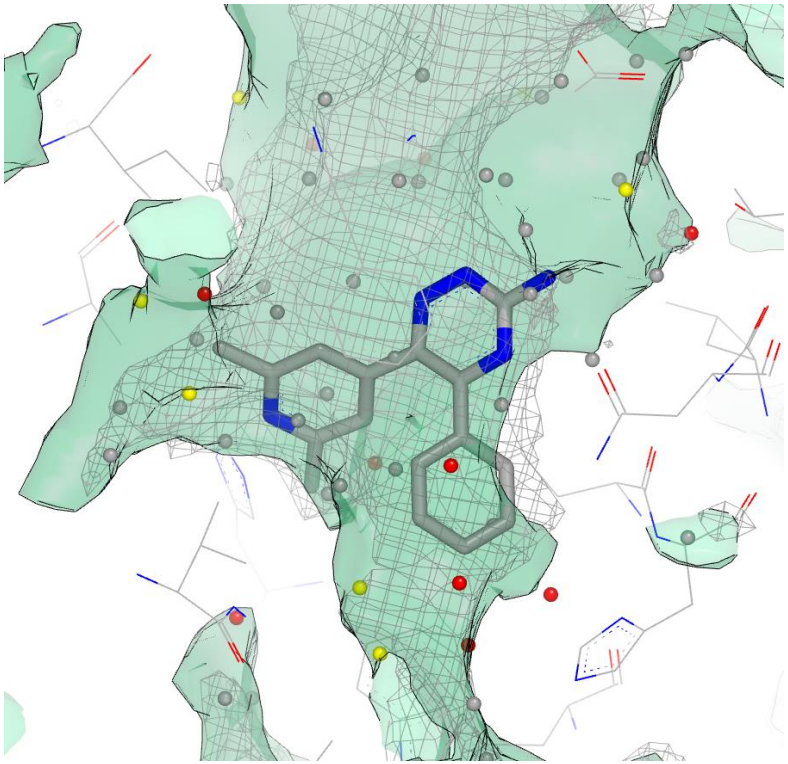
- A_1 with 4g docked



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Selectivity

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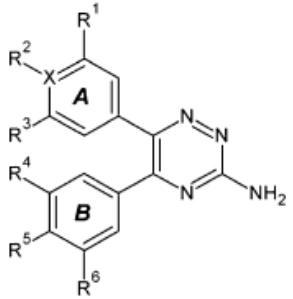
- Adenosine A_{2A} triazines



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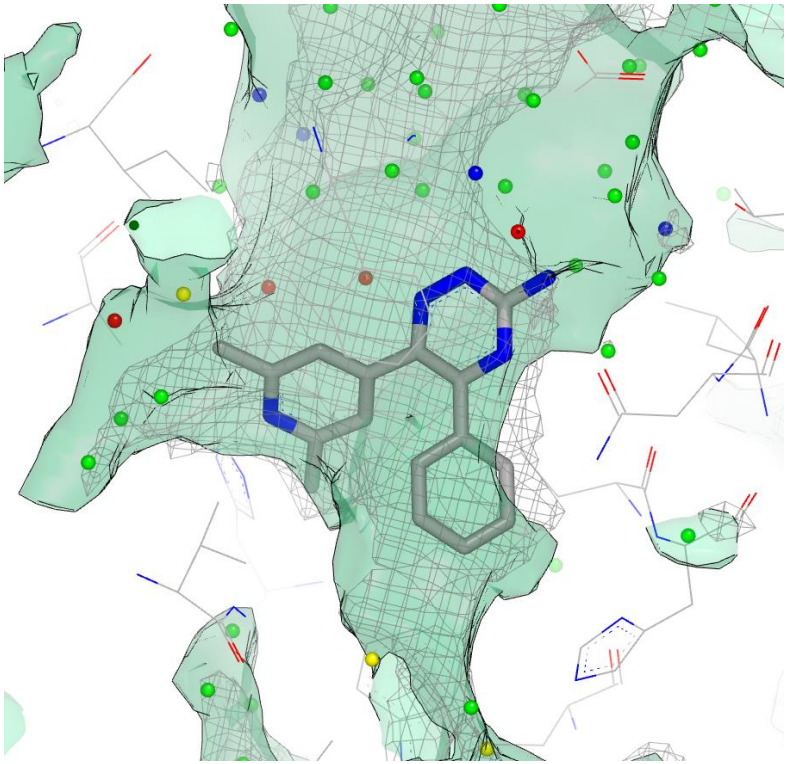
- A_1 with 4g perturbed network



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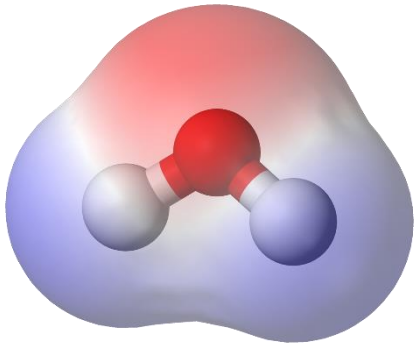
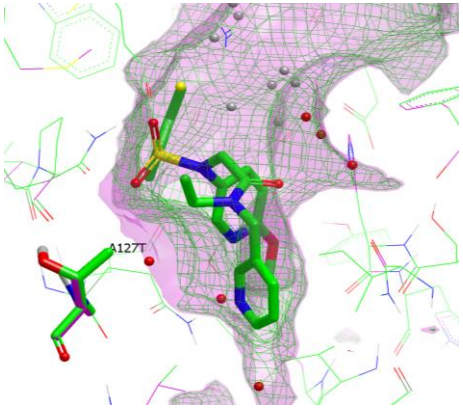
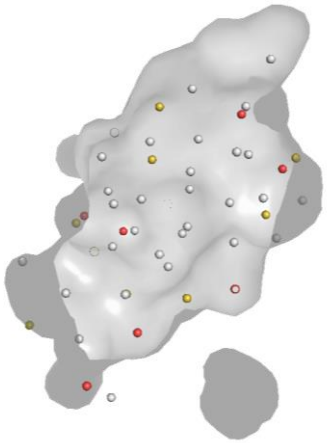
Water

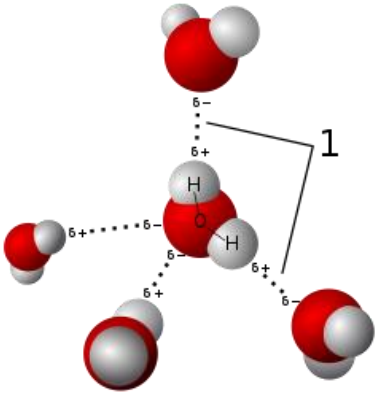




selectivity



SAR issues



quantifying

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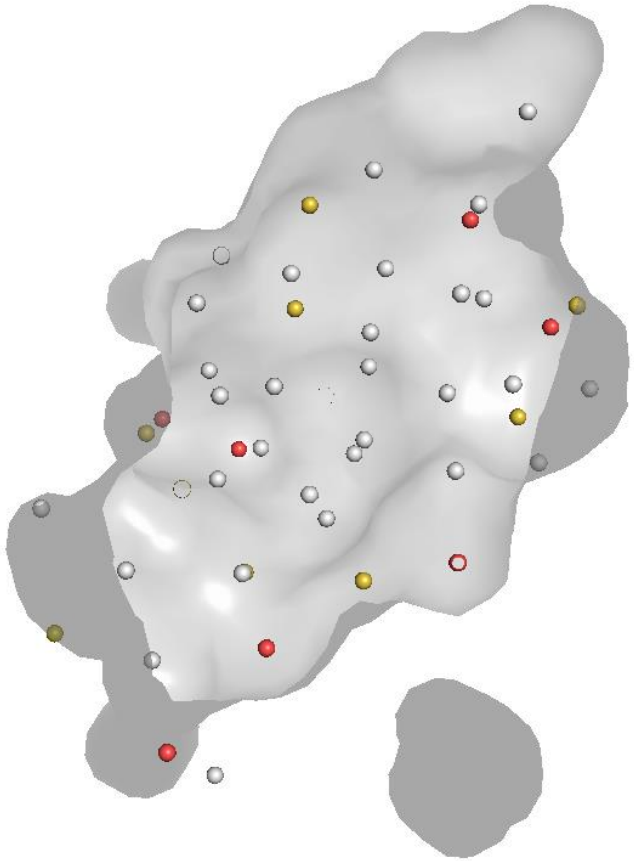
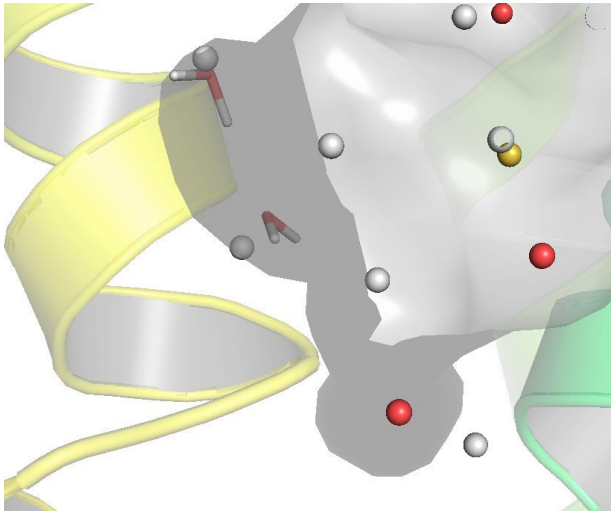
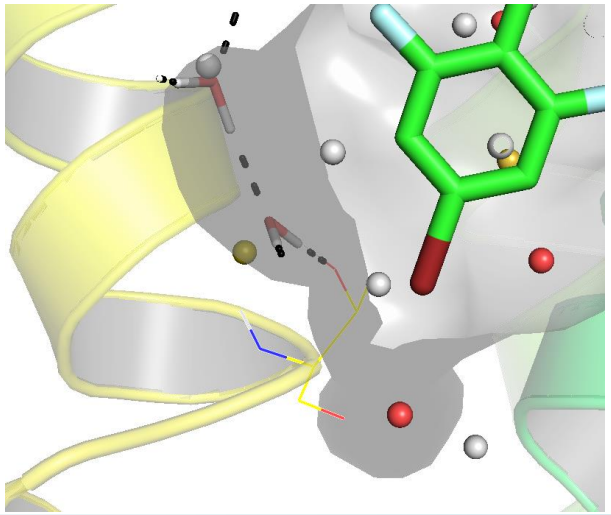
Confounding SAR

- Exploiting Water in the site







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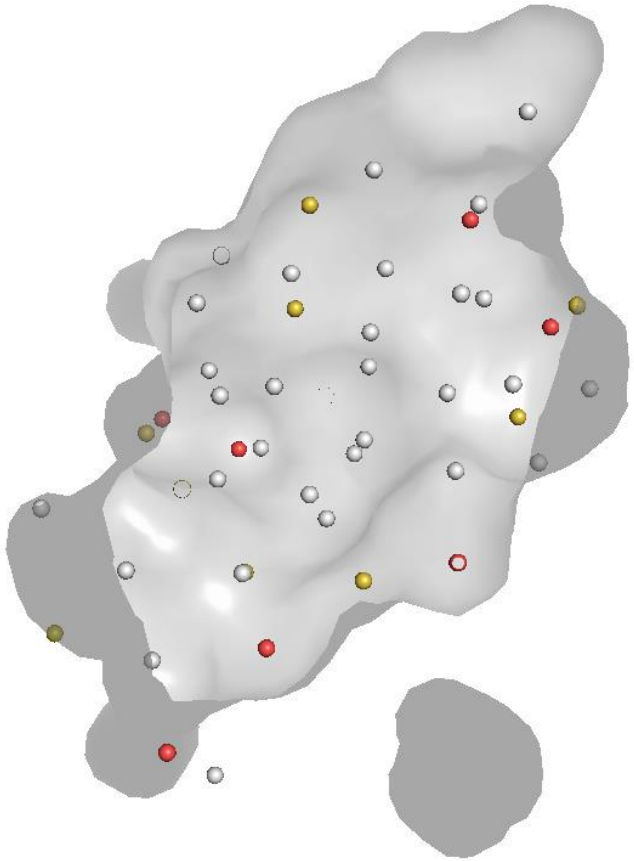
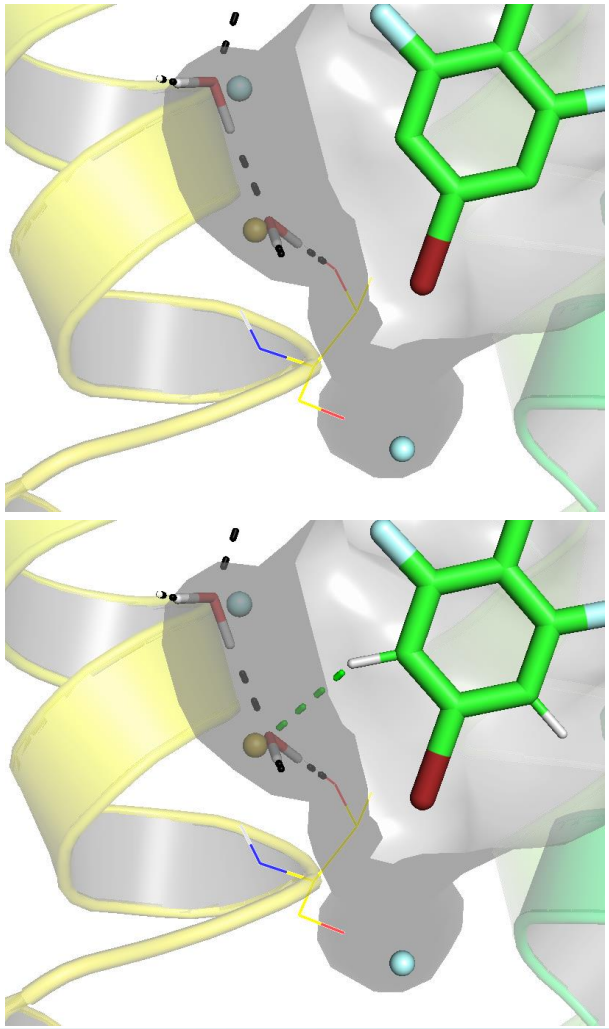
Confounding SAR

- Exploiting Water in the site





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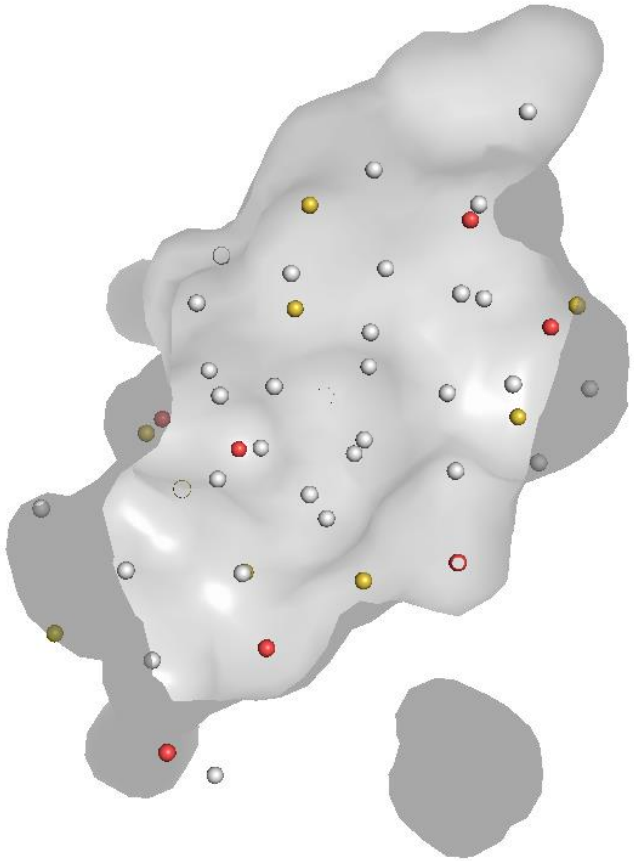

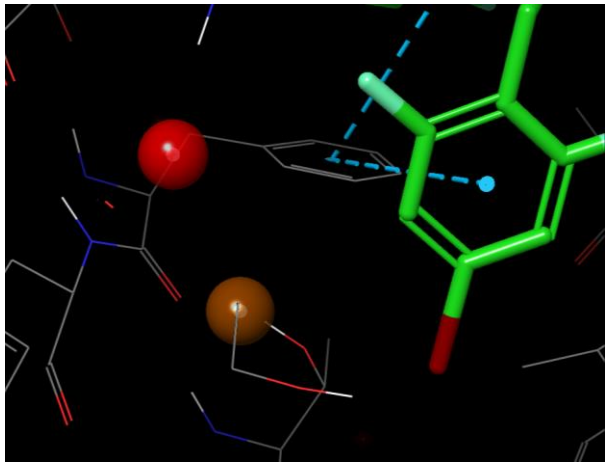

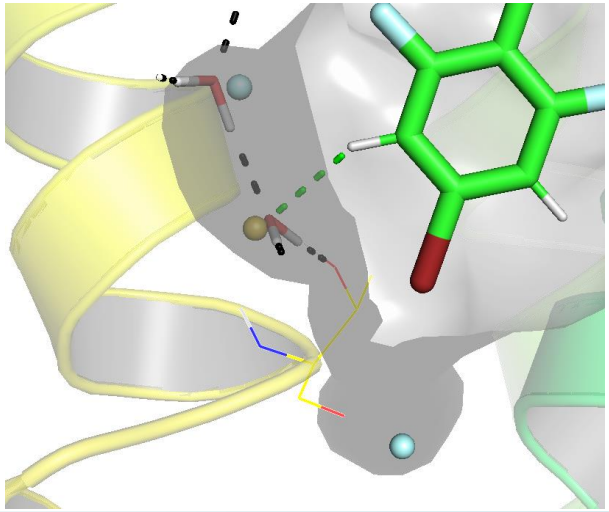

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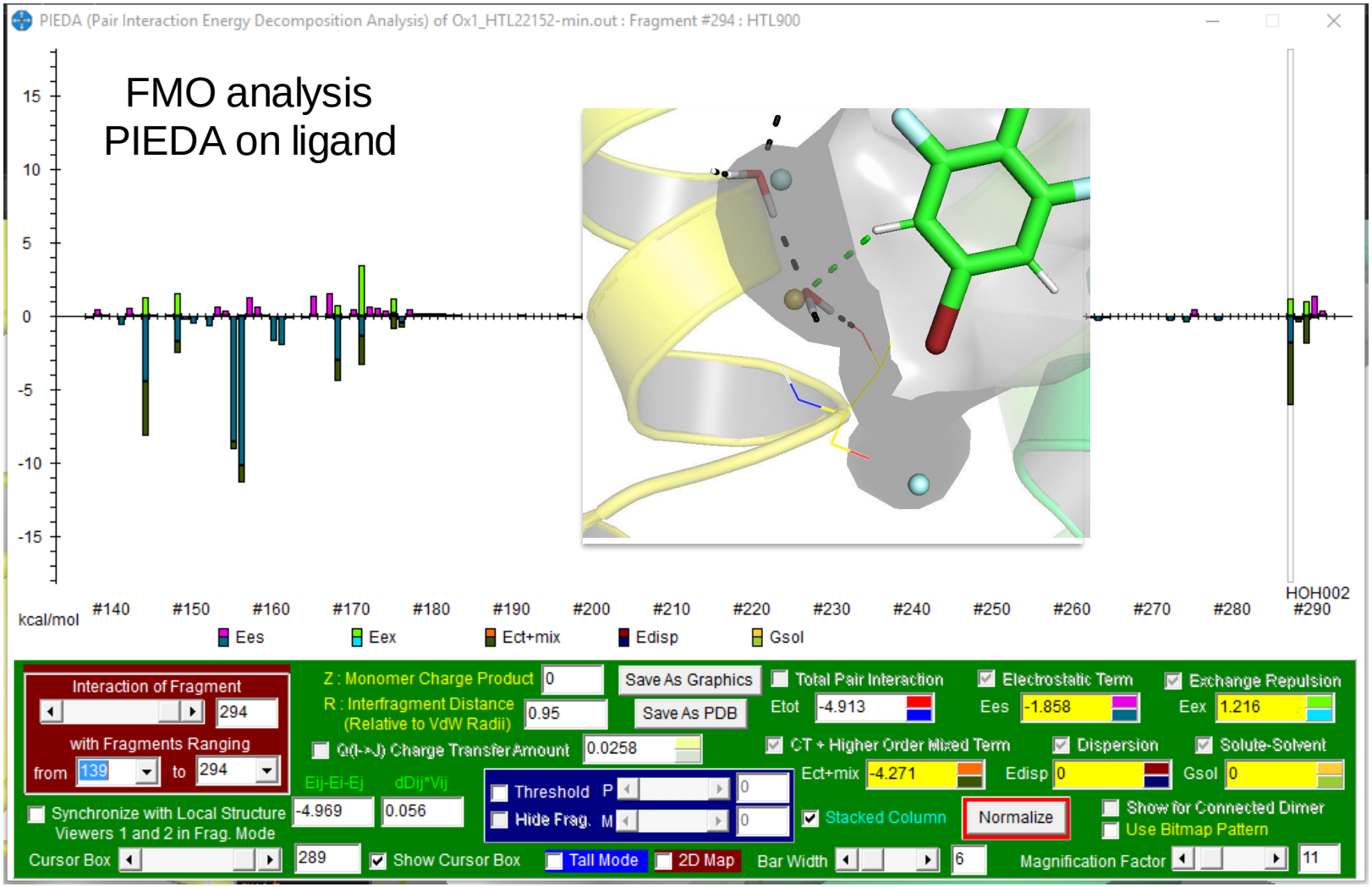

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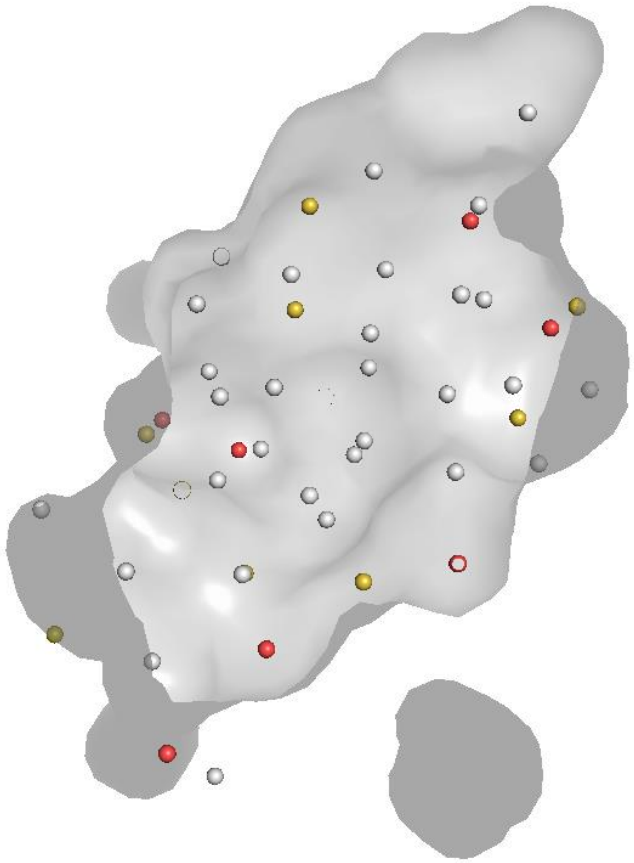
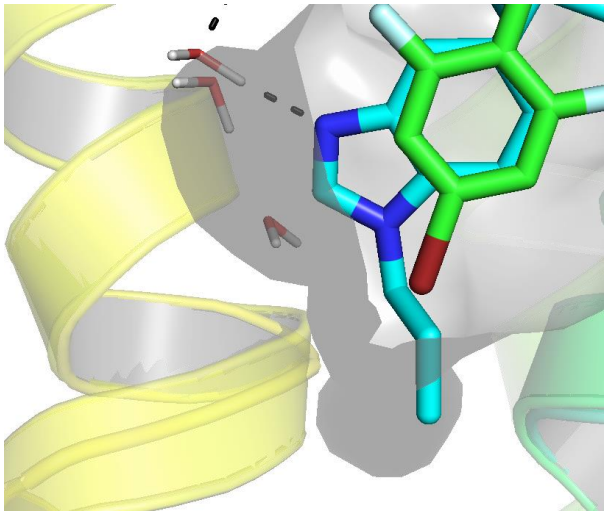
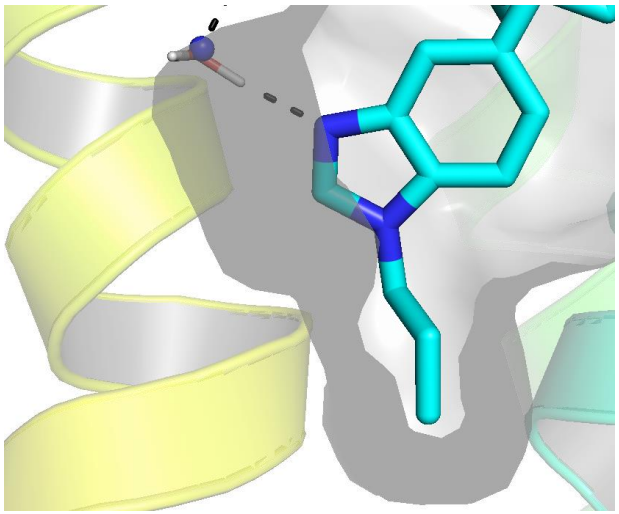
Confounding SAR

- Exploiting Water in the site







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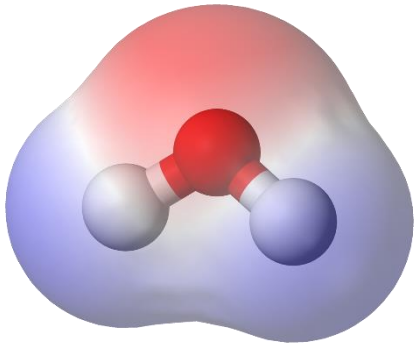
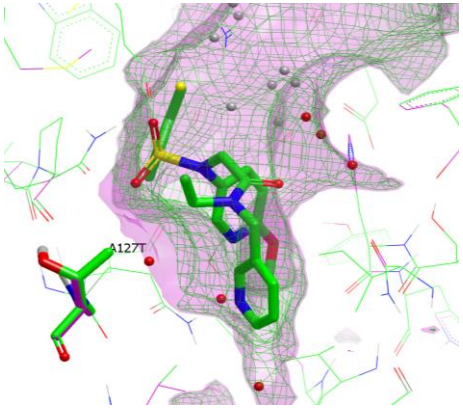
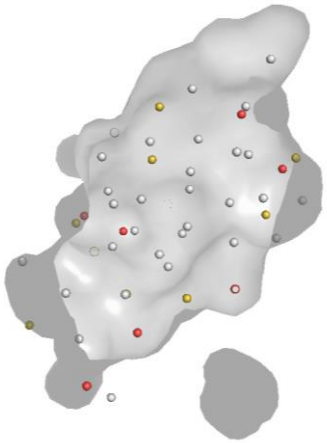
Water

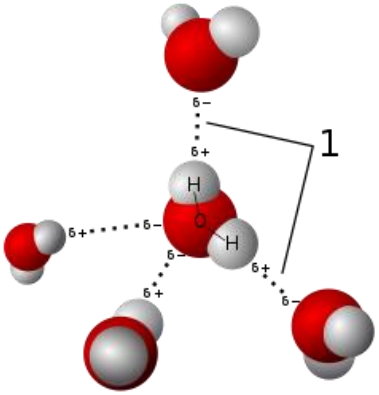




selectivity



SAR issues



quantifying

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Quantifying contributions

Chapter 10

Methodologies for the Examination of Water in GPCRs

Andrea Bortolato, Benjamin G. Tehan, Robert T. Smith,
and Jonathan S. Mason

Abstract

The following chapter examines some of the current "state-of-the-art" tools for predicting, scoring, and examining explicit water molecules in proteins and protein/ligand complexes, highlighting some of the ways information can be readily examined in a manner that is useful in a drug discovery process.

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Quantifying contributions

3.3.2 APO Water Network Generation

Prior to running WaterFLAP on a protein, we first take it through the Protein Preparation Wizard within Maestro (by Schrodinger). After the preparation, we save the protein as protein.pdb, and the ligand as ligand.mol2.

A new automated flag has been introduced by Molecular Discovery, which captures many of the key options we had previously set within our Python scripts, making them redundant. For the more recent versions of WaterFLAP (released during 2016), to generate an apo water network, we run the following command line:

(Path to WaterFLAP)/flapwater -w-auto -i protein.pdb -o WAT_PRED_OCT.pdb -gl ligand.mol2 -gr 6 -cpu 8 >apowaterflap. log 2>apowaterflap.log2

The key command line options we employ are:

-w-auto which runs the automated water protocol, with the default settings.

-i which defines the protein file.

-o which defines where we want the output to be saved.

-gl which defines the ligand that was present within the pocket.

-gr which defines the distance from the ligand to explore using GRID (and the resulting waters).

-cpu which defines how many CPU cores to use for generating the water network (maximum of 8).

3.3.3 Complex Water Network Generation

Alongside apo water network prediction, WaterFLAP also has the ability to optimize and score a water network around a docked ligand. Generally in this process the apo waters overlapping the ligand are displaced, and several iterations of optimization on the remaining water network are carried out.

To re-score or convert the apo water network to a complex water network, we copy the WAT_PRED_OCT_DG_WAT_COMPLEX.pdb (generated previously), the protein.pdb file and the docked ligand (saved as a mol2 file) into a new directory and after changing to that directory, run the following command:

(Path to WaterFLAP)/flapdock -mol2 ligand.mol2 -gl ligand.mol2 -pdb protein.pdb -wat WAT_PRED_OCT_DG_WAT_COMPLEX.pdb -score_wat -refine_wat -wat_iter 20

The key command line options we employ are:

-mol2 which defines the docked ligand we want to optimise the water network around.

-gl which defines the docked ligand we want to optimise the water network around.

-pdb which defines the protein pdb we have docked ligand. mol2 into.

-wat which defines the apo water network with the receptor pdb file (the WAT_PRED_OCT_DG_WAT_COMPLEX.pdb generated during the apo prediction).

-score_wat which informs WaterFLAP that we want to score the

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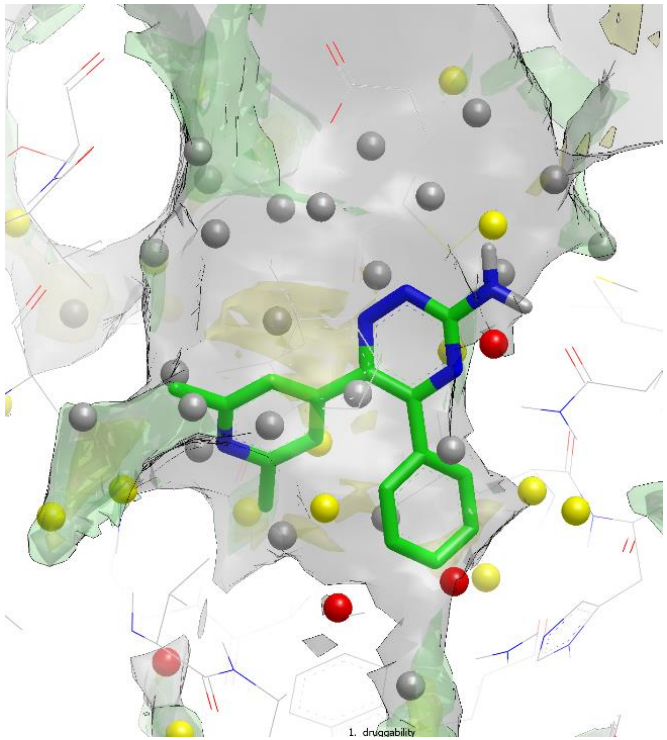
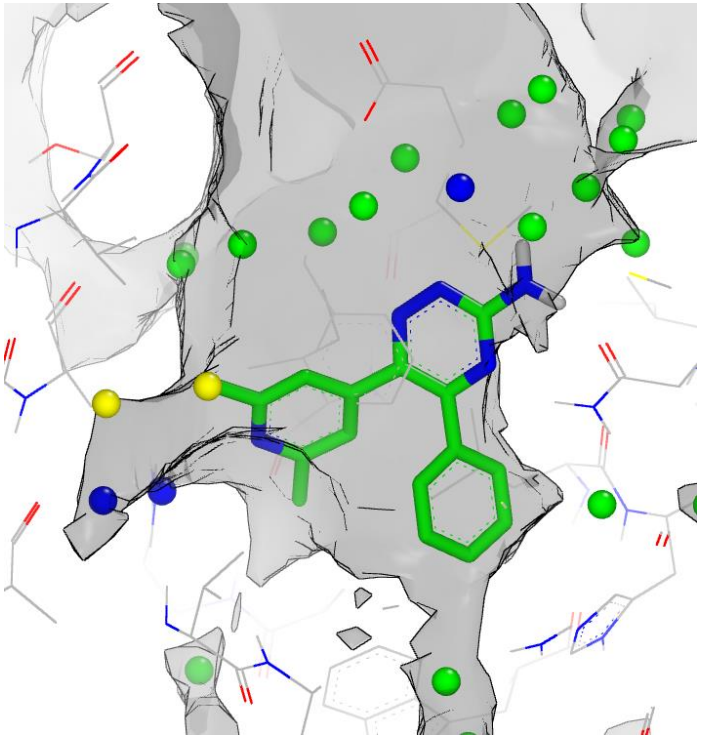
Quantifying contributions

- Pseudo-apo water network
  - (energetically coded: red & yellow unhappy)
- Triazine ligand complex
  - Perturbed water network
  - effect of pyridine





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- flapdock -mol2 l.mol2 -gl l.mol2 -pdb protein.pdb -wat WAT_PRED_OCT_DG_WAT_COMPLEX.pdb **-v 5** -score_wat -refine_wat -wat_iter 20 > scoreWat.log
  - -v 5 => verbose mode

scoreWat.log

WaterFLAP summary of delta DG between apo and complex

Water: 0	DG_APO:	0.014	DG_COMPLEX:	0.091	DDG: 0.076 CLASS: 3
Water: 1	DG_APO:	4.045	DG_COMPLEX:	3.658	DDG: -0.387 CLASS: 3
Water: 2	DG_APO:	-1.177	DG_COMPLEX:	-1.701	DDG: -0.524 CLASS: 3
Water: 3	DG_APO:	-0.947	DG_COMPLEX:	-1.062	DDG: -0.115 CLASS: 3
Water: 4	DG_APO:	0.118	DG_COMPLEX:	0.111	DDG: -0.006 CLASS: 3
Water: 5	DG_APO:	0.615	DG_COMPLEX:	1.542	DDG: 0.927 CLASS: 3
Water: 6	DG_APO:	1.310	DG_COMPLEX:	1.582	DDG: 0.272 CLASS: 3
Water: 7	DG_APO:	-0.802	DG_COMPLEX:	Displaced	
Water: 8	DG_APO:	2.948	DG_COMPLEX:	2.610	DDG: -0.338 CLASS: 3
Water: 9	DG_APO:	1.595	DG_COMPLEX:	Displaced	
Water: 10	DG_APO:	2.546	DG_COMPLEX:	2.691	DDG: 0.144 CLASS: 3
Water: 11	DG_APO:	-0.945	DG_COMPLEX:	-0.935	DDG: 0.010 CLASS: 3
Water: 12	DG_APO:	0.366	DG_COMPLEX:	0.145	DDG: -0.221 CLASS: 3
Water: 13	DG_APO:	4.161	DG_COMPLEX:	Displaced	
Water: 14	DG_APO:	5.071	DG_COMPLEX:	5.992	DDG: 0.921 CLASS: 3
Water: 15	DG_APO:	2.976	DG_COMPLEX:	2.801	DDG: -0.175 CLASS: 3
Water: 16	DG_APO:	4.468	DG_COMPLEX:	Displaced	
Water: 17	DG_APO:	0.620	DG_COMPLEX:	-0.100	DDG: -0.720 CLASS: 3
Water: 18	DG_APO:	4.300	DG_COMPLEX:	1.444	DDG: -2.857 CLASS: 4
Water: 19	DG_APO:	4.372	DG_COMPLEX:	4.162	DDG: -0.210 CLASS: 3
Water: 20	DG_APO:	0.919	DG_COMPLEX:	Displaced	
Water: 21	DG_APO:	1.692	DG_COMPLEX:	Displaced	
Water: 22	DG_APO:	2.768	DG_COMPLEX:	Displaced	
Water: 23	DG_APO:	0.110	DG_COMPLEX:	1.543	DDG: 1.433 CLASS: 2
Water: 24	DG_APO:	0.000	DG_COMPLEX:	Displaced	
Water: 25	DG_APO:	1.317	DG_COMPLEX:	Displaced	

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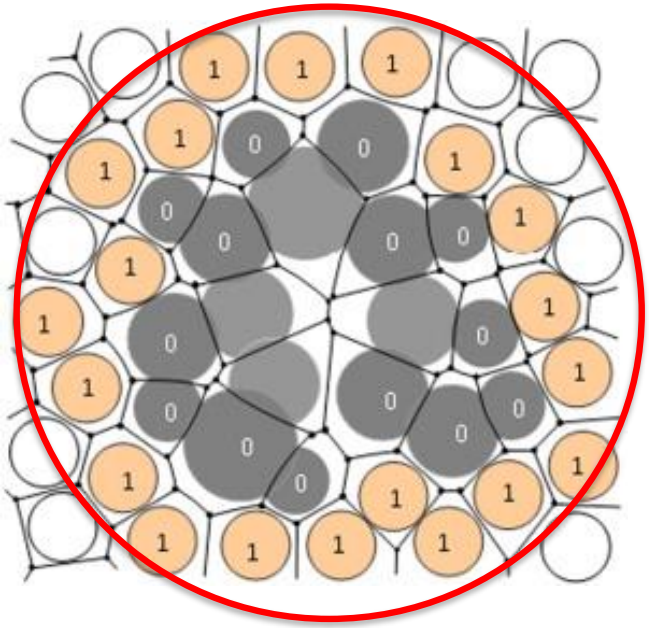
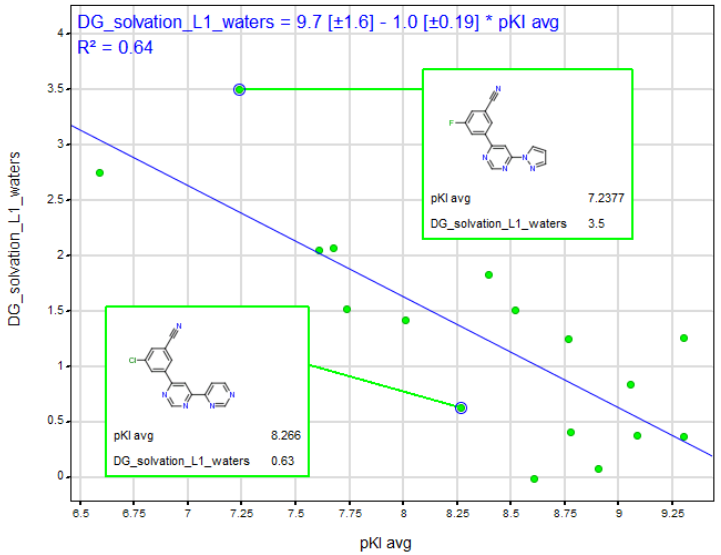
The Role of Water

- Quantifying the effect of water perturbation
  - Analysis of the first voronoi shell
  - Ensures only perturbed waters in contact with ligand are examined



  - 16 cpds from congeneric series of mGlu5 ligands
  - Christopher et al. JMC 2015, *58: 6653-6664*



  - Utilising WaterFlap and voronoi functionality in SciPy

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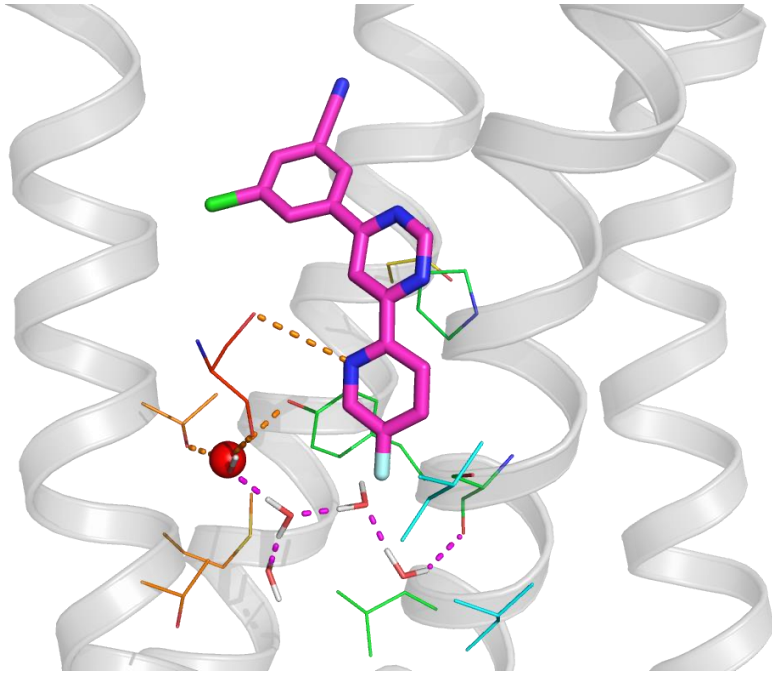
The Role of Water

- Quantifying the effect of water perturbation
  - Analysis of the first voronoi shell
  - Ensures only waters perturbed are examined



TM's 1, 2 & 7 removed

  - 16 cpds from congeneric series of mGlu5 ligands
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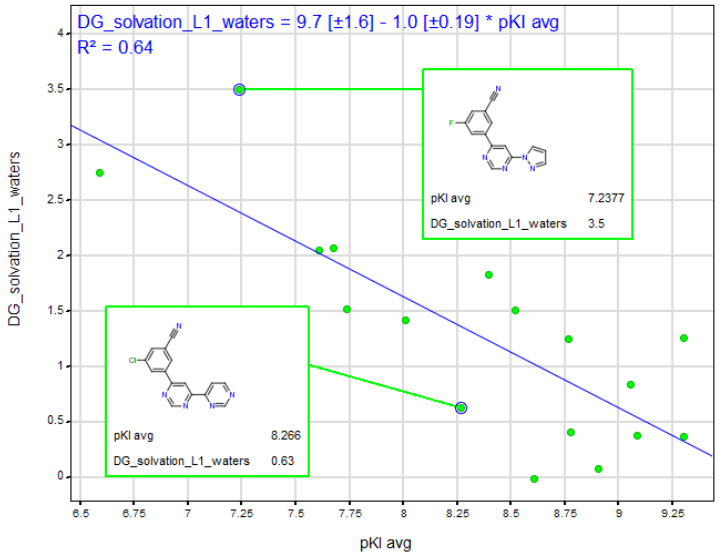

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The Role of Water

- Quantifying the effect of water perturbation
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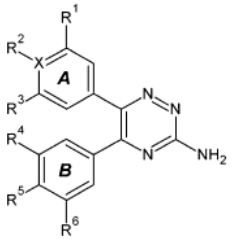
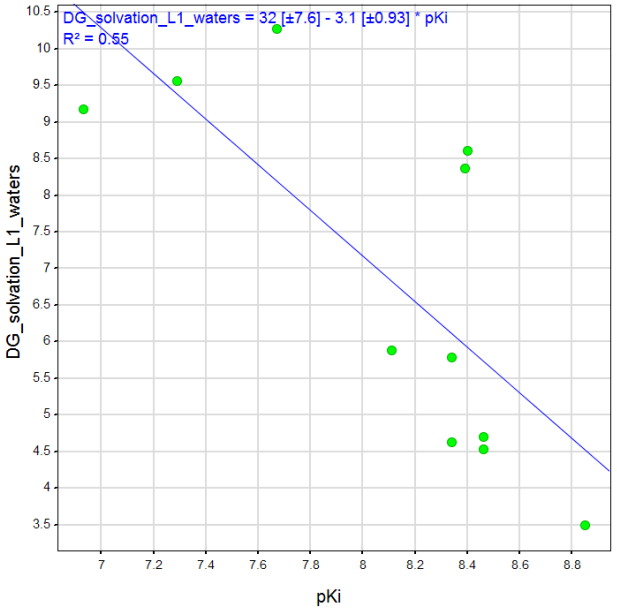
  - A2A congeneric triazines
  - Congreve et al. JMC *2012; 55:1898-1903*



ID	formula	A_{2A} pK_i	A_1 pK_i
4a	X=C; R^{1-6}=H	6.93	6.56
4b	X=C; R^1=Cl; R^{2-6}=H	7.29	7.25
4c	X=C; R^1=R^3=Cl; $R^{2,4,5,6}$=H	8.40	7.36
4d	X=C; R^1=R^3=Me; $R^{2,4,5,6}$=H	7.67	6.71
4e	X=C; R^1=Cl; R^2=OH; $R^{3,4,5,6}$=H	8.85	9.79
4f	X=C; R^1=R^3=Me; R^2=OH; $R^{4,5,6}$=H	8.39	7.78
4g	X=N; R^1=R^3=Me; $R^{4,5,6}$=H	8.11	7.07
4h	X=N; R^1=R^3=Me; R^5=F; $R^{4,6}$=H	7.81	6.40
4i	X=N; R^1=R^3=Me; $R^{4,6}$=F; R^5=H	7.56	6.77
4j	X=N; R^1=R^3=Me; R^4=F; $R^{5,6}$=H	7.98	6.96
4k	X=N; R^1=Me; R^3=CF_3; $R^{4,5,6}$=H	8.46	7.50
4l	X=N; R^1=Me; R^3=CF_3; R^5=F; $R^{4,6}$=H	8.34	6.93



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The Role of Water

- Quantifying the effect of water perturbation
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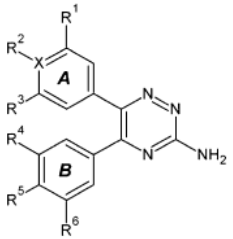
  - A2A congeneric triazines
  - Congreve et al. JMC *2012; 55:1898-1903*



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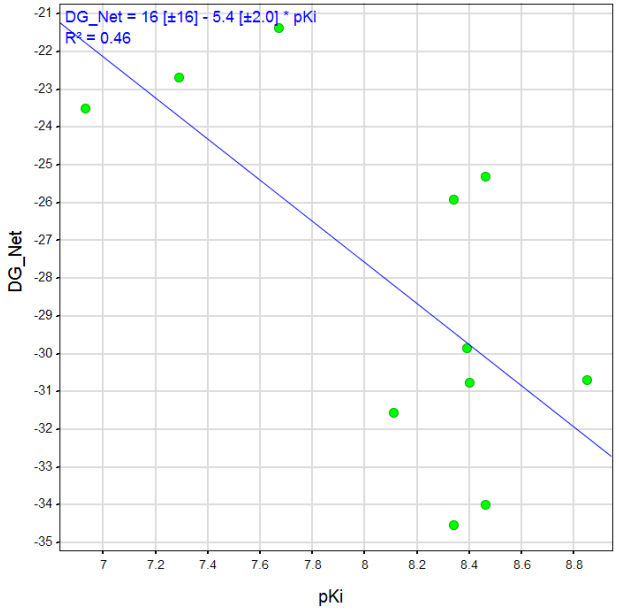
4d	X=C; R^1=R^3=Me; $R^{2,4,5,6}$=H	7.67	6.71
4e	X=C; R^1=Cl; R^2=OH; $R^{3,4,5,6}$=H	8.85	9.79
4f	X=C; R^1=R^3=Me; R^2=OH; $R^{4,5,6}$=H	8.39	7.78
4g	X=N; R^1=R^3=Me; $R^{4,5,6}$=H	8.11	7.07
4h	X=N; R^1=R^3=Me; R^5=F; $R^{4,6}$=H	7.81	6.40
4i	X=N; R^1=R^3=Me; $R^{4,6}$=F; R^5=H	7.56	6.77
4j	X=N; R^1=R^3=Me; R^4=F; $R^{5,6}$=H	7.98	6.96
4k	X=N; R^1=Me; R^3=CF_3; $R^{4,5,6}$=H	8.46	7.50
4l	X=N; R^1=Me; R^3=CF_3; R^5=F; $R^{4,6}$=H	8.34	6.93



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The Role of Water

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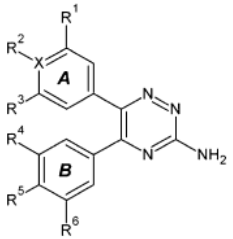
  - Congreve et al. JMC *2012; 55:1898-1903*



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4d	X=C; R^1=R^3=Me; $R^{2,4,5,6}$=H	7.67	6.71
4e	X=C; R^1=Cl; R^2=OH; $R^{3,4,5,6}$=H	8.85	9.79
4f	X=C; R^1=R^3=Me; R^2=OH; $R^{4,5,6}$=H	8.39	7.78
4g	X=N; R^1=R^3=Me; $R^{4,5,6}$=H	8.11	7.07
4h	X=N; R^1=R^3=Me; R^5=F; $R^{4,6}$=H	7.81	6.40
4i	X=N; R^1=R^3=Me; $R^{4,6}$=F; R^5=H	7.56	6.77
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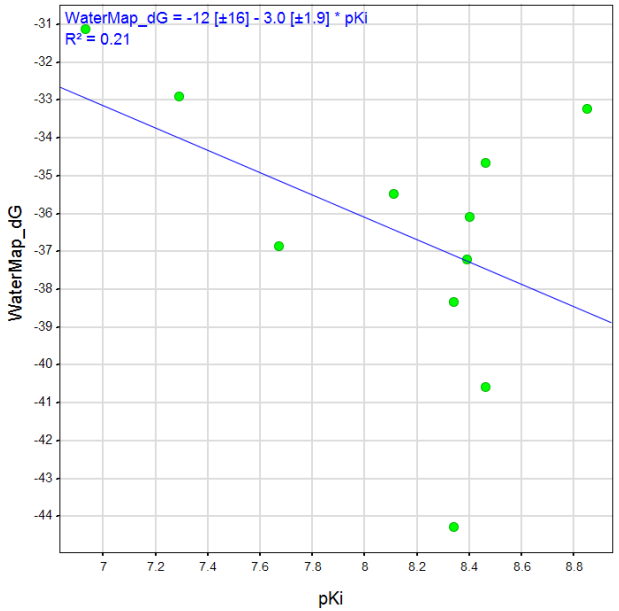

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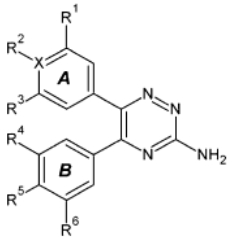

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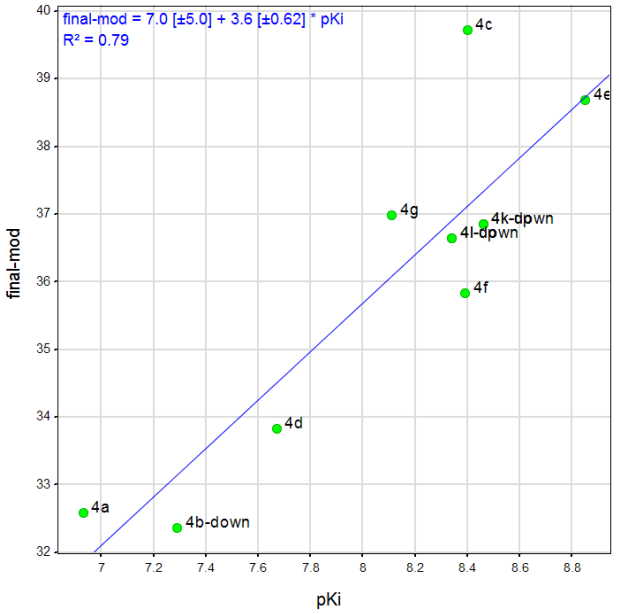

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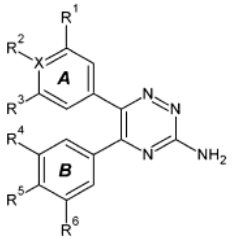

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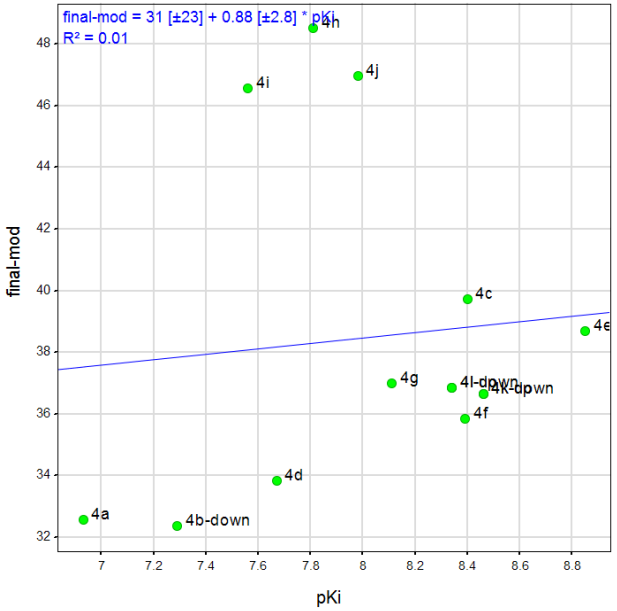

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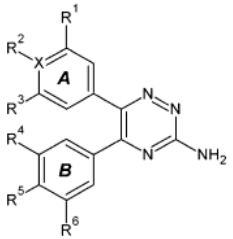

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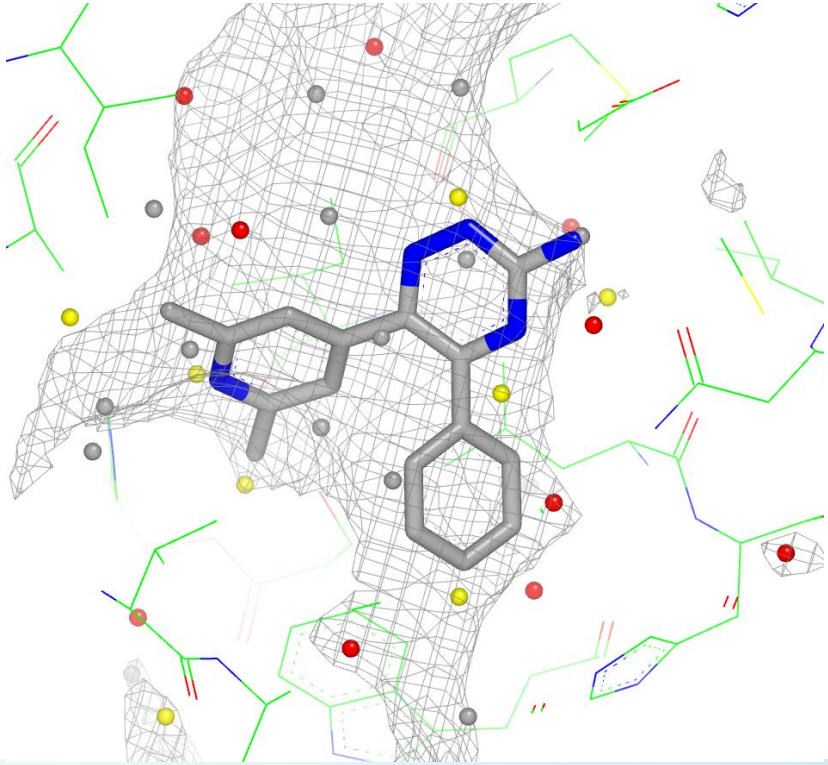

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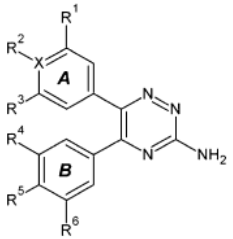

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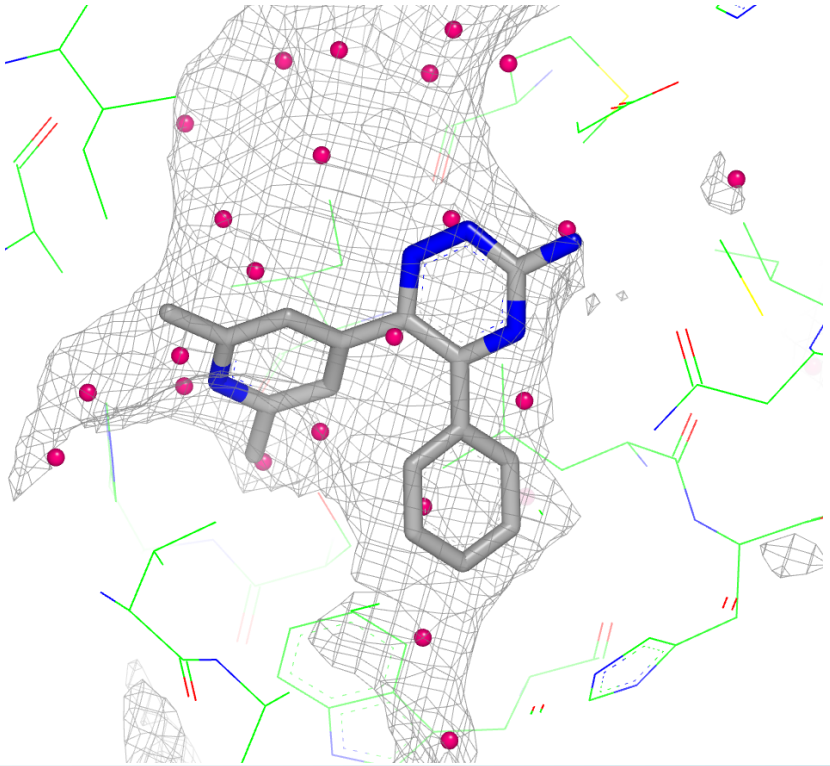

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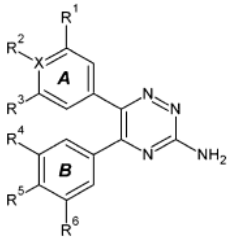

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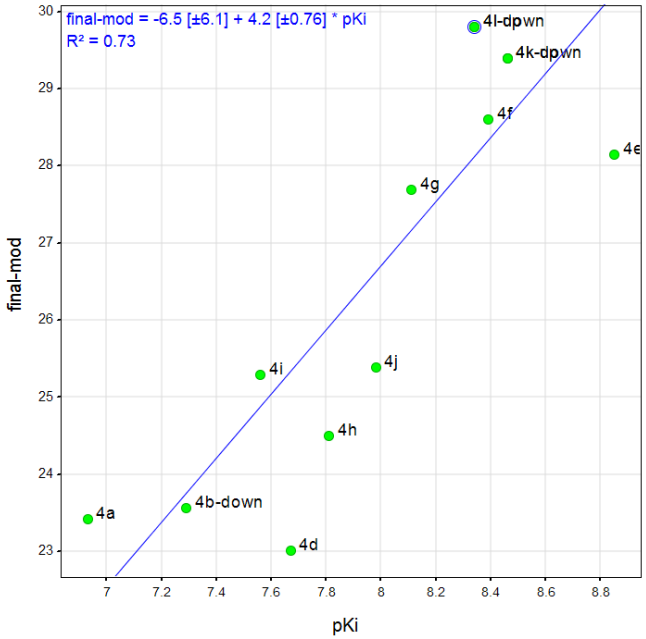

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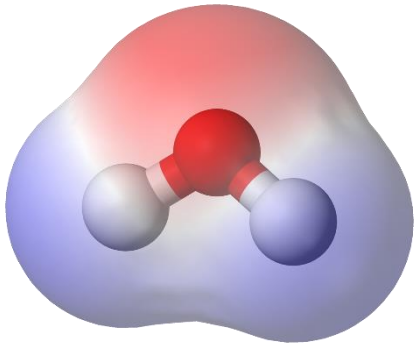
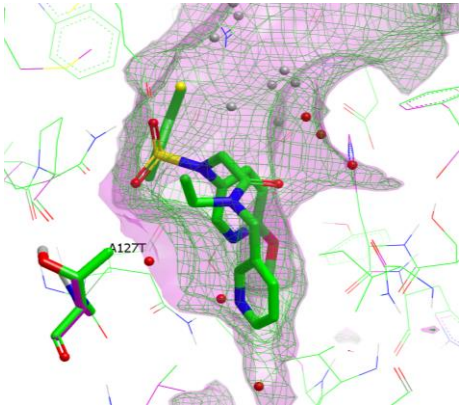
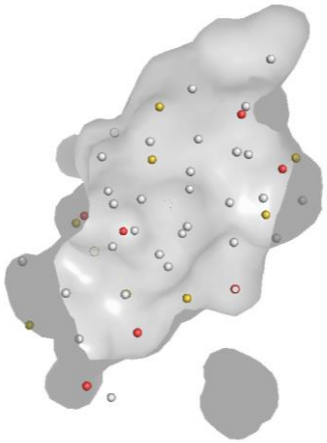
Water

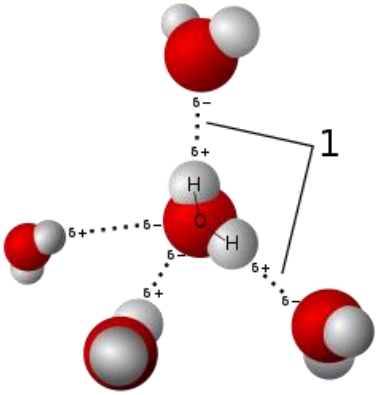




selectivity



SAR issues



quantifying

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Take home message

- Water in Binding
- Significant repercussions for ligand modelling & design
  - Pharmacophore models may be wrong
  - Docking to protein structure may be completely wrong
  - Opportunities to make ligands smaller with different properties, utilising water
- Quantifying waters contribution
  - Sometimes higher level quantum mechanical methods are required
  - No one current standout method
  - Combining methodologies
  - Dewetted regions

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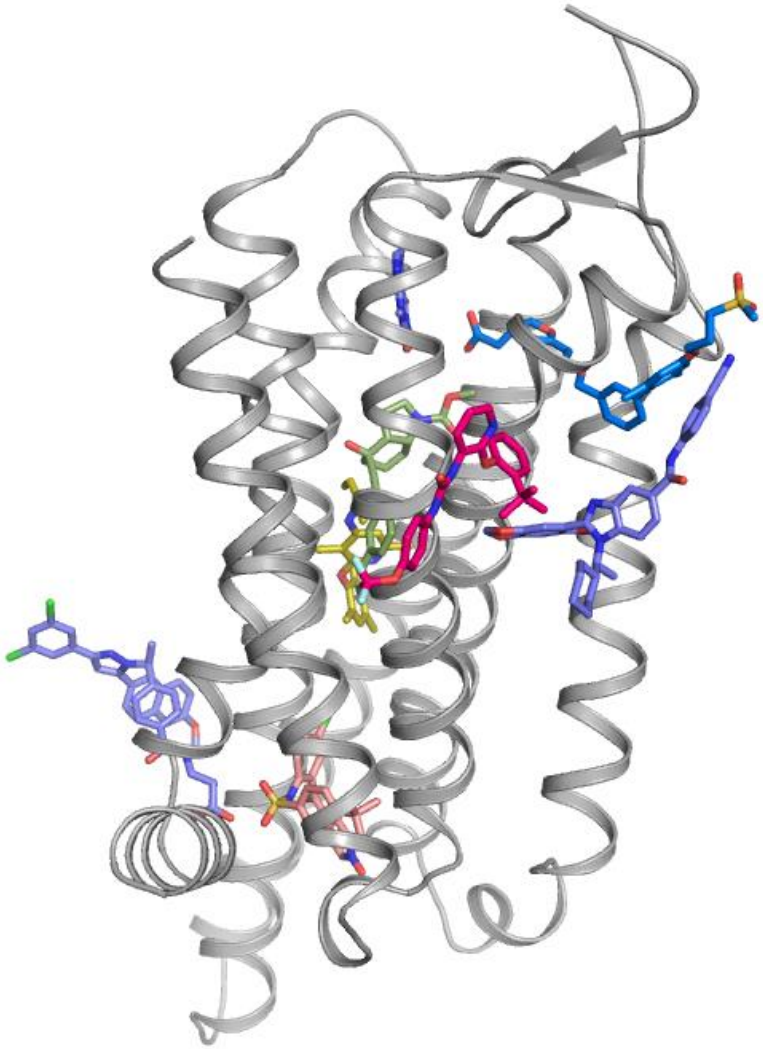
Acknowledgements

- Heptares Therapeutics
  - Computational Chemistry
  - Chemistry
  - Pharmacology
  - Crystallography
  - Molecular Biology

  - Malcolm Weir (CEO)



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