Assessment of Water Energetics and Applications to Drug Discovery

Daniel D. Robinson

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Water Energetics and Drug Discovery

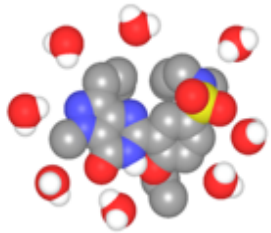
- Water is, of course, everywhere
  - Protein binding sites are largely filled with water
  - Water is a direct competitor when it comes to binding
- And yet, until fairly recently, there were few accessible tools to help us understand the thermodynamic properties of the water molecules that were playing such a critical role
- This situation has changed in recent years, a number of tools have become available that allow us to model the networks of water within protein cavities with a fair degree of accuracy
  - Here, we shall look at some of the results coming from Schrodinger's offering in this area, WaterMap

WaterMap: A Tool for Probing Solvent Thermodynamics

Solvated Ligand

ΔH_{conf} penalty
ΔS_{conf} penalty

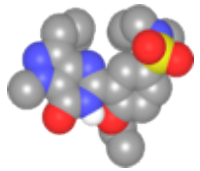
ΔG(1)



Solvated Ligand in Bioactive Conformation

ΔH penalty
ΔS reward

ΔG(3)



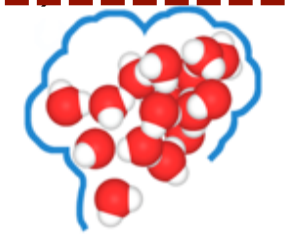
Desolvated Ligand

Solvated Apo Protein

ΔG(2)

ΔH_{conf} penalty
ΔS_{conf} penalty

Solvated Protein in Ligand-Induced Conformation



ΔG(4)

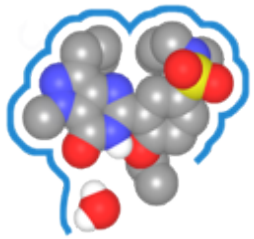
ΔH ?
ΔS ?

Ligand-Induced Desolvated Protein Binding Site

WaterMap explicitly computes this term

ΔG(5)

ΔH reward
$\Delta S_{rot/trans}$ penalty



Protein/Ligand/ Water Complex

$$\Delta G_{bind} = \sum_{i=1}^{5} \Delta G(i)$$

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How Does WaterMap Work?

- WaterMap uses molecular-dynamics (MD) to model the behaviour of solvation
  - ~2ns simulation with explicit solvent and restrained protein
- Hydration sites are located by clustering the water locations from the simulation
  - These show how and where water is localised within the pocket
    - The locations show good agreement with solvent molecules found from crystallography
- Each hydration site can be characterised thermodynamically using inhomogeneous solvation theory*
  - Enthalpy is taken directly from the non-bonded interactions
  - Entropy is taken from a local expansion of spatial and orientational correlation functions

*Lazaridis, T. *J. Phys. Chem. B.* **1998**, 102, *3531-3541*.

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A Survey of Water Molecules Around Proteins

PROTEINS
STRUCTURE ■ FUNCTION ■ BIOINFORMATICS

Thermodynamic analysis of water molecules at the surface of proteins and applications to binding site prediction and characterization

Thijs Beuming,[1] Ye Che,[2] Robert Abel,[1] Byungchan Kim,[1] Veerabahu Shanmugasundaram,[2]* and Woody Sherman[1]*

Proteins, **2012**, 80 (3), *871–883*

A survey of 27 different protein across a range of families
Thermodynamic information (ΔG, ΔH, $-T\Delta S$) characterised for ~32,000 hydration-sites

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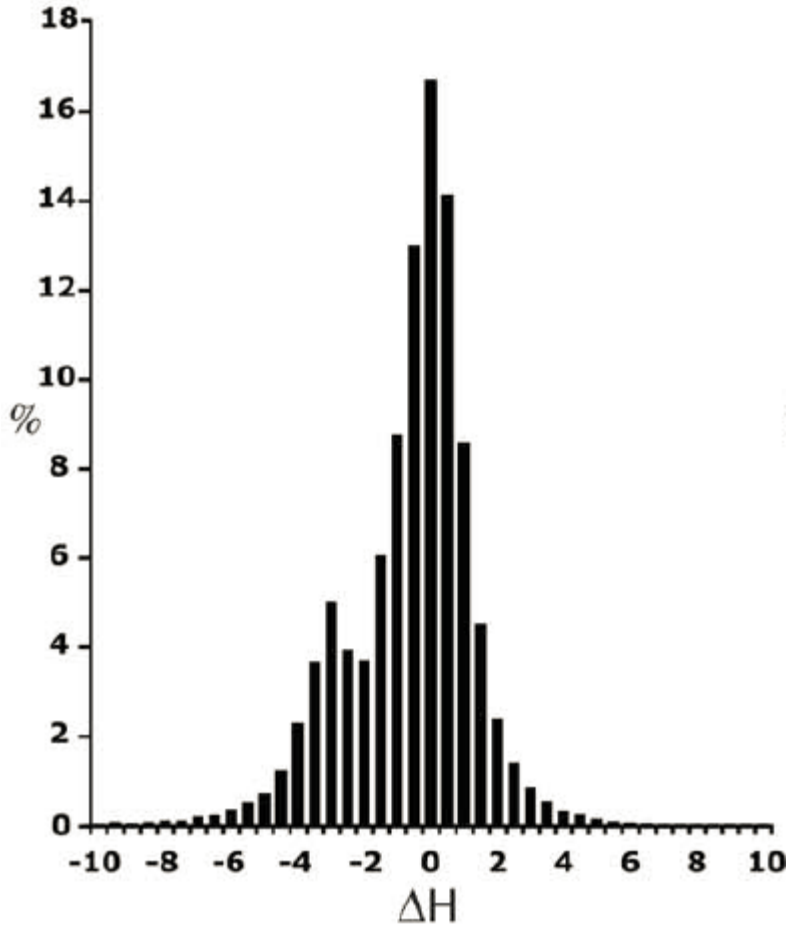
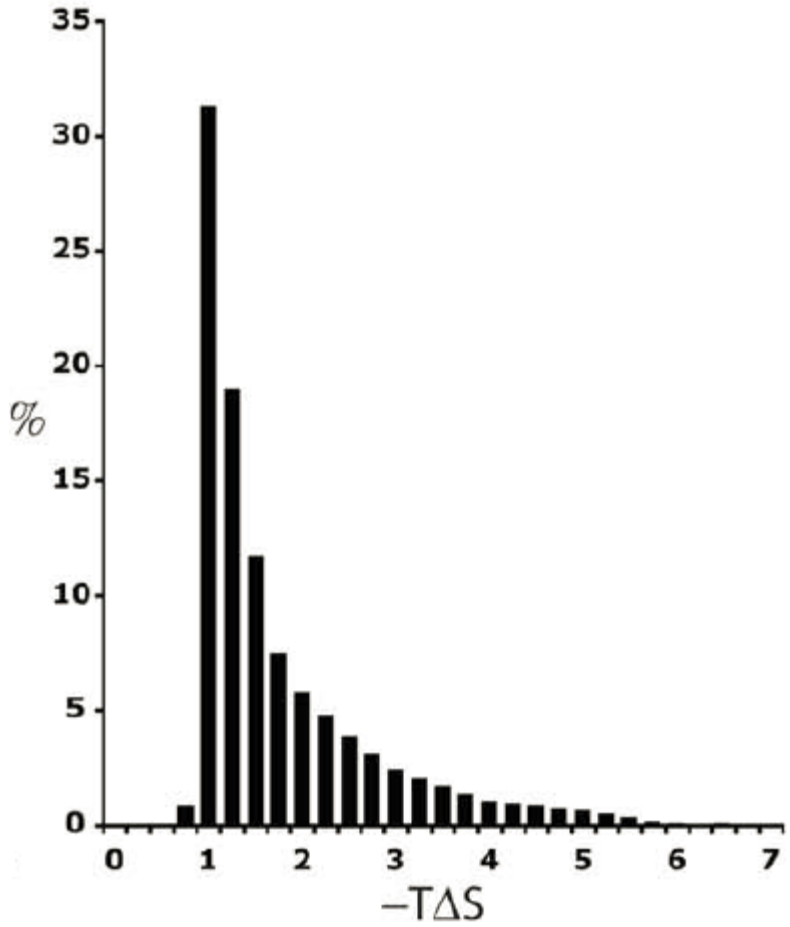
Enthalpy Distribution



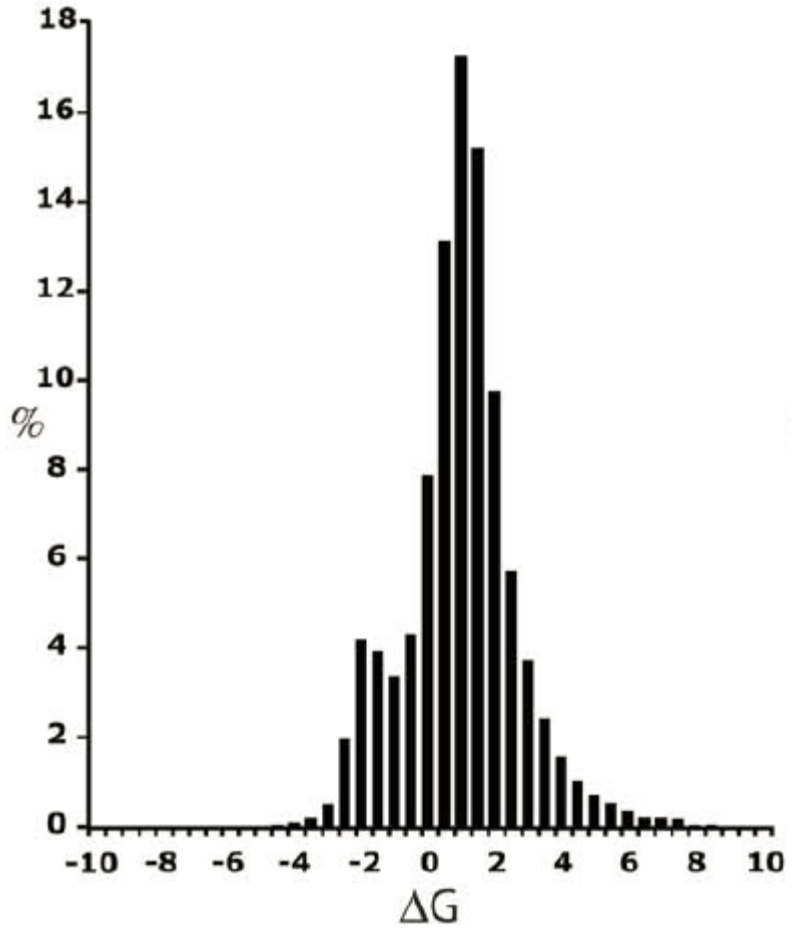
- Careful analysis of the ΔH-values reveals a tri-modal distribution
  - The modes are centred on:
    - -4.0kcal/mol for interactions with acidic-groups
    - -2.0kcal/mol for interactions with basic-groups
    - 0.0kcal/mol for interactions with uncharged-groups

Entropy Distribution



- The –TΔS value are all >0.0kcal/mol
  - This comes from our definition of entropy
    - Any interactions between the hydration-site and the protein will yield some protein-water correlation entropy
- The –TΔS values fall off asymptotically towards 6kcal/mol
  - This maximum value is reasonable
    - The entropy loss of transferring a water-molecule from the gas-phase to an ice-crystal at 298K is estimated to be 6.3kcal/mol

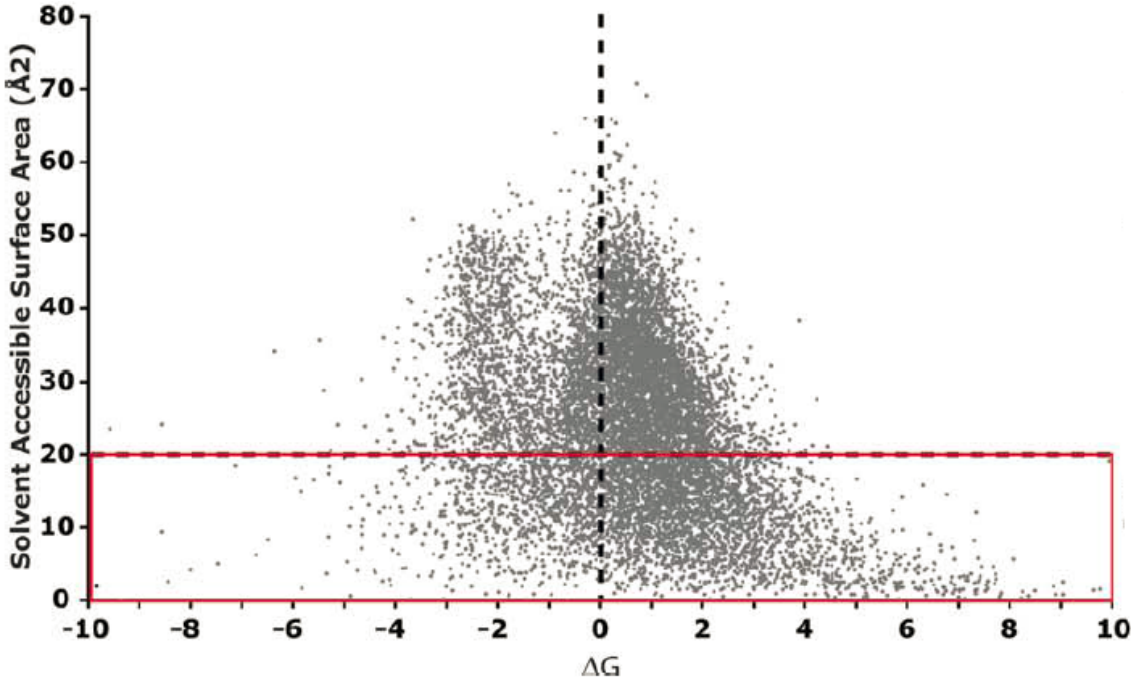
Free-Energy Distribution



- The ΔG-values are, of course, a superposition of the ΔH and –TΔS-values

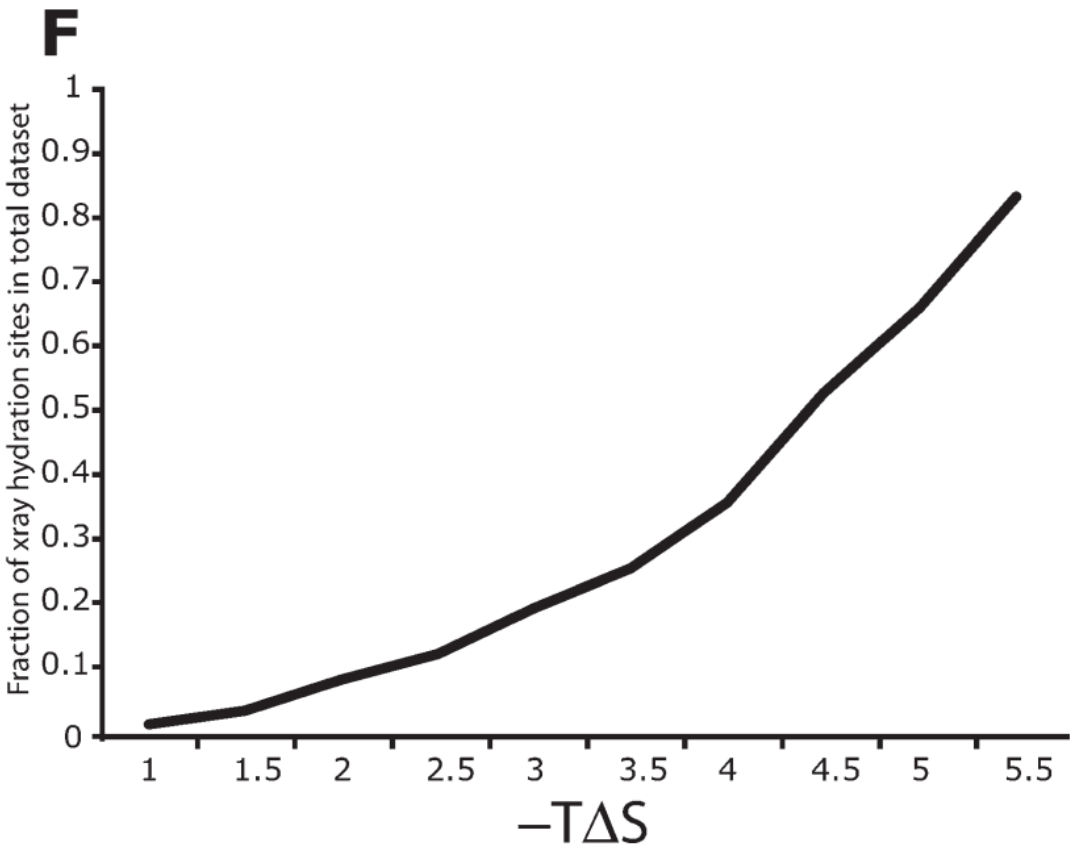
- Here we can see that few hydration-sites have ΔG>8kcal/mol
  - Waters that are destabilised beyond 8kcal/mol have a strong tendency to evacuate the binding site

The Effect of Solvent Accessibility

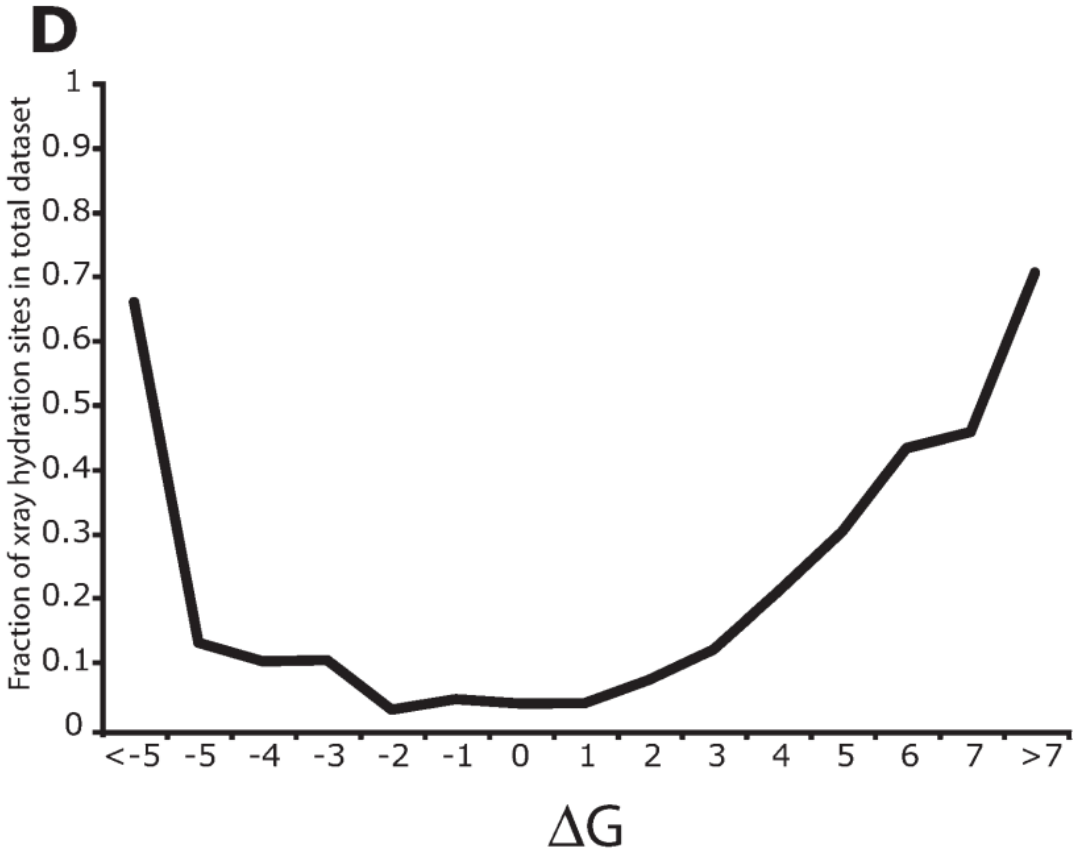


- The maximum SASA for a 1Å-radius hydration-site in 1.4Å-radius water is ~72Å^2
  - Such hydration sites are 'fully' solvent-exposed and should have ΔG=0.0kcal/mol
- As the hydration-site gets more buried we see a divergence in the energy
  - Some become more stable, some less
- The majority of profoundly unstable water-molecules (ΔG>2.0kcal/mol) have a SASA<20Å^2
  - i.e. they are buried
- However, a significant proportion of water-molecules with SASA<20Å^2 are quite stable
  - Being buried in a protein does not necessarily imply that a water-molecule is unstable

Crystallographic Water Molecules



Crystallographic water molecules have a more unfavourable entropy than typical waters. This is obviously a function of their localisation



The localisation either comes from electrostatic stabilisation, or from hydrophobic enclosure.

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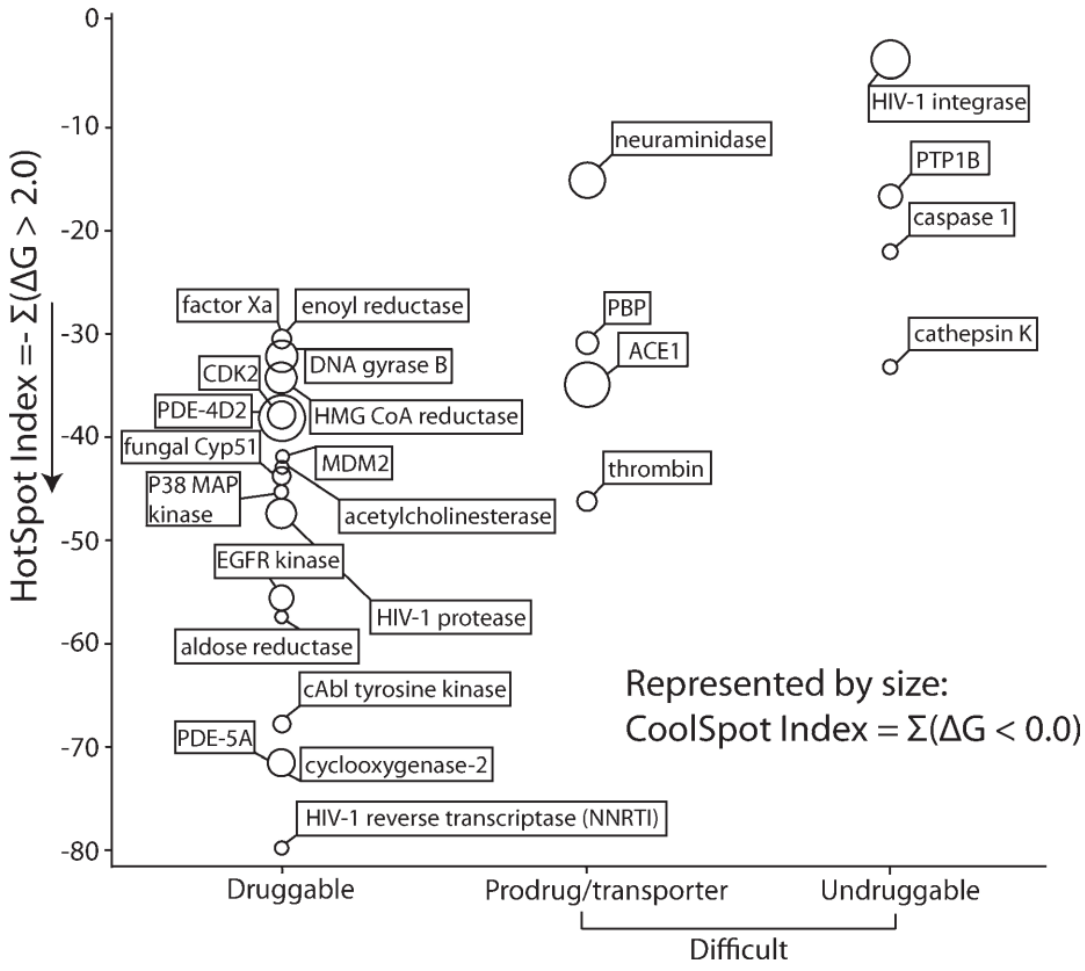
Druggability Assessment

- The stability/instability of water molecules occupying a site dictate the ease of finding a drug like ligand

- Two indices, the 'HotSpot' and 'CoolSpot' give an overall indication of the occupying water's properties
  - **HotSpot** – Characterises the level of hydrophobicity within the site
    - It's generally easier to bind a hydrophobic drug like compound to such a site
  - **CoolSpot** – Characterises the hydrophilicity within the site
    - Excessively numbers of stable water molecules make binding a hydrophobic ligand very difficult



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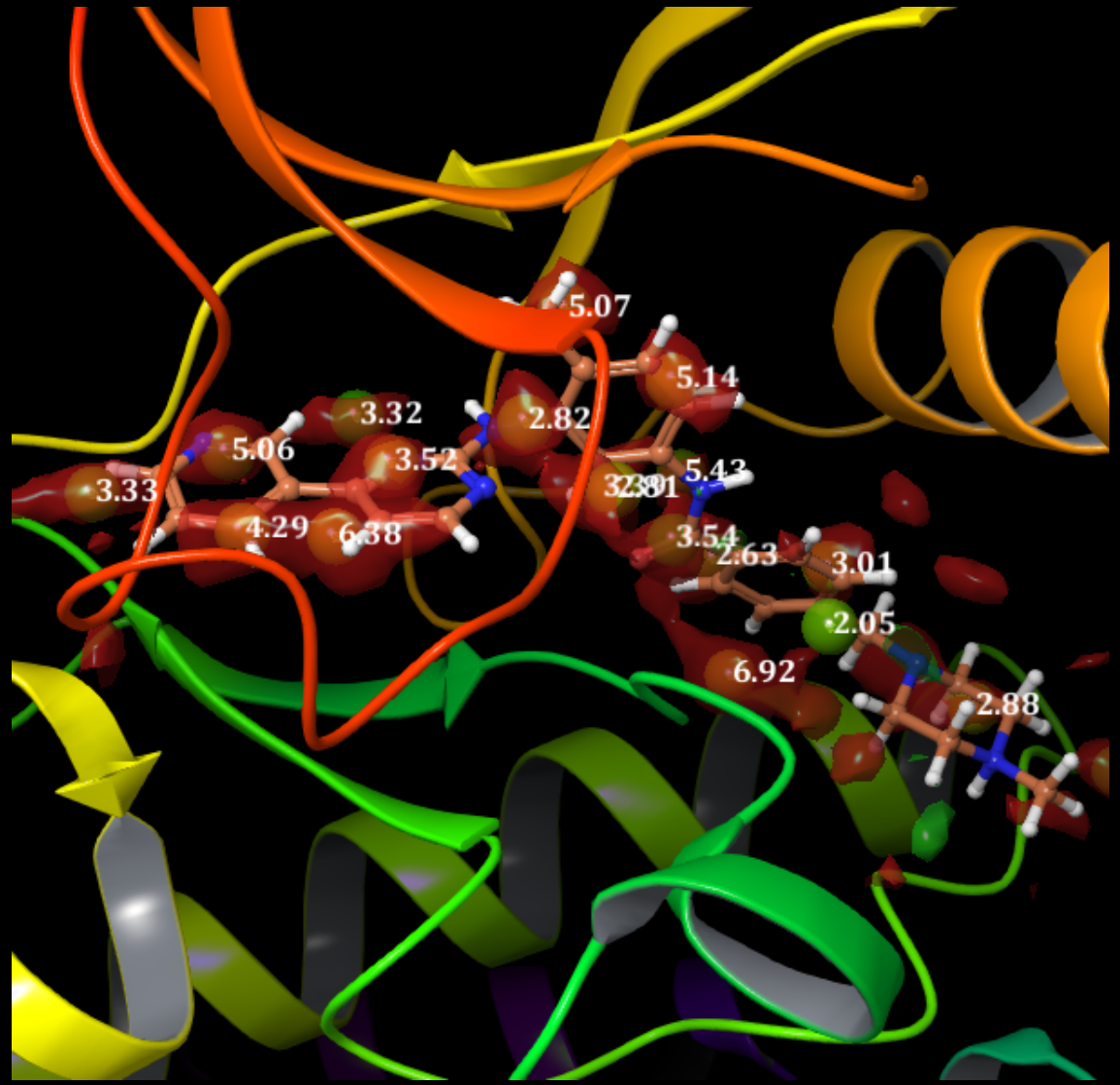
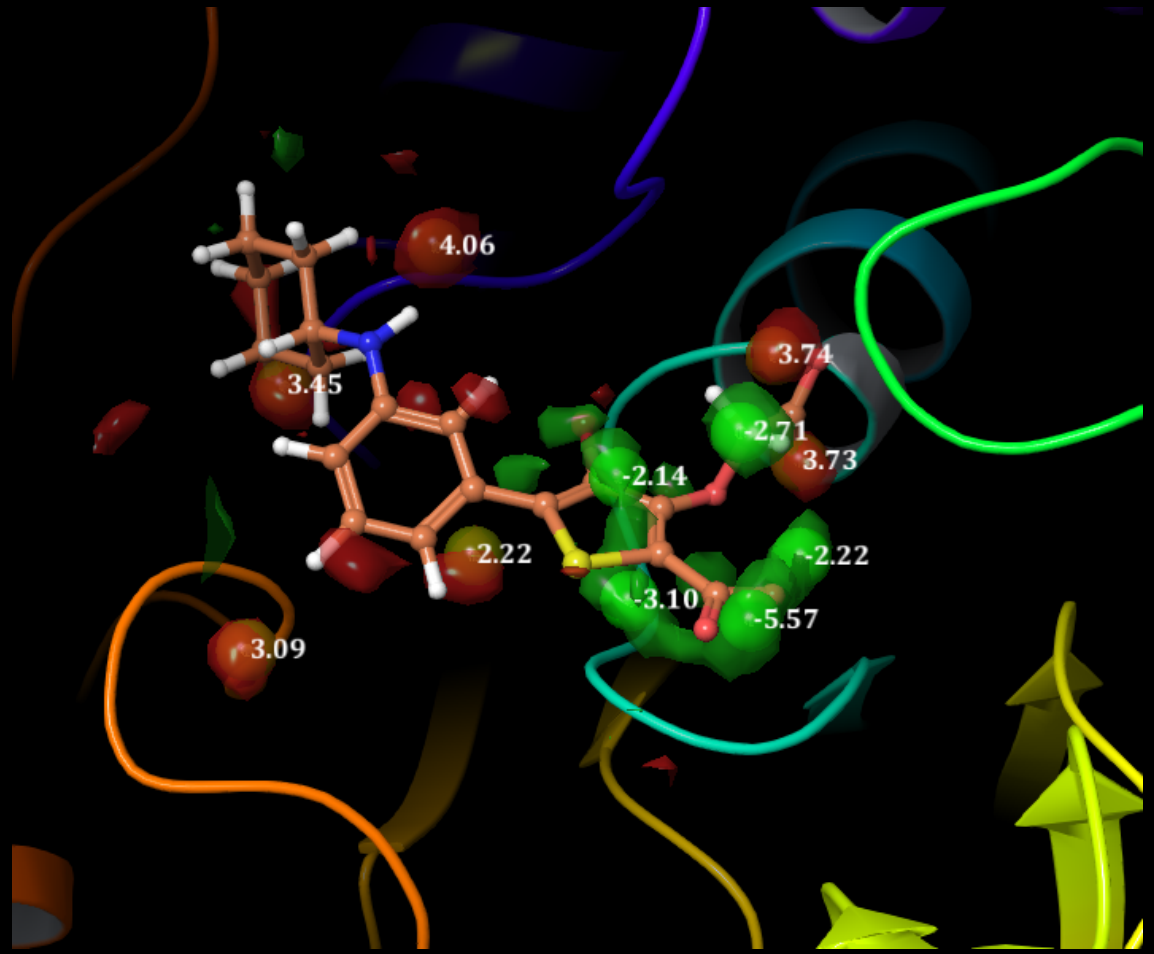
Druggability – Gleevec/Abl



- Abl is obviously a highly druggable target
- The WaterMap shows a complete chain of highly unstable hydration-sites
  - These actually provide a useful indication of the shape of an 'ideal' molecule
  - This is reinforced by looking at the continuous WaterMap, which shows an almost unbroken red-region throughout the binding-site

Druggability – PTP-1b



- PTP-1b provides an excellent example of an undruggable binding-site
- The core of the binding-site contains a large cloud of stable water-molecules
  - Hydrophobic atoms in this region are actually detrimental to binding
  - Only powerfully ionic atoms are capable of replacing some of these water-molecules
    - This naturally limits the drug likeness of any ligand
  - There are a scattering of hydrophobic-regions away from the main binding-site
    - But reaching these requires large, inefficient, ligands

Water Contributions to Ligand Binding

DOI: 10.1002/cmdc.201000533

CHEMMEDCHEM
CHEMISTRY ENABLING DRUG DISCOVERY

Contribution of Explicit Solvent Effects to the Binding Affinity of Small-Molecule Inhibitors in Blood Coagulation Factor Serine Proteases

Robert Abel,[a] Noeris K. Salam,[a] John Shelley,[a] Ramy Farid,[a] Richard A. Friesner,[b] and Woody Sherman*[a]

ChemMedChem, **2011**, 6 (6), *1049–1066*

Demonstrates how explicit water thermodynamics can be included in (semi-)quantitative ligand scoring

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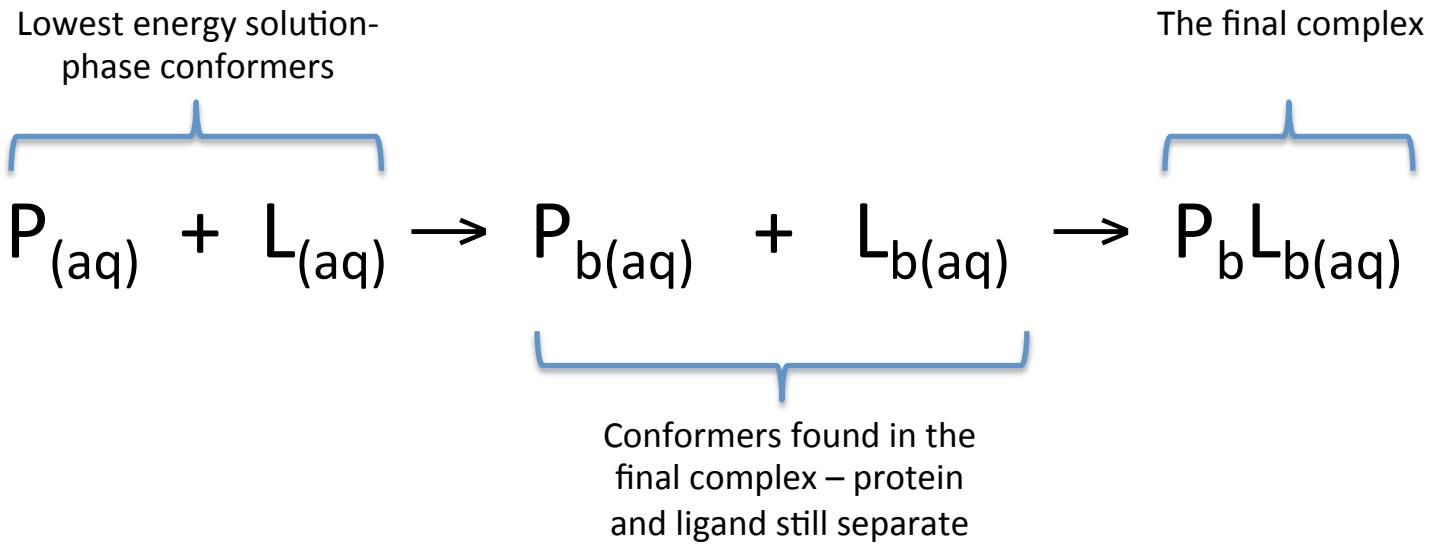
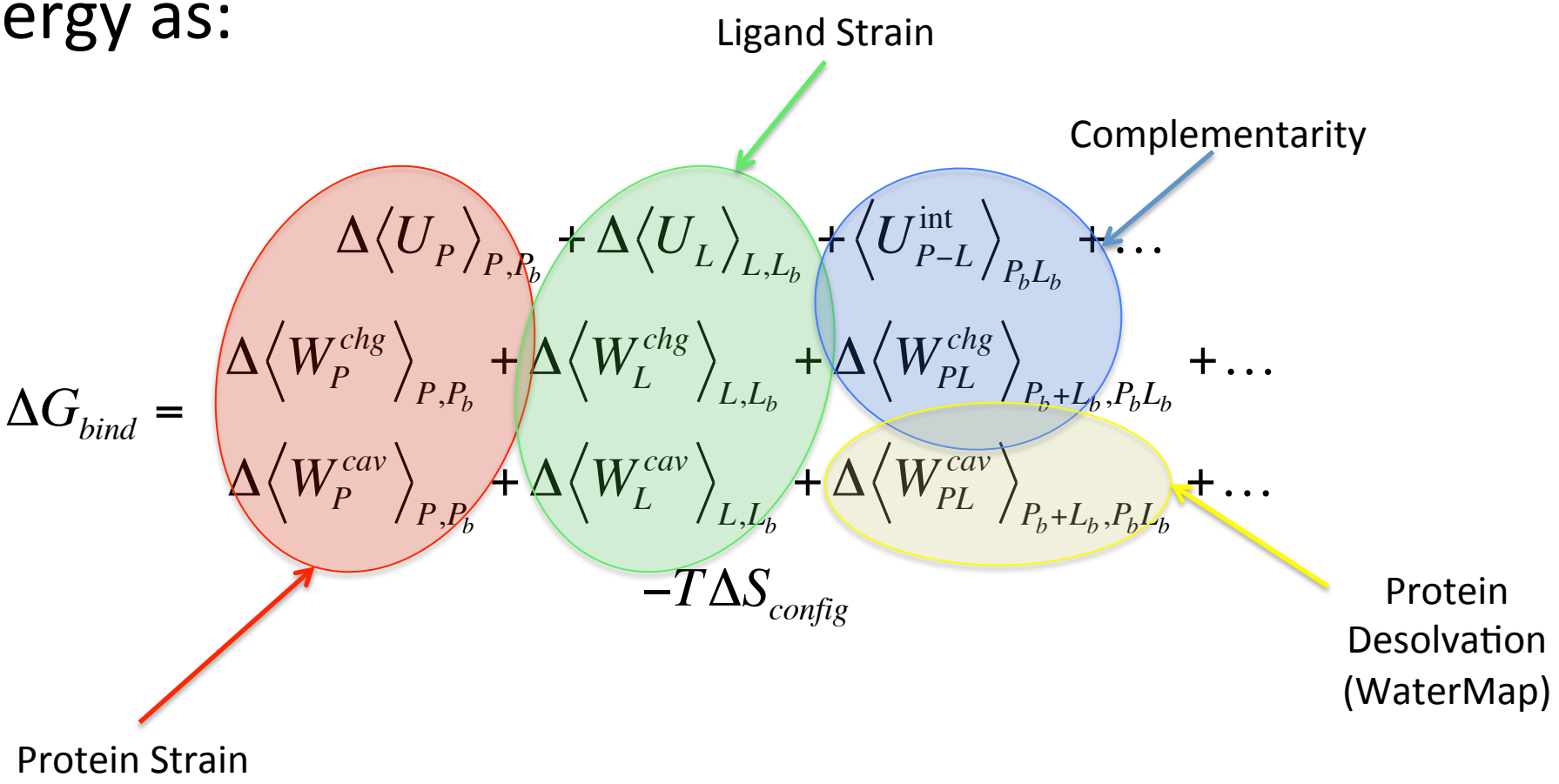
Modelling Protein-Ligand Affinities

- We can decompose protein-ligand binding into a three stage process:

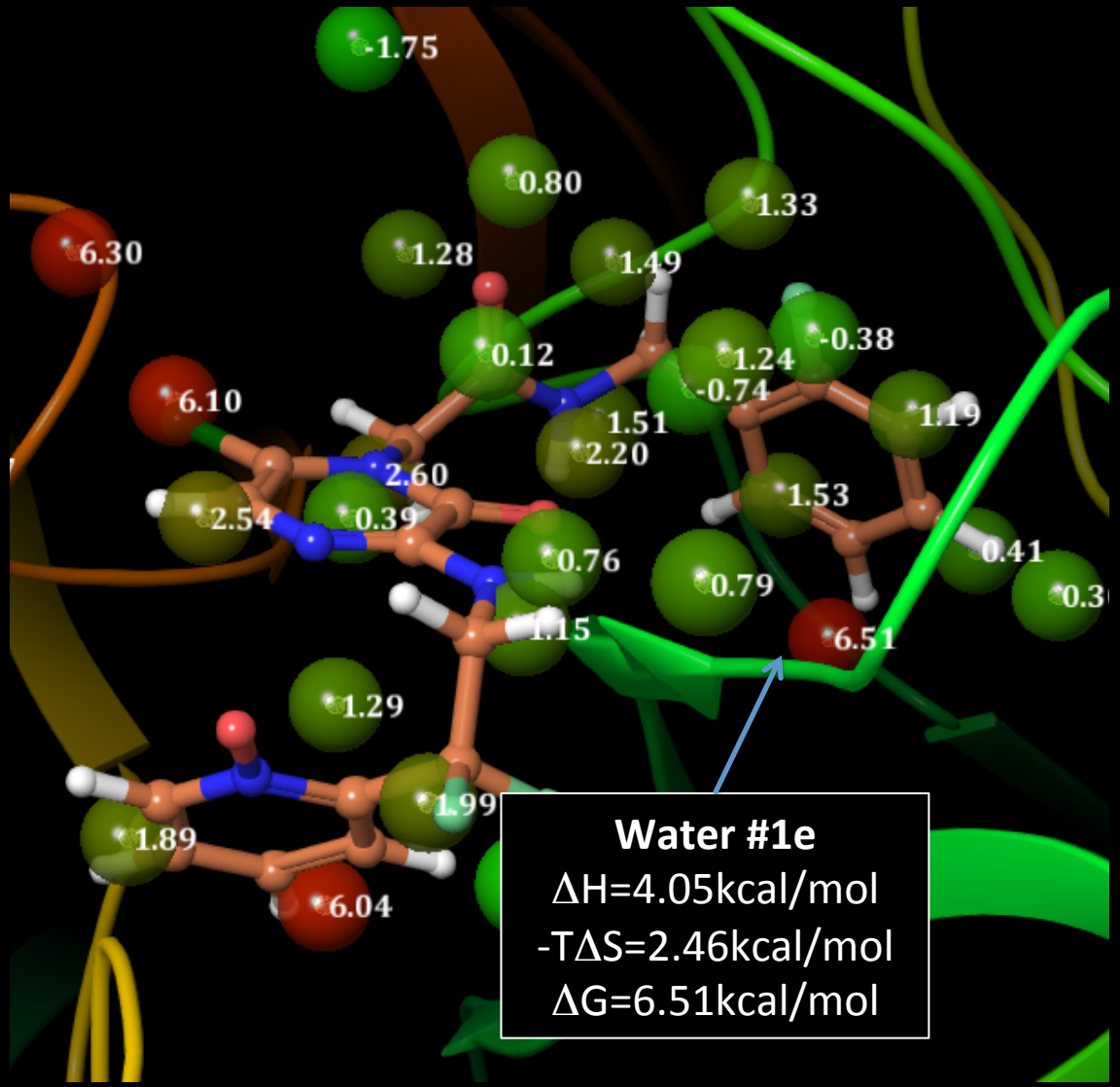


Modelling Protein-Ligand Affinities

- Based on this decomposition we can write down the binding free energy as:



Thrombin…



- Individual water molecules are known to play a significant role in binding to thrombin
- Classical modelling approaches, such as MMGBSA, do a reasonable job of getting the protein-ligand interactions modelled correctly
  - But miss out crucial details, such as the highly unstable water molecule highlighted

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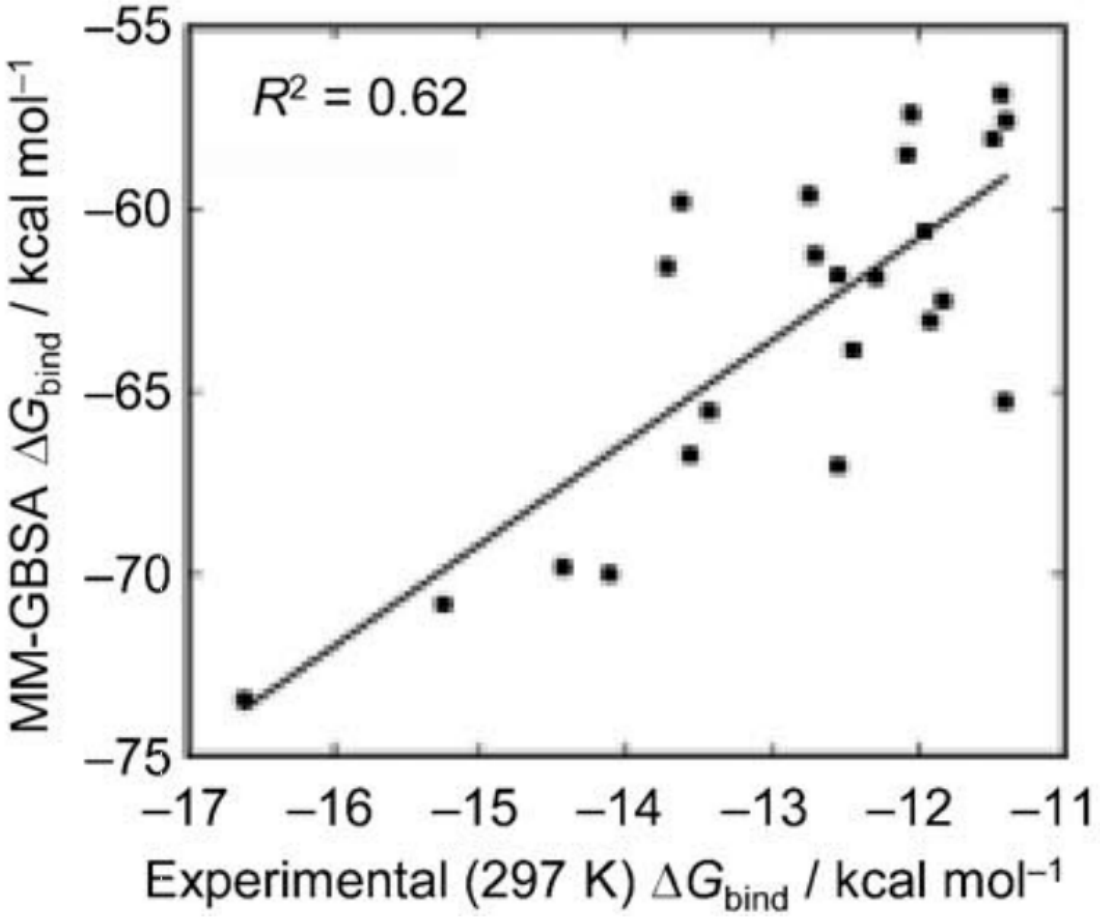
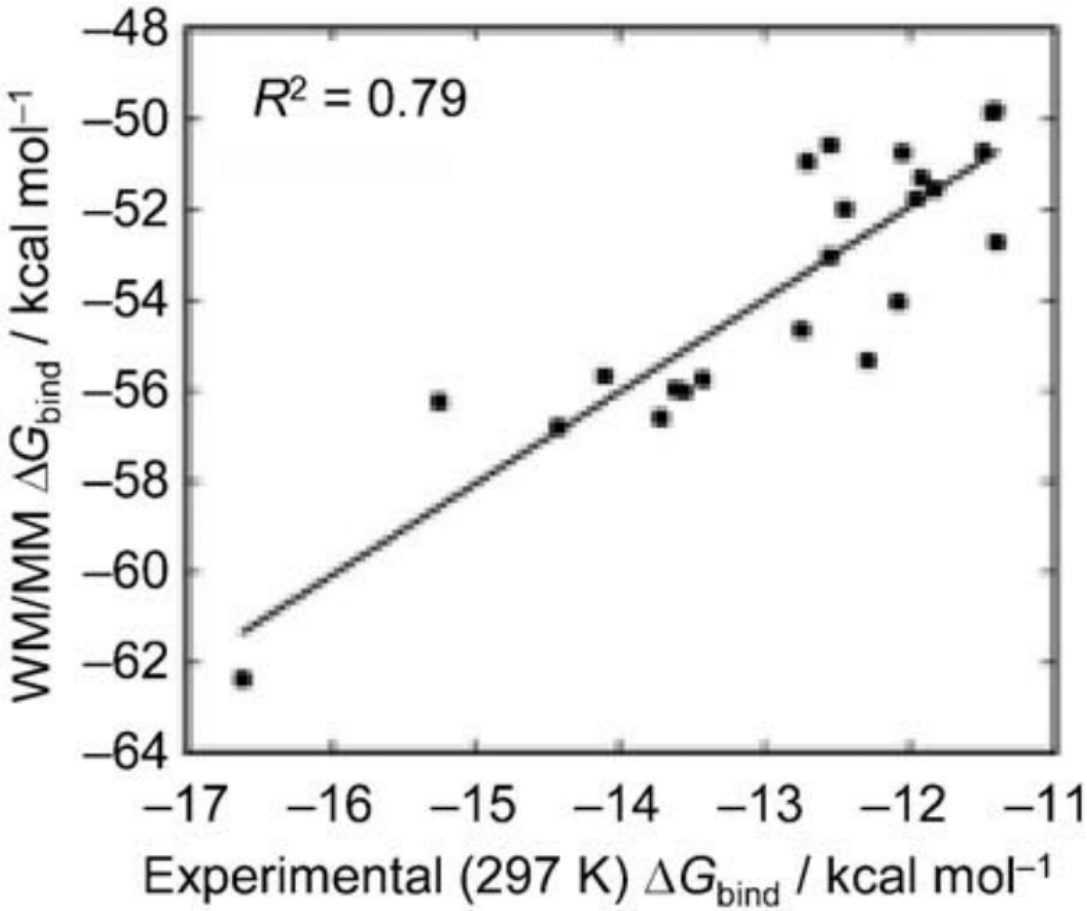
The Effect of Including Water Molecules



The plain MM-GBSA calculation does a passable job at modelling the complete set of congeneric thrombin inhibitors.



However, the inclusion of explicit water energetics, in this case calculated by WaterMap, improve the accuracy considerably.

*R^2(MW)=0.4

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Water Contributions to Selectivity

CHEMMEDCHEM

DOI: 10.1002/cmdc.200900501

Understanding Kinase Selectivity Through Energetic Analysis of Binding Site Waters

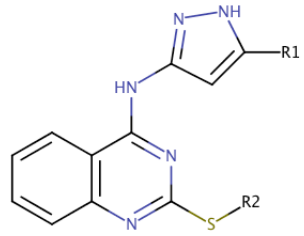
Daniel D. Robinson,[b] Woody Sherman,[a] and Ramy Farid*[a]

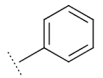
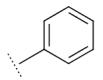
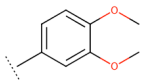
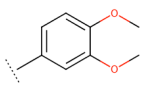
ChemMedChem, **2010**, 5, *618-627*

Examines how subtle differences in binding site solvation can explain significant features of selectivity SAR

Src/GSK3β Selectivity

- This data is taken from patents and publications by Vertex
- Their data shows that adding a cyclopropyl-group to the R_1 position selectively gains activity on Src
- No immediate structural hypothesis can be put forward for this selective 8-20x gain in potency on Src vs. GSK3β



		Kinase Inhibition (μM)	
R_1	R_2	K_i(GSK3β)	K_i(Src)
		0.171	0.8
		<0.1	<0.1
		1.168	2.180
		>1.0	0.1-1.0

Davies, R., et al., *Pyrazole compounds useful as protein kinase inhibitors*. 2003, Vertex Pharmaceuticals Incorporated (Cambridge, MA, US): United States.
Bebbington, D., et al., *The discovery of the potent aurora inhibitor MK-0457 (VX-680).* Bioorg Med Chem Lett, 2009. **19**(13): p. 3586-92.

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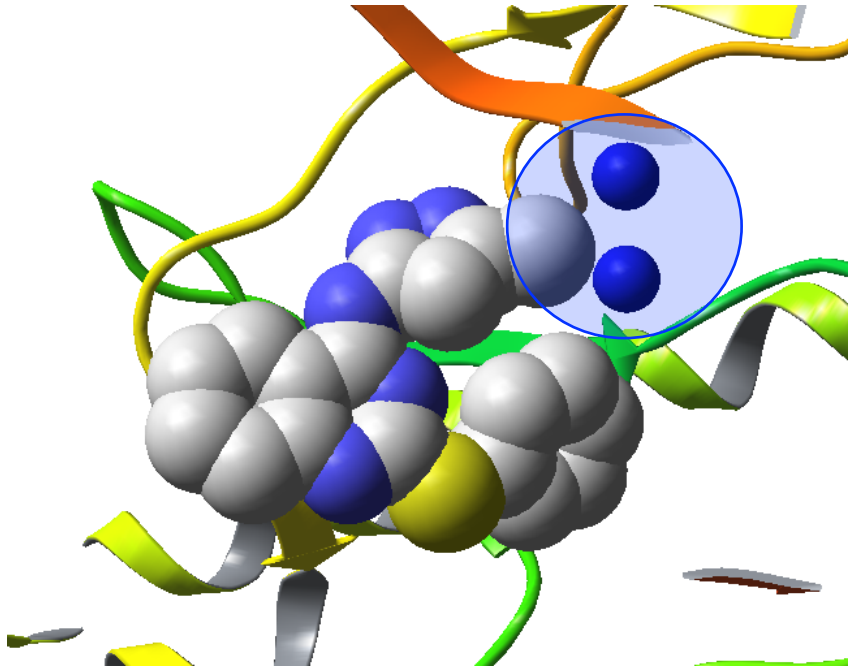
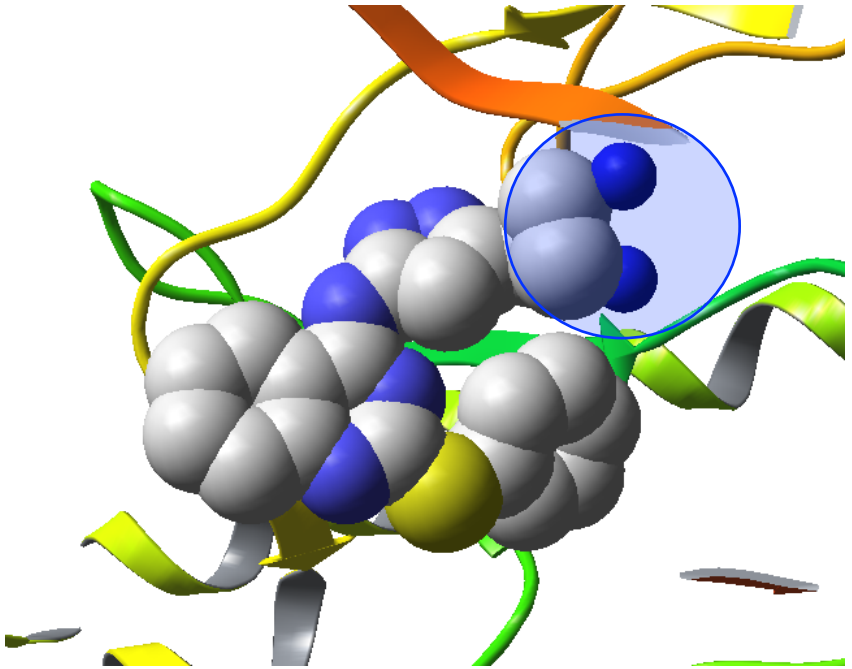
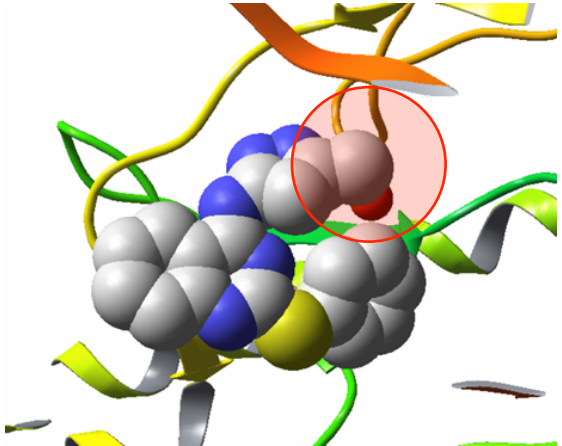
Src/GSK3β Selectivity





High energy water molecules present in Src and not GSK3β (**blue**)



High energy water present in GSK3β (**red**) is displaced by both methyl and cyclopropyl variants

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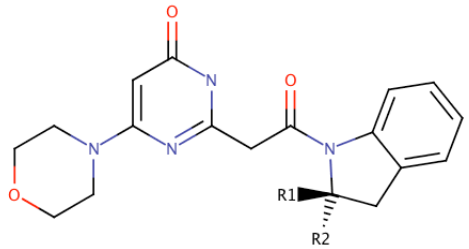
PI3-Kinase Isoform Selectivity

- This data was supplied by Sanofi who were working on the ligand series shown
- A methyl-group was added for reasons other than activity/selectivity
  - Unexpectedly and fortuitously addition of the methyl-group in one position yielded selectivity for PI3β
  - This SAR trend was repeated over a number of variants with the same result
    - R_1 = H: PI3β selective, overall weaker binding
    - R_2 = H: Equipotent PI3β/δ, overall stronger binding
- Crystal structures of the ligands bound showed no differences in protein-ligand contacts



R_1	R_2	Selectivity (PI3β/PI3δ)
Me	H	1x
H	Me	20x

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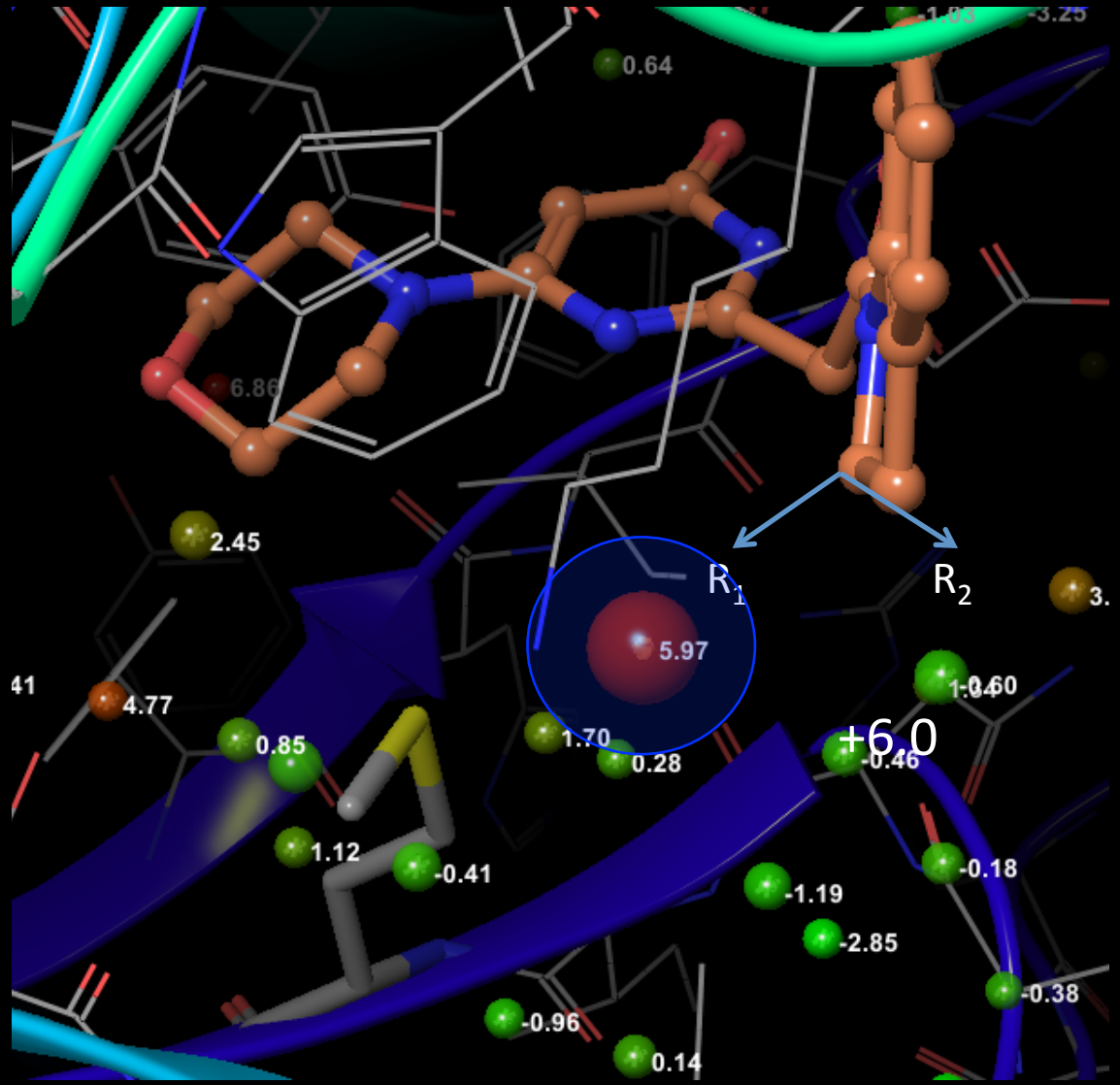
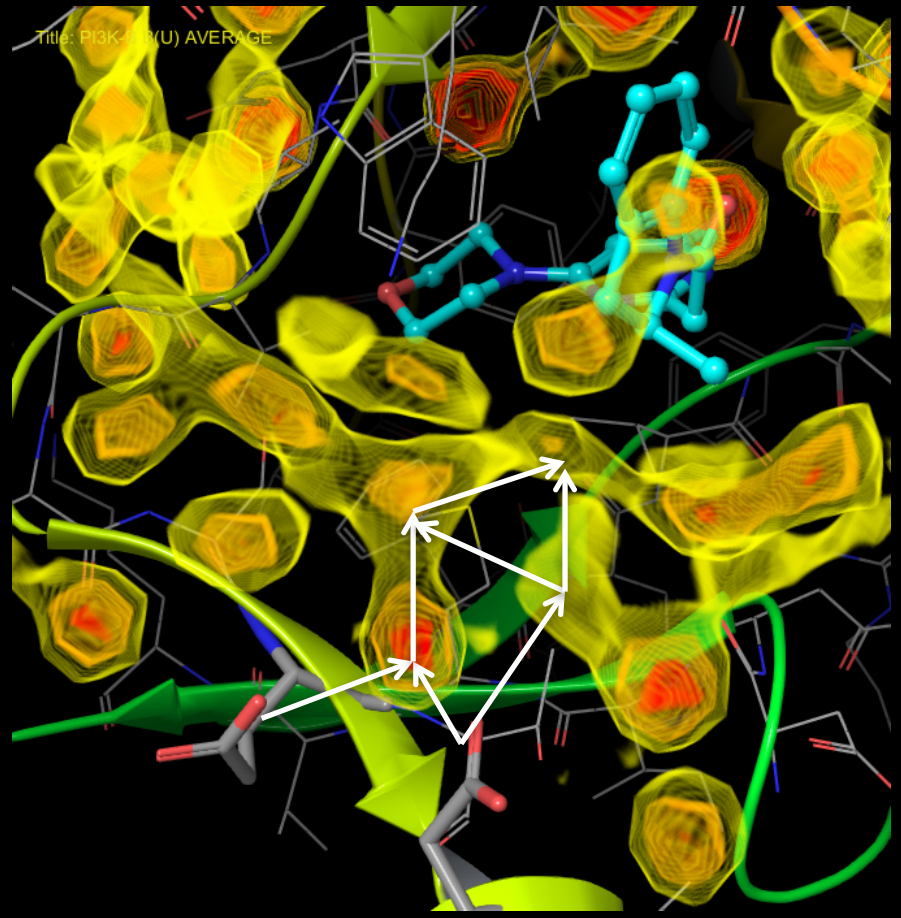
PI3Kβ WaterMap – R_1=R_2=H



- Based on the Sanofi crystal structures we can calculate the properties of the solvent within the pocket in the presence of the ligand
- One obviously 'unhappy' water molecule is found in both PI3Kβ and PI3Kδ
  - This water molecule is trapped between the ligand and the hydrophobic 'base' of the kinase binding site
- Substituents from R_1 are perfectly positioned to displace this water molecule from both isoforms
  - This accounts for the higher potency of the R_1 substituted compounds
- Substituents from R_2 tend to trap and isolate this water molecule further
  - A closer analysis reveals that this trapping is more unfavourable in PI3Kδ than PI3Kβ
  - This accounts for the greater loss of potency in PI3Kδ

A Structural Explanation?

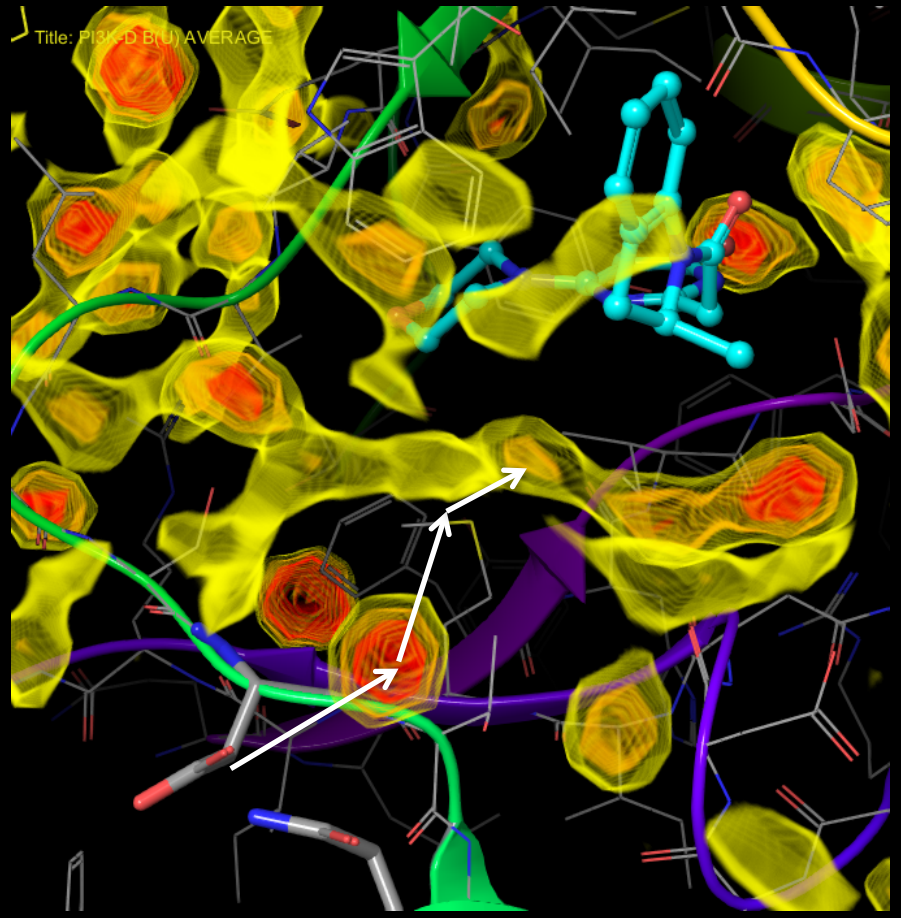


%Occ

60%

70%

80%



Water-Density PI3Kβ R_1=H R_2=Me

Water-Density PI3Kδ R_1=H R_2=Me

Analysis of the water density around PI3Kβ shows a well-structured network of water molecules emanating from ^{852}Glu and ^{856}Asp. These stabilise the trapped water.

A somewhat similar situation appears in PI3δ. However the nature of the residues involved are different (^{836}Asn and ^{832}Asp). This gives much less support to the trapped water molecule causing the greater loss in potency.

The Future?

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

Accurate and Reliable Prediction of Relative Ligand Binding Potency in Prospective Drug Discovery by Way of a Modern Free-Energy Calculation Protocol and Force Field

Lingle Wang,[†] Yujie Wu,[†] Yuqing Deng,[†] Byungchan Kim,[†] Levi Pierce,[†] Goran Krilov,[†] Dmitry Lupyan,[†] Shaughnessy Robinson,[†] Markus K. Dahlgren,[†] Jeremy Greenwood,[†] Donna L. Romero,[‡] Craig Masse,[‡] Jennifer L. Knight,[†] Thomas Steinbrecher,[†] Thijs Beuming,[†] Wolfgang Damm,[†] Ed Harder,[†] Woody Sherman,[†] Mark Brewer,[†] Ron Wester,[‡] Mark Murcko,[†] Leah Frye,[†] Ramy Farid,[†] Teng Lin,[†] David L. Mobley,[⊥] William L. Jorgensen,[∥] Bruce J. Berne,[§] Richard A. Friesner,[§] and Robert Abel*[,†]

J. Am. Chem. Soc, **2015**, 137, *2695-2703*

Introduces a robust, general purpose, FEP protocol for routine relative binding predictions

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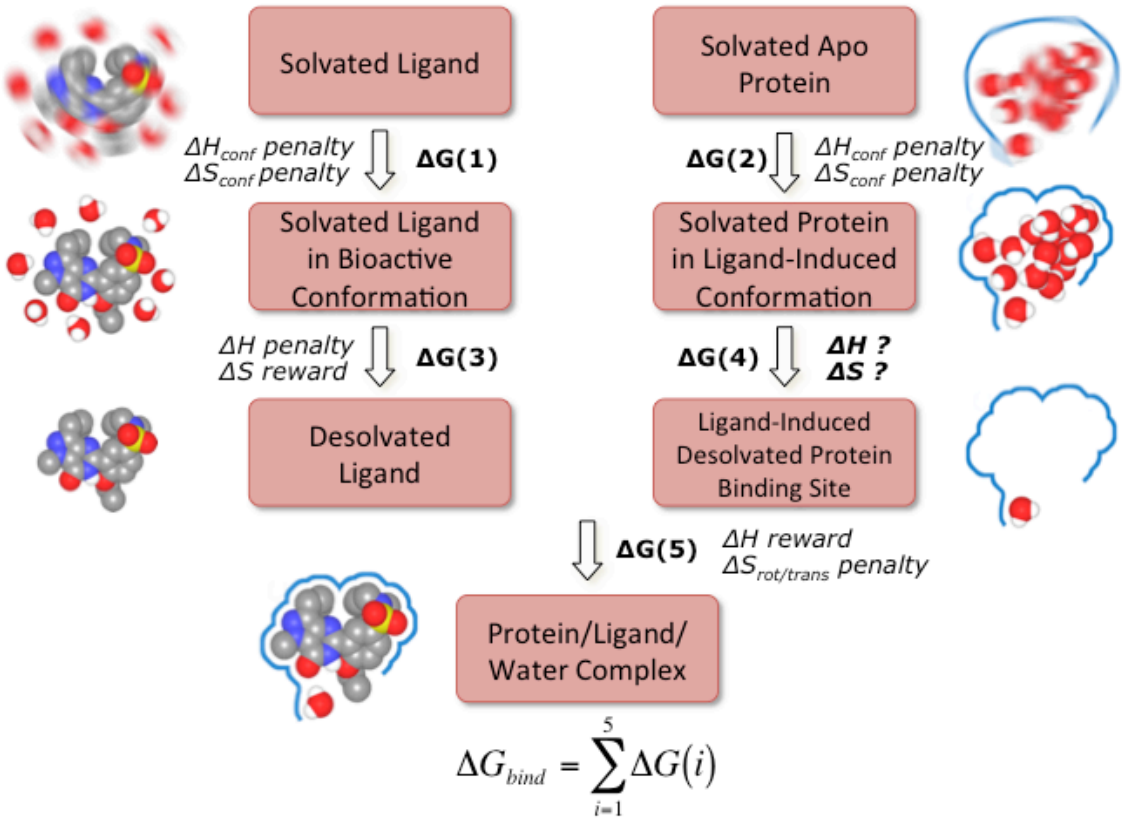
Free-Energy Perturbation

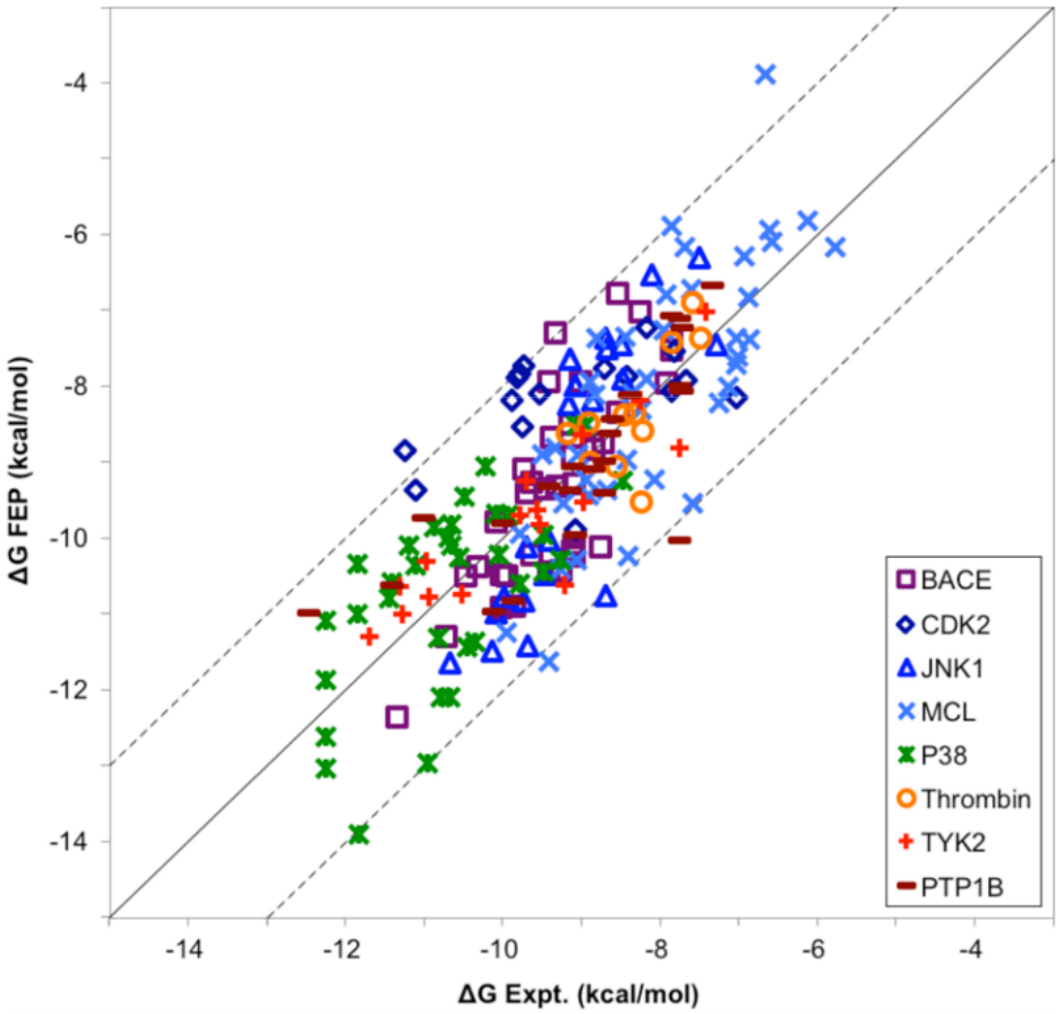


- All of the previous examples looked at isolated parts of the complete protein-ligand binding event
  - Potential protein desolvation for druggability
  - Augmented MM-GBSA for (semi-)quantitative ligand binding estimations
  - Residual solvent stability, particularly in the case of PI3Kβ/δ
- Such a breakdown is useful, but it is also artificial
  - Approaches like FEP allow us to consider the complete binding event in a holistic manner

Free-Energy Perturbation

- The theory of FEP has been around for a long time
  - But only recently do we have all of the pieces needed to give it a chance of functioning in an industrial setting. These include:
    - **Accurate force-fields** – capable of describing both the ligand and the protein
    - **Sufficient compute power** – GPUs provide a 20-100x speed improvement over standard CPUs
    - **Adequate sampling** – Enhanced sampling algorithms, such as replica-exchange, allow us to explore all of the relevant space
    - **Robust, automated setup and analysis** – To ensure consistency and convergence

- Thus far, the results appear promising
  - Retrospective studies show excellent agreement with experiment (RMSE≅1.2kcal/mol)
  - Early prospective studies show similar levels of accuracy



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Summary

- Explicit waters are essential in molecular recognition
- Thermodynamic characterisation of water within a protein cavity can be used to assess druggability, affinity, and selectivity
  - The effect of the ligand on residual solvent is just as important as the water molecules it displaces
- Full simulation approaches, such as FEP, show real promise in providing robust fully quantitative estimates of (relative) ligand potency

Acknowledgements

Schrödinger

- Thijs Beuming
- Goran Krilov

WaterMap

- Robert Abel
- Byungchan Kim
- Jen Knight
- Goran Krilov
- Teng Lin
- Levi Pierce
- Lingle Wang
- Yujie Wu

FEP

Sanofi (PI3K)

- Thomas Bertrand
- Frank Halley
- Andreas Karlsson
- Magali Mathieu
- Herve Minoux
- Laurent Schio

WaterMap
PI3K

Columbia University

- Rich Friesner

FEP

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Assessment of Water Energetics and Applications to Drug Discovery

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