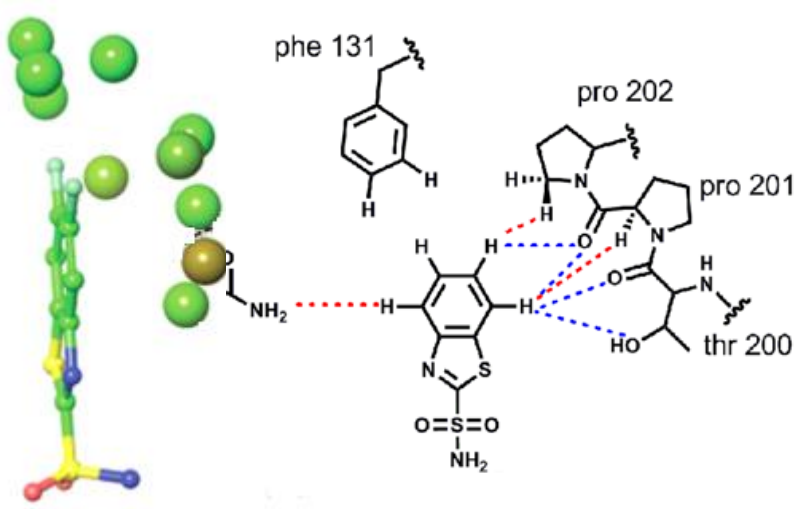
Classification of Water Molecules at The Ligand-Binding Site

Speaker: Xiaoxiao Zhang

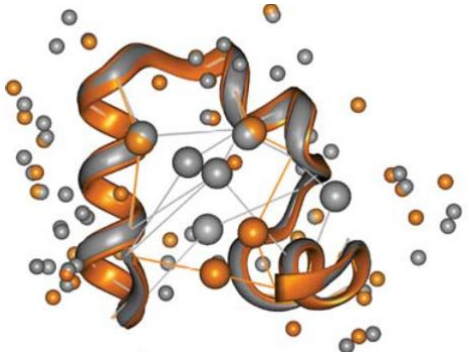
2014.8.20

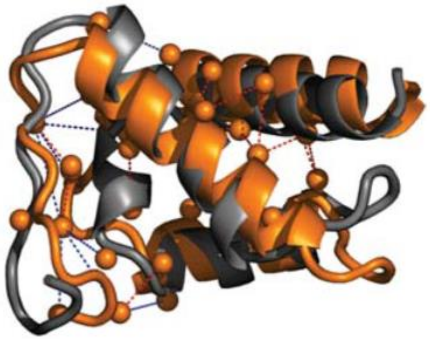
Contents

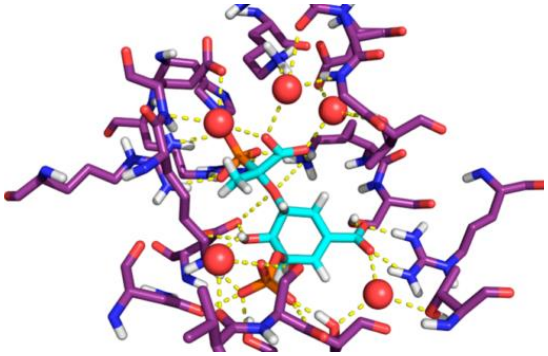
- General introduction: protein-water interaction.
- Classical examples in drug design.
- The main problem of considering wate
- Water in bromodomain.
- Methods of classifying water.
- Summary

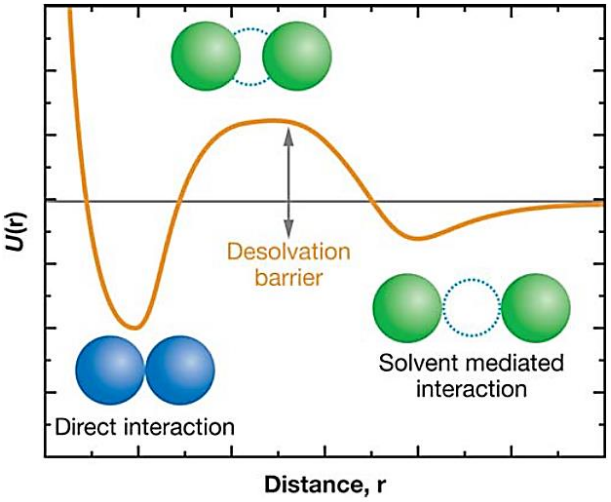


Water Mediation in Protein Dynamics







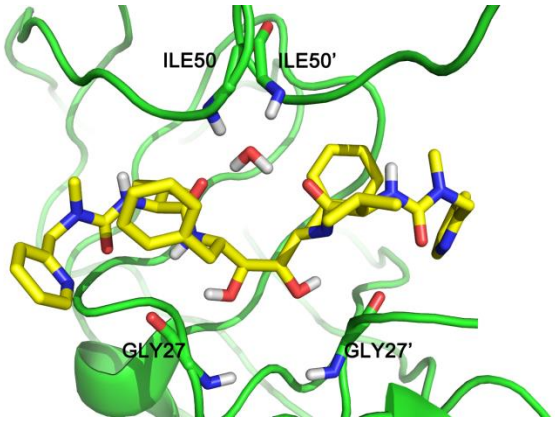


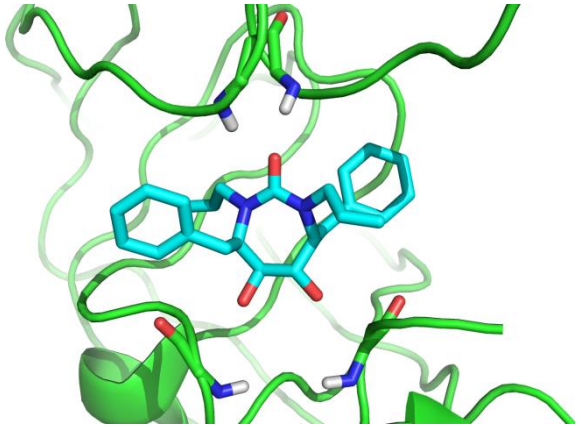
- Contribute to the structure, stability, dynamics, and function of biological macromolecules.
- Guide the conformational search in protein folding by gating hydrophobic residues.
- An active component rather than an inert environment.

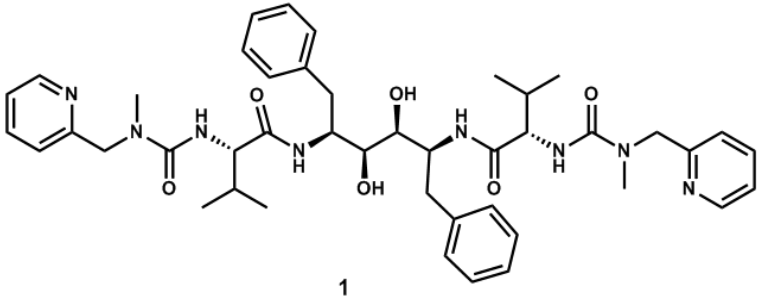
Petrone, P. M. & Garcia, A. E. *J. Mol. Biol.* **338,** 419–35 (2004).

Levy, Y. & Onuchic, N. *Annu. Rev. Biophys. Biomol. Struct.* **35**,389–415, (2006).

Cyclic Urea HIV-1 Protease Inhibitor: Displacing Water







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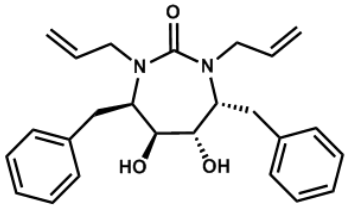


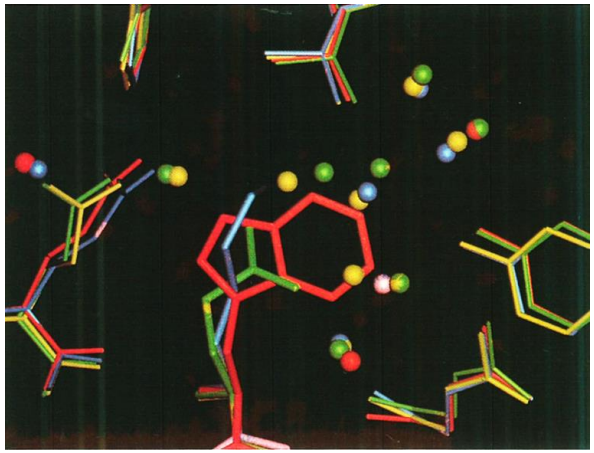
2

Advantages:

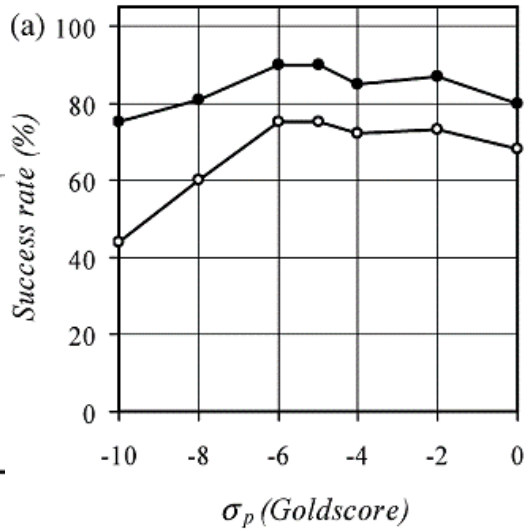
- Better bioavailability.
- Avoid molecular obesity.
- Increased specificity by polar interactions.
- Emphasis on improving enthalpic rather than entropic interactions.

Lam, P. Y, Ericksonviitanen, S. *Science*, **263**, 380-384, (1994).
Ala, P. J. *Journal of Biological Chemistry*, **273**, 12325-12331, (1998).

Decreasing Binding Affinity in Displacing Water



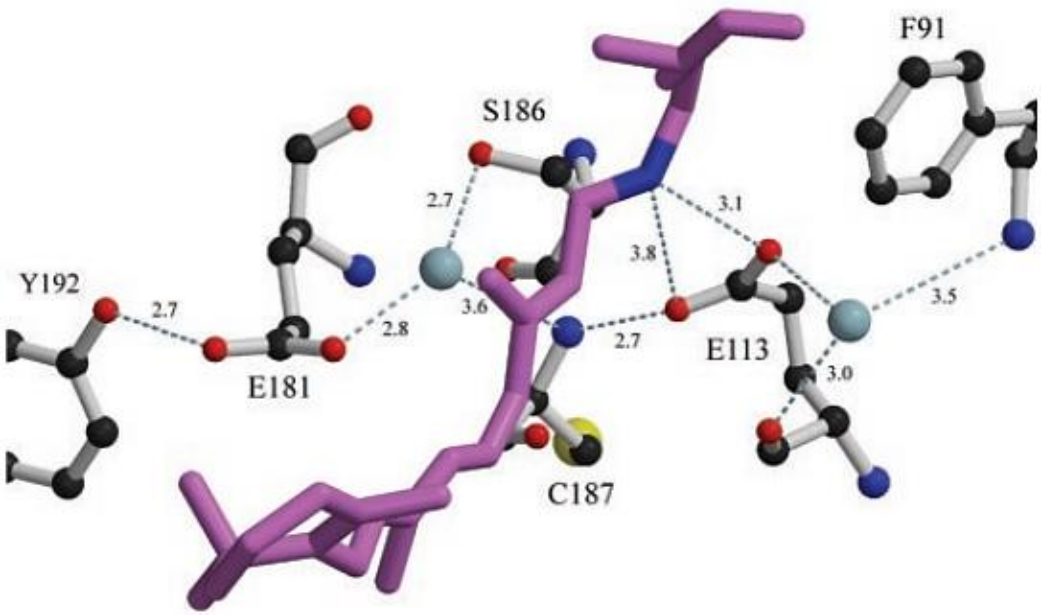
Peptide	Water*	K_d μM	ΔG° kJ mol^{-1}
J mol^{-1} K^{-1}			
KAK	7	0.06	−41.4
KWK	4	0.11	−39.7
$\Delta_{Trp \rightarrow Ala}$	3	–	−1.7
KEK	7	0.15	−38.9
KKK	6	2.0	−32.3
$\Delta_{Lys \rightarrow Glu}$	1	–	−6.6



- On binding the Lys-X-Lys, OppA remains invariant.
- The water network of the binding site changes.
- When X displaces water molecules, the binding affinity responses differently.
- The results describing docking are much more accurate including water molecules.

Ladbury, J. E. *Chem. Biol*. **3**, 973–80, (1996).
Barillari, C., Essex, J. W., *J. Am. Chem. Soc.* **129**, 2577-2587, (2007).

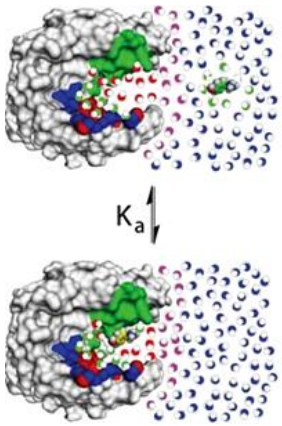
The Main Problem For The Consideration of Water

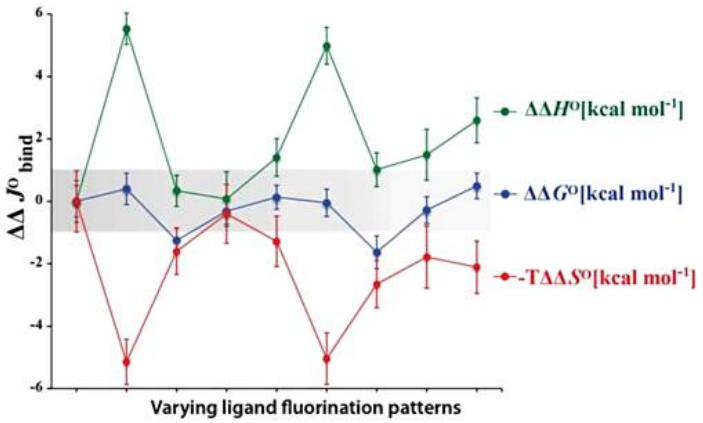


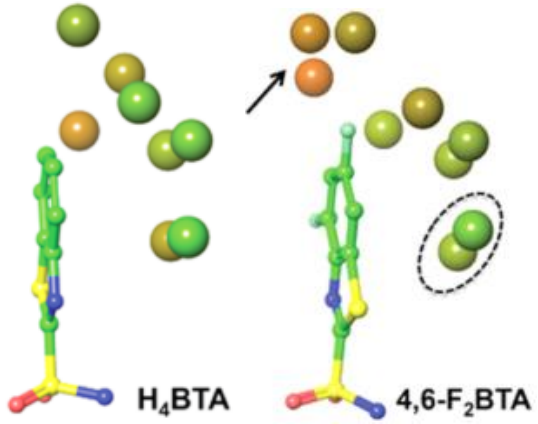
- Understanding the mechanism of water network contribute to the protein-ligand binding.
- Measuring the propensity of a water molecule for displacement.

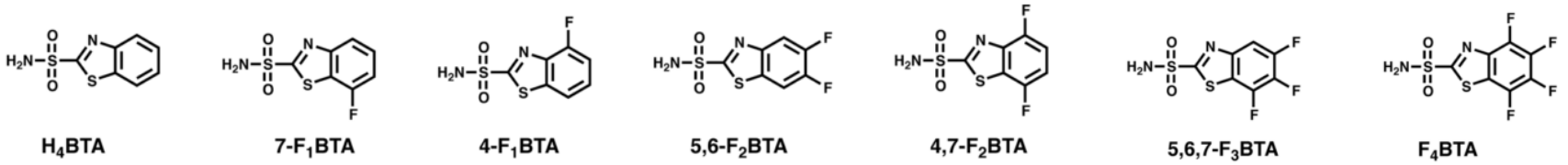
Okada, T. *et al. PNAS* **99,** 5982–5987 (2002).

Water Networks Contributing to H/S Compensation









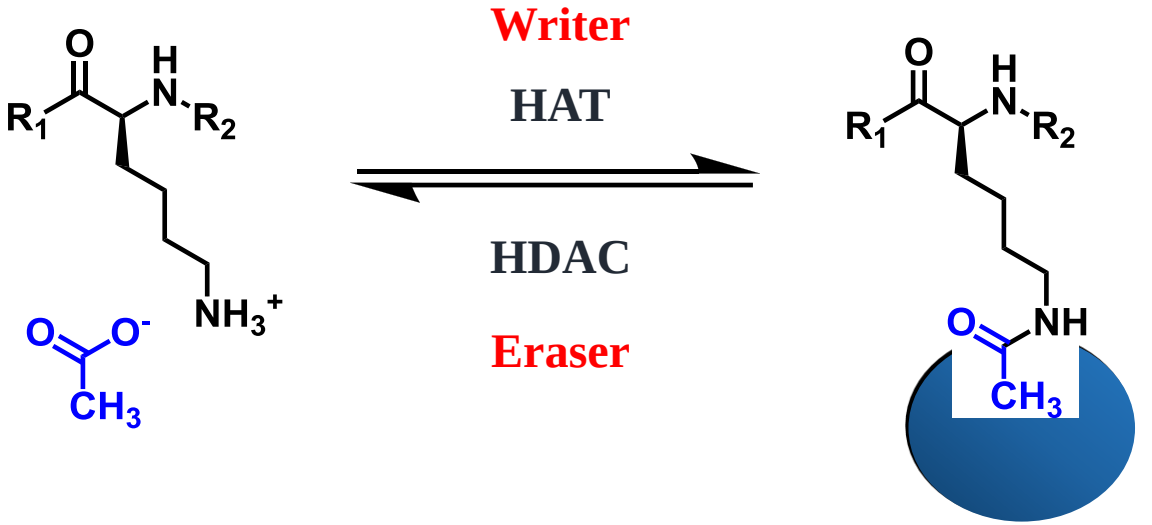
H/S compensation:

- An unfavorable entropy caused by conformational restrictions of the ligand;
- Small conformational changes throughout the protein;
- Reorganization of solvent molecules.

Changes in the ligand result in surprisingly small changes in the $\Delta\Delta G^o$.
The shape of the ligand, thus the related water H-bond networks, determines the $\Delta\Delta G^o$.

Breiten, B. *et al. J. Am. Chem. Soc.* **135**, 15579−15584, (2013).

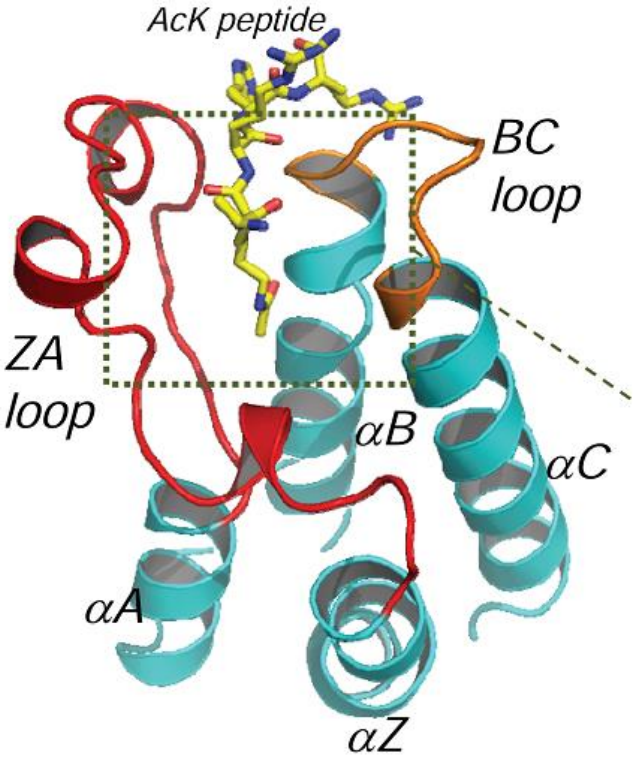
Epigenetic Regulators Involved in Histone Acetylation

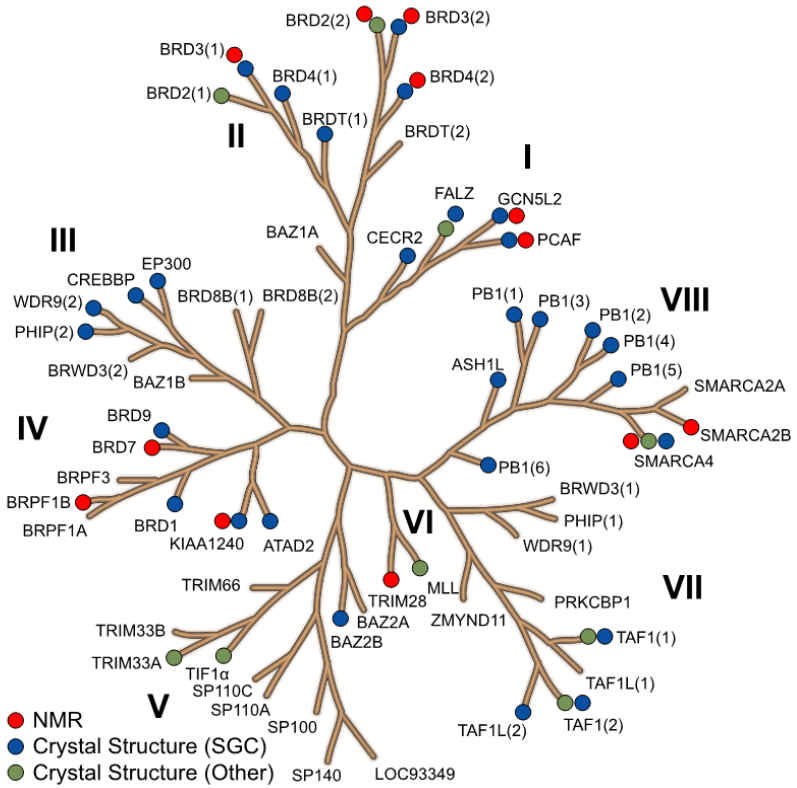


HAT: Histone acetyltransferse
HDAC: Histone deacetylase

Minucci, S.; Pelicci, P. G. *Nature*, **6**, 38, **(**2006).
Ponting, C. P.; Russell, R. R. *Annu. Rev. Biophys. Biomol. Struct.*, **31**, 45, (2002).

The Bromodomain Protein Family

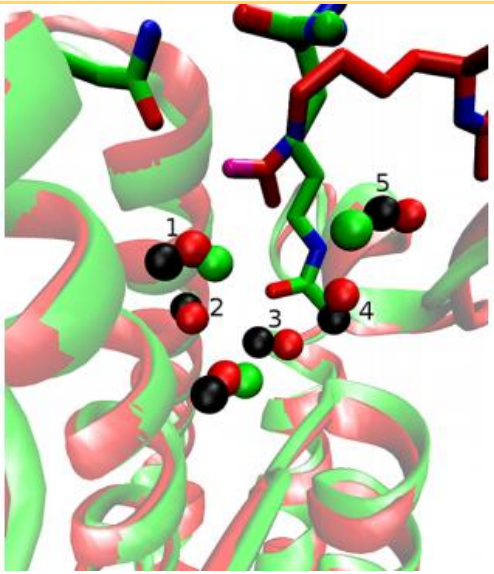




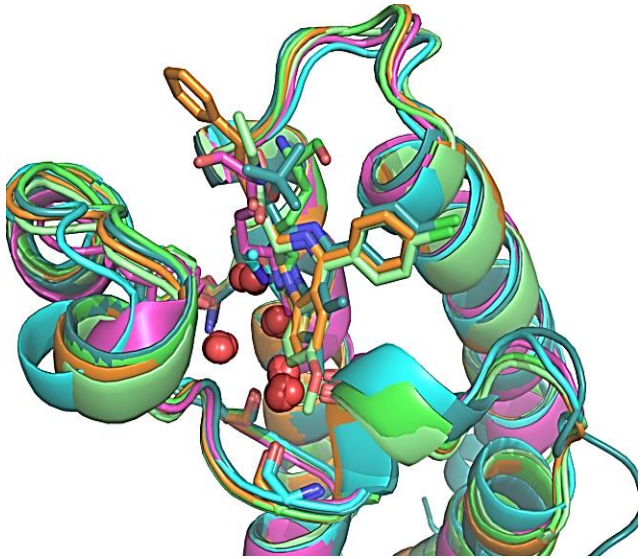
61 BRDs in the human proteome present in 46 diverse proteins.

Filippakopoulos, P.; *et al. Cell*, **149**, 214, (2012).
Chung, C.-W.; *et al. J. Biomol. Screen.*, **16**, 1170, (2011).

Difference in Displacing Water Molecules



- TAF1(2)
- Superposition of the crystal, N-binding mode and P-binding mode.
- In P mode, the wat2-4 was displaced.

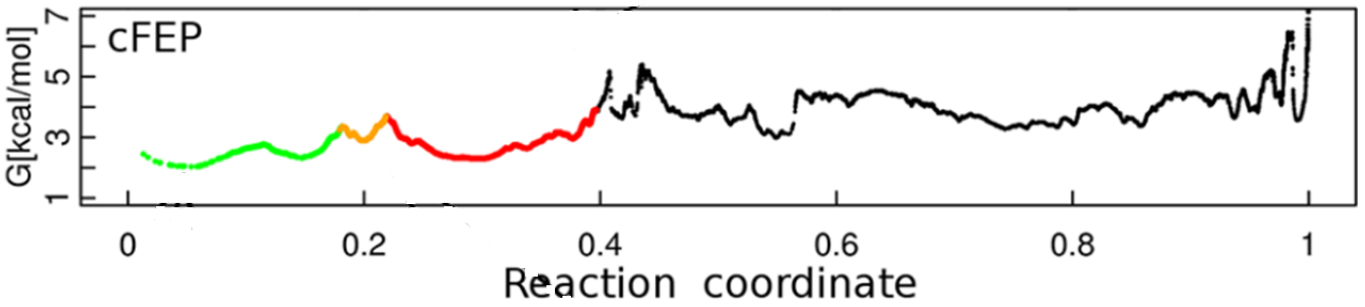


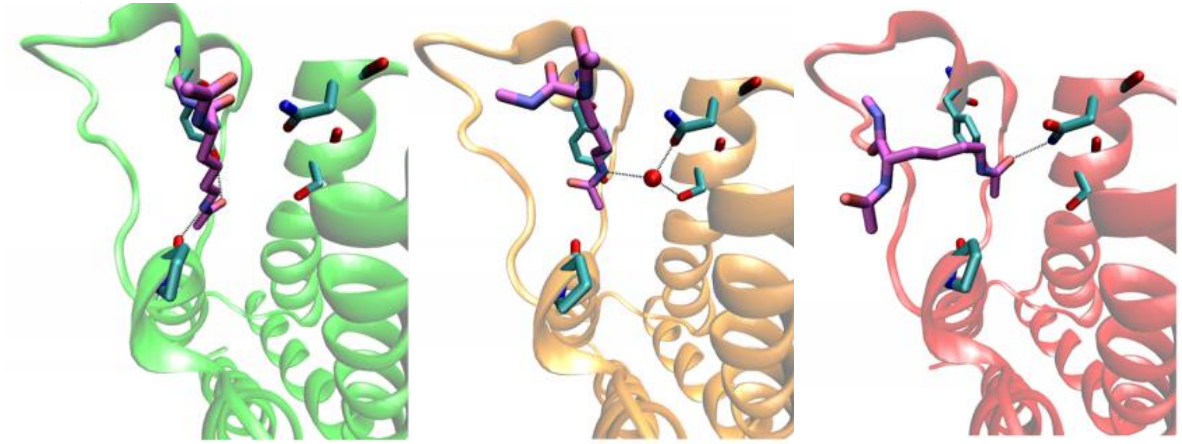
- Superposition of the crystal structures of 6 holo-Bromodomain.
- No water displacement was observed.

- The simulation found a more buried pocket for drug design.
- In the new P-binding mode, water network changes with the ligand, so do their conservation.

A. Magno, A. Caflisch, *J. Chem. Theory Comput.* **9**, 4225−4232. (2013)

Acetyl-Lysine Binding to Bromodomain TAF1(2)





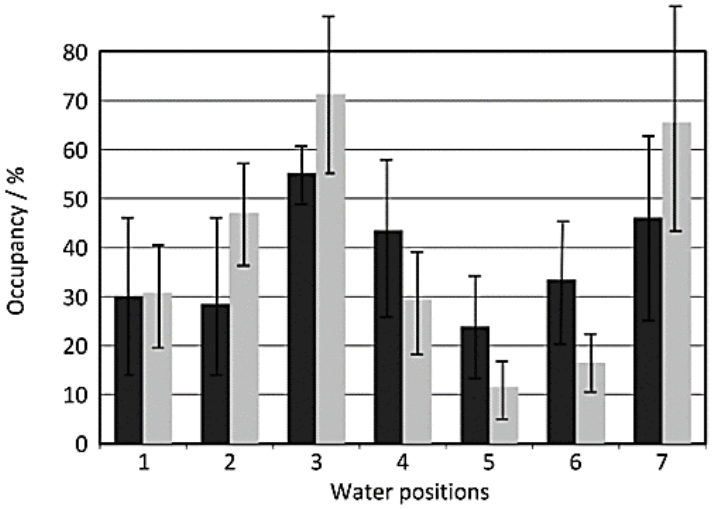
P-binding mode P/N intermediate N-binding mode

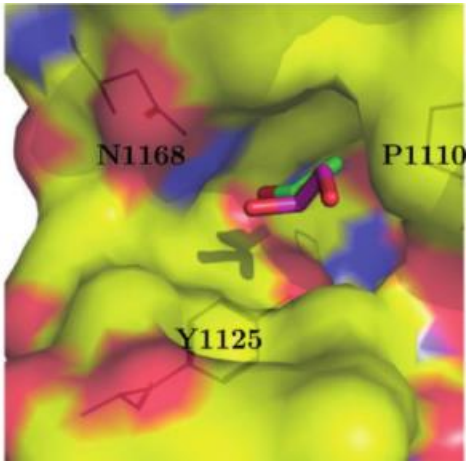
- In 8 parallel trajectories using different force fields, the natural ligand binds with bromodomain in 2 modes.
- The P-binding mode is slightly more favored.
- The N-binding mode is consistent with the crystal structure.

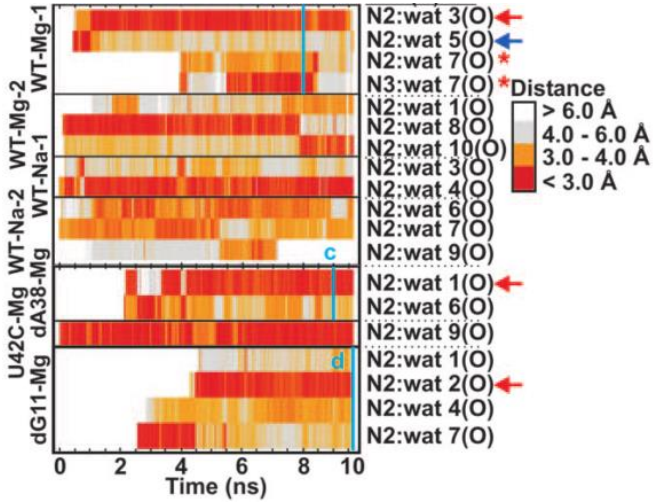
A. Magno, A. Caflisch, *J. Chem. Theory Comput.* **9**, 4225−4232. (2013)

Methods of Water Molecule Classification

Experiment: ITC measuring $\Delta\Delta G^{o}$, water displacement.





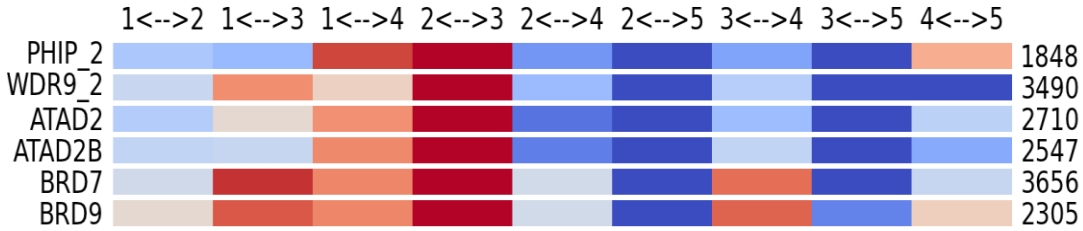


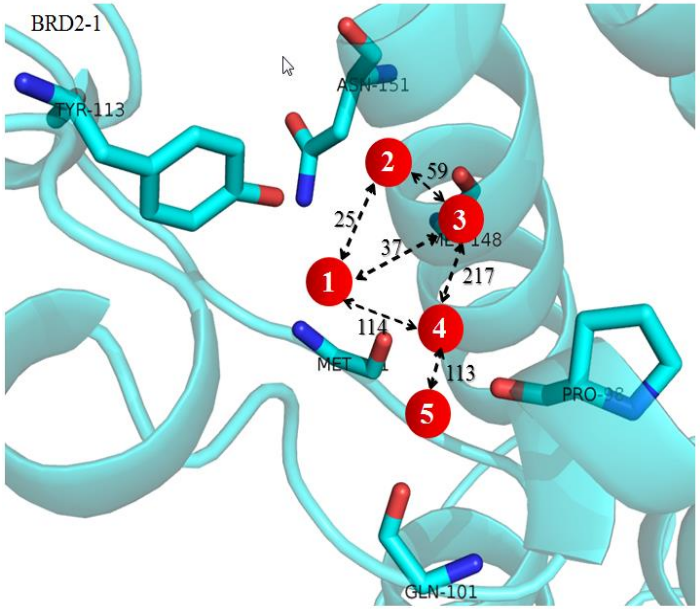
Simulation: Occupancy, Residence time, Co-solvent displacement, H-bond analysis, Binding free energy calculation.

Water in Apo-Bromodomain Structures (500ns)

Occ(%)	Wat1	Wat2	Wat3	Wat4	Wat5
BRD2(1)	99.3	73.3	90.1	91.4	94.2
BRDT(1)	99.4	78.2	84.7	93.3	90.9
TIF1	98.2	31.0	65.0	70.6	86.2
PHIP(2)	98.2	34.4	33.9	51.0	81.5

τ (ns)	Wat1	Wat2	Wat3	Wat4	Wat5
BAZ2B	0.9	0.1	0.2	0.1	0.1
PHIP(2)	1.5	0.1	0.2	0.1	0.1
WDR9(2)	1.5	0.1	0.4	0.2	0.1
CREBBP	0.4	0.1	0.2	0.1	0.1

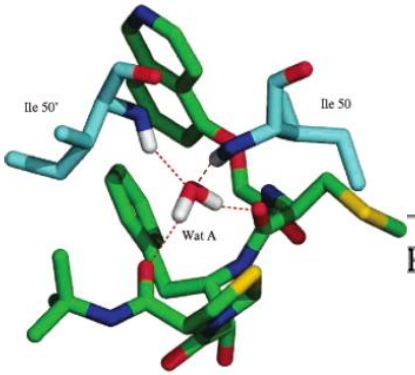




Qualitative conclusions:

a. Wat1 is structurally isolated, conserved in a hydrophilic protein pocket.
b. Wat2-5 form a H-bond network and interchanges frequently.
c. Local difference in the active site could cause different binding affinity of water molecules.

Methods of Water Molecule Classification



$$S_{sol} \longrightarrow S_{gas} \qquad \Delta G_{hyd}$$

$$RS_{sol} \longrightarrow R_{sol} + S_{gas} \qquad \Delta G_{dec}$$

$$R_{sol} + S_{sol} \longrightarrow RS_{sol} \qquad \Delta G_{abs} = \Delta G_{hyd} - \Delta G_{dec}$$

$$P(C|x) = \frac{p(x|C)\,P(C)}{p(x)}$$

$$p(x|C) = \frac{1}{\sigma\sqrt{2\pi}} \exp(-((x-\mu)^2/2\sigma^2))$$

water[a]	ΔG_{abs}[b]	ΔG_{abs}^{lit}[c]
412	-2.2 ± 0.5	-0.4 ± 2
417	-6.3 ± 0.5	-6.3 ± 2
418	-1.0 ± 0.5	-2.6 ± 2
804	$+0.4 \pm 0.5$	$+0.1 \pm 2$

water[a]	ΔG_{abs}[b]	$P(C\|x)$	$P(D\|x)$
107	−2.2	0.21	0.79
71	−2.4	0.22	0.78
108	3.1	0.10	0.90

- Study holo-structures instead of apo-structures.
- Use the double-decoupling method to calculate the water binding free energy.
- Then calculate the displacement/conservation probabilities.

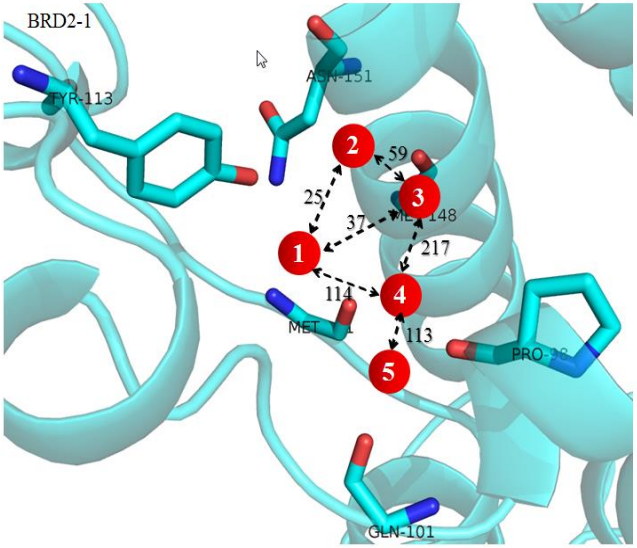
Barillari, C. *.et al. J. Am. Chem. Soc. 2007*, **129,** 2577–2587 (2007).

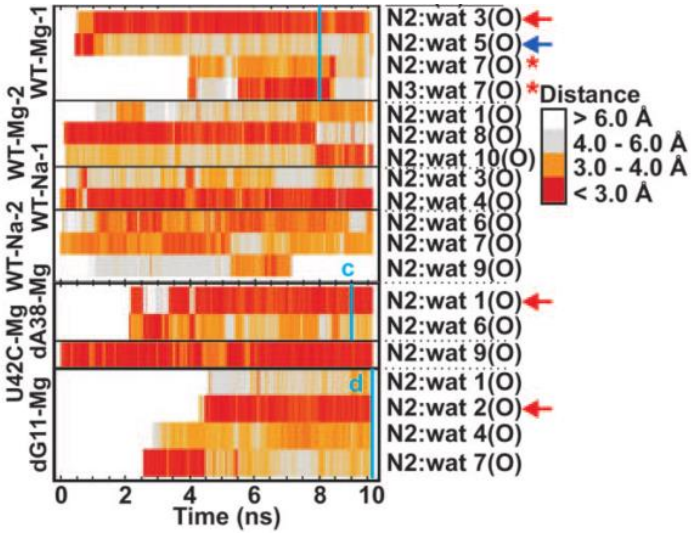
Summary

- Water need to be considered in protein-ligand binding event, especially highly-structured ones.
- Water molecules in the ligand-binding site could be classified into conserved and flexible, to facilitate inhibitor design and understanding binding mechanisms.
- Local difference in the active site could cause different binding affinity of water molecules.

Thank You !!!

What to do next?





We may try:

Investigate the residence time, occupancy and transition map through holo-structures.

Survey the interaction of the ligand, water and the protein active site.

Add H-bond analysis

Water in protein active sites
H/S compensation
HIV-1 protease
Classification of water molecules
Conserved tight waters should not be replaced
How to identify different kind of water
Bromodomain conserved water
Different binding mode, different replaced water
Why in crystal structure, the 5 water still remain, while in the simulation, the waters 2-4 were replaced?!
What did Kai do---- residency time, occupancy, indirect evidence.
What can we do now?

If we have a new water molecule, not belonging to the dataset, with binding free energy x, then the probability that the water molecule belongs to the class of conserved water molecules can be calculated using Bayes' formula as follows:[54]

$$P(C|x) = \frac{p(x|C)\,P(C)}{p(x)} \tag{4}$$

where $P(C|x)$ is the probability of the water molecule being conserved; $P(C)$ is the a priori probability, given by the ratio between the number of conserved water molecules in the training set (18) and the total number of observations in the training set (54); $p(x)$ is the marginal density of the binding free energy; $p(x|C)$ is the conditional density function of the binding free energy for conserved water molecules, which is taken to be the Gaussian density as follows:

$$p(x|C) = \frac{1}{\sigma\sqrt{2\pi}} \exp(-((x-\mu)^2/2\sigma^2)) \tag{5}$$

where μ is the mean free energy of conserved water molecules, σ is the standard deviation, and x is the free energy of the new water molecule.

water[a]	ΔG_{abs}[b]	ΔG_{abs}^{lit}[c]
412	-2.2 ± 0.5	-0.4 ± 2
417	-6.3 ± 0.5	-6.3 ± 2
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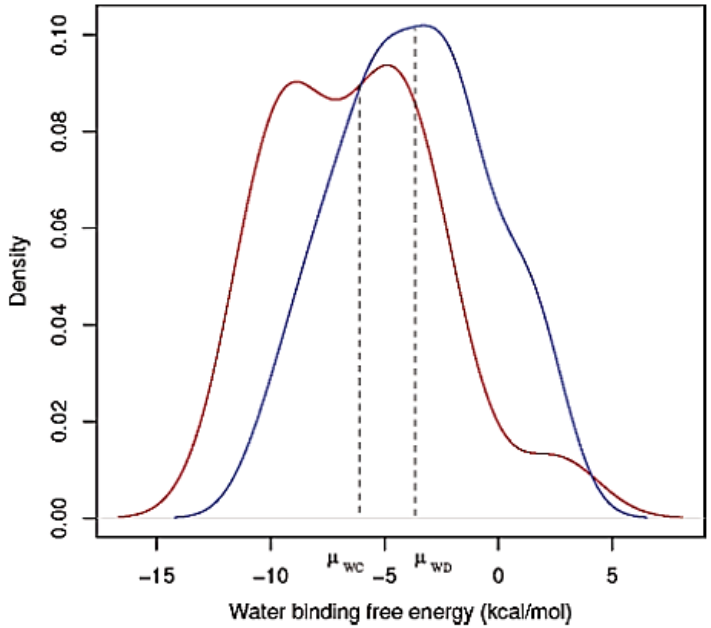
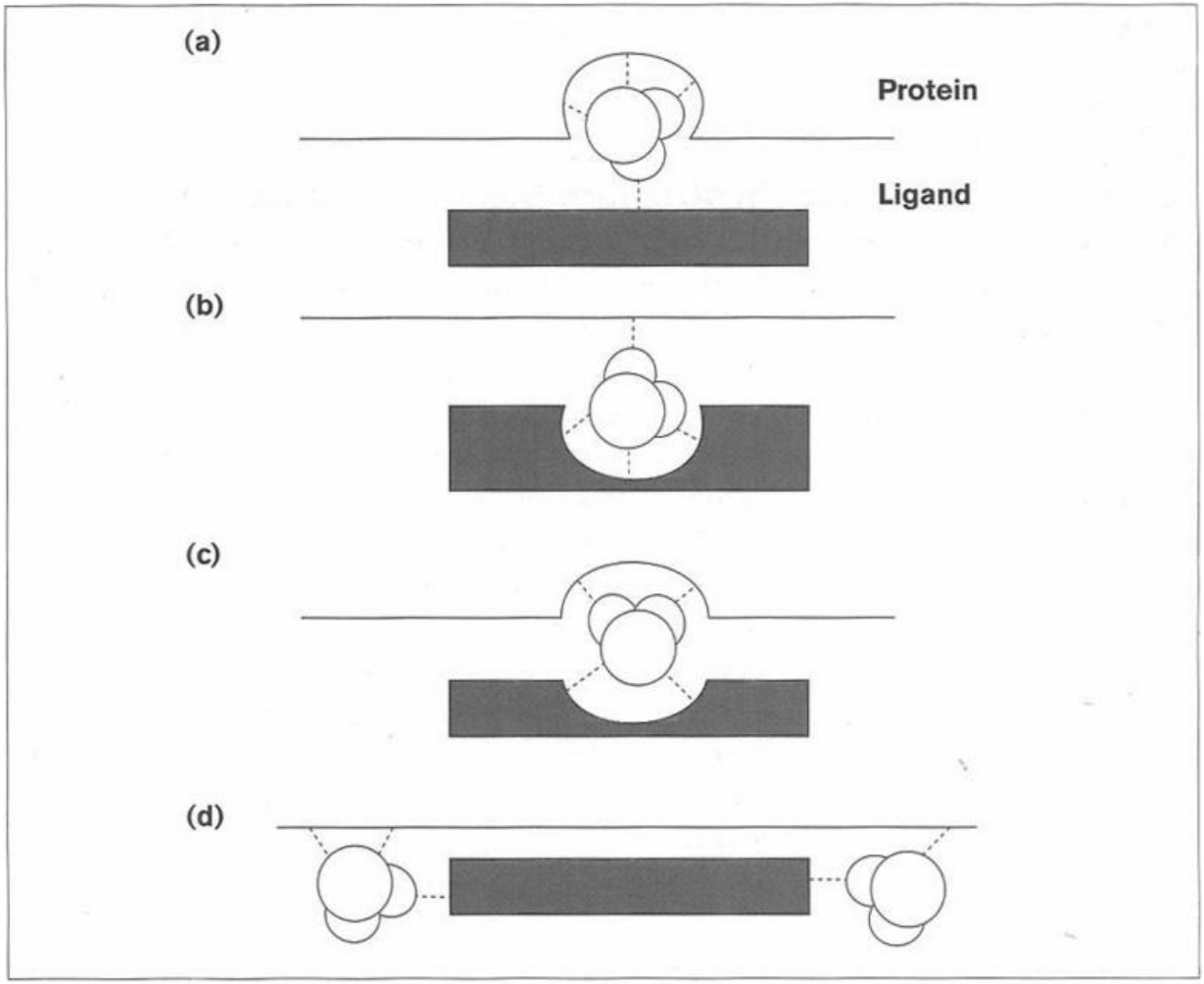
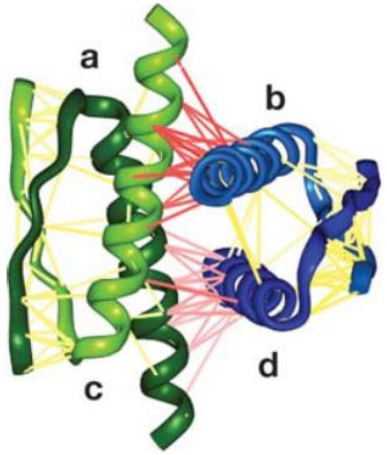


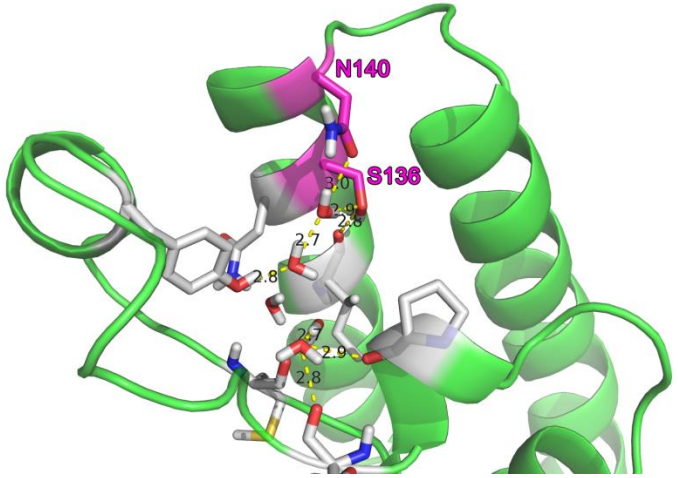


Schematic of the modes in which water can be incorporated into a binding site. The water molecules and hydrogen bonds (broken lines) are arbitrarily positioned. The water can bind in several different ways; it may be largely bound to **(a)** the protein or **(b)** the ligand, or may bind approximately equally to both, either **(c)** in the binding site or **(d)** at the periphery o the binding site.

SUMMARY POINTS

1. Water is highly acknowledged for playing an important role in the structure, stability, dynamics, and function of biological macromolecules. Yet only recently has water been considered an active component rather than an inert environment.
2. Water guides the conformational search in protein folding by gating hydrophobic residues. Several studies reported the existence of wet native-like intermediates. Thus water has a dynamic role in mediating the collapse of the chain and the search for the native topology through a funneled energy landscape.
3. Water can enhance the stability of biological macromolecules.Water-mediated interactions are favorably enthalpic. Alternatively, water residing in hydrophobic pockets can stabilize entropically because it has higher entropy than in bulk water. Both cases indicate that water molecules are not just “filling spaces” but are integral components of the structure.
4. Water can mediate recognition by discriminating between specific and nonspecific binding.
5. Giving attention to water will shed light on the physics of self-assembly and advance our understanding of the natural design of proteins and nucleic acids.


a
b
c
d


N140
S136
3.0
2.9
2.8
2.7
2.8
2.7
2.9
2.8