# The role of water in ligand binding

Balázs Zoltán Zsidó and Csaba Hetényi

Exploration of the complex modulatory role of water in ligand–target binding is a current challenge of drug design. This review reports on recent advances of prediction of water structure and function in the context of ligand engineering. The surveyed theoretical approaches showed remarkable progress in the past years. Beyond complementing experiments, they also supplied unmeasurable data. For example, thermodynamic calculations improved ligand binding by the selection of certain water molecules for structural replacement. Molecular dynamics and explicit solvent models remained indispensable to achieve precise results. Topographical analyses of hydration networks proved useful for the prediction of the stabilizing role of interconnected water clusters mediating target–ligand interactions.

**Address**

Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Szigeti út 12, 7624 Pécs, Hungary

Corresponding author: Hetényi, Csaba (hetenyi.csaba@pte.hu)

## Introduction

Water plays various roles on both macroscopic [1] and microscopic [2–4] stages of life. The present review focuses on the microscopic roles of water during the binding of a ligand to a target molecule. The precise understanding and prediction of ligand binding are essential in drug design projects. Ligands possess various sizes ranging between small organic compounds [5,6], and large proteins [7,8]. Water molecules mediate the binding of ligands of any sizes, and can be sorted roughly into four functional categories [2,4,9,10] (Figure 1).

Experimental determination of water positions requires atomic resolution techniques. A number of permanent limitations of experimental structure determination [11] impose a challenge on the elucidation of water function in biological complexes. Drug designers answer this

'hydration challenge' with the help of computational approaches surveyed in our present review. We focus mainly on the results of the past two years concerning structure, binding affinity, and networking roles of water in ligand binding.

## Water structure

Biomolecular crystallography is the primary experimental source [12,13] of atomic resolution structures of target–ligand complexes. There is a continuous development of X-ray [14] and joint neutron [15] crystallographic methods. Promising combined methods were also introduced [16] with quantum chemical refinements of experimental structures. However, the determination of water positions remains an Achilles' heel [11,17] of crystallography. It is also difficult to assess the quality of assigned water positions. An analysis [18] of 2.3 million experimental water positions concluded that high resolution of a system does not guarantee proper assignation of the hydration structure.

The experimental limitations have motivated the development and application of dynamic and static computational methods for the prediction of water molecules affecting (Figure 1) ligand binding. Dynamic methods supply water positions by clustering snapshots of short molecular dynamics (MD) simulations of explicit water molecules hydrating the solute (target, ligand, or their complex). Static methods use knowledge-based grid maps or geometric rules to build up the hydration structure around a given solute.

Interfacial water molecules can be captured at high precision as they are strongly bound in a relatively tight and buried crevice between the target and the ligand (Figure 1). In a recent report [19], a commercial, dynamic method WaterMap [20] found 90 % of the 41 crystallographic water positions in the interfaces of bromodomain targets and aromatic ligands at a 1.5 Å match level. An open-source software MobyWat [17] showed the same performance on 344 interfacial water molecules in various complexes of peptide and protein ligands [11]. A geometry-based method WarPP [21••] applies an iterative shifting-clustering algorithm. WarPP was validated on almost 20 000 experimental water positions of protein–ligand interfaces of 1500 complexes, and showed a success rate of the above dynamic methods. Other research groups also developed new static approaches, like HydraMap [22] and Splash'Em [23].

The determination of surface waters (Figure 1) is slightly more demanding. An analysis using the EDIA (Electron

**Figure 1**
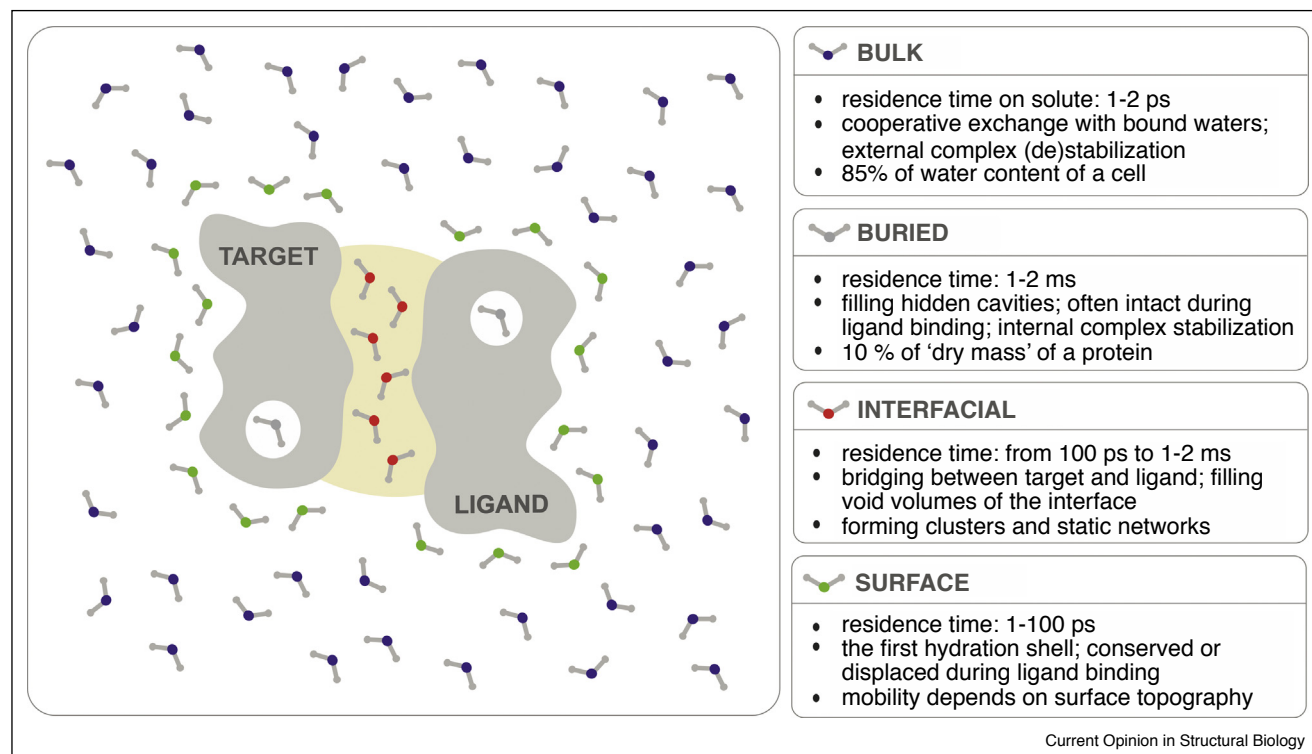


Functional categories of water in ligand binding.

Density for Individual Atoms) index showed [18] that 90% of insufficiently resolved crystallographic water molecules are positioned on the surface. However, there are conserved surface water molecules of low B-factors which can be captured relatively easily. They remain bound to the target surface during ligand binding [17,24], and make up 77% of bridging waters between the target and the ligand according to an MD study [25]. Other, mobile surface waters of uncertain positions (higher B-factors) cause a plausible drop in overall success rates of prediction methods from 90% (see the previous paragraph) to ca. 80% [17] calculated for all surface waters.

Buried water molecules (Figure 1) occupy hidden binding pockets with a challenging geometry to predict. A combination of the static 3D-RISM [26] and dynamic WAT-site [27] methods produced [28•] successful predictions. JAL, an explicit solvent MD-based method also managed to compute buried water positions in tumour suppressor protein p53 and a translation initiation factor [29]. The application of MD can be recommended for the difficult cases of buried waters. Dynamic methods have general applicability in all three categories (interface, surface, and buried) discussed above as they take care of both solute–water and water–water interactions and allow cooperative water exchange [10] with the bulk (Figure 1) [11,29], as

well. They provide accurate [30•] and reproducible [31] results, and the necessary MD snapshots can be produced in short simulations by high performance, parallelized open-source software [32].

The generation of water structure during the computational docking of a ligand to a target would be an attractive technique for virtual (high throughput) screening [33,34] projects. While there are several promising advances [35,36] of this direct methodology, its full automation remains a challenge. Another (indirect) approach, the comparison of end-points of ligand binding seems fairly manageable by available tools. The above-mentioned methods supply surface and interfacial water positions for the hydration structures of the initial (apo, ligand-free) and the final (holo, ligand-bound) stages, respectively. Pairwise comparisons of holo and apo structures or holo structures with similar ligands [37••] (Figure 2) help the identification of conserved and displaced waters, and optimization of ligand–target interactions (see also next Section).

## Contribution of water molecules to binding affinity

The ligand–target binding affinity is expressed as the free energy change of the binding reaction ($\Delta G_b$). The $\Delta G_b$ can

**Figure 2**
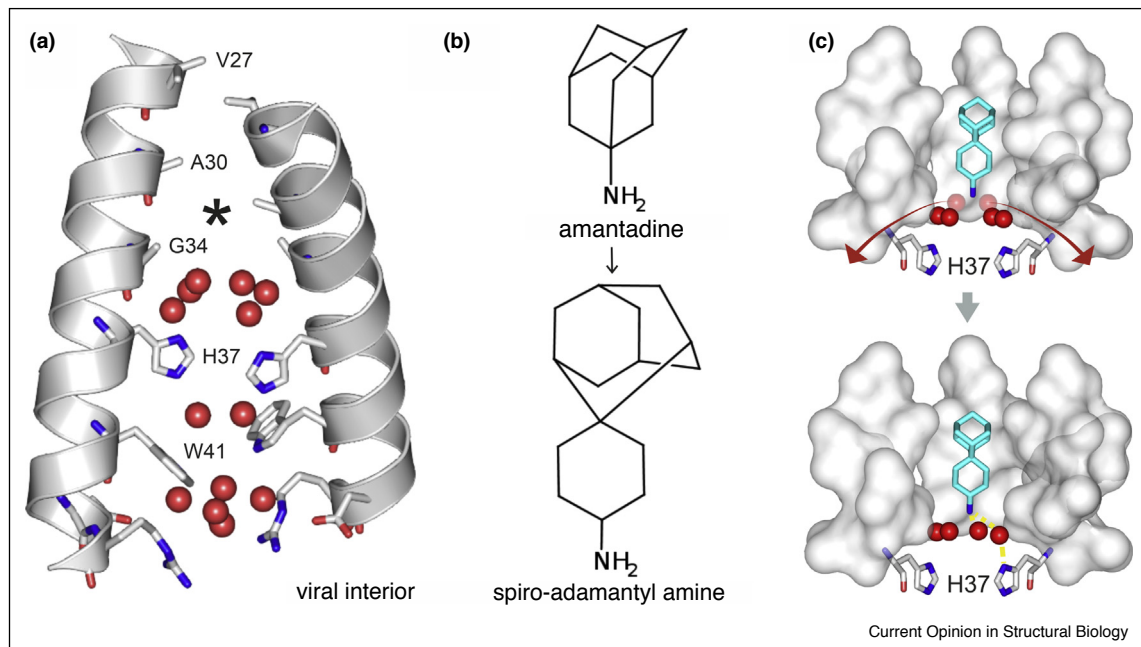


Conserved and displaced water molecules during binding of spiro-adamantyl amine in the influenza M2 transmembrane (TM) proton channel. The structure of the proton channel **(a)** was constructed as a homotetramer of the TM helices (grey cartoon, only a dimer is shown for clarity, PDB code 3lbw) containing residues 22–46. Water molecules and side-chains inside the channel are shown as red spheres, and sticks, respectively. The black asterisk marks the center of the binding pocket of amantadine, a drug in clinical use. Spiro-adamantyl amine preserved **(b)** the main pharmacophores including the amine group and the bulky hydrophobic ring system. Having a larger size than amantadine (b), it displaces some of the water molecules (light red in **(c)**) observed in the amantadine-bound pocket (PDB code 3lbw; at the top in (c)) [37**]. Other water molecules above the H37 side-chains remain conserved in the spiro-adamantyl amine-bound pocket (PDB code 6bmz; at the bottom in (c)) and involved in H-bonding interaction (yellow dashes in (c)) with the amine group of the inhibitor pointing toward the viral interior. To feature the steric conflict with the positions of displaced water molecules, the structure of spiro-adamantyl amine was created in the amantadine-bound pocket (at the top in (c)) by superimposition of PDB structure 6bmz on 3lbw. Programs PyMol [71] and Marvin Sketch [72] were used for drawing of spatial and Lewis structures, respectively.

be engineered via ligand modifications affecting the hydration structure [17,24,30•,34,37••,38–42,43•,44•,45,46,47•,48]. For example, the target–ligand complex can be stabilized by inserting H-bonding functional groups that interact with or replace (Figure 3a) interfacial water molecules resulting in a favourable contribution to binding enthalpy ($\Delta H_b$) [43•]. New functional groups may increase the ligand's ability to expel surface waters into the bulk (Figure 3b) increasing binding entropy ($\Delta S_b$) [46] and affinity.

The determination of thermodynamic stability and the prediction of the contributions of individual water positions to binding affinity (Figure 3) is a key to ligand design. However, experimental methods like isothermal titration calorimetry (ITC) cannot partition $\Delta G_b$ values into individual contributions per water molecule [18,39,49]. Theoretical methods with explicit solvent models help to overcome this limitation. For example, the inhomogeneous fluid solvation theory (IFST) [50] has gained application for thermodynamic characterization of individual hydration sites. IFST with explicit

solvent MD calculations was used [47•] to investigate various modifications of ligand structures that led to the displacement (see e.g. Figure 2) of binding site water molecules. The IFST calculations were useful [47•] in guiding water replacements in lead optimization but did not improve the prediction of the corresponding differences in $\Delta G_b$. Such differences of $\Delta G_b$ were successfully correlated with solvent displacement on sets of similar ligands in another study [51] presenting new functionals for grid inhomogeneous solvation theory (GIST) [52].

Nevertheless, there is some controversy in the literature on the usefulness of the above solvation theories for the prediction of $\Delta G_b$. Initial evaluations of IFST (in WaterMap [20]), and GIST [51] performed better for prediction of $\Delta G_b$ than other calculators based on implicit solvent models [53]. Indeed, GBSA (PBSA) methods or their combination with explicit water molecules showed limited [30•] or occasional [35] success for $\Delta G_b$ calculations, due to their theoretical limitations [54,55]. However,

**Figure 3**
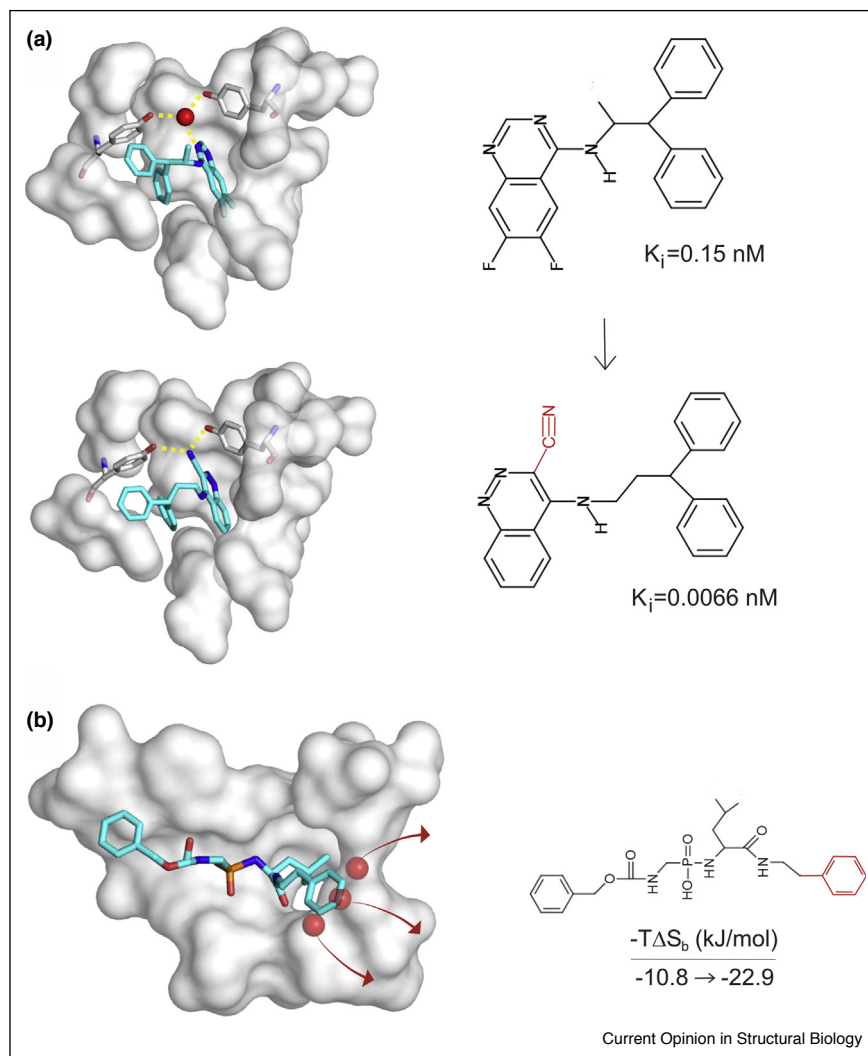


Water structure helps enthalpic and entropic optimization of ligands.

The binding free energy ($\Delta G_b$) of ligand molecules can be optimized by modification of the enthalpic ($\Delta H_b$) or entropic ($\Delta S_b$) contributions according to $\Delta G_b = \Delta H_b - T\Delta S_b$ (where T is the thermodynamic temperature). Targets and ligands are shown in surface and stick representations, respectively. **(a)** Ligand (6,7-difluoro-quinazolin-4-yl)-(1-methyl-2,2-diphenyl-ethyl)-amine shows a good, subnanomolar binding to scytalone-dehydratase stabilized by a bridging water molecule. The isosteric displacement of the bridging water molecule with a nitrile group (red in the Lewis structure) further lowered the $K_i$ and contributed to the enthalpic optimization of ligand binding. The direct hydrogen bonding between the nitrile group of the ligand and the tyrosine residues of the target provides a stronger target–ligand contact (more negative $\Delta H_b$) than the indirect hydrogen bond system with bridging water molecule [43•]. **(b)** The growing of ligand UBTLN46 by addition of a larger phenyl group (red in the Lewis structure), resulted in the displacement of water molecules from the binding pocket of thermolysin. The leaving water molecules increased $\Delta S_b$ [46] which resulted in a more favourable negative contribution to $\Delta G_b$.

other reports [19,30•] comparing grid-based SZMAP [56], WaterFLAP [57], 3D-RISM [26], and WaterMap did not show significant improvement of $\Delta G_b$ calculations with IFST. Assessment of the general applicability of solvation theories in $\Delta G_b$ calculations will require additional validations on large and diverse test sets. At present, the above methods seem more useful [30•] for selecting key waters for planned ligand modifications (Figure 3).

An increasing number of studies suggest that the use of appropriately positioned explicit water molecules is required in binding thermodynamics calculations. For example, relative $\Delta G_b$ calculations on small organic ligands showed that the free energy perturbation method [48] is very sensitive to the choice of initial hydration structure possibly due to water molecules trapped in and/ or insufficiently filling buried cavities. Another study [58]

also involved large peptide ligands and applied a combination of predicted, explicit interfacial water molecules with the COnductor-like Screening MOdel (COSMO) [59] in end-point calculations. The combined water model resulted in good correlations with experimental $\Delta H_b$ values at a PM7 semi-empirical quantum mechanics level.

## The mobility of water networks

In addition to their individual contributions (see previous Sections), water molecules often participate in molecular networks at various locations (Figure 1). Exploration of networking of waters may open a new pathway of ligand design likewise to discoveries in other complex (data) systems [60]. In the cases of small ligands [61•], network changes may be discovered by manual comparisons of the end-points (Figure 2). In the case of large water networks of for example, protein–protein or protein–DNA [62] (Figure 4) complexes, the comparisons should be automatized using graph representations [11,63•].

However, there are relatively few methods offering graph theoretical approaches of hydration networks. Brysbaert et al. analyzed [63•] the changes of residue interaction networks (RIN) of interfaces of protein complexes using RINspector [64]). Adding water molecules to the RIN graphs helped the identification of interface residues involved in the water-mediated binding of the protein partners. The mobility of water nodes was used to distinguish between static and dynamic hydration networks in another graph-based study [11]. Static networks of low mobility contain numerous solute–water and water–water H-bonds stabilizing the target–ligand complex [41,65,66]. Dynamic networks contribute to complex destabilization [11,61•] and binding diverse ligands [67] via cooperative water exchange mechanisms [10] with the bulk.

Ligand binding can be fine-tuned by surrounding water networks. The stabilizing role of static networks was demonstrated by the analysis of the changes in hydration graphs [11] of a histone-chaperone complex [68] following amino acid mutations in the interface region. A similar networking situation was explored [44•] in the case of mutated protein–glycan complexes. The study showed the dominating contribution of a static hydration network of a few, core water molecules to binding thermodynamic signatures. Similarly, only a few stable water positions were identified in ligand binding pockets of G-protein coupled receptors (GPCRs) [67]. Although the conserved GPCR binding pockets are filled mostly by mobile

**Figure 4**
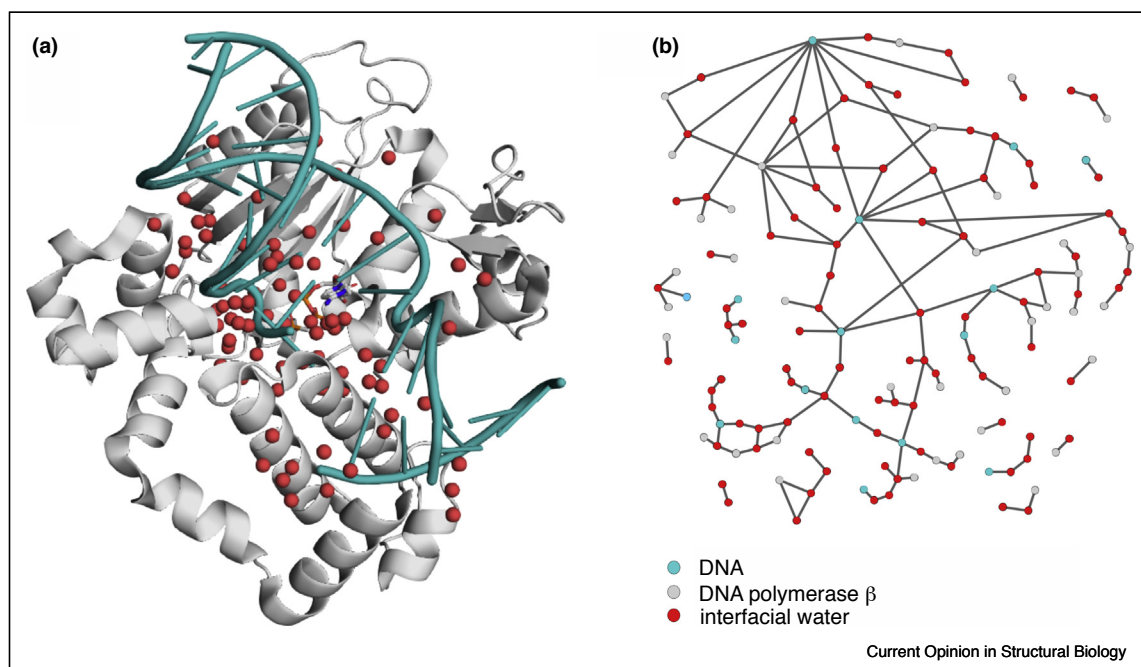


The complexity of the interfacial hydration network of the DNA polymerase β (cyan) in complex with DNA (light blue cartoon). A small molecule inhibitor dCMPP(CH2)P and crystallographic (PDB code 6w2m) water positions are shown **(a)** as sticks red spheres, respectively. The large polymerase-DNA interface holds numerous water molecules mediating between the binding partners. The two-dimensional graph of the interfacial hydration network **(b)** shows a high complexity due to several water-solute and water–water connections. The graph representation allows quick visualization, automated analysis and comparisons of complex hydration networks between large macromolecules. The graph in panel (b) was generated from the PDB structure using the NetDraw function of program MobyWat [11] with a 3 Å distance cut-off, and visualized by Gephi [73].

waters, the stable waters and conserved water-networks are involved in the binding of structurally diverse ligands.

Reorganization or replacement of water networks can be often observed during ligand binding. Water networks of the binding pocket of human carbonic anhydrase II show fast μs-time-scale dynamics according to NMR measurements combined with MD simulations [69]. The effect of an inhibitor ligand on the disruption of such intra-pocket water–water networking and enzymatic activity was analyzed [69]. Another study combining crystallography and MD [37$^{\bullet\bullet}$] showed how amantadyl-amine ligands disrupt key segments of water networks in the influenza A virus matrix 2 proton channel. An earlier MD study [70] of the same system also suggested the replacement of water clusters for the design of new ligands (see also Figure 2) with an ammonium group mimicking the effect of oxonium ions in proton transport. The effect of the dynamic reorganization of water networks on ligand binding affinity was quantified [61$^{\bullet}$] involving a crystallographic structure set of Haemophilus influenzae virulence protein SiaP mutants in complex with sialic acid ligands. Relative $\Delta G_b$ values were calculated [61$^{\bullet}$] using B-factors of water molecules involved in the interaction network around the ligand. Although the approach is probably applicable only for similar complexes, further tests with experimental data or extension using calculated B-factors might be interesting.

Some of the above studies [44$^{\bullet}$,68] report on mutations of target amino acids not directly interacting with the ligand. In these examples, mutations affect ligand binding indirectly, via concerted changes in the interfacial water network. Exploration of such 'hidden' features of a complex (Figure 4b) hydration network is a key to the prediction of binding affinity of large ligands.

## Conclusions
Drug designers often complain of incomplete experimental hydration structures. They could make good use of quantifying thermodynamic contributions of individual water molecules to the overall binding process which cannot be supplied by experiments. Computational techniques have supplied solutions to these requests and performed well in the calculation of the water structure of biomolecules participating in target–ligand binding. Ligand design has benefited from structure-based thermodynamic calculations comparing hydration structures of the apo and holo stages. Molecular dynamics and explicit solvent models have become the gold standard of simulations accounting for water–water interactions often observed in extended hydration networks of for example, protein ligands. Like any other approach, the surveyed theoretical methods and applications have their technical limitations which can be overcome in the not-too-distant future. Potential improvements of polarizable water models (force fields), new quantum mechanical

applications, and topographical analyses of water networks will further increase the efficiency of prediction of the role of water in ligand binding.

## Conflict of interest statement
Nothing declared.

## Acknowledgements

## References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as

- • of special interest
- •• of outstanding interest

1. Brini E, Fennell CJ, Fernandez-Serra M, Hribar-Lee B, Lukšič M, Dill KA: **How water's properties are encoded in its molecular structure and energies**. *Chem Rev* 2017, **117**:12385-12414.

2. Ball P: **Water as an active constituent in cell biology**. *Chem Rev* 2008, **108**:74-108.

3. Bellissent-Funel MC, Hassanali A, Havenith M, Henchman R, Pohl P, Sterpone F, Van Der Spoel D, Xu Y, Garcia AE: **Water determines the structure and dynamics of proteins**. *Chem Rev* 2016, **116**:7673-7697.

4. Laage D, Elsaesser T, Hynes JT: **Water dynamics in the hydration shells of biomolecules**. *Chem Rev* 2017, **117**:10694-10725.

5. Trisciuzzi D, Nicolotti O, Miteva MA, Villoutreix BO: **Analysis of solvent-exposed and buried co-crystallized ligands: a case study to support the design of novel protein–protein interaction inhibitors**. *Drug Discov Today* 2019, **24**:551-559.

6. Schneider P, Walters WP, Plowright AT, Sieroka N, Listgarten J, Goodnow RA, Fisher J, Jansen JM, Duca JS, Rush TS et al.: **Rethinking drug design in the artificial intelligence era**. *Nat Rev Drug Discov* 2020, **19**:353-364.

7. Caskey M, Klein F, Nussenzweig MC: **Broadly neutralizing anti-HIV-1 monoclonal antibodies in the clinic**. *Nat Med* 2019, **25**:547-553.

8. Navarro S, Ventura S: **Computational re-design of protein structures to improve solubility**. *Expert Opin Drug Discov* 2019, **14**:1077-1088.

9. Stephens AD, Kaminski Schierle GS: **The role of water in amyloid aggregation kinetics**. *Curr Opin Struct Biol* 2019, **58**:115-123.

10. Halle B, Helliwell JR, Kornyshev A, Engberts JBFN: **Protein hydration dynamics in solution: a critical survey**. *Philos Trans R Soc B Biol Sci* 2004, **359**:1207-1224.

11. Jeszenői N, Bálint M, Horváth I, Van Der Spoel D, Hetényi C: **Exploration of interfacial hydration networks of target-ligand complexes**. *J Chem Inf Model* 2016, **56**:148-158.

12. Susannah S, Ando N: **X-rays in the cryo-EM era: structural biology's dynamic future**. *Biochemistry* 2018, **57**:277-285.

13. Zsidó BZ, Hetényi C: **Molecular structure, binding affinity, and biological activity in the epigenome**. *Int J Mol Sci* 2020, **21**:1-40.

14. Weichenberger CX, Afonine PV, Kantardjieff K, Rupp B: **The solvent component of macromolecular crystals**. *Acta Crystallogr Sect D Biol Crystallogr* 2015, **71**:1023-1038.

15. Koruza K, Mahon BP, Blakeley MP, Ostermann A, Schrader TE, McKenna R, Knecht W, Fisher SZ: **Using neutron crystallography to elucidate the basis of selective inhibition of carbonic anhydrase by saccharin and a derivative**. *J Struct Biol* 2019, **205**:147-154.

16. Malaspina LA, Wieduwilt EK, Bergmann J, Kleemiss F, Meyer B, Ruiz-López MF, Pal R, Hupf E, Beckmann J, Piltz RO *et al.*: **Fast and accurate quantum crystallography: from small to large, from light to heavy**. *J Phys Chem Lett* 2019, **10**:6973-6982.

17. Jeszenői N, Horváth I, Bálint M, Van Der Spoel D, Hetényi C: **Mobility-based prediction of hydration structures of protein surfaces**. *Bioinformatics* 2015, **31**:1959-1965.

18. Nittinger E, Schneider N, Lange G, Rarey M: **Evidence of water molecules - a statistical evaluation of water molecules based on electron density**. *J Chem Inf Model* 2015, **55**:771-783.

19. Nittinger E, Gibbons P, Eigenbrot C, Davies DR, Maurer B, Yu CL, Kiefer JR, Kuglstatter A, Murray J, Ortwine DF *et al.*: **Water molecules in protein–ligand interfaces. Evaluation of software tools and SAR comparison**. *J Comput Aided Mol Des* 2019, **33**:307-330.

20. Abel R, Young T, Farid R, Berne BJ, Friesner RA: **Role of the active-site solvent in the thermodynamics of factor Xa ligand binding**. *J Am Chem Soc* 2008, **130**:2817-2831.

21. Nittinger E, Flachsenberg F, Bietz S, Lange G, Klein R, Rarey M:
•• **Placement of water molecules in protein structures: from large-scale evaluations to single-case examples**. *J Chem Inf Model* 2018, **58**:1625-1637.
A thoroughly validated, geometry-based prediction tool of interfacial waters with iterative shifting cycles for positional refinements.

22. Li Y, Gao YD, Holloway MK, Wang R: **Prediction of the favorable hydration sites in a protein binding pocket and its application to scoring function formulation**. *J Chem Inf Model* 2020 http://dx.doi.org/10.1021/acs.jcim.9b00619.

23. Wei W, Luo J, Waldispühl J, Moitessier N: **Predicting positions of bridging water molecules in nucleic acid-ligand complexes**. *J Chem Inf Model* 2019, **59**:2941-2951.

24. García-Sosa AT, Mancera RL, Dean PM: **WaterScore: a novel method for distinguishing between bound and displaceable water molecules in the crystal structure of the binding site of protein-ligand complexes**. *J Mol Model* 2003, **9**:172-182.

25. Rudling A, Orro A, Carlsson J: **Prediction of ordered water molecules in protein binding sites from molecular dynamics simulations: the impact of ligand binding on hydration networks**. *J Chem Inf Model* 2018, **58**:350-361.

26. Truchon JF, Pettitt BM, Labute P: **A cavity corrected 3D-RISM functional for accurate solvation free energies**. *J Chem Theory Comput* 2014, **10**:934-941.

27. Hu B, Lill AM: **WATsite: hydration site prediction program with PyMOL interface**. *J Comput Chem* 2014, **35**:1255-1260.

28. Masters MR, Mahmoud AH, Yang Y, Lill MA: **Efficient and
• accurate hydration site profiling for enclosed binding sites**. *J Chem Inf Model* 2018, **58**:2183-2188.
A hybrid method with a dynamic step for prediction of water positions in occluded binding sites.

29. Pradhan MR, Nguyen MN, Kannan S, Fox SJ, Kwoh CK, Lane DP, Verma CS: **Characterization of hydration properties in structural ensembles of biomolecules**. *J Chem Inf Model* 2019, **59**:3316-3329.

30. Bucher D, Stouten P, Triballeau N: **Shedding light on important
• waters for drug design: simulations versus grid-based methods**. *J Chem Inf Model* 2018, **58**:692-699.
An expert account with realistic conclusions comparing solvent mapping tools.

31. Jeszenői N, Schilli G, Bálint M, Horváth I, Hetényi C: **Analysis of the influence of simulation parameters on biomolecule-linked water networks**. *J Mol Graph Model* 2018, **82**:117-128.

32. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJC: **GROMACS: fast, flexible, and free**. *J Comput Chem* 2005, **26**:1701-1718.

33. Zhong H, Wang Z, Wang X, Liu H, Li D, Liu H, Yao X, Hou T: **Importance of a crystalline water network in docking-based virtual screening: a case study of BRD4**. *Phys Chem Chem Phys* 2019, **21**:25276-25289.

34. Hu X, Maffucci I, Contini A: **Advances in the treatment of explicit water molecules in docking and binding free energy calculations**. *Curr Med Chem* 2019, **26**:7598-7622.

35. Maffucci I, Hu X, Fumagalli V, Contini A: **An efficient implementation of the Nwat-MMGBSA method to rescore docking results in medium-throughput virtual screenings**. *Front Chem* 2018, **6**:1-14.

36. Lu Q, Qi LW, Liu J: **Improving protein-ligand binding prediction by considering the bridging water molecules in autodock**. *J Theor Comput Chem* 2019, **18**:1-12.

37. Thomaston JL, Polizzi NF, Konstantinidi A, Wang J, Kolocouris A,
•• Degrado WF: **Inhibitors of the M2 proton channel engage and disrupt transmembrane networks of hydrogen-bonded waters**. *J Am Chem Soc* 2018, **140**:15219-15226.
A textbook example on exploration of the role of water networks in inhibitor design.

38. Bhattarai S, Pippel J, Scaletti E, Idris R, Freundlieb M, Rolshoven G, Renn C, Lee SY, Abdelrahman A, Zimmermann H *et al.*: **2-substituted α,β-methylene-ADP derivatives: potent competitive ecto-5′-nucleotidase (CD73) inhibitors with variable binding modes**. *J Med Chem* 2020, **63**:2941-2957.

39. Geschwindner S, Ulander J: **The current impact of water thermodynamics for small-molecule drug discovery**. *Expert Opin Drug Discov* 2019, **14**:1221-1225.

40. Bodnarchuk MS: **Water, water, everywhere . . . it's time to stop and think**. *Drug Discov Today* 2016, **21**:1139-1146.

41. Maurer M, Oostenbrink C: **Water in protein hydration and ligand recognition**. *J Mol Recognit* 2019, **32**:1-19.

42. Ratkova EL, Dawidowski M, Napolitano V, Dubin G, Fino R, Ostertag MS, Sattler M, Popowicz G, Tetko IV: **Water envelope has a critical impact on the design of protein-protein interaction inhibitors**. *Chem Commun* 2020, **56**:4360-4363.

43. Chen D, Li Y, Zhao M, Tan W, Li X, Savidge T, Guo W, Fan X:
• **Effective lead optimization targeting the displacement of bridging receptor-ligand water molecules**. *Phys Chem Chem Phys* 2018, **20**:24399-24407.
An in-depth thermodynamic study on the replacement of bridging water molecules for lead optimization.

44. Kunstmann S, Gohlke U, Broeker NK, Roske Y, Heinemann U,
• Santer M, Barbirz S: **Solvent networks tune thermodynamics of oligosaccharide complex formation in an extended protein binding sit**. *J Am Chem Soc* 2018, **140**:10447-10455.
A mobility analysis and identification of static water networks influencing the thermodynamic signature of ligand binding.

45. Li A, Gilson MK: **Protein-ligand binding enthalpies from near-millisecond simulations: analysis of a preorganization paradox**. *J Chem Phys* 2018, **149**:1-15.

46. Krimmer SG, Betz M, Heine A, Klebe G: **Methyl, ethyl, propyl, butyl: futile but not for water, as the correlation of structure and thermodynamic signature shows in a congeneric series of thermolysin inhibitors**. *ChemMedChem* 2014, **9**:833-846.

47. Wahl J, Smieško M: **Thermodynamic insight into the effects of
• water displacement and rearrangement upon ligand modifications using molecular dynamics simulations**. *ChemMedChem* 2018, **13**:1325-1335.
A systematic MD study and thermodynamic analysis on the role of the displacement of water molecules in ligand binding.

48. Wahl J, Smieško M: **Assessing the predictive power of relative binding free energy calculations for test cases involving displacement of binding site water molecules**. *J Chem Inf Model* 2019, **59**:754-765.

49. Kairys V, Baranauskiene L, Kazlauskiene M, Matulis D, Kazlauskas E: **Binding affinity in drug design: experimental and computational techniques**. *Expert Opin Drug Discov* 2019, **14**:755-768.

50. Lazaridis T: **Inhomogeneous fluid approach to solvation thermodynamics. 1. Theory**. *J Phys Chem B* 1998, **102**:3531-3541.

51. Hüfner-Wulsdorf T, Klebe G: **Protein-ligand complex solvation thermodynamics: development, parameterization, and testing of GIST-based solvent functionals**. *J Chem Inf Model* 2020, **60**:1409-1423.

52. Balius TE, Fischer M, Stein RM, Adler TB, Nguyen CN, Cruz A, Gilson MK, Kurtzman T, Shoichet BK: **Testing inhomogeneous solvation theory in structure-based ligand discovery**. *Proc Natl Acad Sci U S A* 2017, **114**:6839-6846.

53. Sitkoff D, Sharp KA, Honig B: **Accurate calculation of hydration free energies using macroscopic solvent models**. *J Phys Chem* 1994, **98**:1978-1988.

54. Zhang H, Yin C, Yan H, Van Der Spoel D: **Evaluation of generalized born models for large scale affinity prediction of cyclodextrin host-guest complexes**. *J Chem Inf Model* 2016, **56**:2080-2092.

55. Genheden S, Ryde U: **The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities**. *Expert Opin Drug Discov* 2015, **10**:449-461.

56. Bayden AS, Moustakas DT, Joseph-McCarthy D, Lamb ML: **Evaluating free energies of binding and conservation of crystallographic waters using SZMAP**. *J Chem Inf Model* 2015, **55**:1552-1565.

57. Pastor M, Cruciani G, Watson KA: **A strategy for the incorporation of water molecules present in a ligand binding site into a three-dimensional quantitative structure - activity relationship analysis**. *J Med Chem* 1997, **40**:4089-4102.

58. Horváth I, Jeszenői N, Bálint M, Paragi G, Hetényi C: **A fragmenting protocol with explicit hydration for calculation of binding enthalpies of target-ligand complexes at a quantum mechanical level**. *Int J Mol Sci* 2019, **20**:4384-4403.

59. Klamt A, Schüürmann G: **COSMO: a new approach to dielectric screening in solvents with explicit expressions for the screening energy and its gradient**. *J Chem Soc Perkin Trans 2* 1993, **5**:799-805.

60. Cheng F, Kovács IA, Barabási AL: **Network-based prediction of drug combinations**. *Nat Commun* 2019, **10**:1197-1208.

61. Darby JF, Hopkins AP, Shimizu S, Roberts SM, Brannigan JA,
   • Turkenburg JP, Thomas GH, Hubbard RE, Fischer M: **Water networks can determine the affinity of ligand binding to proteins**. *J Am Chem Soc* 2019, **141**:15818-15826.

A structure-based approach linking mobility of water networks to ligand binding affinity.

62. Batra VK, Wilson SH: **Structure of a DNA polymerase abortive complex with the 8OG:dA base pair at the primer terminus**. *Commun Biol* 2020, **3**:8-11.

63. Brysbaert G, Blossey R, Lensink MF: **The inclusion of water
   • molecules in residue interaction networks identifies additional central residues**. *Front Mol Biosci* 2018, **5**:1-7.

A timely paper on graph theoretical analysis of residue interaction networks extended with water nodes.

64. Brysbaert G, Lorgouilloux K, Vranken WF, Lensink MF: **RINspector: a cytoscape app for centrality analyses and DynaMine flexibility prediction**. *Bioinformatics* 2018, **34**:294-296.

65. Majewski M, Ruiz-Carmona S, Barril X: **An investigation of structural stability in protein-ligand complexes reveals the balance between order and disorder**. *Commun Chem* 2019, **2**:110-118.

66. Schiebel J, Gaspari R, Wulsdorf T, Ngo K, Sohn C, Schrader TE, Cavalli A, Ostermann A, Heine A, Klebe G: **Intriguing role of water in protein-ligand binding studied by neutron crystallography on trypsin complexes**. *Nat Commun* 2018, **9**:3559-3574.

67. Venkatakrishnan AJ, Ma AK, Fonseca R, Latorraca NR, Kelly B, Betz RM, Asawa C, Kobilka BK, Dror RO: **Diverse GPCRs exhibit conserved water networks for stabilization and activation**. *Proc Natl Acad Sci U S A* 2019, **116**:3288-3293.

68. Elsässer SJ, Huang H, Lewis PW, Chin JW, Allis CD, Patel DJ: **DAXX envelops a histone H3.3-H4 dimer for H3.3-specific recognition**. *Nature* 2012, **491**:560-565.

69. Singh H, Vasa SK, Jangra H, Rovó P, Päslack C, Das CK, Zipse H, Schäfer LV, Linser R: **Fast microsecond dynamics of the protein-water network in the active site of human carbonic anhydrase II studied by solid-state NMR spectroscopy**. *J Am Chem Soc* 2019, **141**:19276-19288.

70. Gianti E, Carnevale V, DeGrado WF, Klein ML, Fiorin G: **Hydrogen-bonded water molecules in the M2 channel of the influenza A virus guide the binding preferences of ammonium-based inhibitors**. *J Phys Chem B* 2015, **119**:1173-1183.

71. DeLano WL: *The PyMOL Molecular Graphics System; Version 2.0*. New York, NY, USA: Schrödinger LLC; 2002.

72. Marvin 19.18., 2019, ChemAxon. (http://www.chemaxon.com).

73. Bastian M, Heymann S, Jacomy M: **Gephi: an open source software for exploring and manipulating networks**. *Int AAAI Conf Weblogs Soc Media* 2009.