Effects of Water Deuteration on Thermodynamic and Structural Properties of Proteins and Biomembranes

Carmelo Tempra, Victor Cruces Chamorro, and Pavel Jungwirth*

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic

E-mail: pavel.jungwirth@uochb.cas.cz

January 23, 2023

Abstract

Light and heavy water are often used interchangeably in spectroscopic experiments with the tacit assumption that the structure of the investigated biomolecule does not depend too much on employing one or the other solvent. While this may often be a good approximation, we demonstrate here using molecular dynamics simulations incorporating nuclear quantum effects via modification of the interaction potential that there are small but significant differences. Namely, as quantified and discussed in the present study, both proteins and biomembranes tend to be slightly more compact and rigid in D_2O than in H_2O, which reflects the stronger hydrogen bonding in the former solvent.

Introduction

Properties of light (H_2O) and heavy (D_2O) water are very similar to each other, save for a trivial ∼10 % difference in density due to the higher mass of the D over the H isotope[1]. This similarity is the rationale behind using the two water isotopes interchangeably as biomolecular solvents in spectroscopic experiments[2,3]. Nevertheless, concerning non-trivial diferences the two solvents vary by several degrees in melting points, by 0.4 pH (or pD) units in the autoionization equilibrium constant, and by about 20 % in viscosity. Also, the number density (i.e., the number of molecules per unit volume) of D_2O is not equal to, but actually lower than that of H_2O. These variations in turn translate to a slightly different behavior of biomolecules dissolved in H_2O vs in D_2O. In particular, soluble proteins tend to be somewhat more compact and rigid in heavy water and so do phospholipid bilayers[4,5]. As we have discussed recently, and intriguing consequence of nuclear quantum effects in water is the observed sweet taste of heavy water, as contrasted to a taste-neutral light water[6]. More precisely, it is the reduction of nuclear quantum effects upon moving from H_2O to D_2O that triggers the activation of the human sweet taste receptor.

Small differences between light and heavy water can be related to slightly stronger hydrogen bonds in the latter liquid. These in turn can be traced back to nuclear quantum effects. Namely, zero point motions along as well as perpendicular to the direction of the water-water hydrogen bond are more pronounced in H_2O over in D_2O with a net effect of a slight hydrogen bond destabilization[7]. Rigorously, computationally demanding quantum simulations such as path integral molecular dynamics (PIMD) should be employed to recover these effects. While feasible for neat water, such simulations become prohibitively expensive when large biomolecules are added to the solution. However, as already demonstrated by Feynmann and Hibbs, zero point energy effects can effectively be incorporated into classical simulations by modifying the interaction potential[8,9]. We have recently employed this approach to develop, based on an earlier model[10], a classical force field for heavy water. Here, we use this approach to quantify the differences in thermodynamic and structural properties of amino acids, proteins, and phospholipid membranes in light vs heavy water, comparing the simulation result to experiment whenever possible and providing a molecular interpretation of the observed phenomena. Focusing on diferences between bulk properties of light vs heavy water, secondary effects of deuteration of exchangable hydrogens of the biomolecules in heavy water have been neglected in the present study.

Methods

Force fields

The force fields used here to simulate H_2O and D_2O are the commonly used SPC/E model[11] for the former and our recently developed SPCE-HW parameterization[10] for the latter. Amino acids, proteins, lipids and ions were modeled using the CHARMM36 topology generated by the CHARMM-GUI web-interface[12,13]. Classical equations of motion was solved numerically with a 2 fs integration timestep using the Verlet-list algorithm[14]. Long-range electrostatic interaction were accounted for using the Particle Mesh Ewald scheme[15,16]

employing a short-range cut-off of 1.2 nm. For Van der Walls interaction a force-switching algorithm from 1.0 of 1.2 nm was employed. Simulations were run in the isothermal-isobaric (NpT) ensemble with the velocity-rescale thermostat[17] and the Parrinello-Rahaman barostat[18] imposing temperature of 298 K and pressure of 1 atm, with coupling constants of 5 and 1 ps, respectively.

Free energy of amino acid transfer

A box 6 x 6 x 12 nm^3 unit cell was filled with adjacent equally sized slabs of light (SPCE) and heavy (SPCE-HW) water, each containing 7203 water molecules. A flat-bottomed potential along the long (Z) axis (see Fig. 1) with a force constant of 100 kJ/mol and a distance from the center of each slabs R_i of 3 nm was applied to each of the cubes to keep the light and heavy water molecules separated from each other. The system was energy minimized and then equilibrated in the NpT ensemble for 10 ns. All the essential amino acids were one by one placed in the center of the SPC/E water slab and energy minimized. The umbrella sampling technique was then applied to compute the Potential of Mean Force (PMF) along the Z axis (moving from H_2O slab to D_2O), and the free energy of transfer is extracted as the difference of the PMF at the bulk of the two solvents. Thirty windows were generated along the z-axis, separated by 0.2 nm each and a force constant of 1000 kJ $mol^{-1}nm^{-2}$ was applied in each window. Free energies in the individual windows were connected using the weighted histogram analysis method (WHAM), with the associated statistical error evaluated using the bootstrap method[19,20].

Protein and phospholipid membrane simulations

Three representative globular proteins have been chosen for the present study – azurine[21], lactoglobuline[22], and ribonuclease T1[23]. The initial PDB structures were processed and solvated in a water box extending at least 2 nm from the protein to the edges of the unit cell using the CHARMM-GUI web server[12,13]. The CHARMM-GUI default water model

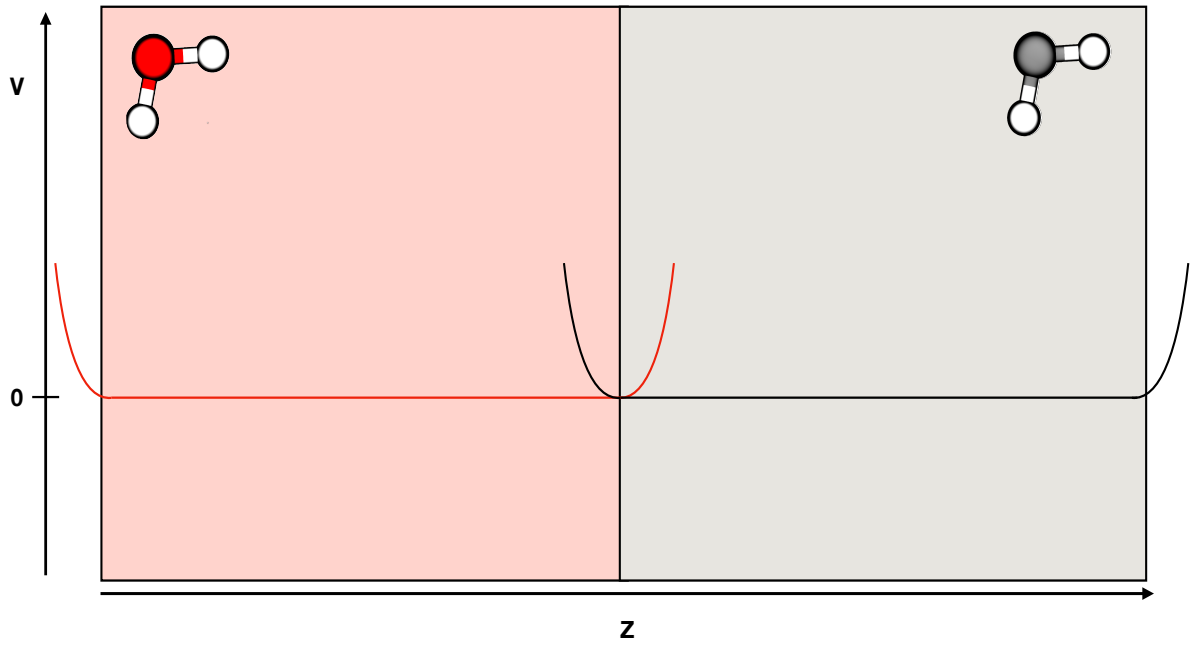


Figure 1: Simplified description of the set-up used for calculating the free energy of transfer from H_2O to D_2O. Red (gray) color indicates D_2O (H_2O). The flat-bottomed potentials are also indicated in the figure.

(i.e., TIP3P) was changed to SPC/E or SPCE-HW. Sodium or chloride counter ions[13] were added to neutralize the systems. The obtained systems were then energy minimized and equilibrated in the NpT ensemble for 10 ns, after which a production run of 1 µs followed for each of the three proteins. In addition, for ribonuclease a set of extra simulations in a range of different temperature was performed in order to simulate melting of the protein. For each temperature, a 1.7 µs trajectory was generated with the first µs taken as equilibration and discarded from the analysis (for further details see SI, tables S2 and S3).

To explore the effect of water deuteration on biological membranes, a bilayer containing 200 phospholipids (POPC) was constructed using CHARMM-GUI[12,13]. The total amount of water molecules (SPC/E or SPCE-HW) added was 15180. After energy minimization

and equilibration in the NpT ensemble of 10 ns, the systems were run for 200 ns. Furthermore, a patch of a dipalmitoylphosphatidylcholine (DPPC) membrane was build using CHARMM-GUI[12,13] and simulated to evaluate the effect of the employed water models on the temperature of phase transition from the gel phase to the liquid phase. A bilayer composed of 64 lipids was solvated with a total of 2600 water molecules and simulated with temperature annealing from 325 K to 305 K in 2 µs.

Results and discussion

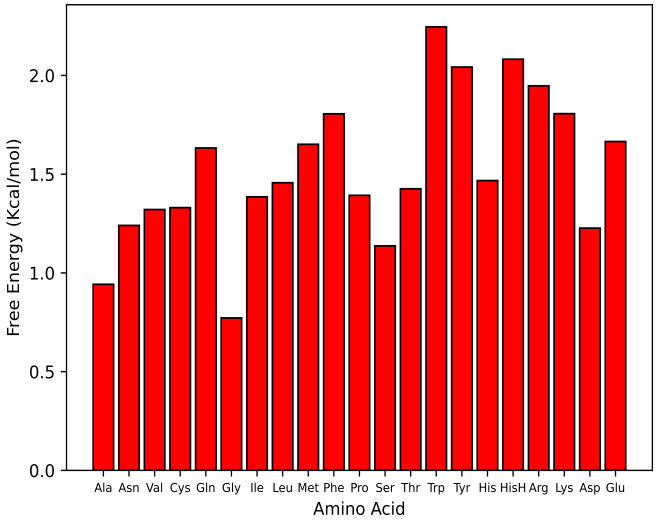


Figure 2: Free energy of transfer from SPC/E (H_2O) to SPCE-HW (D_2O). Errors are not reported because too small. A table of the values with associated errors can be found in the SI (table S1)

The free energies of transfer ΔG from H_2O to D_2O are summarized for all the amino acids in Fig. 2. All the calculated free energies are positive, which means the amino acids are less stable in the heavy water than in light water. It is worth noting that the ΔG values, which vary between 0.7 and 2.2 kcal/mol do not follow the hydrophobicity scale of amino acids. The results presented in Fig. 2 rather point to the molecular size as the main factor governing the ΔG values – the larger the amino acid the more unfavorable the transfer from

H_2O to D_2O. The always positive free energy of transfer between the two solvents indicates that - compared to H_2O - D_2O has a higher propensity to form water-water hydrogen bonds than water-amino acid hydrogen bonds. This explains why the free energy of hydration depends on the excluded volume, which for small molecules like amino acids correlates well with the molecular weight. We indeed see a very good linear correlation between the molar mass and the free energy of transfer (Fig. 3).

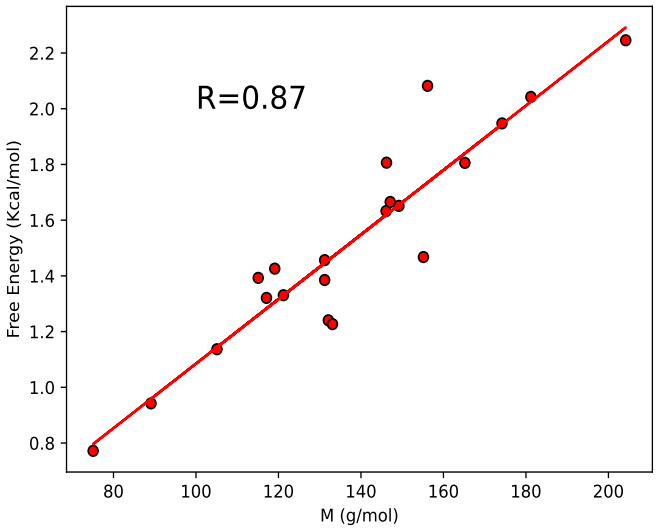
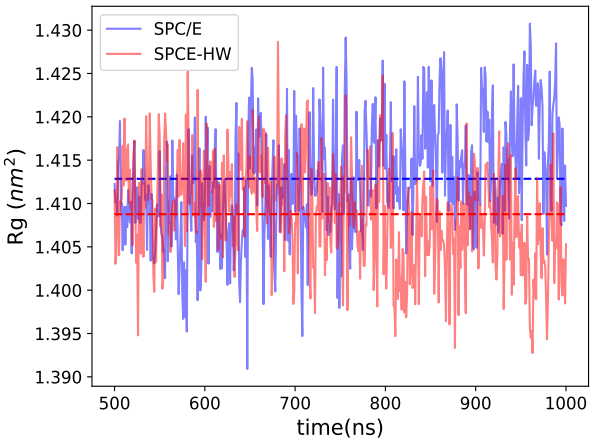
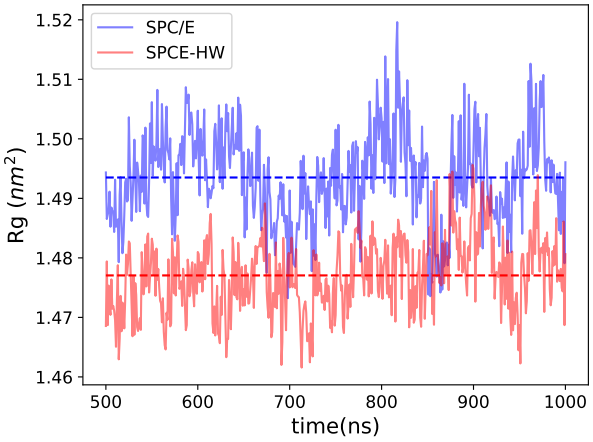


Figure 3: Free energy of transfer from SPC/E (H_2O) to SPCE-HW (D_2O) as a function of the amino acid molar mass showing a very good linear correlation.

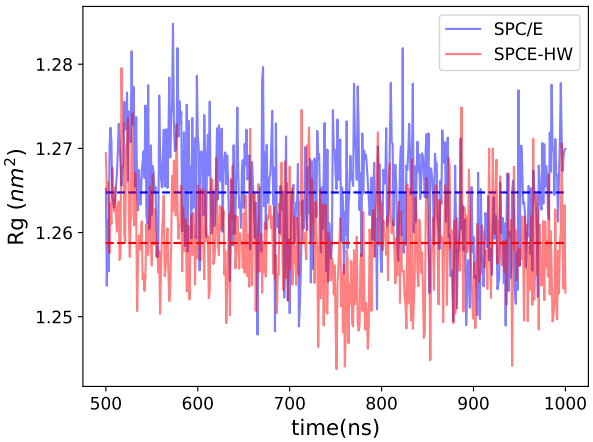
The above results concerning amino acids indicate that D_2O may be a somewhat worse solvent than H_2O for proteins, hence inducing also more compact structures with a reduced radius of gyration. To test this, we modelled and analyzed the behaviour of three grobular proteins – azurine, lactoglobuline and ribonuclease – in D_2O vs H_2O. All three proteins show a small but consistent decrease in the radius of gyration when moving from H_2O to D_2O, see Fig. 4. The same trend is also observed for the solvent accessible surface area (SASA), see figure S1 in the SI . Simulations thus show that water deuteration is making the proteins tighter, which is consistent with the positive free energy of transfer presented above, as well as with the generally tightening effect of D_2O found in experiment[24].



(A)



(B)



(C)

Figure 4: Radius of gyration of azurine (A), lactoglobuline (B) and ribonuclease (C) in SPC/E (H_2O, blue line) and SPCE-HW (D_2O, red line). The dashed lines represent average values over the production runs of 500 ns.

For ribonuclease, we also modelled the effect of deuteration on the protein melting temperature. From the results presented in Fig. 5, we see that the tightening of the protein structure upon water deuteration also leads to stabilization and increase of the protein melting temperature. This is in line with experimental observations[27,28] and it is consistent with

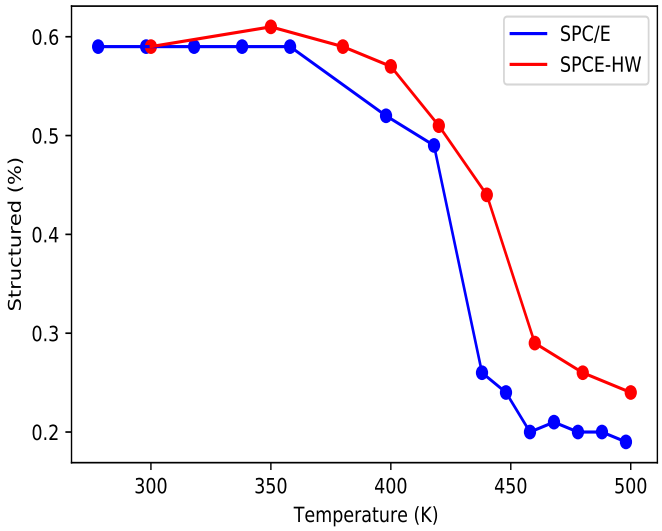


Figure 5: Annealing of ribonuclease. The percentage of structured protein is plotted versus the temperature. The percentage of structured protein was calculater using the gromacs tool "gmx do dssp" , which uses the DSSP algorithm[25,26]. Blu line is SPC/E (H_2O), and red line is SPCE-HW (D_2O). Error bars are not reported as they are too small to be visible in the figure (i.e., below 1%).

the sign of the free energies of transfer from H_2O to D_2O of individual amino acids in Fig. 2.

The tightening effect of the heavy water is not limited to proteins as demonstrated on the areas per lipid (APL) calculated for a POPC bilayer, yielding a value of 0.63 nm for H_2O and 0.59 nm for D_2O. The effect of the solvent on the POPC membrane APL is qualitatively in line (albeit more pronounced) with previous simulations[29]. At the same time, we observe a small (about 3 %) bilayer thickening, as deduced from the density profile of phosphate (see SI). These results are consistent with experimental findings[30].

In addition, the behaviour of a DPPC bilayer around the melting point was investigated

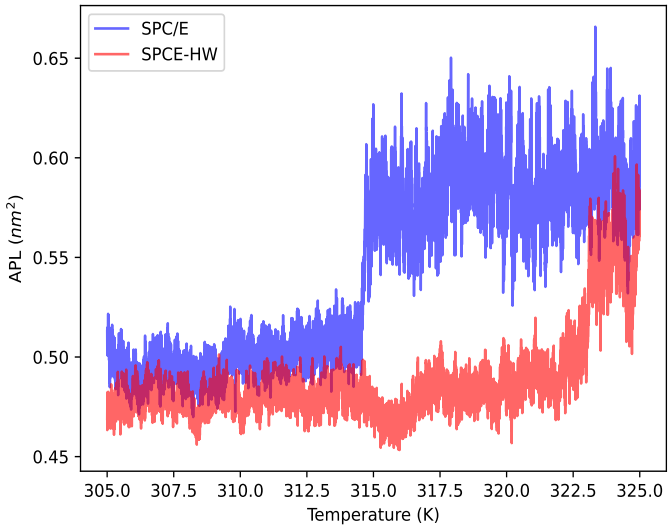


Figure 6: Area per lipid of a DPPC bilayer as a function of temperature in SPC/E H_2O (blue) and SPCE-HW D_2O (red).

by monitoring the APL as a function of temperature. The results are shown in Fig. 6. The first thing to notice is that the APL in D_2O is systematically smaller that that in H_2O, which is consistent with the above results for POPC. DPPC in H_2O exhibits a melting temperature of 314.5 K, which is in very good agreement both with the experimentally determined value of 314.15 K[31] and the value from MD simulations using the default TIP3P charmm model[32]. The system simulated in D_2O using the present model shows a clear upward shift of the melting temperature of almost 10 K. Qualitatively, this is in accord with experimental findings[31], although the measured shift in melting temperature is smaller (less then 1 K).

Conclusion

In this work we quantified the effect of water deuteration on amino acids, proteins, and phospholipid membranes. This was done using classical molecular dynamics simulations employing models that account for differences between H_2O to D_2O in an effective way,

incorporating nuclear quantum effects into the intermolecular potential. In particular, we focused on differences in structural properties such as the compactness of the biomolecules and thermodynamic effects like melting temperatures and free energies of transfer of solutes from light to heavy water. To the former, our results reveal small but systematic structural effects on proteins. Namely, we observe a decrease in radii of gyration of less then 1% upon moving from H_2O to D_2O. Interestingly, structural effect on phospolipid membranes are larger than on proteins, in particular, upon deuteration we observed a decrease of the area per lipid by more than 10% and thickening of the bilayer by about 3%. To the latter, our results show that all amino acids are slightly less soluble in heavy vs light water. Also, moving from H_2O to D_2O we observe an upward shift by several degrees of the melting point of a model protein – ribonuclease. The same affect of increasing the melting temperature is found for DPPC. Altogether the simulations show that structural effect on globular protein might be small, but thermodynamic effect (melting) on protein and membranes can be important, especially if an experiment is conducted close to the phase transition temperature, where even a small shift can change the physical-chemical properties. Comparison to available experimental data shows that our simple models capture well the principal effect, namely that D_2O is a somewhat worse solvent for biomolecules that H_2O. This also implies that association between proteins or between a protein and a biomembrane may be positively affected by water deuteration. Finally, protein domains that are intrinsically disordered and thus very sensitive to the balance between protein-solvent and solvent-solvent interaction, may show a high sensitivity to the H_2O to D_2O substitution.

Supporting Information

Free energy of transfer (with associated error) between water and heavy water for each single amino acid. Solvent Accessible Surface Area (SASA) of azurine, lactoglobuline and ribonuclease in water and heavy water. Detailed data about annealing of ibonuclease T1.

Phosphate density profile of POPC in water and heavy water.

Acknowledgment

P.J. thanks the Czech Science Foundation (EXPRO grant no. 19-26854X). C.T. and V.C.C. are grateful for support from the Faculty of Science of the Charles University, Prague, where they are enrolled as PhD. students. C.T. and V.C.C. thanks the International Max Planck Research School for Many-Particle Systems in Structured Environments hosted by the Max Planck Institute for the Physics of Complex Systems, Dresden, Germany.

References

(1) Ceriotti, M.; Fang, W.; Kusalik, P. G.; McKenzie, R. H.; Michaelides, A.; Morales, M. A.; Markland, T. E. Nuclear Quantum Effects in Water and Aqueous Systems: Experiment, Theory, and Current Challenges. *Chemical Reviews* **2016**, *116*, 7529–7550.

(2) De Meutter, J.; Goormaghtigh, E. Evaluation of protein secondary structure from FTIR spectra improved after partial deuteration. *European Biophysics Journal* **2021**, *50*, 613–628.

(3) Hume, S.; Hithell, G.; Greetham, G. M.; Donaldson, P. M.; Towrie, M.; Parker, A. W.; Baker, M. J.; Hunt, N. T. Measuring proteins in H 2 O with 2D-IR spectroscopy. *Chemical science* **2019**, *10*, 6448–6456.

(4) Cioni, P.; Strambini, G. B. Effect of heavy water on protein flexibility. *Biophysical journal* **2002**, *82*, 3246–3253.

(5) Beranová, L.; Humpolíčková, J.; Sỳkora, J.; Benda, A.; Cwiklik, L.; Jurkiewicz, P.; Gröbner, G.; Hof, M. Effect of heavy water on phospholipid membranes: experimental confirmation of molecular dynamics simulations. *Physical Chemistry Chemical Physics* **2012**, *14*, 14516–14522.

(6) Ben Abu, N.; Mason, P. E.; Klein, H.; Dubovski, N.; Ben Shoshan-Galeczki, Y.; Malach, E.; Pražienková, V.; Maletínská, L.; Tempra, C.; Chamorro, V. C. et al. Sweet taste of heavy water. *Communications biology* **2021**, *4*, 1–10.

(7) Ceriotti, M.; Fang, W.; Kusalik, P. G.; McKenzie, R. H.; Michaelides, A.; Morales, M. A.; Markland, T. E. Nuclear quantum effects in water and aqueous systems: Experiment, theory, and current challenges. *Chemical reviews* **2016**, *116*, 7529–7550.

(8) Guillot, B.; Guissani, Y. Quantum effects in simulated water by the Feynman–Hibbs approach. *The Journal of chemical physics* **1998**, *108*, 10162–10174.

(9) Sesé, L. M. Feynman-Hibbs quantum effective potentials for Monte Carlo simulations of liquid neon. *Molecular Physics* **1993**, *78*, 1167–1177.

(10) Chamorro, V. C.; Tempra, C.; Jungwirth, P. Heavy Water Models for Classical Molecular Dynamics: Effective Inclusion of Nuclear Quantum Effects. *The Journal of Physical Chemistry B* **2021**, *125*, 4514–4519.

(11) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. The missing term in effective pair potentials. *The Journal of Physical Chemistry* **1987**, *91*, 6269–6271.

(12) Jo, S.; Kim, T.; Iyer, V. G.; Im, W. CHARMM-GUI: a web-based graphical user interface for CHARMM. *Journal of computational chemistry* **2008**, *29*, 1859–1865.

(13) Lee, J.; Cheng, X.; Swails, J. M.; Yeom, M. S.; Eastman, P. K.; Lemkul, J. A.; Wei, S.; Buckner, J.; Jeong, J. C.; Qi, Y. et al. CHARMM-GUI input generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM simulations using the CHARMM36 additive force field. *Journal of chemical theory and computation* **2016**, *12*, 405–413.

(14) Swope, W. C.; Andersen, H. C.; Berens, P. H.; Wilson, K. R. A computer simulation method for the calculation of equilibrium constants for the formation of physical clusters of molecules: Application to small water clusters. *The Journal of chemical physics* **1982**, *76*, 637–649.

(15) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: An N log (N) method for Ewald sums in large systems. *The Journal of chemical physics* **1993**, *98*, 10089–10092.

(16) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. A

smooth particle mesh Ewald method. *The Journal of Chemical Physics* **1995**, *103*, 8577–8593.

(17) Bussi, G.; Donadio, D.; Parrinello, M. Canonical sampling through velocity rescaling. *The Journal of chemical physics* **2007**, *126*, 014101.

(18) Parrinello, M.; Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *Journal of Applied Physics* **1981**, *52*, 7182–7190.

(19) Kumar, S.; Rosenberg, J. M.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A. The weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. *Journal of computational chemistry* **1992**, *13*, 1011–1021.

(20) Hub, J. S.; De Groot, B. L.; Van Der Spoel, D. g_wham A Free Weighted Histogram Analysis Implementation Including Robust Error and Autocorrelation Estimates. *Journal of chemical theory and computation* **2010**, *6*, 3713–3720.

(21) Nar, H.; Messerschmidt, A.; Huber, R.; Van de Kamp, M.; Canters, G. W. Crystal structure of Pseudomonas aeruginosa apo-azurin at 1.85 Å resolution. *Febs Letters* **1992**, *306*, 119–124.

(22) Brownlow, S.; Cabral, J. H. M.; Cooper, R.; Flower, D. R.; Yewdall, S. J.; Polikarpov, I.; North, A. C.; Sawyer, L. Bovine β-lactoglobulin at 1.8 Å resolution—still an enigmatic lipocalin. *Structure* **1997**, *5*, 481–495.

(23) Kostrewa, D.; Choe, H. W.; Heinemann, U.; Saenger, W. Crystal structure of guanosine-free ribonuclease T1, complexed with vanadate (V), suggests conformational change upon substrate binding. *Biochemistry* **1989**, *28*, 7592–7600.

(24) Cioni, P.; Strambini, G. B. Effect of heavy water on protein flexibility. *Biophysical journal* **2002**, *82*, 3246–3253.

(25) Joosten, R. P.; Te Beek, T. A.; Krieger, E.; Hekkelman, M. L.; Hooft, R. W.; Schneider, R.; Sander, C.; Vriend, G. A series of PDB related databases for everyday needs. *Nucleic acids research* **2010**, *39*, D411–D419.

(26) Kabsch, W.; Sander, C. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers: Original Research on Biomolecules* **1983**, *22*, 2577–2637.

(27) Pica, A.; Graziano, G. Effect of heavy water on the conformational stability of globular proteins. *Biopolymers* **2018**, *109*, e23076.

(28) Makhatadze, G. I.; Clore, G. M.; Gronenborn, A. M. Solvent isotope effect and protein stability. *Nature structural biology* **1995**, *2*, 852–855.

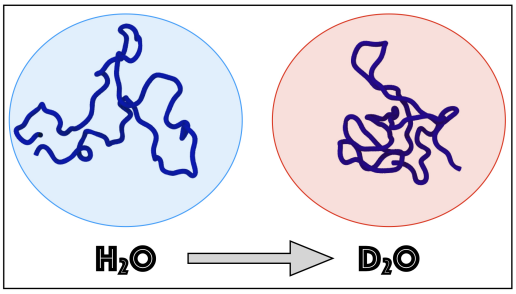
(29) Rog, T.; Murzyn, K.; Milhaud, J.; Karttunen, M.; Pasenkiewicz-Gierula, M. Water isotope effect on the phosphatidylcholine bilayer properties: a molecular dynamics simulation study. *The Journal of Physical Chemistry B* **2009**, *113*, 2378–2387.

(30) Matsuki, H.; Okuno, H.; Sakano, F.; Kusube, M.; Kaneshina, S. Effect of deuterium oxide on the thermodynamic quantities associated with phase transitions of phosphatidylcholine bilayer membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2005**, *1712*, 92–100.

(31) Matsuki, H.; Okuno, H.; Sakano, F.; Kusube, M.; Kaneshina, S. Effect of deuterium oxide on the thermodynamic quantities associated with phase transitions of phosphatidylcholine bilayer membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2005**, *1712*, 92–100.

(32) Khakbaz, P.; Klauda, J. B. Investigation of phase transitions of saturated phosphocholine lipid bilayers via molecular dynamics simulations. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2018**, *1860*, 1489–1501.

Table of Contents graphic



TOC Graphic