Sweet taste of heavy water

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Abstract:

Hydrogen to deuterium isotopic substitution has only a minor effect on physical and chemical properties of water and, as such, is not supposed to influence its neutral taste. Here we conclusively demonstrate that humans are, nevertheless, able to distinguish D_2O from H_2O by taste. Indeed, highly purified heavy water has a distinctly sweeter taste than same-purity normal water and adds to perceived sweetness of sweeteners. In contrast, mice do not prefer D_2O over H_2O, indicating that they are not likely to perceive heavy water as sweet. For humans, the sweet taste of D_2O is suppressed by lactisole, which is a known sweetness inhibitor acting via the TAS1R3 monomer of the TAS1R2/TAS1R3 sweet taste receptor. HEK 293T cells transfected with the TAS1R2/TAS1R3 heterodimer and the chimeric Gα16gust44 G-protein are activated by D_2O but not by H_2O. The present study resolves a long-standing controversy about the taste of heavy water, confirms that its sweet taste is mediated by the human TAS1R2/TAS1R3 taste receptor, and opens way to future studies of potential sites and modes of action.

One sentence summary:

Heavy water elicits sweet taste for humans via the TAS1R2/TAS1R3 taste receptor.

Main:

Heavy water, D_2O, has fascinated researchers since the discovery of deuterium by Urey in 1931(*1, 2*). The most notable difference in physical properties between D_2O and H_2O is the roughly 10% higher density of the former liquid, which is mostly a trivial consequence of deuterium being about twice as heavy as hydrogen. A more subtle effect of deuteration is the formation of slightly stronger hydrogen (or deuterium) bonds in heavy water as compared to the normal (light) water(*3, 4*). This results in a small increase of the freezing and boiling points by 3.8°C and 1.4°C, respectively, and in a slight increase of 0.44 in pH (or pD) of pure water upon deuteration(*5*). In comparison, a mere dissolution of atmospheric CO_2 and subsequent formation of dilute carbonic acid in open containers has a significantly stronger influence on the pH of water, changing it by more than one unit(*6*).

Biological effects are observable for high doses of heavy water. While bacteria can function in practically pure heavy water, albeit with somewhat hindered growth rate(*7, 8*), for eukaryotes, damaging effects on cell division and general metabolism occur at around 25% deuteration, with lethal conditions for plants and animals typically occurring at ~40-50% deuteration of the body water (*2, 9, 10*). Small levels of deuteration are, nevertheless, harmless. This is understandable given the fact that about 1 in every 6400 hydrogens in nature is found in its stable isotope form of deuterium(*11*). Oral doses of several milliliters of D_2O are safe for humans(*12*) and are being routinely used together with another stable isotopic form of water, $H_2{}^{18}O$, for metabolic measurements in clinical praxis(*13*). Probably the best-established effect of heavy water is the increase of the circadian oscillation length upon its administration to both animals and plants. This

has been attributed to a general slowdown of metabolism upon deuteration, although the exact mechanism of this effect is unknown(*14, 15*).

A long-standing unresolved puzzle concerns the taste of heavy water. There is anecdotal evidence from the 1930s that the taste of pure D_2O is distinct from the neutral one of pure H_2O, being described mostly as "sweet"(*16*). However, Urey and Failla addressed this question in a short article published in Science in 1935 concluding authoritatively that upon tasting "neither of us could detect the slightest difference between the taste of ordinary distilled water and the taste of pure heavy water"(*17*). This had, with a rare exception(*18*), an inhibitive effect on further human studies, with research concerning effects of heavy water focusing primarily on animal or cell models. Experiments in animals indicated that rats developed aversion toward D_2O when deuteration of their body water reached harmful levels, but there is conflicting evidence to whether they can taste heavy water or use other cues to avoid it(*19, 20*).

Within the last two decades, the heterodimer of the taste receptor of the TAS1Rs type of G-protein coupled receptor (GPCRs), denoted as TAS1R2/TAS1R3, was established as the main receptor for sweet taste(*21*). The human TAS1R2/TAS1R3 heterodimer recognizes diverse natural and synthetic sweeteners(*22*). The binding sites of the different types of sweeteners include orthosteric (sugar-binding site in the extracellular Venus-Fly Domain of TAS1R2) and several allosteric sites, including sites in the extra-cellular regions of the TAS1R2 and TAS1R3 subunits and in the transmembrane domain of TAS1R3(*23, 24*) (Figure 1). Additional pathways for sweet taste recognition have also been suggested, involving glucose transporters and ATP-gated K+ channel(*25, 26*).

Interestingly, not all artificial sweeteners are recognized by rodents(*27*). Differences in human and rodent responses to tastants, as well as sweetness inhibitors such as lactisole, have

been useful for delineating the molecular recognition mechanism of sweet compounds – using human-mouse chimeric receptors, it was shown that the trans membrane domain (TMD) of human TAS1R3 is required for the activating effects of cyclamate(*28*) and for the inhibitory effect of lactisole(*29*).

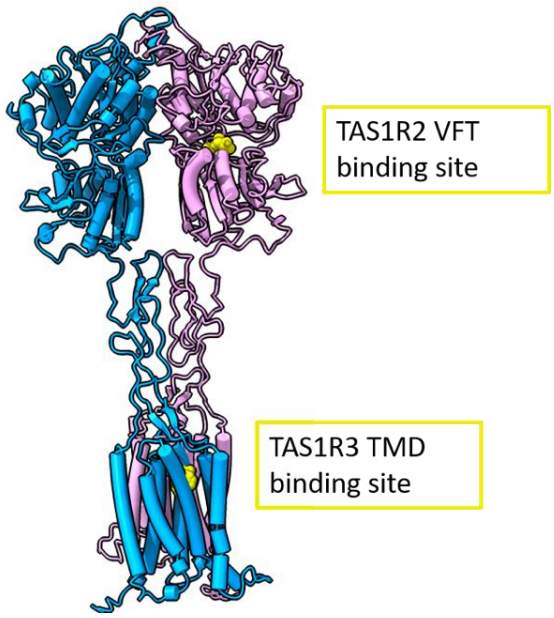

Figure 1. Full human sweet taste TAS1R2/TAS1R3 receptor model with the TAS1R2 monomer colored in pink and the TAS1R3 monomer in cyan. Binding sites are represented in yellow. The full receptor heterodimer was prepared with the I-Tasser web server(*30*) based on multiple published structures (i.e. 6N51, 5X2M, and 5K5S). The structures were aligned to a Metabotropic Glutamate Receptor 5 Cryo-EM structure (PDB: 6N51) and minimized using Schrödinger Maestro 2019-1. The binding site of TAS1R2 is based on coordinates of docked D-glucose to a Venus flytrap (VFT) model that was previously validated (*31*) (modeling based on template PDB ID: 5X2M, docking performed with Schrödinger Maestro 2019-1, Glide XP), and the TAS1R3 binding site is based on a lactisole molecule docked to the TAS1R3 TMD model (template PDB IDs: 4OR2 and 4OO9, Schrödinger Maestro 2018-2, Glide SP). The figure was made using ChimeraX (version 0.93)(*32*).

A combination of TAS1R3 with another member of the TAS1R family, TAS1R1, results in a dimer that mediates umami taste, elicited by molecules such as glutamate or its sodium form monosodium glutamate (MSG)(*33*). Bitter taste is mediated by the taste receptors type 2 (TAS2R) gene family(*34*), a branch of Family A GPCRs(*23*). The human genome has 25 TAS2R

subtypes and over a thousand of bitter compounds are currently known(*35*) and numerous additional bitterants are predicted (*36*).

In this study, we systematically address the question of sweet taste of heavy water by a combination of sensory experiments in humans, behavioral experiments in mice, tests on sweet taste receptor-transfected cell lines, and computational modeling, including molecular dynamics (MD) simulations. This combined approach consistently leads to a conclusion that the sweet taste of pure D_2O is a real effect for human subjects due to activation of the TAS1R2/TAS1R3 sweet taste receptor. While present simulations show, in accord with previous experiments(*37*), that proteins are systematically slightly more rigid and compact in D_2O than in H_2O, the specific molecular mechanism of the heavy water effect on the TAS1R2/TAS1R3 receptor in particular remains to be established.

Water purity

We have paid great attention to the purity of the water samples, further degassing and redistilling under vacuum the purest commercially available D_2O and H_2O. Lack of non-negligible amounts of organic impurities was subsequently confirmed by gas chromatography with mass spectrometry analysis and by experiments with water samples at different levels of purification, see Supporting Material (SM). This is extremely important – note in this context that "the vibrational theory of olfaction", which suggested distinct perception of deuterium isotopes of odorants due to difference in their vibrational spectra(*38*), has been refuted with some of the observed effects turning out to be due to impurities(*39, 40*).

Experiments with a human sensory panel

A human sensory panel was employed to study the D_2O taste. Triangle tests based on two samples of H_2O and one sample of D_2O (or vice versa), with random success rate of ⅓, were

presented to the panelists in randomized order. Panelists were asked to pick the odd sample out - to smell only, to taste only (with nose clips), or to taste with open nose. Our results show that humans perceive D_2O as being clearly distinguishable from H_2O based on its taste: In open nose taste test 22 out of 28 participants identified the odd sample correctly ($p=0.001$), and in taste-only test 14 out of 26 identified the odd sample correctly ($p=0.03$). However, in smell-only triangle test, only 9 out of 25 panelists chose the odd sample correctly ($p>0.05$). Data are summarized in Figure S1A-C in SM.

Next, the perceived sweetness of D_2O in increasing proportion to H_2O was reported using a 9-point scale. D_2O sweetness was shown to increase in dose-dependent manner (Figure 2A). The perceived sweetness of increasing concentrations of glucose (Figure 2B), sucrose (Figure 2C) and an artificial sweetener cyclamate (Figure 2D) was tested when dissolved in H_2O or in D_2O. Pure D_2O was again perceived as slightly sweet and significantly sweeter than H_2O. Furthermore, D_2O adds to the sweetness of glucose, cyclamate, and low concentrations of sucrose.

We then checked whether the effect of D_2O is sweetness-specific or general, whereby D_2O might also add intensity to savory taste of umami compounds (MSG) and to bitter taste of bitter compounds (quinine), since these taste modalities are also mediated by GPCR receptors expressed in taste cells. It was established here that the intensity of savory taste of MSG in D_2O did not differ from that in H_2O (Figure 2E), while the perceived bitterness of quinine was in fact slightly reduced in D_2O compared to quinine in H_2O (Figure 2F). This is in agreement with the known effect of sweeteners as maskers of bitter taste, that can be due to both local interactions and sensory integration effects(*41-43*). Thus, we have ascertained that D_2O is sweet, and adds to sweetness of other sweet molecules, but not to intensity of other GPCR-mediated taste modalities.

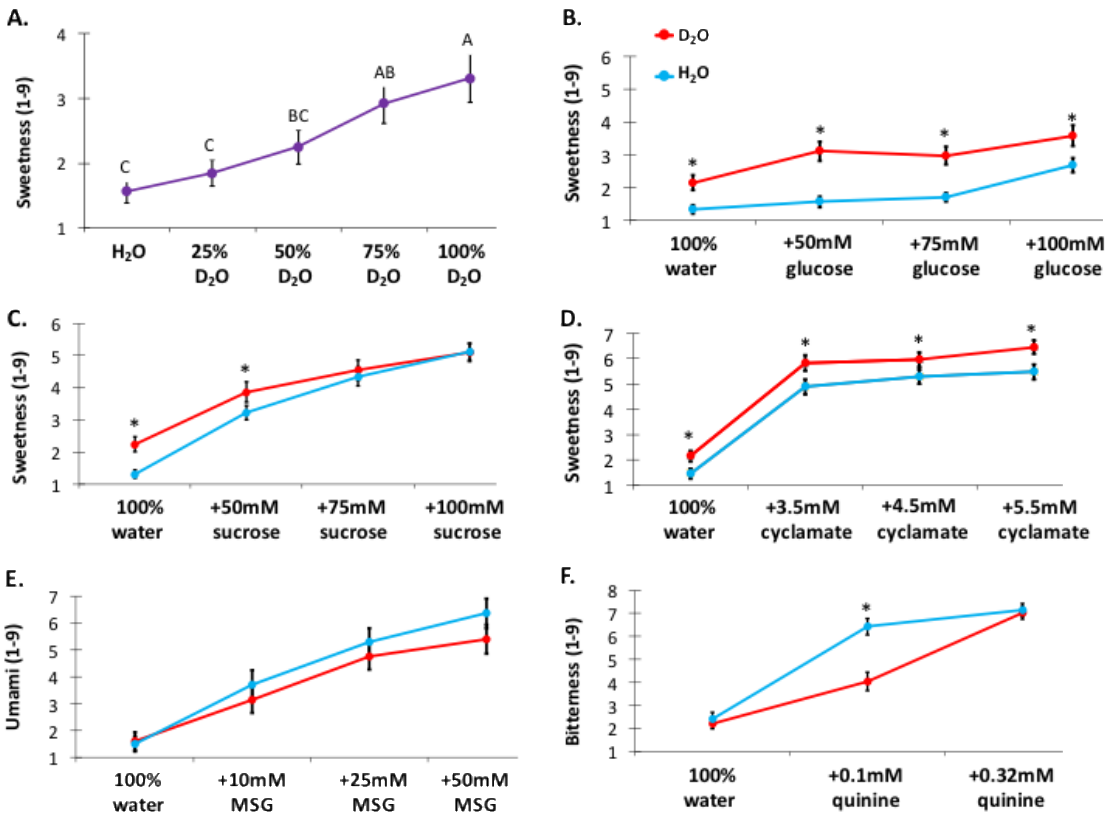

Figure 2. D_2O sweetness and its effect on different tastants. (A) Sweetness of D_2O at increasing ratios when mixed with H_2O. Treatments not connected by the same letters are significantly different ($p<0.05$ in Tuckey Kramer test). (B)-(F) The effect of D_2O (red) compared to H_2O (blue) on glucose (B), sucrose (C), cyclamate (D), quinine (E), and MSG (F) taste-specific intensity. Asterisks indicate a significant ($p < 0.05$) difference between water types using the two-way analysis of variance (ANOVA) with a pre-planned comparison t-test. All data are presented as the mean ± the Standard Error of Measurement (SEM); n=15-30 (4-12 males). The y axis shows the response for individual modalities, while the x axis is labeled with different water samples. Scale for each modality is labeled as 1 = no sensation, 3 = slight, 5 = moderate, 7 = very much, and 9 = extreme sensation.

Experiments with mice

Next, we addressed the question whether the sweetness of D_2O is perceived also by rodents. Lean mice of the C57BL/6J strain were drinking pure H_2O, D_2O, or a 43 mmol/l H_2O sucrose solution for 16h during a night period. Namely, each of the three groups of mice had a choice from two bottles containing i) H_2O and D_2O, ii) H_2O and sucrose solution, or iii) H_2O and H_2O (as a control). The food intake was unaffected in all groups (see SM).

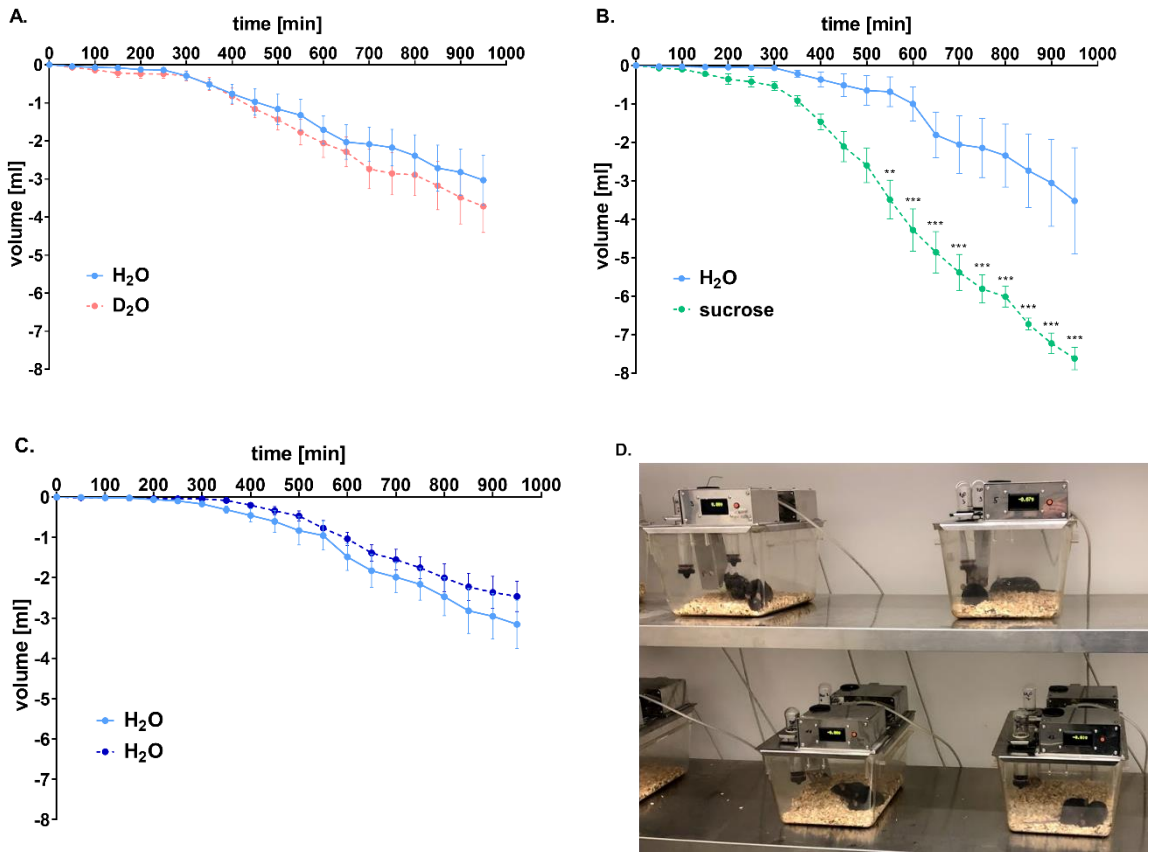

Figure 3. Time-resolved volumes of water consumption by mice. (A) Volume consumption of D_2O is not different from that of H_2O (n = 12). (B) Mice show strong preference to sucrose solution (n = 10). Significance is ** $p < 0.01$, *** $p < 0.001$. (C) Volume consumption of the control group drinking H_2O only (n = 12). (D) Snapshot of the automatic drinking monitoring system. Mice were placed in groups of two in individual cages. Data are presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using two-way ANOVA with a Bonferroni's multiple comparisons test.

The results of the drinking experiments are presented in Figures 3A-C, with a snapshot of the experimental setup shown in Figure 3D. In cages where mice were offered both normal water and heavy water (Figure 3A) consumption of D_2O was within statistical error the same as that of H_2O. Previous reports have shown that on longer timescales than those reported here mice learned to avoid D_2O, as it is poisonous to them in larger quantities(*9*). It is not clear what is the cue that enables the avoidance learning, but it is evident that the early response to D_2O is not attractive, suggesting that it is not eliciting sweet taste in mice.

By contrast, mice exhibit a strong preference for sucrose solution over H_2O. Indeed, the consumed volume was significantly increased in line with the predilection of mice for sucrose

solutions (Figure 3B). The amount of H_2O consumed by the control group from either of the two bottles, both containing H_2O, is depicted in Figure 3C. Overall, the data shows that in all three experiments mice consumed comparable amounts of H_2O, as well as D_2O, with significant increase of consumption of the sucrose solution. Differences between human and rodents in their responses to non-sugar sweeteners are quite common(*27*), and will be further explored for D_2O in the future.

Assessing involvement of TAS1R2/TAS1R3 receptor using human sensory panel

The chemical dissimilarity of D_2O from sugars and other sweeteners raises the question whether the effect we observed in human subjects is mediated by TAS1R2/TAS1R3, which is the major receptor for sweet taste(*21*). This was first explored by combining water samples with lactisole, an established TAS1R2/TAS1R3 inhibitor(*29*). Using the two-alternative forced choice (2AFC) method, in which the participant must choose between two samples, 18 out of 25 panelists chose pure D_2O as sweeter than D_2O + 0.9 mM lactisole solution ($p<0.05$, Figure 4A). In an additional experiment, the sweetness of pure D_2O was scored significantly higher than that of D_2O + 0.9 mM lactisole solution ($p=0.0003$), while the same amount of lactisole had no effect on the perception of sweetness of H_2O that served as control (Figure 4B). These results suggest that D_2O elicits sweetness via the TAS1R2/TAS1R3 sweet taste receptor.

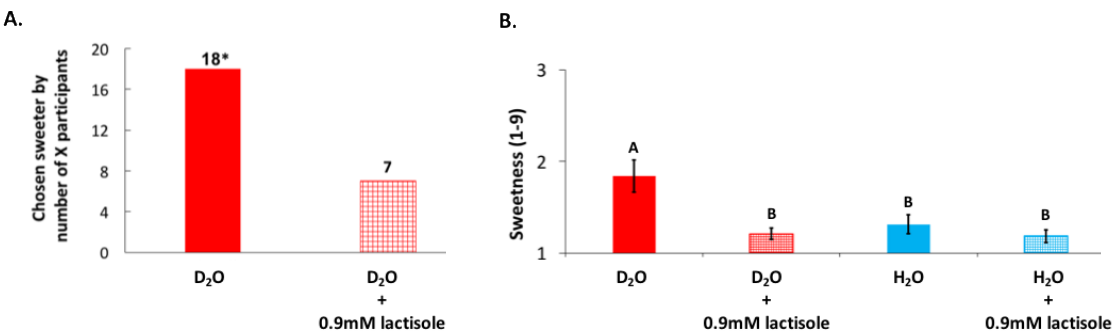

Figure 4. Lactisole reduces sweetness of D_2O. (A) 2AFC test. Pure D_2O was chosen to be sweeter ($p<0.05$) than the sample with lactisole by 18 participants (n=25; 11 males). (B) Effect of 0.9mM lactisole on sweetness intensity using the 9-Likert scale. Statistical analysis was performed using ANOVA with a Tuckey Kramer test (n=27; 9 males); results outside the error bars are significantly different ($p<0.05$). Data are presented as the mean ± SEM. The y axis shows the

response for sweetness on a 9-point scale, while the x axis is labeled with different water samples. Scale for sweetness is labeled as 1 = no sensation, 3 = slight, 5 = moderate, 7 = very much, and 9 = extreme sensation.

Cell-based experiments for establishing the role of TAS1R2/TAS1R3

To confirm the involvement of the sweet taste receptor TAS1R2/TAS1R3 in D_2O signaling we used HEK 293T cells. These cells do not endogenously express TAS1R2/TAS1R3, while expressing the muscarinic receptor (M3). Using an IP1 assay(*44, 45*) on the HEK 293T cells, we observed that dose-dependent curves of carbachol, which is an agonist of M3(*46*), did not differ between H_2O and D_2O-based medium (Figure 5A) and that cell medium that had either 10 % or 100 % D_2O, did not activate HEK 293T basal IP1 accumulation (Figure 5B). The functionality of HEK 293T transiently expressing the TAS1R2/TAS1R3 receptor along with the chimeric Gα16gust44 subunit(*47, 48*) was next illustrated by dose-dependent response to D-glucose (Figure 5C). We further found that 10 % D_2O and, more significantly, 100 % D_2O activated these cells as well (Figure 5D).

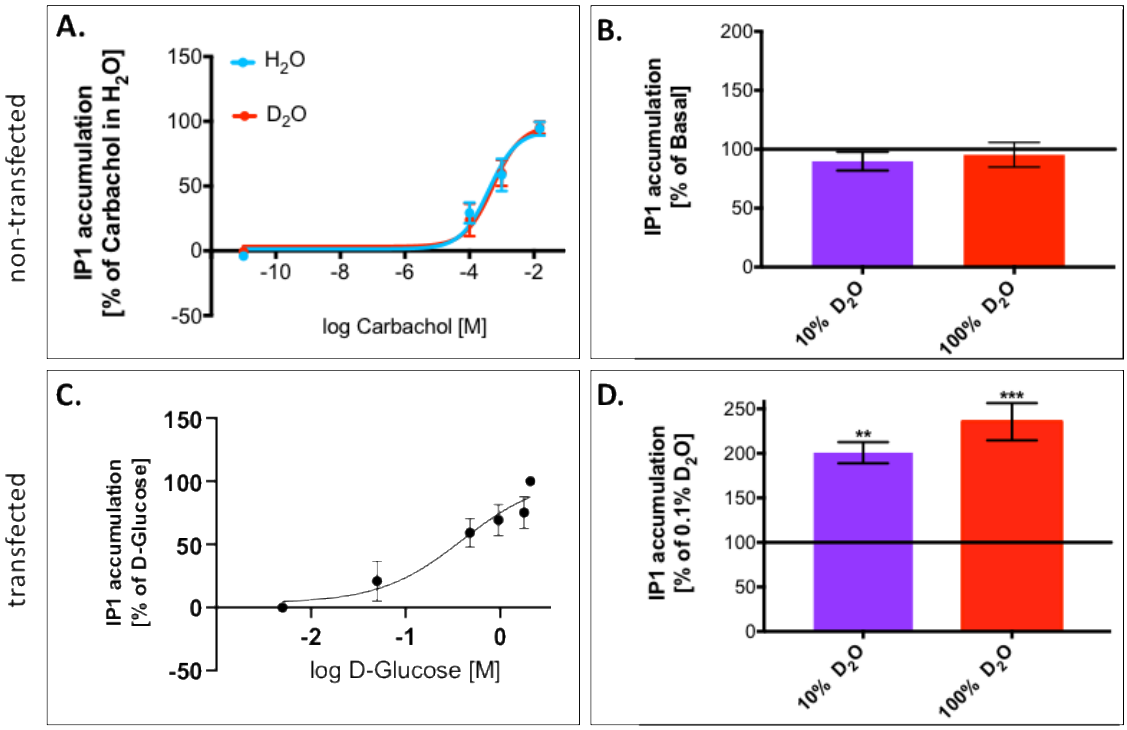

Figure 5. IP1 accumulation in HEK 293T cells following exposure to different ligands dissolved in powder-based DMEM medium. (A) Non-transfected HEK 293T cells respond similarly to raising concentrations of carbachol dissolved in D_2O (red) as in H_2O (blue). (B) D_2O caused no elevation of IP1 levels in non-transfected HEK 293T cells. (C) HEK 293T cells transiently expressing TAS1R2/TAS1R3 respond positively to D-glucose. (D) Transfected HEK 293T cells are activated by D_2O. Values represent the mean ± SEM of at least 3 replicates. The horizontal black line represents the basal values of controls. Significant differences in IP1 values from control values are marked with ** for $p \leq 0.005$ and *** for $p \leq 0.0005$ using Dunnett's multiple comparisons test.

Molecular modelling

These cellular response results further support the hypothesis that the sweet taste of D_2O is mediated via the TAS1R2/TAS1R3 receptor. Multiple sites of interactions and various mechanisms governing this effect can be envisioned. As a potential suspect, we focus on the TAS1R3 TMD (see Figure 1), as it is already known to be a modulation site with functional differences between humans and rodents(*22, 28, 29*). Furthermore, water-binding sites were discovered at the TMD of many GPCRs(*49, 50*), suggesting a potential target for D_2O binding. We modeled the human TAS1R3 (hTAS1R3) TMD using the I-TASSER server(*30*). Positions of H_2O molecules were compared among mGluR5 structures (PDB: 4OO9, 5CGC, and 5CGD), and two conserved positions were found. The H_2O molecules in these two positions were merged with hTAS1R3 model and minimized (Figure 6A). The water mapping protocol from OpenEye(*51*) enables mapping of water positions based on the energetics of water, and ~40 water molecules were predicted in the binding site using this protocol (Figure 6A). Water densities of H_2O and D_2O in the TMD of the TAS1R2/TAS1R3 receptor were calculated from MD simulations as described below. Overall, all three methods suggest the possibility for at least some internal molecules (trapped in the TMD bundle) in addition to water that surrounds the extracellular and intracellular loops (Figure 6A).

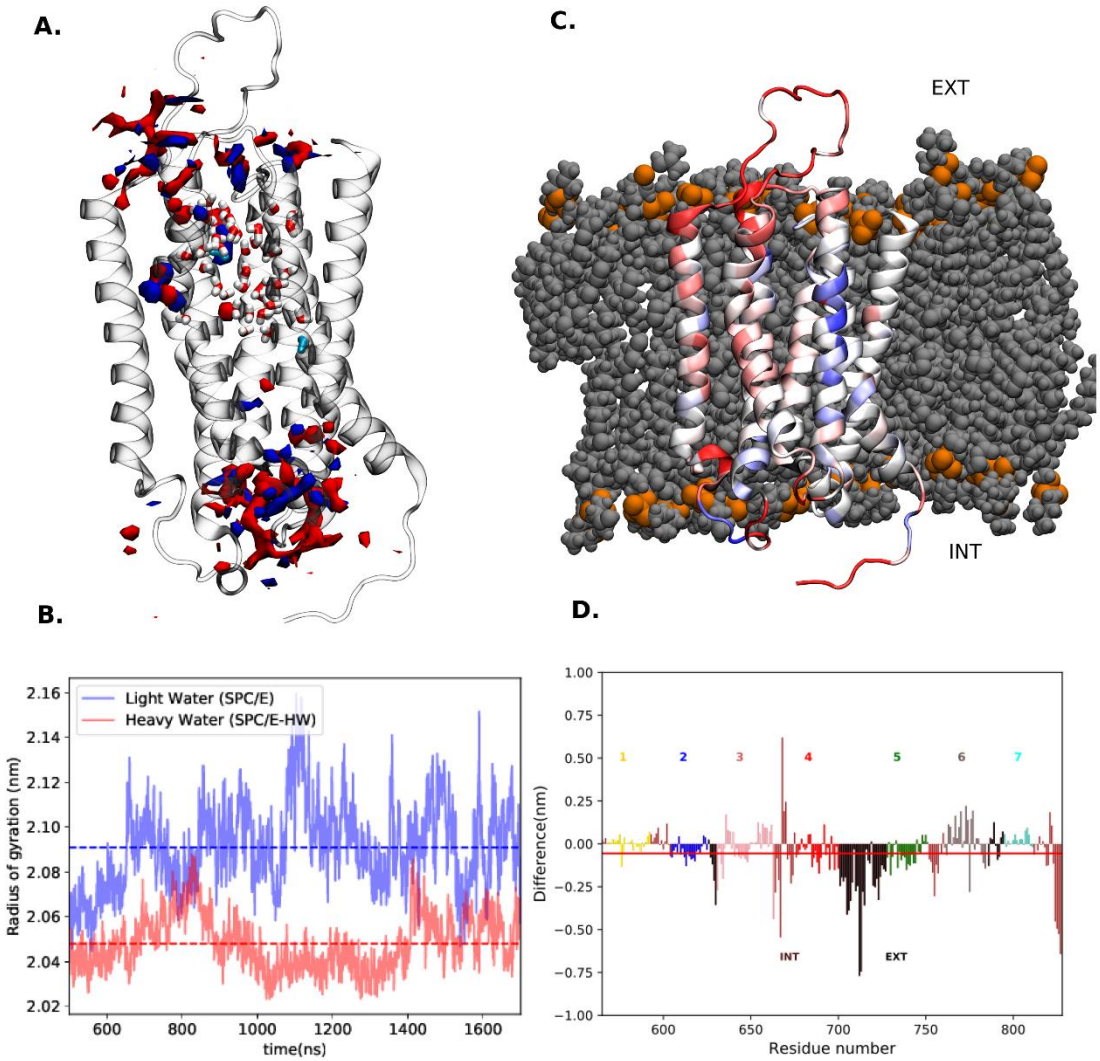

Figure 6: Differences between the behavior of the trans-membrane part of the human sweet taste receptor in H_2O vs D_2O. (A) Structure of the TMD of the TAS1R2/TAS1R3 receptor with the probability density (volumetric map) of H_2O (blue) or D_2O (red) molecules within 10 Å from the protein evaluated using the VMD VolMap tool from the MD simulations at an isovalue of 0.5. The conserved water molecules in the x-ray templates are shown in cyan color. Water molecules predicted with the software OpenEye(*51*) are shown in licorice representation. (B) Time evolution of the radii of gyration in H_2O (blue) and D_2O (red) with mean values as dashed lines, showing that the protein is more compact in heavy water. (C) Representative snapshot of the trans-membrane part of the human sweet taste receptor color-coded that red/blue represents parts more/less rigid in D_2O vs H_2O. The embedding lipid membrane is represented in gray. (D) Difference in root mean square fluctuations in MD trajectories. Negative/positive values mean that structures are more/less rigid in D_2O than in H_2O. The red line represents the sum over all residues.

Microsecond MD simulations of the TMD embedded in a phospatidylcholine (POPC) bilayer in either H_2O or D_2O were carried out (for details see SM). We note that water molecules

have entered the TMD domain and have clustered at positions that overlap with the modeled water positions, see Figure 6A. These internal positions may have a differential effect between H_2O and D_2O, though differences between the averaged water densities are not very pronounced. Figure 6B shows the time evolution of the radius of gyration of the TMD domain, while Figures 6D presents the root mean square fluctuations (RMSF) of individual residues of the proteins superimposed on its structure and plotted in a graph together with the mean value of RMSF. A small but significant difference is apparent in the behavior of the protein in H_2O vs D_2O. Namely, structural fluctuations of most residues and of the protein as a whole are slightly attenuated in D_2O, in which environment the protein is also somewhat more compact than in H_2O (Figure 6B). Additional simulations on other representative systems show that the rigidifying effect of heavy water is apparent also in small soluble proteins (see SM).

Summary and outlook

In summary, we have systematically addressed the question of sweet taste of heavy water. Importantly, by employing gas chromatography/mass spectrometry analysis we demonstrate that the effect is not due to impurities. Being only isotopically different from H_2O, in principle, D_2O should be indistinguishable from H_2O with regard to taste, namely it should have no taste of its own. Regular water was shown to elicit sweet taste when sweet-taste inhibitors are rinsed away, both in human sensory and in cell-based studies. This was explained in terms of a two-state model, where the receptor shifts to its activated state when released from inhibition by rinsing with water (*52*). Here we studied the direct taste of D_2O and H_2O, not related to washing away of sweet taste inhibitors. Using psychophysics protocols, we show that humans differentiate between D_2O and H_2O based on taste. Next, we illustrate that human subjects consistently perceive D_2O as being mildly sweet, and significantly sweeter than H_2O.

Moreover, D_2O added to the sweetness of some sweeteners; the sweetness of glucose and cyclamate appears to be directly additive while in the case of sucrose the additive effect was observed only at 50 mM sugar concentration. Furthermore, D_2O did not affect the umami taste perception, and slightly reduced the perceived bitterness of quinine, in agreement with the known effect of bitterness suppression by sweet molecules.

A further important funding is that lactisole, an established blocker of the TAS1R2/TAS1R3 sweet taste receptor that acts at the TAS1R3 transmembrane domain(*29*), suppresses the sweet perception of D_2O. In support of the observations on humans, cell-based experiments showed that HEK 293T cells transfected with TAS1R2/TAS1R3 and Gα16gust44 chimera, but not the non-transfected cells, are activated by D_2O, as measured by IP1 accumulation compared to control values. Finally, taste experiments on mice showed that they do not prefer D_2O over H_2O.

Our findings point to the human sweet taste receptor TAS1R2/TAS1R3 as the target of D_2O activation. Molecular dynamics simulations show, in agreement with experiment(*37*), that proteins in general are slightly more rigid and compact in D_2O than in H_2O. At a molecules level, this general behavior may be traced back to the slightly stronger hydrogen bonding in D_2O vs H_2O, which is due to differences in zero-point energy, as a demonstration of a nuclear quantum effect(*3, 4*). Future studies should be able to elucidate the precise site and mechanism of action, as well as the reason why D_2O activates TAS1R2/TAS1R3 in particular, resulting in sweet (but not other) taste. To this end, site directed mutagenesis as well as determination of the precise structure of the TAS1R2/TAS1R3 receptor would be of a key importance.

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References

1. H. C. Urey, F. G. Brickwedde, G. M. Murphy, A Hydrogen Isotope of Mass 2. *Physical Review* **39**, 164-165 (1932).
2. M. Francl, The weight of water. *Nature Chemistry* **11**, 284-285 (2019).
3. T. Clark, J. Heske, T. D. Kühne, Opposing Electronic and Nuclear Quantum Effects on Hydrogen Bonds in H2O and D2O. *ChemPhysChem* **20**, 2461-2465 (2019).
4. F. Paesani, G. A. Voth, The Properties of Water: Insights from Quantum Simulations. *The Journal of Physical Chemistry B* **113**, 5702-5719 (2009).
5. F. Macdonald, D. R. Lide, CRC handbook of chemistry and physics: From paper to web. *Abstr Pap Am Chem S* **225**, U552-U552 (2003).
6. R. A. J. Bogan, S. Ohde, T. Arakaki, I. Mori, C. W. McLeod, Changes in Rainwater pH associated with Increasing Atmospheric Carbon Dioxide after the Industrial Revolution. *Water, Air, and Soil Pollution* **196**, 263-271 (2009).
7. E. Van Horn, G. C. Ware, Growth of Bacterium coli and Staphylococcus albus in Heavy Water. *Nature* **184**, 833-833 (1959).
8. O. Mosin, I. Ignatov, D. Skladnev, V. Shvets, Studying of Phenomenon of Biological Adaptation to Heavy Water. *European Journal of Molecular Biotechnology* **6**, (2014).
9. H. G. Barbour, The Basis of the Pharmacological Action of Heavy Water in Mammals. *Yale J Biol Med* **9**, 551-565 (1937).
10. D. M. Czajka, A. J. Finkel, C. S. Fischer, J. J. Katz, Physiological effects of deuterium on dogs. *American Journal of Physiology-Legacy Content* **201**, 357-362 (1961).
11. W. Dansgaard, STABLE ISOTOPES IN PRECIPITATION. *Tellus* **16**, 436-468 (1964).
12. P. J. H. Jones, S. T. Leatherdale, Stable isotopes in clinical research: safety reaffirmed. *Clinical Science* **80**, 277-280 (1991).
13. J. Speakman, Speakman JR. The history and theory of the doubly labeled water technique. Am J Clin Nutr 68, 932S-938S. *The American journal of clinical nutrition* **68**, 932S-938S (1998).
14. C. S. Pittendrigh, P. C. Caldarola, E. S. Cosbey, A Differential Effect of Heavy Water on Temperature-Dependent and Temperature-Compensated Aspects of the Circadian System of <em>Drosophila pseudoobscura</em&gt. *Proceedings of the National Academy of Sciences* **70**, 2037 (1973).
15. C. P. Richter, Heavy water as a tool for study of the forces that control length of period of the 24-hour clock of the hamster. *Proc Natl Acad Sci U S A* **74**, 1295-1299 (1977).
16. K. Hansen, E. Rustung, Untersuchen uber die Biologische Wirkungen von "Schwerem Wasser" bei Warmblutigen Tieren. *Klinische Wochenschrift* **14**, 104-108 (1935).
17. H. C. Urey, G. Failla, Concerning the taste of heavy water. *Science* **81**, 273 (1935).
18. R. H. Kirby, D. F. Pick, M. S. Riddick, Discrimination between heavy water and deionized water using gustation vs. olfaction in humans. *Physiological Psychology* **4**, 102-104 (1976).
19. C. P. Richter, STUDY OF TASTE AND SMELL OF HEAVY-WATER (99.8PERCENT) IN RATS. *Proceedings of the Society for Experimental Biology and Medicine* **152**, 677-684 (1976).
20. I. J. Miller, G. Mooser, TASTE RESPONSES TO DEUTERIUM-OXIDE. *Physiology & Behavior* **23**, 69-74 (1979).
21. G. Nelson, M. A. Hoon, J. Chandrashekar, Y. Zhang, N. J. P. Ryba, C. S. Zuker, Mammalian Sweet Taste Receptors. *Cell* **106**, 381-390 (2001).
22. X. Li, L. Staszewski, H. Xu, K. Durick, M. Zoller, E. Adler, Human receptors for sweet and umami taste. *Proceedings of the National Academy of Sciences* **99**, 4692 (2002).
23. A. Di Pizio, M. Y. Niv, Computational Studies of Smell and Taste Receptors. *Israel Journal of Chemistry* **54**, 1205-1218 (2014).
24. R. Gutierrez, E. Fonseca, S. A. Simon, The neuroscience of sugars in taste, gut-reward, feeding circuits, and obesity. *Cellular and Molecular Life Sciences*, (2020).
25. S. Damak, M. Rong, K. Yasumatsu, Z. Kokrashvili, V. Varadarajan, S. Zou, P. H. Jiang, Y. Ninomyia, R. F. Margolskee, Detection of Sweet and Umami Taste in the Absence of Taste Receptor T1r3 *Science* **301**, 850-853 (2003).
26. K. K. Yee, S. K. Sukumaran, R. Kotha, T. A. Gilbertson, R. F. Margolskee, Glucose transporters and ATP-gated K+ (KATP) metabolic sensors are present in type 1 taste receptor 3 (T1r3)-expressing taste cells. *Proc Natl Acad Sci U S A* **108**, 5431-5436 (2011).
27. A. A. Bachmanov, M. G. Tordoff, G. K. Beauchamp, Sweetener preference of C57BL/6ByJ and 129P3/J mice.

Chem Senses **26**, 905-913 (2001).
28. P. Jiang, M. Cui, B. Zhao, L. A. Snyder, L. M. J. Benard, R. Osman, M. Max, R. F. Margolskee, Identification of the Cyclamate Interaction Site within the Transmembrane Domain of the Human Sweet Taste Receptor Subunit T1R3. *Journal of Biological Chemistry* **280**, 34296-34305 (2005).
29. P. Jiang, M. Cui, B. Zhao, Z. Liu, L. A. Snyder, L. M. J. Benard, R. Osman, R. F. Margolskee, M. Max, Lactisole Interacts with the Transmembrane Domains of Human T1R3 to Inhibit Sweet Taste. *Journal of Biological Chemistry* **280**, 15238-15246 (2005).
30. J. Yang, Y. Zhang, I-TASSER server: new development for protein structure and function predictions. *Nucleic Acids Res* **43**, W174-181 (2015).
31. Y. Ben Shoshan-Galeczki, M. Y. Niv, Structure-based screening for discovery of sweet compounds. *Food Chemistry* **315**, 126286 (2020).
32. T. D. Goddard, C. C. Huang, E. C. Meng, E. F. Pettersen, G. S. Couch, J. H. Morris, T. E. Ferrin, UCSF ChimeraX: Meeting modern challenges in visualization and analysis. *Protein Sci* **27**, 14-25 (2018).
33. G. Q. Zhao, Y. Zhang, M. A. Hoon, J. Chandrashekar, I. Erlenbach, N. J. P. Ryba, C. S. Zuker, The Receptors for Mammalian Sweet and Umami Taste. *Cell* **115**, 255-266 (2003).
34. N. Chaudhari, S. D. Roper, The cell biology of taste. *J Cell Biol* **190**, 285-296 (2010).
35. A. Dagan-Wiener, A. Di Pizio, I. Nissim, M. S. Bahia, N. Dubovski, E. Margulis, M. Y. Niv, BitterDB: taste ligands and receptors database in 2019. *Nucleic Acids Research* **47**, D1179-D1185 (2019).
36. A. Dagan-Wiener, I. Nissim, N. Ben Abu, G. Borgonovo, A. Bassoli, M. Y. Niv, Bitter or not? BitterPredict, a tool for predicting taste from chemical structure. *Scientific Reports* **7**, 12074 (2017).
37. P. Cioni, G. B. Strambini, Effect of heavy water on protein flexibility. *Biophysical Journal* **82**, 3246-3253 (2002).
38. S. Gane, D. Georganakis, K. Maniati, M. Vamvakias, N. Ragoussis, E. M. C. Skoulakis, L. Turin, Molecular Vibration-Sensing Component in Human Olfaction. *PLOS ONE* **8**, e55780 (2013).
39. E. Block, S. Jang, H. Matsunami, V. S. Batista, H. Zhuang, Reply to Turin et al.: Vibrational theory of olfaction is implausible. *Proceedings of the National Academy of Sciences* **112**, E3155 (2015).
40. L. B. Vosshall, Laying a controversial smell theory to rest. *Proceedings of the National Academy of Sciences* **112**, 6525 (2015).
41. J. A. Mennella, D. R. Reed, P. S. Mathew, K. M. Roberts, C. J. Mansfield, "A Spoonful of Sugar Helps the Medicine Go Down": Bitter Masking by Sucrose Among Children and Adults. *Chem Senses* **40**, 17-25 (2014).
42. N. Ben Abu, D. Harries, H. Voet, M. Y. Niv, The taste of KCl – What a difference a sugar makes. *Food Chemistry* **255**, 165-173 (2018).
43. N. Dubovski, E. Ert, M. Niv, Bitter mouth-rinse affects emotions. *Food Quality and Preference* **60**, (2017).
44. E. Trinquet, M. Fink, H. Bazin, F. Grillet, F. Maurin, E. Bourrier, H. Ansanay, C. Leroy, A. Michaud, T. Durroux, D. Maurel, F. Malhaire, C. Goudet, J.-P. Pin, M. Naval, O. Hernout, F. Chrétien, Y. Chapleur, G. Mathis, d-myo-Inositol 1-phosphate as a surrogate of d-myo-inositol 1,4,5-tris phosphate to monitor G protein-coupled receptor activation. *Analytical Biochemistry* **358**, 126-135 (2006).
45. R. Zhang, X. Xie, Tools for GPCR drug discovery. *Acta Pharmacol Sin* **33**, 372-384 (2012).
46. G. P. Hussmann, R. P. Yasuda, Y. Xiao, B. B. Wolfe, K. J. Kellar, Endogenously expressed muscarinic receptors in HEK293 cells augment up-regulation of stably expressed α4β2 nicotinic receptors. *J Biol Chem* **286**, 39726-39737 (2011).
47. T. Ueda, S. Ugawa, H. Yamamura, Y. Imaizumi, S. Shimada, Functional interaction between T2R taste receptors and G-protein alpha subunits expressed in taste receptor cells. *Journal of Neuroscience* **23**, 7376-7380 (2003).
48. M. Behrens, K. Blank, W. Meyerhof, Blends of Non-caloric Sweeteners Saccharin and Cyclamate Show Reduced Off-Taste due to TAS2R Bitter Receptor Inhibition. *Cell Chemical Biology* **24**, 1199-+ (2017).
49. J. S. Mason, A. Bortolato, M. Congreve, F. H. Marshall, New insights from structural biology into the druggability of G protein-coupled receptors. *Trends Pharmacol Sci* **33**, 249-260 (2012).
50. A. J. Venkatakrishnan, A. K. Ma, R. Fonseca, N. R. Latorraca, B. Kelly, R. M. Betz, C. Asawa, B. K. Kobilka, R. O. Dror, Diverse GPCRs exhibit conserved water networks for stabilization and activation. *Proc Natl Acad Sci U S A* **116**, 3288-3293 (2019).
51. A. S. Bayden, D. T. Moustakas, D. Joseph-McCarthy, M. L. Lamb, Evaluating Free Energies of Binding and Conservation of Crystallographic Waters Using SZMAP. *Journal of Chemical Information and Modeling* **55**, 1552-1565 (2015).
52. V. Galindo-Cuspinera, M. Winnig, B. Bufe, W. Meyerhof, P. A. S. Breslin, A TAS1R receptor-based explanation of sweet 'water-taste'. *Nature* **441**, 354-357 (2006).
53. V. Galindo-Cuspinera, P. A. S. Breslin, The Liaison of Sweet and Savory. *Chem Senses* **31**, 221-225 (2005).
54. S. Sutton, Encyclopedia of Research Design. *Libr J* **135**, 105-106 (2010).

55. S. E. Kemp, T. Hollowood, J. Hort, *Sensory evaluation : a practical handbook*. (Ames Wiley-Blackwell, Chichester, U.K. ;, 2009), pp. xi, 196 p.
56. B. G. Green, G. S. Shaffer, M. M. Gilmore, Derivation and Evaluation of a Semantic Scale of Oral Sensation Magnitude with Apparent Ratio Properties. *Chem Senses* **18**, 683-702 (1993).
57. G. G. G. G. Krivov, M. V. Shapovalov, J. R. L. Dunbrack, R.L. , Improved Prediction of Protein Side-Chain Conformations With SCWRL4 *Proteins* **77**, 778-795 (2009).
58. J. Huang, S. Rauscher, G. Nawrocki, T. Ran, M. Feig, B. L. de Groot, H. Grubmuller, A. D. MacKerell, CHARMM36m: an improved force field for folded and intrinsically disordered proteins. *Nature Methods* **14**, 71-73 (2017).
59. S. Pronk, S. Páll, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M. R. Shirts, J. C. Smith, P. M. Kasson, D. van der Spoel, B. Hess, E. Lindahl, GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics* **29**, 845-854 (2013).
60. W. G. Hoover, Canonical dynamics: Equilibrium phase-space distributions. *Physical Review A* **31**, 1695-1697 (1985).
61. M. Parrinello, A. Rahman, Polymorphic transitions in single crystals: A new molecular dynamics method. *Journal of Applied Physics* **52**, 7182-7190 (1981).
62. J. R. Grigera, An effective pair potential for heavy water. *Journal of Chemical Physics* **114**, 8064-8067 (2001).
63. B. Hess, Determining the shear viscosity of model liquids from molecular dynamics simulations. *The Journal of Chemical Physics* **116**, 209-217 (2002).
64. H. J. C. Berendsen, J. R. Grigera, T. P. Straatsma, The Missing Term in Effective Pair Potentials. *Journal of Physical Chemistry* **91**, 6269-6271 (1987).