# T1R3 subunit of the sweet taste receptor is activated by $D_2O$ in transmembrane domain-dependent manner

Natalie Ben Abu, Yaron Ben Shoshan-Galeczki, Einav Malach and Masha Y. Niv#

The Institute of Biochemistry, Food Science and Nutrition, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel
# correspondence to masha.niv@mail.huji.ac.il

## Abstract

Deuterium oxide ($D_2O$) is a water molecule in which both hydrogens are replaced by the heavier and rare isotope deuterium. We have previously shown that $D_2O$ has distinct sweet taste, which is mediated by the T1R2/T1R3 sweet taste receptor. Here we explore the effect of heavy water on T1R2 and T1R3 subunits. We show that $D_2O$ activates T1R3 transfected HEK293T cells similarly to T1R2/T1R3 transfected cells. The response to glucose dissolved in $D_2O$ is higher than to glucose dissolved in water. Mutations of Phenylalanine at position $730^{5.40}$ in the transmembrane domain of T1R3 to Alanine, Leucine or Tyrosine, impair or diminish activation by $D_2O$, suggesting a critical role for T1R3 TMD domain in relaying the heavy water signal.

**Key words:** Deuterium oxide, sweetness, T1R3, TMD, GPCRs, family C

## 1. Introduction

Deuterium Oxide ($D_2O$), commonly referred to as "heavy water'' is a naturally occurring, but extremely rare molecule [7] that differs from regular water ($H_2O$) by H-D isotopic substitution. This results in 10% increase in molecular weight, small differences in pH (+0.44 for $D_2O$), and higher melting and boiling points [5, 24, 27, 34]. Cellular function of mitosis was shown to be inhibited by $D_2O$ in animal and plant cells [26]. In algae, $D_2O$ has been observed to affect membrane depolarization and activation of $Ca^{2+}$ channels [1]. Protozoa, bacteria and yeasts are able to tolerate 70% -100% $D_2O$ [22]. In whole organism studies, small amounts of $D_2O$, usually lower than 20% of body weight, were tolerated well over short time periods [36]. In fact, $D_2O$ and deuterated drugs are used in drugs metabolism studies in humans and other animals, and recently also as novel versions of existing drugs [33]. Effects of deuteration on drugs in enzymatic reactions [38], as well as on interactions with receptors, are studied using cell-based and computational approaches [16, 21]. For example, a recent molecular dynamics simulations study indicates that $D_2O$ has a higher ability to form water−water hydrogen bonds than water−amino acid hydrogen bonds [32]. However, detailed effects of $D_2O$ on protein function remain to be explored.

Here we investigate the effect of heavy water on the human sweet taste receptor, T1R2/T1R3, in cell-based functional assays. The debate regarding the potential taste of heavy water has begun almost 90 years ago [15, 34]. We have recently shown, using an integrated sensory, cell-based and computational approaches study, that humans differentiate between $D_2O$ and $H_2O$ based on taste alone, that $D_2O$ is sweeter than same-purity $H_2O$ and that $D_2O$ may add to the perceived sweetness of sweeteners [4]. Recognition of sweetness is related to T1R subfamily which contains three taste receptors; T1R1, T1R2, and T1R3. The main sweet taste receptor is the T1R2/T1R3 heterodimer of Class C GPCRs [23, 40], though stimulation of sodium-dependent glucose transporter (SGLT1) expressed in sweet taste may also participate in signal transduction of sugars [25]. Lactisole, a known sweetness inhibitor acting via the T1R3 monomer of the T1R2/T1R3 [18], suppressed the sweetness of $D_2O$ for humans, suggesting T1R2/T1R3 as $D_2O$ target. Accordingly, T1R2/T1R3-transfected HEK293T

cells were activated by $D_2O$ and this cellular response was also inhibited by lactisole [4]. Interestingly, similarly to the lack of murine response to the artificial sweetener cyclamate [2], mice did not show preference for $D_2O$ over regular water, raising the hypothesis that the site of interaction of $D_2O$ with the receptor may be close to the binding site of cyclamate [4].

T1Rs proteins have three main domains: the large N-terminal "Venus flytrap" domain (VFT) which was shown as the orthosteric site for sugar binding by numerous site-directed mutagenesis studies [13]; the Cysteine rich domain (CRD) that contains nine highly conserved cysteines and is targeted mainly by sweet proteins, and the seven helical transmembrane domain (TMD)[6]. The TMD binding site is analogous to the orthosteric site in Family A GPCRs, and in human hT1R3 was shown to accommodate cyclamate, with F730 (5.40 BW position [3] of the T1R3 (http://www.gpcrdb.org/)) established as important for activation [19]. Previous highlighted the impact of T1R2 and T1R3 single nucleotide polymorphisms (SNPs) on consumption of sugars as well as on sweet products [11, 29]. Examining activation of the receptors expressed in HEK293T cells, Dubovski et al. showed differences in sensitivity comparing two sequences of T1R2: the older reference sequence (NM_152232.1) which contains four of the SNPs - I191V, R317G, I486V and S9C and an updated reference sequence (NM_152232.4). Residue R317 in the VFT domain of T1R2, was shown to be responsible for this difference. Furthermore, they showed that site-directed mutagenesis of Serine at position 147 in the binding site of T1R3 VFT domain to Alanine, abolished receptor activation by D and L glucose enantiomers [10].

Interestingly, T1R3 without T1R2 (possibly functioning as a homodimer) was shown to respond to monosaccharides and disaccharides [10, 39]. This is of physiological relevance, since T1R3 (but not T1R2) expression was shown in the gastrointestinal tract [25] pancreas and liver [9, 31].

Here we investigate whether T1R2 or T1R3 may react to $D_2O$ when expressed without the other unit, and test our hypothesis regarding the key role of T1R3 TMD domain in sensitivity to heavy water.

## 2. Experimental Procedures

*Materials* – Cyclamate, D-glucose, powder Dulbecco's Modified Eagle's Medium (DMEM), were purchased from Sigma-Aldrich (St. Louis, Missouri, United States). Deuterium Oxide ($D_2O$) was purchased from Tzamal D-Chem Laboratories Ltd (Kiryat-Matalon, Petach Tikva, Israel). Unless otherwise noted, the typical concentration of tested solutions was as follows: cyclamate (0.01M), $D_2O$ (49.9M) and D-glucose (0.96M).

*Cell Culture and IP - One HTRF Assay* – Human embryonic kidney 293T (HEK293T from ATCC) cells were cultured, maintained, and transiently transfected as described previously [4]. All treatments were done in triplicate, and all experiments were repeated at least three times. In general, cells were grown to a confluency of approximately 85-90 % and transiently transfected with plasmids encoding T1R proteins (T1R2, T1R3) and Gα16gust44 (a chimeric G protein and subunit containing the last 44 amino acids of gustducin). After 24h, cells were seeded in 24-well culture plates (0.5 ml cells per well) and maintained for 8-12 h at 37 °. Next, cells were "starved" overnight by changing the medium to 0.1% DMEM (containing 0.1% FBS) without glucose, aiming to reduce the basal activity of the cells. After an additional 18 h, cells were exposure to tested compounds by addition of 0.5ml of each with 50 mM Lithium Chloride (LiCl) needed for IP-One accumulation (based on Cisbio IP-One HTRF assay) and dissolved in 0.1 % DMEM (without glucose), for 5 minutes. In case of monitoring the effect of heavy water, 0.1 % DMEM medium was made using $D_2O$ instead of $D_2O$ water. Upon exposure time, tastant solution was replaced with fresh medium (0.1 % DMEM) containing 50 mM LiCl for another 1h and then washed with 100µl cold phosphate buffered saline (PBS) + Triton X-100, and kept at -80◦C for at least 30min, in order to dissolve the cell membrane. Cell lysate was mixed with the IP-One HTRF detection reagents (IP1-d2 conjugate and Anti-IP1 cryptate TB conjugate), and added to each well in a 384-well plate for 60min incubation at room temperature. Changes in IP-One levels were read using Clariostar plate reader (665nm/620nm emission ratio).

*Statistical analysis* – Dose – response curves were fitted by non-linear regression using the algorithms of PRISM 7 (GraphPad Software, San Diego, CA, USA). All responses are

presented as the means ± SEM of IP1 accumulation (%) and were normalized based on maximum response of the full sweet taste heterodimer receptor T1R2/T1R3 to 0.96M glucose (Emax=100%), as well as tested in comparison to basal activity.

*Mutants*— Site-directed mutagenesis in T1R3 was performed by Transfer-PCR (TPCR), as described previously [12]. All mutants were activated by glucose, indicating that amino acids substation at these positions did not affect protein expression.

*Water mapping* – Mapping of water molecules was calculated on the AlphaFold2 [20] model of hT1R3 (Uniprot ID: Q7RTX0). The model was downloaded from the AlphaFold2 EBI database (https://alphafold.ebi.ac.uk), and then prepared and minimized with Schrödinger Maestro protein preparation panel (Maestro Version 12.7.161, MMshare Version 5.3.161, Release 2021-1, Platform Windows-x64). The minimized receptor model was then used to map possible water molecule positions near F730 using OpenEye SZMAP with default settings (SZMAP 1.6.5.2: OpenEye Scientific Software, Santa Fe, NM. http://www.eyesopen.com, OpenEye Applications & Toolkits 2022.1.2).

## 3. Results

### *$D_2O$ activates the T1R3 subunit*

To assess which of the T1R subunits of the heterodimeric human sweet receptor is required for sensing $D_2O$. Hence, we expressed separately each one of the human T1R subunits; T1R2 or T1R3, by transient transfection in HEK293T cells, along with Gα16gust44. Activation of the receptor was monitored by IP1 accumulation. 960mM of D-glucose was used here as control for each transfection, based on previous work [4, 10].

As seen in Figure 1A, T1R3 expressed without T1R2 responded to $D_2O$ similarly to the response of T1R2/T1R3 whereas the T1R2 expressed without T1R3 did not (p.v ≤0.0001). As expected, D-glucose caused IP1 elevation in all types of transfections. Figure 1B illustrated a similar experiment in functional cell assay: we find that 960mM D-glucose dissolved in $D_2O$ elicited significantly higher (p.v ≤ 0.005) IP1 values

compared to the same concentration dissolved in $H_2O$, whereas no significant difference was observed for cyclamate. This result raises the possibility that cyclamate and heavy water share a common binding region.

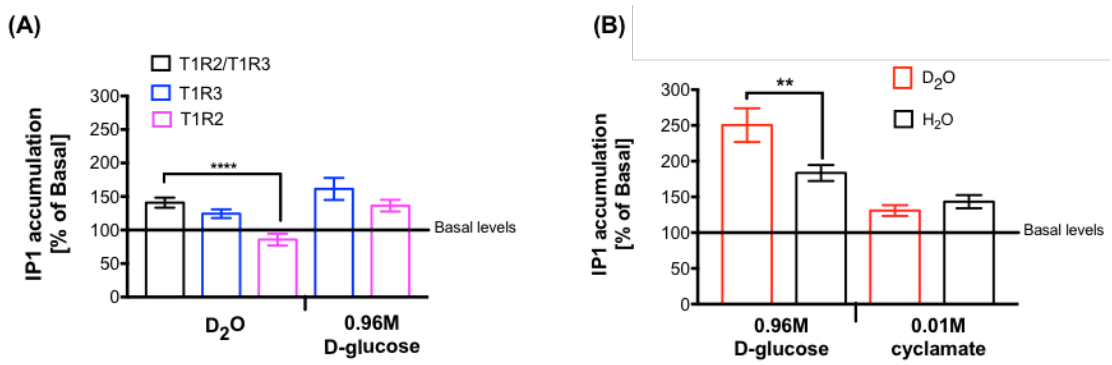


**Fig. 1. $D_2O$ signaling requires T1R3 subunit. (A)** The receptors composed of T1R2(NM_152232.1)/T1R3(black), T1R3(blue) or T1R2(NM_152232.1, pink) were co-expressed by transient transfection in HEK293T cells along with Gα16gust44 and tested toward their responses to $D_2O$ in comparison to the positive control of D-glucose. **(B)** The effect of $D_2O$ (red) on T1R2/T1R3 activation elicited by different sweet molecules. On y-axis, changes in IP1 accumulation upon stimulus application are shown as % of basal – pure $H_2O$; x-axis,100% $D_2O$, 0.96M glucose or 0.01M cyclamate. Asterisks indicate IP1 changes that are significantly different from basal (* for $p \leq 0.05$, ** for $p \leq 0.005$ and **** for $p \leq 0.0001$) using t-test. All tested solutions were done in at least triplicate; each experiment (transfection) was repeated three to five times.

We next tested varying concentrations of heavy water for different types of transfections. $D_2O$ dose response curves were obtained for cells expressing T1R3 (Emax=50% of activation by 0.96M glucose, which was used as reference), while cells transfected with T1R2 subunit were not activated (Fig. 2A). Dubovski et al. showed that the T1R2 (NM_152232.4) is more sensitive to presence of D-glucose [10]. In order to understand whether these SNPs affect the sensitivity of activation by $D_2O$, we compared the dose response curves of HEK293T cells, transfected with either the T1R2 (NM_152232.1) or the more sensitive version (NM_152232.4), along with T1R3. As shown in Fig. 2B, we observed only minor differences in responses to $D_2O$ by these two versions of T1R2 (NM_152232.1 Emax = 60% and Emax = 50% for NM_152232.4), confirming that T1R3 subunit play the dominant role in $D_2O$ activation.

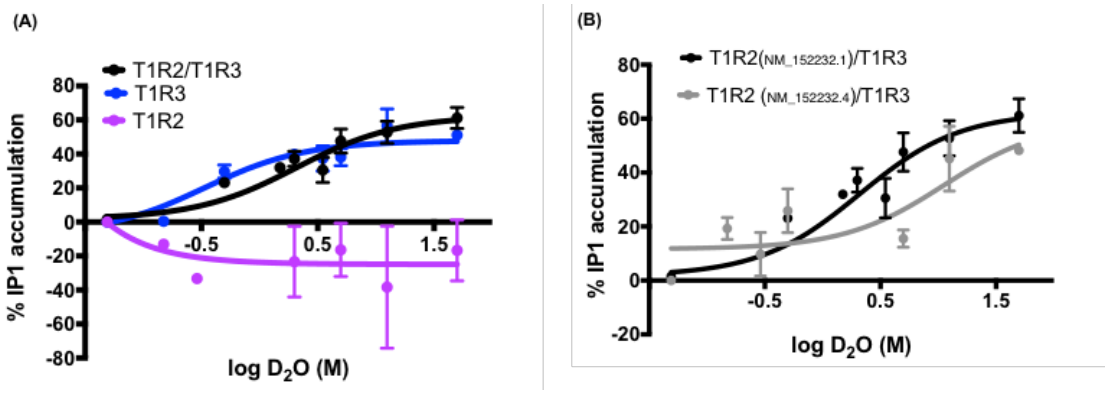


**Fig. 2. $D_2O$ dose response curve characterization. (A)** T1R3 transfected HEK293T cells achieved $D_2O$ dose-responsive manner. Varying concentrations of deuterium Oxide were tested among different transfections; T1R2(NM_152232.1)/T1R3(black), T1R3/T1R3(blue) or T1R2/T1R2(NM_152232.1, pink). **(B)** Dose-response curves of HEK293T cells transiently transfected with T1R2(NM_152232.1)/T1R3 (black); or T1R2(NM_152232.4)/T1R3 (grey); All data were normalized based on maximum response of the full sweet taste heterodimer receptor [10]T1R2/T1R3 to 0.96M glucose. All tested solutions were done in at least triplicate; each experiment (transfection) was repeated three to five times.

### *Transmembrane domain of hT1R3 is involved in $D_2O$ response*

Once we have established that the T1R3 is essential and sufficient for activation by $D_2O$, we aimed to identify the responsible sub-domain in T1R3. First, we tested the effect of alanine mutations in residues: 730 (F730A, the TMD domain) and 147 (S147A, VFT domain, see Fig 3A for location on the receptor and 3B for human-mouse sequence alignment in the relevant regions on T1R3 activation by $D_2O$. T1R3 plasmids of T1R3F730A or T1R3S147A were transfected together with Gα16gust44 (without T1R2), and compared to WT T1R3. (Fig. 3C). None of the mutants was activated by $D_2O$; yet IP1 levels for F730A were significantly lower than WT basal activity ($p.v \leq 0.0001$) while the activation of S147A was similar to WT basal activity. Importantly, cells transfected with either one of the alanine mutants of T1R3, were activated by 0.96M of D-glucose ($p.v<0.05$; Fig. SI.2).

As seen in dose response curves in Fig. 3D, both F730 and S147A mutants dramatically reduced Emax values, but cells transfected with F730A mutant had completely abolished the activation by $D_2O$, while S147A mutant was still active.

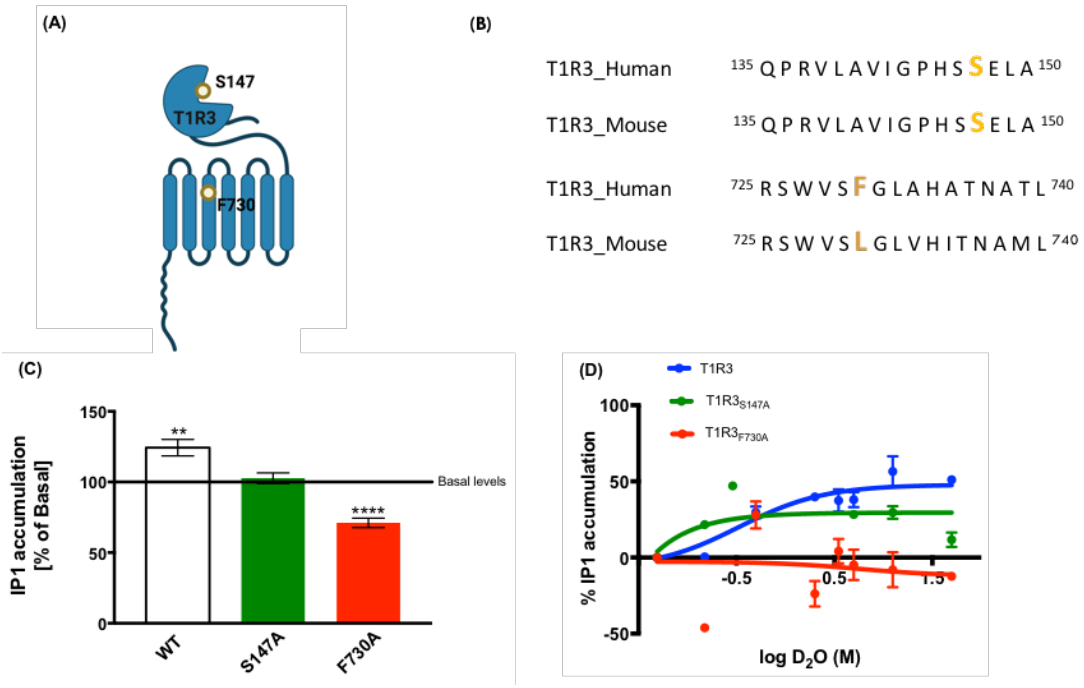


**Fig. 3. Involvement of TM helix 5 of hT1R3 in $D_2O$ binding. (A)** Schematic picture of T1R3 subunit with domain-specific tested residues. **(B)** The alignment of human and mouse T1R3 sequences with an emphasis (orange) on the tested residues. **(C)** The effect of T1R3 F730A or T1R3 S147A mutant on activation by 100% $D_2O$. T1R3 mutated human-receptors were co-expressed by transient transfection in HEK293T cells along with Gα16gust44. Asterisks indicate IP1 changes that are significantly different from basal levels (*$p \leq 0.05$ **$p \leq 0.005$ and ****$p \leq 0.0001$) using *t*-test. **(D)** The effect of alanine mutants in putative $D_2O$-binding areas in the hT1R3 on $D_2O$ response. All data were normalized based on maximum response of the full sweet taste heterodimer receptor T1R2/T1R3 to 0.96M glucose.

### *Exploring the 5.40 position*

S147A mutant retained significant responsiveness to $D_2O$, while F730A mutant showed no activity. The alignment of human and mouse T1R3 sequences shows that, at position 147 (VFT domain) both human and mouse have Serine, while at position 730 (5.40 TMD differs between these two species (Fig. 3B). We have also established a different behavioral effect to $D_2O$ of human and mice [4] and therefore focused on residue 730. In human, this residue is an aromatic amino acid Phenylalanine, which is predicted to interact with additional aromatic residues (Fig. 4A), while in mice this position harbors Leucine. Hence, we aimed to check the importance of aromaticity in this area. We mutated Phenylalanine (aromatic and hydrophobic) to Leucine (aliphatic and hydrophobic) and Tyrosine (aromatic). F730L mutant showed a complete loss of

responsiveness to $D_2O$, similarly to F730A, while F730Y still had some activity (Emax=20%) (Fig. 3B). We next confirmed that the effect of 730 T1R3 residue remains also in the framework of the full transfection (T1R2/T1R3) as seen in Figure 3C.

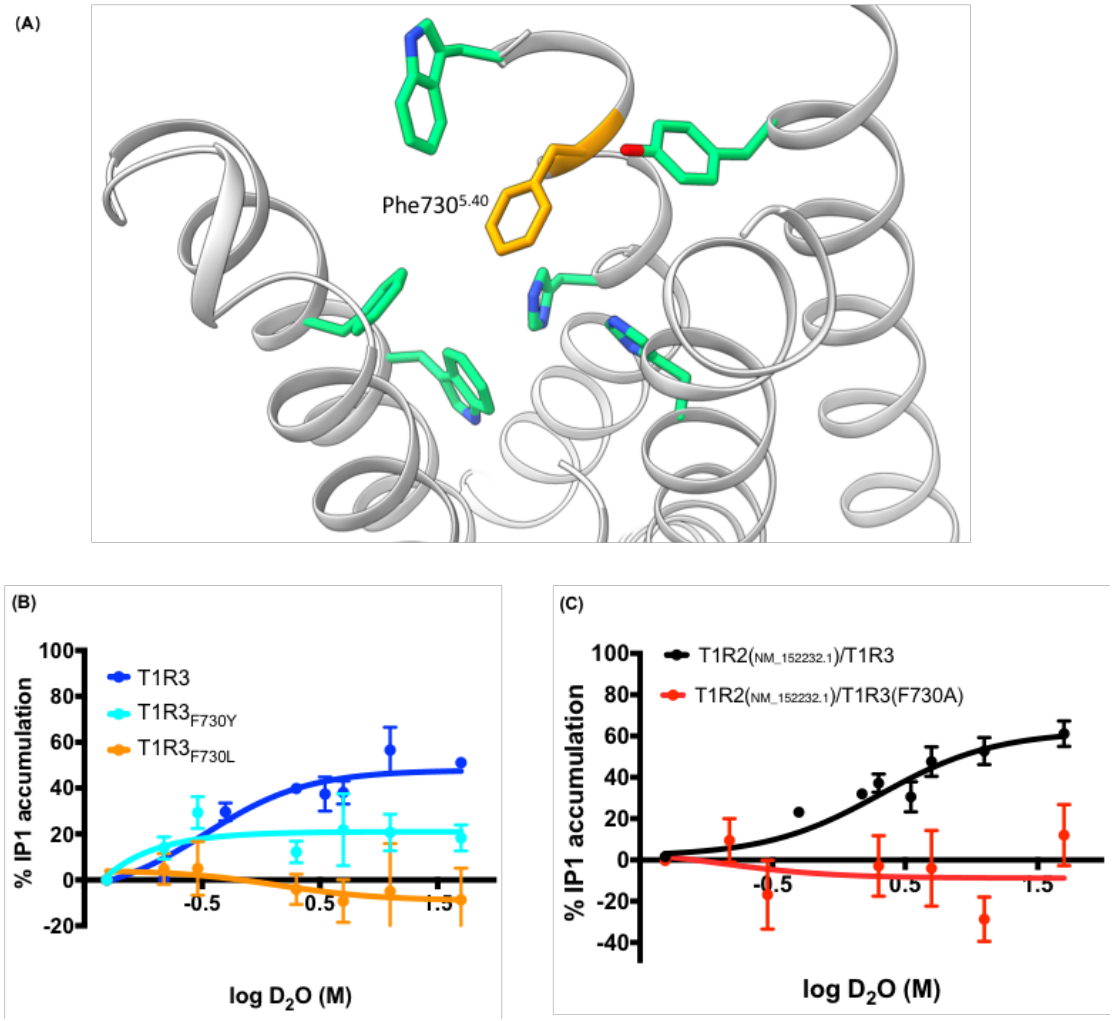


**Fig. 3. The importance of residue 730 in TMD pocket of T1R3. A.** Snapshot of potential biding area of $D_2O$ to T1R3 based on AlphaFold model in which we found out that surrounding residues (within 4 angstrom) of F730$^{5.40}$ are mainly aromatic amino acids. **B.** Dose-response profiles toward $D_2O$ of T1R3 F730$^{5.40}$ mutants – substitution to tyrosine (cyan) and leucine (orange). **C.** The effect of F730A mutant of T1R3 on full transfected cells; T1R2(NM_152232.1)/T1R3(black), T1R2/T1R3F730A (red). T1R3 mutated human-receptors were co-expressed by transient transfection in HEK293T cells along with Gα16gust44 alone or with T1R2 (NM_152232.1) for full transfected cells. All data were normalized based on maximum response of the full sweet taste heterodimer receptor T1R2/T1R3 to 960mM glucose

## 4. Discussion

In this study, we continued to characterize the effects of $D_2O$ on human sweet taste receptor. This followed our previous work that showed $D_2O$ is mildly sweet to humans

(and not to mouse) and that this sweetness is mediated via T1R2/T1R3 heterodimer [4]. In the present study, we showed that $D_2O$ can also active T1R3 expressed HEK293T cells without the T1R2 monomer. The responses of HEK293T cells were characterized and showed similar dose-response curves and Emax values. The more sensitive variant of T1R2 (NM_152232.4) did not affect the $D_2O$-elicited dose response. These outcomes pointed to T1R3 as the essential subunit for receptor activation by $D_2O$. Furthermore, $D_2O$ had an added effect to the response elicited by 0.96M D-glucose, which had been shown as recognized by the VFT domain of T1R3 [10]. However, $D_2O$ did not have the same effect on the activation elicited by 0.01M cyclamate, a sweetener that had been shown to be detected by the TMD of T1R3 (Jiang, Cui et al. 2005). This is similar to our previous finding that mice were not attracted to heavy water [4]. Together, these indications supported the hypothesis that the interaction site of $D_2O$ with the T1R3 receptor should be near the binding site of cyclamate in the TMD of T1R3.

Here, we measured D-glucose to define which mutations (F730A in the T1R3 TMD or S147A in the T1R3 VFT) may cause lower activity. Both mutants were activated by D-glucose, thus, indicating that T1R3 remained an active protein, but to a lower degree, even though D-glucose is recognized by VFT domain, but probably not the TMD of T1R3. The indirect effect could be explained by reduced trafficking to the cell membrane, reduced receptor stability [14, 30, 37] or indirect allosteric effects [28]. Importantly, our experimental results showed that for $D_2O$, mutation F730A completely abolished activation by $D_2O$, while S147A remained active, albeit with dramatically reduced Emax value. We have therefore concluded that the TMD region of T1R3 is critically important for the activation of this receptor by heavy water.

A CryoEM structure of class C orphan GPCR, GPR15, was recently solved [17]. Residue $D579^{5.40}$, equivalent to $F730^{5.40}$ in T1R3, as well as in other class C GPCRs, belongs to ionic locks network and takes part in stabilization of transmembrane region. This position had been shown to be part of the allosteric binding sites in class C GPCR metabotropic glutamate receptor 5 [8, 28]. Due to different residues in position 730 of T1R3 in human (Phenylalanine - aromatic and hydrophobic) and in mouse (Leucine - non-aromatic and hydrophobic), we explored additional mutations there. Indeed,

F730L mutation completely abolished the response to $D_2O$, just like the F730A mutation. F730Y however, had a similar effect to S147A, with only reduced $D_2O$ activity. F730A and F730L mutations also abolished the response to cyclamate [19].

Computational water mapping near residue F730, indicated that water molecules can potentially get into the TMD pocket (Fig. SI2). This is in accord with other studies of GPCRs that confirmed that many of these receptors have transmembrane water molecules [35]. Hence, substitution of normal water molecules with $D_2O$ in this region could lead to stabilization of the active conformation, thus explaining the difference in receptor activity compared to regular water. Furthermore, both proteins and biomembranes tend to be slightly more compact and rigid in $D_2O$ that has stronger hydrogen bonding, than in $H_2O$ [32], suggesting a possibility for additional indirect effects of heavy water. How exactly heavy water affects T1R3 conformations, and how the effects differ in other T1Rs, present intriguing topics for further studies.

In summary, here we demonstrate that heavy water elicits functional response of the T1R3 subunit, but not through the T1R2 subunit of the sweet taste receptor, and that T1R3 activation depends on the residue found in position 5.40. Our findings are important since heavy water is used in clinical procedures, and T1R3 is expressed not only on the tongue but also in extra-oral tissues. Moreover, elucidation of effects of $D_2O$ on some but not other Family C GPCRs will help in understanding of the role of water in family C GPCRs function.

## 5. Acknowledgments

The authors thank Dr. R. F. Margolskee for the pcDNA3 of chimeric Gα16gust44and Dr. Maik Behrens for the pcDNA3 of T1R3 and for the pcDNA5FRT PM of T1R2S9C, I191V, R317G, I486V. We thank Dr. Yoav Peleg for help with sequencing and site directed mutagenesis; and for the construction of pcDNA5 of NM_152232.4, pcDNA5 of T1R3F730A, pcDNA5 of T1R3 F730L, pcDNA5 of T1R3F730Y, and pcDNA3 of TIR3S147A, and Prof. Michael Naim for helpful discussions. The study was supported by ISF grant #1129/19.

## 6. References


1 P. R. Andjus, and D. Vučelić, 'D2o-Induced Cell Excitation', *The Journal of Membrane Biology,* 115 (1990), 123-27.

2 Alexander A. Bachmanov, Michael G. Tordoff, and Gary K. Beauchamp, 'Sweetener Preference of C57bl/6byj and 129p3/J Mice', *Chemical Senses,* 26 (2001), 905-13.

3 Juan A. Ballesteros, and Harel Weinstein, '[19] Integrated Methods for the Construction of Three-Dimensional Models and Computational Probing of Structure-Function Relations in G Protein-Coupled Receptors', in *Methods in Neurosciences*, ed. by Stuart C. Sealfon (Academic Press, 1995), pp. 366-428.

4 Natalie Ben Abu, Philip E. Mason, Hadar Klein, Nitzan Dubovski, Yaron Ben Shoshan-Galeczki, Einav Malach, Veronika Pražienková, Lenka Maletínská, Carmelo Tempra, Victor Cruces Chamorro, Josef Cvačka, Maik Behrens, Masha Y. Niv, and Pavel Jungwirth, 'Sweet Taste of Heavy Water', *Communications Biology,* 4 (2021), 440.

5 Robert AJ Bogan, Shigeru Ohde, Takeshi Arakaki, Ikuko Mori, and Cameron W McLeod, 'Changes in Rainwater Ph Associated with Increasing Atmospheric Carbon Dioxide after the Industrial Revolution', *Water, air, and soil pollution,* 196 (2009), 263-71.

6 Jean-Baptiste Chéron, Jérôme Golebiowski, Serge Antonczak, and Sébastien Fiorucci, 'The Anatomy of Mammalian Sweet Taste Receptors', *Proteins: Structure, Function, and Bioinformatics,* 85 (2017), 332-41.

7 W. Dansgaard, 'Stable Isotopes in Precipitation', *Tellus,* 16 (1964), 436-68.

8 A. S. Doré, K. Okrasa, J. C. Patel, M. Serrano-Vega, K. Bennett, R. M. Cooke, J. C. Errey, A. Jazayeri, S. Khan, B. Tehan, M. Weir, G. R. Wiggin, and F. H. Marshall, 'Structure of Class C Gpcr Metabotropic Glutamate Receptor 5 Transmembrane Domain', *Nature,* 511 (2014), 557-62.

9 N. Dubovski, F. Fierro, E. Margulis, Y. Ben Shoshan-Galeczki, L. Peri, and M. Y. Niv, 'Taste Gpcrs and Their Ligands', *Prog Mol Biol Transl Sci,* 193 (2022), 177-93.

10 Nitzan Dubovski, Yaron Ben-Shoshan Galezcki, Einav Malach, and Masha Y Niv, 'Sensitivity of Human Sweet Taste Receptor Subunits T1r2 and T1r3 to Activation by Glucose Enantiomers', *Chemical Senses* (2023).

11 K. M. Eny, T. M. Wolever, P. N. Corey, and A. El-Sohemy, 'Genetic Variation in Tas1r2 (Ile191val) Is Associated with Consumption of Sugars in Overweight and Obese Individuals in 2 Distinct Populations', *Am J Clin Nutr,* 92 (2010), 1501-10.

12 A. Erijman, A. Dantes, R. Bernheim, J. M. Shifman, and Y. Peleg, 'Transfer-Pcr (Tpcr): A Highway for DNA Cloning and Protein Engineering', *J Struct Biol,* 175 (2011), 171-7.

13 Ranier Gutierrez, Esmeralda Fonseca, and Sidney A. Simon, 'The Neuroscience of Sugars in Taste, Gut-Reward, Feeding Circuits, and Obesity', *Cellular and Molecular Life Sciences,* 77 (2020), 3469-502.

14 M. K. Hahn, M. S. Mazei-Robison, and R. D. Blakely, 'Single Nucleotide Polymorphisms in the Human Norepinephrine Transporter Gene Affect Expression, Trafficking, Antidepressant Interaction, and Protein Kinase C Regulation', *Mol Pharmacol,* 68 (2005), 457-66.

15 Klaus Hansen, and Erling Rustung, 'Untersuchungen Über Die Biologischen Wirkungen Von „Schwerem Wasser "Bei Warmblütigen Tieren', *Klinische Wochenschrift,* 14 (1935), 1489-93.

16 Lucija Hok, Janez Mavri, and Robert Vianello, 'The Effect of Deuteration on the H2 Receptor Histamine Binding Profile: A Computational Insight into Modified Hydrogen Bonding Interactions', *Molecules,* 25 (2020), 6017.

17 Eunyoung Jeong, Yoojoong Kim, Jihong Jeong, and Yunje Cho, 'Structure of the Class C Orphan Gpcr Gpr158 in Complex with Rgs7-Gβ5', *Nature Communications,* 12 (2021), 6805.

18 Peihua Jiang, Meng Cui, Baohua Zhao, Zhan Liu, Lenore A. Snyder, Lumie M. J. Benard, Roman Osman, Robert F. Margolskee, and Marianna Max, 'Lactisole Interacts with the Transmembrane Domains of Human T1r3 to Inhibit Sweet Taste*', *Journal of Biological Chemistry,* 280 (2005), 15238-46.

19 Peihua Jiang, Meng Cui, Baohua Zhao, Lenore A. Snyder, Lumie M. J. Benard, Roman Osman, Marianna Max, and Robert F. Margolskee, 'Identification of the Cyclamate Interaction Site within the Transmembrane Domain of the Human Sweet Taste Receptor Subunit T1r3 * [Boxs]', *Journal of Biological Chemistry,* 280 (2005), 34296-305.

20 John Jumper, Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger, Kathryn Tunyasuvunakool, Russ Bates, Augustin Žídek, Anna Potapenko, Alex Bridgland, Clemens Meyer, Simon A. A. Kohl, Andrew J. Ballard, Andrew Cowie, Bernardino Romera-Paredes, Stanislav Nikolov, Rishub Jain, Jonas Adler, Trevor Back, Stig Petersen, David Reiman, Ellen Clancy, Michal Zielinski, Martin Steinegger, Michalina Pacholska, Tamas Berghammer, Sebastian Bodenstein, David Silver, Oriol Vinyals, Andrew W. Senior, Koray Kavukcuoglu, Pushmeet Kohli, and Demis Hassabis, 'Highly Accurate Protein Structure Prediction with Alphafold', *Nature,* 596 (2021), 583-89.

21 Mojca Kržan, Robert Vianello, Aleksandra Maršavelski, Matej Repič, Maja Zakšek, Kristina Kotnik, Estera Fijan, and Janez Mavri, 'The Quantum Nature of Drug-Receptor Interactions: Deuteration Changes Binding Affinities for Histamine Receptor Ligands', *PLOS ONE,* 11 (2016), e0154002.

22 D. J. Kushner, A. Baker, and T. G. Dunstall, 'Pharmacological Uses and Perspectives of Heavy Water and Deuterated Compounds', *Can J Physiol Pharmacol,* 77 (1999), 79-88.

23 Xiaodong Li, Lena Staszewski, Hong Xu, Kyle Durick, Mark Zoller, and Elliot Adler, 'Human Receptors for Sweet and Umami Taste', *Proceedings of the National Academy of Sciences,* 99 (2002), 4692-96.

24 F Macdonald, and DR Lide, 'Crc Handbook of Chemistry and Physics: From Paper to Web', in *Abstracts of Papers of the American Chemical Society* (Amer Chemical Soc 1155 16TH ST, NW, WASHINGTON, DC 20036 USA, 2003), pp. U552-U52.

25 R. F. Margolskee, J. Dyer, Z. Kokrashvili, K. S. Salmon, E. Ilegems, K. Daly, E. L. Maillet, Y. Ninomiya, B. Mosinger, and S. P. Shirazi-Beechey, 'T1r3 and Gustducin in Gut Sense Sugars to Regulate Expression of Na+-Glucose Cotransporter 1', *Proc Natl Acad Sci U S A,* 104 (2007), 15075-80.

26 Douglas Marsland, Lewis G. Tilney, and Michael Hirshfield, 'Stabilizing Effects of D2o on the Microtubular Components and Needle-Like Form of Heliozoan Axopods: A Pressure-Temperature Analysis', *Journal of Cellular Physiology,* 77 (1971), 187-93.

27 Francesco Paesani, and Gregory A. Voth, 'The Properties of Water: Insights from Quantum Simulations', *The Journal of Physical Chemistry B,* 113 (2009), 5702-19.

28 Margherita Persechino, Janik Björn Hedderich, Peter Kolb, and Daniel Hilger, 'Allosteric Modulation of Gpcrs: From Structural Insights to in Silico Drug Discovery', *Pharmacology & Therapeutics,* 237 (2022), 108242.

29 M. B. Pioltine, M. E. de Melo, and A. S. Santos, 'Genetic Variations in Sweet Taste Receptor Gene Are Related to Chocolate Powder and Dietary Fiber Intake in Obese Children and Adolescents', 8 (2018).

30 Christopher M. Tan, Ashley E. Brady, Hilary Highfield Nickols, Qin Wang, and Lee E. Limbird, 'Membrane Trafficking of G Protein–Coupled Receptors', *Annual Review of Pharmacology and Toxicology,* 44 (2004), 559-609.

31 K. Taniguchi, 'Expression of the Sweet Receptor Protein, T1r3, in the Human Liver and Pancreas', *J Vet Med Sci,* 66 (2004), 1311-4.

32 Carmelo Tempra, Victor Cruces Chamorro, and Pavel Jungwirth, 'Effects of Water Deuteration on Thermodynamic and Structural Properties of Proteins and Biomembranes', *The Journal of Physical Chemistry B,* 127 (2023), 1138-43.

33 Graham S. Timmins, 'Deuterated Drugs: Where Are We Now?', *Expert Opinion on Therapeutic Patents,* 24 (2014), 1067-75.

34 HC Urey, and G Failla, 'Concerning the Taste of Heavy Water', *Science,* 81 (1935), 273-73.

35 A. J. Venkatakrishnan, A. K. Ma, R. Fonseca, N. R. Latorraca, B. Kelly, R. M. Betz, C. Asawa, B. K. Kobilka, and R. O. Dror, 'Diverse Gpcrs Exhibit Conserved Water Networks for Stabilization and Activation', 116 (2019), 3288-93.

36 S. A. Wallace, J. N. Mathur, and B. J. Allen, 'The Influence of Heavy Water on Boron Requirements for Neutron Capture Therapy', *Medical Physics,* 22 (1995), 585-90.

37 Stuart D. C. Ward, Fadi F. Hamdan, Lanh M. Bloodworth, and Jürgen Wess, 'Conformational Changes That Occur During M3muscarinic Acetylcholine Receptor Activation Probed by the Use of an in Situ Disulfide Cross-Linking Strategy*', *Journal of Biological Chemistry,* 277 (2002), 2247-57.

38 S. K. Yang, R. Tang, and Pu Quanlong, 'Synthesis of 3-Deuterated Diazepam and Nordiazepam 4-Oxides and Their Use in the Synthesis of Other 3-Deuterated Derivatives', *Journal of Labelled Compounds and Radiopharmaceuticals,* 38 (1996), 753-60.

39 Grace Q Zhao, Yifeng Zhang, Mark A Hoon, Jayaram Chandrashekar, Isolde Erlenbach, Nicholas JP Ryba, and Charles S Zuker, 'The Receptors for Mammalian Sweet and Umami Taste', *Cell,* 115 (2003), 255-66.

40 Grace Q. Zhao, Yifeng Zhang, Mark A. Hoon, Jayaram Chandrashekar, Isolde Erlenbach, Nicholas J. P. Ryba, and Charles S. Zuker, 'The Receptors for Mammalian Sweet and Umami Taste', *Cell,* 115 (2003), 255-66.