

# Dielectric response of frustrated water down to a single-molecule contribution

Ariel Fernández\* 

Received 17 November 2016, revised 3 February 2017, accepted 3 April 2017

Published online 12 May 2017

A theory of dielectric response of water under nanoscale confinement was long overdue. This work addresses the problem by establishing a relation between dielectric response and hydrogen-bond frustration subsumed in a non-Debye polarization term. The results hold down to the single-molecule contribution and are validated *vis-à-vis* experimental measurements on a system where dielectric modulation entails removal of a single water molecule. The frustrated dielectric response down to molecular scales is assessed by contrasting two enantiomeric ligands in association with the same protein, with the complexes differing in the removal of a single interfacial water molecule.

## 1 Introduction

It is often argued that water confined in nanotubes, nanocavities, or interfacing with surfaces endowed with nanometer topographic detail, behaves fundamentally differently from bulk solvent, especially in its dielectric response [1–5]. Thus, a nanoscale theory of water dielectrics has been long overdue. Recent work revealed that the nanoscale behavior is attributable in good measure to water frustration [1, 2, 6–11]. Accordingly, this work introduces a frustration-related non-Debye polarization term to account for the nanoscale dielectric response. The results are validated experimentally down to molecular dimensions by applying the methods to a system where dielectric modulation entails removal of a single water molecule.

Frustration, described by the scalar field  $\phi = \phi(\mathbf{r})$ , refers to the reduction in the expected number of hydrogen-bond coordinations of a water molecule with the oxygen atom placed within a ball centered at position  $\mathbf{r}$  [1, 2, 11]. The dynamic nature of liquid water and the limitations of the force fields adopted to reproduce this behavior [2, 6–9] prompts us to average over relaxation

time intervals ( $\tau \approx 10$  ps) and space (ball radius  $B$  fixed at  $4 \text{ \AA}$ ) to generate a scalar field of expected values. These values are less informative than instant fine grained snapshots but more reliable given dynamic and orientation uncertainty at sub-picosecond levels and single position [2, 6–9, 11]. Furthermore, the discrete nature of water hydrogen bond patterns clashes with the differentiability demanded by semiclassical electrostatic treatments, so the averaging is required to smooth out the scalar field. The parametrization  $\tau = 10$  ps,  $B = 4 \text{ \AA}$  is known to be the finest grain capable of yielding a smooth scalar field as required for analytic derivations [2]. We get  $\phi = 0$  for bulk water and  $\phi \geq 0$  for constrained water under nanoscale confinement. Thus, interfacial water near a surface with nanoscale topographical detail realizes the  $\phi \geq 0$  case [1, 2] illustrated by complex protein/water interfaces [6–9]. In this context, the local dielectric properties of frustrated water remain unexplored, yet expected to be related to biomolecular associations [10, 12]. Thus, a nanoscale dielectric theory is required and such a theory must account for frustration effects. This work addresses this need and presents two cross-validated analytic derivations and a computational strategy to assess the effect of frustration on dielectric response. The results are satisfactorily contrasted against two independent experimental measurements [13, 14] and hold down to sub-nanoscales, yielding the contribution of a single frustrated water molecule.

## 2 Theory

The scalar field  $\phi = \phi(\mathbf{r})$  informs of local distortions of the dynamic tetrahedral hydrogen-bond network

\* Corresponding author E-mail: ariel@jafinnovation.com  
CONICET-National Research Council, Buenos Aires 1033; INQUISUR Chemistry Institute, UNS/CONICET, Bahía Blanca 8000; IAM Argentine Institute of Mathematics, CONICET, Buenos Aires 1083, Argentina; Collegium Basilea, Institute for Advanced Study, Hochstrasse, 51, Basel 4056, Schweiz

representative of bulk water, and of uncompensated partial charges, with the latter located on any of the four molecular orbitals utilized for hydrogen bonding by a generic water molecule. Thus, uncompensated charges resulting from water confinement yield a “non-Debye” polarization term  $\mathbf{P}^\#$ , uncoupled from the electrostatic field  $\mathbf{E}$  [1, 2]. To delineate the dielectric response of frustrated water, we start by noting that water polarization  $\mathbf{P}$  cannot be identified in this case with the “Debye component”  $\mathbf{P}^\parallel$  aligned with  $\mathbf{E}$ . A more general treatment is required.<sup>1</sup> Thus, we expect a breakdown of the Debye *ansatz* typically used to define dielectric susceptibility ( $\chi$ ):  $\mathbf{P}(t) = \mathbf{P}^\parallel(t) = \varepsilon_0 \int_{-\infty}^t \chi(t-t') \mathbf{E}(t') dt'$  ( $\varepsilon_0 =$  vacuum permittivity). Adopting a higher level of generality, we also include the orthogonal polarization component  $\mathbf{P}^\#$  in the dielectric picture. Since this component arises from the uncompensated partial charges resulting from frustration, it follows the structural distortion of the water hydrogen-bond network, thus fulfilling the experimentally validated relation  $\mathbf{P}^\# = -\xi \nabla \phi$ , where  $\xi = (\omega \varepsilon_0)^{1/2}$  and  $\omega = 9.18 \times 10^{-20} \text{ mJ \AA}^{-1}$  [2, 11]. Thus, the uncompensated charge  $\gamma^\#$  induced by frustration is determined by the curvature of  $\phi$ :  $\gamma^\# = -\nabla \cdot \mathbf{P}^\# = \xi \nabla^2 \phi$ , and the following relation holds:  $\mathbf{P}^\parallel = \mathbf{P} - \mathbf{P}^\# = \mathbf{P} + \xi \nabla \phi$ .

We now determine the frequency ( $\nu$ )-dependent parallel permittivity coefficient  $\varepsilon_\parallel(\nu)$  defined by  $\mathbf{P}^\parallel = (\varepsilon_\parallel - 1) \varepsilon_0 \mathbf{E}$ , a relation valid in the limit  $t \rightarrow \infty$  ( $\nu = 0$ ). Spatial location is taken to be fixed throughout the derivation, so we drop the spatial coordinate vector. We first define the green function  $G^\parallel(t)$  determining the parallel polarization response induced by a pulse  $(\Delta E \delta(t))$  at  $t = 0$ :

$$\Delta \mathbf{p}^\parallel(t) = \chi_0^\parallel \varepsilon_0 \int_{-\infty}^t \Delta \mathbf{E}(t') G^\parallel(t-t') dt' \quad (1)$$

with  $\chi_0^\parallel = \chi^\parallel(\nu = 0) = \lim_{t \rightarrow \infty} \chi^\parallel$ . Assuming  $\Delta \mathbf{E}(t) = \Delta \mathbf{E}_0 e^{i2\pi \nu t}$ , integrating by parts Eq. (1), and finally comparing the result with  $\Delta \mathbf{p}^\parallel(t) = \chi^\parallel \Delta \mathbf{E}(t)$ , we obtain the frequency-dependent complex parallel susceptibility

$$\chi^\parallel(\nu) = \chi_0^\parallel \int_0^\infty e^{-2\pi i \nu t} G^\parallel(t) dt. \quad (2)$$

The Green function  $G^\parallel(t)$  becomes readily accessible from the fluctuation-dissipation computation [2, 11]:

$$\chi_0^\parallel \varepsilon_0 G^\parallel(t) = -\frac{\beta}{2} \partial / \partial t \langle \mathbf{p}^\parallel(0) \cdot \mathbf{P}^\parallel(t) \rangle, \quad (3)$$

where “ $\langle \rangle$ ” indicates the average over trajectories in phase space,  $\beta = (k_B T)^{-1}$  and  $\mathbf{p}^\parallel =$  denotes parallel polarization density.

Since  $\varepsilon_\parallel(\nu) = 1 + \chi^\parallel(\nu)$ , we get

$$\varepsilon_\parallel(\nu) = 1 - \beta(2\varepsilon_0)^{-1} \int_0^\infty e^{-2\pi i \nu t} \partial / \partial t \langle \mathbf{p}^\parallel(0) \cdot \mathbf{P}^\parallel(t) \rangle dt, \quad (4)$$

In the limit  $t \rightarrow \infty$ , Eq. (4) yields:

$$\varepsilon_\parallel = 1 + \beta(2\varepsilon_0)^{-1} \left[ \langle \mathbf{p}^\parallel(0) \cdot \mathbf{P}^\parallel(0) \rangle - \lim_{t \rightarrow \infty} \langle \mathbf{p}^\parallel(0) \cdot \mathbf{P}^\parallel(t) \rangle \right] \quad (5)$$

In this limit, the Debye parallel polarization  $\mathbf{P}^\parallel = \mathbf{P} + \xi \nabla \phi$  constitutes also the projection of  $\mathbf{P}$  along  $\mathbf{E}$ , hence the frustrated water polarization  $\mathbf{P}$  satisfies the fundamental linear relation:

$$\xi \nabla \phi = \left[ \mathbf{P}, \frac{\mathbf{E}}{\|\mathbf{E}\|} \right] \frac{\mathbf{E}}{\|\mathbf{E}\|} - \mathbf{P} = \underline{\mathbf{L}} \mathbf{P}, \text{ with } \underline{\mathbf{L}} = \underline{\pi}_E - \underline{\mathbf{I}}, \quad (6)$$

where  $\underline{\mathbf{L}}$  is a linear operator,  $\underline{\pi}_E$  denotes projection along  $\mathbf{E}$ -direction and  $\underline{\mathbf{I}}$  is the identity matrix.

The linear system given by Eq. (6) enables the direct computation of  $\mathbf{P}$  at any point in space by linearly transforming the frustration gradient in an  $\mathbf{E}$ -dependent manner, according to the relation  $\mathbf{P} = \underline{\mathbf{L}}^{-1} \xi \nabla \phi$ . Thus, Eq. (5) becomes computationally accessible by solving the linear system in Eq. (6).

It is also possible to cross-validate Eq. (5) by calculating  $\varepsilon_\parallel$  directly from the relation:

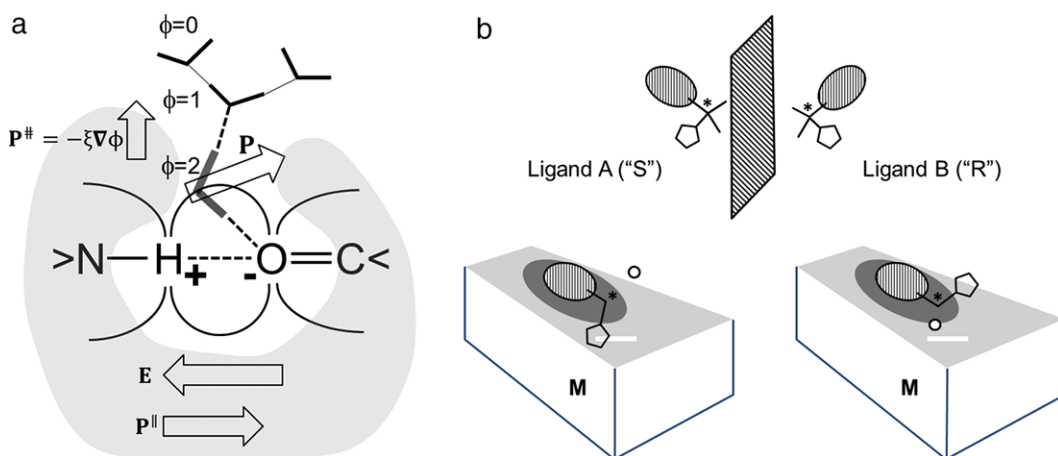
$$\underline{\pi}_E \mathbf{P} = \mathbf{P}^\parallel = \underline{\pi}_E \left[ \underline{\mathbf{L}}^{-1} \xi \nabla \phi \right] = (\varepsilon_\parallel - 1) \varepsilon_0 \mathbf{E} \quad (7)$$

### 3 Results

To validate the physical picture described by Eqs. (5)–(7), we compute the energetic increment  $\Delta U$  associated with removal of frustrated water adjacent to a preformed hydrogen bond (Fig. 1a):

$$\begin{aligned} \Delta U &= U_{C,0} [1 - \varepsilon_\parallel^{-1}] \\ &= U_{C,0} \left\{ 1 - \left[ 1 + \beta(2\varepsilon_0)^{-1} \left( \langle \mathbf{p}^\parallel(0) \cdot \mathbf{P}^\parallel(0) \rangle \right. \right. \right. \\ &\quad \left. \left. \left. - \lim_{t \rightarrow \infty} \langle \mathbf{p}^\parallel(0) \cdot \mathbf{P}^\parallel(t) \rangle \right) \right]^{-1} \right\} \quad (8) \end{aligned}$$

Here  $\mathbf{P}^\parallel$  refers to the Debye  $\mathbf{E}$ -aligned polarization of interfacial water surrounding the preformed hydrogen bond,  $U_{C,0}$  is the Coulomb energy of the electrostatic



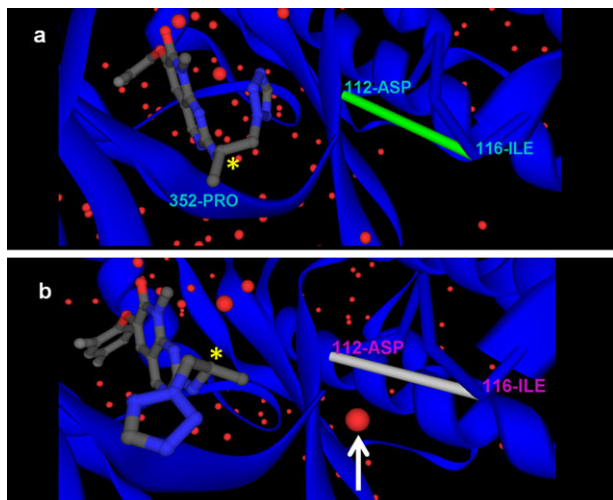
**Figure 1** a. Interaction of nanoscale-confined frustrated water interfacing with a preformed amide-carbonyl hydrogen bond weakens the latter.  $\mathbf{P}$  results from two contributions: a) dipole alignment along field lines of  $\mathbf{E}$  created by the preformed hydrogen bond, with the water molecule also hydrogen-bonding to the paired carbonyl, and b) nanoscale confinement of the water dipole, yielding a frustrated state. The projection of  $\mathbf{P}$  along  $\mathbf{E}$  yields the Debye polarization component  $P^{\parallel}$  that opposes  $\mathbf{E}$ . On the other hand, water frustration yields the non-Debye component  $P^{\#} = -\xi \nabla \phi$ . Through a suitable molecular association, the water molecule yielding  $\mathbf{E}$ -opposing  $P^{\parallel}$  gets removed, strengthening the preformed hydrogen bond. b. Associations of a protein with two *enantiomeric* ligands differing in the removal of a single interfacial water molecule. The chiral carbon is marked with an asterisk. Protein M is represented by a box with binding site indicated as a shaded region. A solvent-exposed preformed hydrogen bond is represented by a white segment and the vicinal water molecule (Fig. 1a), is displayed as a ball. Upon binding, the water molecule is displaced by ligand A but not by B.

interaction in vacuum, and  $\Delta U$  quantifies the strengthening of the electrostatic interaction as frustrated water gets removed from the vicinity. The full dielectric response to the electrostatic field emanates from the polarization component  $\mathbf{P}^{\parallel}$  that aligns with the field, hence it is enshrined in  $\epsilon_{\parallel}$ . At a variance with Debye's *ansatz*, we do not assume  $\mathbf{P}^{\parallel} = \mathbf{P}$  but the transversal polarization component does not contribute to charge screening. The latter only depends on the projection of polarization along field lines.

To compute  $\Delta U$  and validate the results against experimental measurements we sought to compute the dielectric effect of removal of a *single* frustrated water molecule. To that effect, we identified a protein with an intramolecular water-exposed hydrogen bond capable of associating with two ligands that are non-superimposable mirror images (*enantiomers*), hence differing only in their optical activity. In this way, any confounding factors arising from differences in structural adaptation of the protein upon association with the ligands were minimized, while the frustrated water interface differed solely in a *single* water molecule (Fig. 1b). Thus, the respective protein-ligand associations differ only in that one ligand is capable of sealing the preformed hydrogen bond by expelling a single frustrated water molecule, while the other ligand is incapable of do-

ing so. We sought a system fulfilling the following conditions (Fig. 1b):

- The protein molecule (M) contains a preformed amide-carbonyl (>N-H—O = C<) hydrogen bond partially exposed to water.
- M has affinity for two *enantiomeric* binding partners (A, B), so that no significant difference exists between the induced structures of M within complexes MA or MB (Fig. 1b).
- The 3D structures of the complexes are reported and thermodynamic data on both protein-ligand affinities is available.
- The preformed intramolecular hydrogen bond is water-sealed in MA but water-exposed in MB. In other words, ligand A, and only ligand A, promotes removal of frustrated water from the vicinity of the preformed hydrogen bond, so  $\Delta U = U_C(MA) - U_C(MB)$  may be computed from Eqs. (5)–(8) applied to complexes MA, MB.
- The following estimation holds:  $\Delta U \approx \Delta \Delta G(A, B)$ , where  $\Delta \Delta G(A, B) = \Delta G(MA) - \Delta G(MB)$  is the difference in free energy of association of M with A and B.



**Figure 2** Structural comparison between two protein-ligand complexes differing in the exclusion of a single interfacial water molecule (arrow). A ribbon rendering is used for the protein chain, with stick representation for the backbone in the 112–116 region, and interfacial water identified by spatial locations of oxygen atoms (red balls). a. Complex with initial coordinates from PDB.3FMK resulting from association of the p38 kinase protein and the “S”-ligand 6-(2,4-Difluoro-phenoxy)-8-methyl-2-((S)-1-methyl-2-tetrazol-2-yl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one. The backbone hydrogen bond (green segment) pairing residues Asp112, Ile116 is exposed to water, and upon association the ligand expels the frustrated water molecule nearby, sealing the hydrogen bond. Hence the underlying Coulomb contribution corresponds to *in vacuo* electrostatics. b. Structural rendering of p38 kinase with “R” ligand named 6-(2,4-Difluoro-phenoxy)-8-methyl-2-((R)-1-methyl-2-tetrazol-2-yl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one, with initial coordinates from PDB.3FMH. The single molecule (arrow) hydrating the A112-Ile116 backbone hydrogen bond is retained upon “R”-ligand binding.

A unique system was found fulfilling constraints a–e (Fig. 1b). It consists of  $M =$  p38 kinase [14], containing the backbone solvent-exposed hydrogen bond pairing residues Asp112 and Ile116 (Fig. 2), with MA and MB initial structures reported in PDB files 3FMK and 3FMH, respectively. The structures represent the protein association with ligands  $A =$  “S”-isomer and  $B =$  “R”-isomer, respectively, with “R” and “S” denoting the optical activity (Fig. 2) [14]. Direct examination of the MA complex reveals that, upon binding to  $M$ , the “S” isomer (ligand A) expels the frustrated water molecule vicinal to the Asp112-Ile116 hydrogen bond, sealing off the intramolecular hydrogen bond (Fig. 2a), whereas the “R”-isomer retains the water molecule upon binding (Fig. 2b). Thus, the two ligands

have a different impact on the dielectric environment, quantifiable using Eq. (8).

The difference in Coulomb energy for the Asp112-Ile116 hydrogen bond was first computed as  $\Delta U = U_C(MA) - U_C(MB)$ , using Eq. (8) while resolving for  $\mathbf{P}$  in the MB complex using Eq. (6):  $\mathbf{P} = \underline{\underline{\epsilon}}^{-1} \xi \nabla \phi$ . Water polarization  $\mathbf{P}$  was evaluated within an 8 Å-sphere centered at the proton in the preformed hydrogen bond as dependent on the local electrostatic field  $\mathbf{E}$  and frustration gradient  $\nabla \phi$ . Ten 800 ps-trajectories were generated for each of the solvated MA, MB complexes with initial conditions represented by the structural coordinates obtained from PDB files 3FMK and 3FMH. A TIP3P explicit solvent [15] within an AMBER package [16] and Coulombic interactions evaluated with the Ewald summation scheme [17] were adopted for MD simulation of an isothermal/isobaric ensemble at  $T = 298$  K. The two complexes were solvated in a water box extending at least 9 Å beyond any atom in the protein-ligand complexes and were first thermalized for 100 ps with RMSD between the protein chains in the MA and MB complexes reaching the steady value  $\text{RMSD} = (0.11 \pm 0.01)$  Å for  $t > 100$  ps. Following initial relaxation, positional restraints determined by the force constant  $= 20$  kJ/Å<sup>2</sup> were imposed on all non-hydrogen atoms. For the MA complex we obtained  $\mathbf{P}^{\parallel} = \mathbf{0}$  at all times, implying that the intramolecular hydrogen bond remained sealed from water.

The computation using Eqs. (6) and (8) yielded  $\Delta U = \frac{4.02 \text{ kJ}}{\text{mol}}$ . This value is in satisfactory agreement with an independent experimental determination of the strengthening of a preformed hydrogen bond due to removal of interfacial water upon protein adsorption in a hydrodynamic cuvette [13]. The latter measurement yielded  $\Delta U = \frac{3.91 \text{ kJ}}{\text{mol}}$ . Thirdly,  $\Delta U$  is recalculated from a computation of  $\epsilon_{\parallel}$  obtained by using Eq. (7), yielding  $\Delta U = \frac{3.97 \text{ kJ}}{\text{mol}}$ . Finally, both theoretical figures and the experimentally measured  $\Delta U$  provide a satisfactory estimation of the experimentally measured difference in free energy of association  $\Delta \Delta G(A, B) = \Delta G(MA) - \Delta G(MB) = \frac{4.18 \text{ kJ}}{\text{mol}}$  [14], thus fulfilling  $\Delta U \approx \Delta \Delta G(A, B)$ . This relation again corroborates that both complexes differ solely in the dielectric modulation resulting from removal of a single water molecule interfacing with a preformed backbone hydrogen bond.

## 4 Conclusion

The agreement between independent computations and experimental measurements validates the nanoscale theory of dielectric response of frustrated water and



supports its legitimacy down to single-molecule contributions.

The anisotropy of the dielectric response and its tensorial nature have been derived for water confined to nano-slabs flanked by flat homogeneous surfaces [3–5]. Since the frustration pattern in these cases is relatively simple ( $\phi = 1$  at interface,  $\phi = 0$  beyond second layer), these systems became tractable through linear response theory with polarization correlations obtained from molecular dynamics. Because their frustration pattern is nontrivial, the complex interfaces studied here forces us to incorporate frustration into the dielectric derivation. The results show some similarities with [3–5]. For example, longitudinal polarization at the interface is opposite in sign to the electrostatic field (cf. Fig. 1a and [3]), while the perpendicular component vanishes beyond a few molecular diameters as  $\nabla\phi \rightarrow 0$  (cf. [5]). More importantly, through the relationship between frustration and transversal polarization, we shed light on a term branded “unphysical” and “ill defined” in earlier treatments [3].

**Key words.** interfacial water, nano-scale confinement, hydrogen bond, frustrated water, dielectric response, dehydron, Debye polarization.

## References

- [1] A. Fernández, *J. Chem. Phys.* **139**, 085101 (2013).
- [2] A. Fernández, *Physics at the Biomolecular Interface* (Springer International Publishing AG, Switzerland, 2016), chapter 1.
- [3] V. Ballenegger and J. P. Hansen, *J. Chem. Phys.* **122**, 114711 (2005).
- [4] D. J. Bonhuis, S. Gekle and R. R. Netz, *Phys. Rev. Lett.* **107**, 166102 (2011).
- [5] A. Schlaich, E. W. Knapp and R. R. Netz, *Phys. Rev. Lett.* **117**, 048001 (2016).
- [6] Y. K. Cheng and P. J. Rossky, *Nature* **392**, 696–699 (1998).
- [7] L. Wang, B. J. Berne and R. A. Friesner, *Proc. Natl. Acad. Sci. USA* **108**, 1326–1330 (2011).
- [8] N. Giovanbattista, C. F. Lopez, P. J. Rossky and P. G. Debenedetti, *Proc. Natl. Acad. Sci. USA* **105**, 2274–2278 (2008).
- [9] G. Stirnemann, P. J. Rossky, J. T. Hynes and D. Laage, *Faraday Discuss.* **146**, 263–281 (2010).
- [10] A. Fernández, *Phys. Rev. Lett.* **108**, 188102 (2012).
- [11] A. Fernández, *FEBS Lett.* **590**, 3481–3491 (2016).
- [12] T. Beuming, Y. Che, R. Abel, B. Kim, V. Shanmugasundaram and W. Sherman, *Proteins Str. Func. Bioinf.* **80**, 871–883 (2012).
- [13] A. Fernandez and L. R. Scott, *Phys. Rev. Lett.* **91**, 18102 (2003).
- [14] D. M. Goldstein, M. Soth, T. Gabriel, N. Dewdney, A. Kuglstatter, H. Arzeno, J. Chen, W. Bingenheimer, S. A. Dalrymple, J. Dunn, R. Farrell, S. Frauchiger, J. La Fargue, M. Ghate, B. Graves, R. J. Hill, F. Li, R. Litman, B. Loe, J. McIntosh, D. McWeeney, E. Papp, J. Park, H. F. Reese, R. T. Roberts, D. Rotstein, B. San Pablo, K. Sarma, M. Stahl, M. L. Sung, R. T. Suttman, E. B. Sjogren, Y. Tan, A. Trejo, M. Welch, P. Weller, B. R. Wong and H. Zecic, *J. Med. Chem.* **54**, 2255–2265 (2011).
- [15] W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey and M. L. Klein, *J. Chem. Phys.* **79**, 926–934 (1983).
- [16] R. Salomon-Ferrer, D. A. Case and R. C. Walker, *WIREs Comput. Mol. Sci.* **3**, 198–210 (2013).
- [17] T. Darden, D. York and L. J. Pedersen, *J. Chem. Phys.* **98**, 10089 (1993).