

Stickiness of the Hydrogen Bond

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The dielectric response of bulk water follows laws of continuum electrostatics, a scheme often extrapolated without justification to treat confined interfacial water, where the Debye polarization ansatz breaks down and discrete effects matter. Reconciling the discrete behavior with the continuum equations requires a conceptual leap, all the more so when assessing the electrostatic impact of exclusion of individual water molecules. This work takes up the challenge and identifies the nanoscale stickiness of a preformed water-embedded hydrogen bond as phenomena not encompassed by continuum laws but quantitatively predictable when adopting a nanoscale theory of dielectric response holding down to molecular dimensions. Nanoscale stickiness is known to drive basic cellular events and has been measured using a molecular force probe but its physical underpinnings and computation have been lacking so far. The findings reported may impact molecular design in bio-nanotechnology and shed light on standing challenges in biophysics, especially on the protein folding problem, where organized compaction of the protein chain following nucleating intramolecular hydrogen bonding demands explanation.

dielectric response requires a nontrivial extension of the latter.^[6] Accordingly, this work identifies the nanoscale stickiness of water-embedded preformed hydrogen bonds^[7] as the kind of phenomena that is not encompassed by the classical continuum laws governing dielectric response but can be quantitatively predicted and interpreted within a nanoscale dielectric theory holding down to molecular dimensions. Nanoscale stickiness refers to the attractive drag exerted by the preformed hydrogen bond on nonpolar groups when the system is embedded in water. It has long been recognized as a crucial force driving biomolecular associations,^[8] the basic events in biology, but its physical foundations and quantitative computation have been lacking, constituting the thrust of this work. Our findings are likely to enable the molecular understanding and manipulation of fundamental biological processes and the design of materials for nanotechnology applications.^[9–11]

1. Introduction

Since Debye's days, the dielectric response of water has been treated based on the laws of continuum electrostatics under the *ansatz* that polarization (\vec{P}) is proportional to the electrostatic field (\vec{E}).^[1] This scheme, subsumed into the permittivity coefficient ϵ , has been often adopted with scant justification to treat water partially confined in nanocavities at the interface with protein structure or with other materials featuring nano-detailed surfaces.^[2] The extrapolation is probably unwarranted since the Debye *ansatz* breaks down under nanoscale confinement^[3] mostly due to the frustration of water hydrogen bonding possibilities.^[4–6] Reconciling the discrete behavior of nano-confined water with the continuum equations that govern

An adequate theory of dielectric response holding at nanoscales and further down to molecular dimensions should yield a reliable estimation of the dielectric modulation and resulting in strengthening of a preformed hydrogen bond that takes place as water molecules are expelled from the surroundings of the interaction. Since this dehydration results from the approach of a test nonpolar sphere (hydrophobe), we predict that the test hydrophobe should experience a “wrapping drag,” the mechanical equivalent of the dehydration propensity of the hydrogen bond, realized as a tendency to pull a test hydrophobe toward its center.^[7] Heuristically, this motion is predicted to lower the “local dielectric permittivity” with the net effect of enhancing the underlying electrostatics. Locating the center of coordinates at the hydrogen-bond shared proton, the wrapping drag $f(\vec{R})$, exerted by a hydrogen bond on a test hydrophobe at position \vec{R} , is orthogonal to the Coulombic field (**Figure 1**) and can be heuristically written as $f(\vec{R}) = -\nabla_{\vec{R}}(\epsilon^{\parallel})^{-1} U_{C,0}$, where $U_{C,0}$ is the in vacuo Coulomb energy of the hydrogen-bond underlying electrostatic interaction and ϵ^{\parallel} is the nanoscale Debye component of the dielectric permittivity along the direction of the electrostatic field \vec{E} .^[6] The standard continuum treatment of electrostatics does not enable the computation of the wrapping drag, since the \vec{R} -dependence of the permittivity is not available. Yet, the effective wrapping drag exerted by the hydrogen bond down to the effect of a single-molecule removal has been measured experimentally utilizing a molecular force probe, as shown in **Figure 2**,^[7] and lies within the confidence band of a

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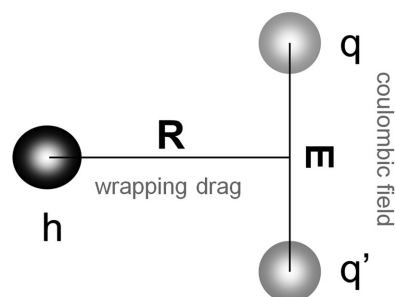


Figure 1. Scheme of the wrapping drag exerted on a test hydrophobe (h) by a pair of opposite charges (q, q') creating a Coulombic field (E) embedded in an aqueous dielectric environment. The test hydrophobe (CH_4) is placed at position R from the barycenter of the charge pair.

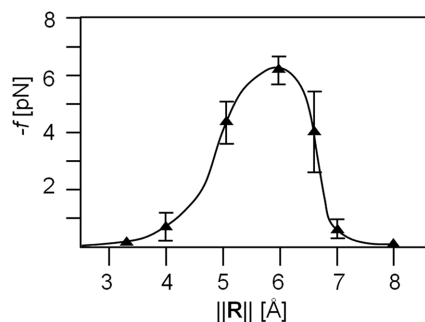


Figure 2. Wrapping drag exerted by a preformed hydrogen bond extracted from the retraction measurements of penetration–retraction hysteresis cycles generated by an MFP of the water–monolayer interface. Adapted with permission.^[7] Copyright 2005, IOP Publishing Ltd.

previous experimental estimation.^[12] To validate the nanoscale dielectric theory,^[6] our scope here is to compute the wrapping drag, and thus interpret the experimental underpinning of the nanoscale stickiness of the hydrogen bond.

2. Experimental Section

The wrapping drag exerted by a preformed hydrogen bond is extracted from penetration–retraction cycles obtained by a molecular force probe (MFP). The MFP interrogates the aqueous interface of a packed monolayer of fatty alcohol chains coating a gold foil and forming a 2D array of hydroxyl hydrogen bonds.^[7] The nanomaterial (hydroxylated alkylthiol $\text{SH}-(\text{CH}_2)_{11}-\text{OH}$) probed generates a half-exposed $\text{H}-\text{O}-\text{H}-\text{O}$ hydrogen-bond monolayer patch susceptible to direct examination. A monolayer nanopatch of the hydrophobic alkylthiol chain $\text{SH}-(\text{CH}_2)_8-\text{CH}_3$ was immobilized on the gold-coated silicon nitride tip of the cantilever through $\text{Au}-\text{S}$ links. The nanopatch is susceptible to being stretched as the cantilever tip retracts from the hydrogen-bond monolayer. Thus, a self-assembling monolayer (SAM), capable of forming a hydrogen-bond monolayer at the water interface was probed using the hydrophobic cantilever tip to determine the magnitude of the drag exerted on the wrapping methyl groups. The penetration–retraction hysteric cycles in the displacement–deflection force plane were obtained and the deflection force measured during cantilever retraction determines the effective wrapping drag that translates mesoscopically into the stickiness

of the hydroxyl-capped monolayer. The detection of an uncoupled wrapping drag is enabled during the last stage of retraction corresponding to a $\approx 3.3-8$ Å distance range between the extremities of SAM and cantilever. At such distances, the methyl groups at the extremities of the cantilever can only expel or immobilize water molecules solvating the surface $\text{H}-\text{O}-\text{H}-\text{O}$ hydrogen bonds in the SAM. Thus, the differences in the final dip along the retraction profile correspond to an attractive force exerted by the $\text{OH}-\text{OH}$ hydrogen bonds on the aliphatic tips of the cantilever coat identified as the wrapping drag. The wrapping drag causative of the stickiness of the hydrogen-bond monolayer has been normalized to a single methyl group (single cantilever molecule). The dispersion error bars (Figure 1) correspond to the aforementioned retraction measurements on 30 penetration/retraction hysteresis cycles.

We extract from experiment the free energy $\Delta G(\vec{R})$ associated with unwrapping a preformed hydrogen bond by pulling/transferring to bulk solvent a test hydrophobe (CH_4) initially located at position \vec{R} with center of coordinates located at the hydrogen-bond shared proton (Figure 1). The hydrogen-bond unwrapping requires work $W(\vec{R})$ performed on the system to overcome the wrapping drag exerted on the test hydrophobe at position \vec{R} . This work is obtained by integrating the opposite of the effective wrapping force, $f(\vec{R})$, measured experimentally (Figure 2) and harvesting pulling trajectories that yield numerical values of the irreversible work $W(\vec{R})$. Then, $\Delta G(\vec{R})$ is obtained by applying Jarzynski's relation:^[13] $\Delta G(\vec{R}) = -k_B T \ln(\langle \exp(-\beta \int_{\vec{R}}^{\infty} f(\vec{R}') d\vec{R}') \rangle)$, where $\langle \dots \rangle$ denotes average over all MFP penetration/retraction cycles previously obtained.^[7]

3. Results

With the advent of a nanoscale theory of dielectric modulation holding down to the molecular level, this thermodynamic result now becomes theoretically accessible, as shown in this work. The computation of the nanoscale stickiness exploits a local molecular descriptor of the water fluxional structure. To that effect, we introduce a “frustration scalar field” $\phi = \phi(\vec{R}, \vec{r}, t)$, parametrically dependent on test hydrophobe position \vec{R} , that indicates the expected number of missed hydrogen-bond opportunities of a test water molecule at spatial location \vec{r} relative to the quasi-tetrahedral saturating coordination associated with bulk water.^[6] The frustration field is defined as $\phi(\vec{R}, \vec{r}, t) = 4 - g(\vec{R}, \vec{r}, t)$, where $g(\vec{R}, \vec{r}, t) \leq 4$ is the expected number of hydrogen bonds engaging a water molecule visiting a sphere of radius $r = 4$ Å centered at position \vec{r} for a minimum time period $\tau = 1$ ps around time t , a permanence time typically associated with the relaxation timescale for a decoupled water lattice.^[14] The hydrogen bond is operationally defined via geometric constraints: $\text{O}-\text{O}$ distance < 3.2 Å and $\text{O}-\text{H}-\text{O}$ angle α_{HB} satisfying $120^\circ \leq \alpha_{\text{HB}} \leq 180^\circ$.^[6]

Water frustration is caused by partial nanoscale confinement and hence introduces non-Debye component $\vec{P}^\#$ of the polarization vector \vec{P} that is uncoupled from the electrostatic field \vec{E} and orthogonal to the Debye polarization component \vec{P}^\parallel . Thus, the electrostatic energy resulting from water frustration as the test

hydrophobe is positioned at \vec{R} becomes

$$\begin{aligned} U^{\#}(\vec{R}) &= \frac{1}{2} \varepsilon_0^{-1} \int \left\| \vec{P}^{\#}(\vec{R}, \vec{r}, \infty) \right\|^2 d\vec{r} \\ &= \frac{1}{2} \int \omega \left\| \vec{\nabla}_{\vec{r}} \phi(\vec{R}, \vec{r}, \infty) \right\|^2 d\vec{r} \end{aligned} \quad (1)$$

Equation (1) follows the relation: $\vec{P}^{\#} = -\xi \vec{\nabla}_{\vec{r}} \phi$,^[15,16] with $\xi = (\omega \varepsilon_0)^{1/2}$, $\varepsilon_0 =$ vacuum permittivity, which points to water frustration, with its lack of partial charge cancellation, as causative of the non-Debye polarization. The latter component becomes then a measure of the extent of departure from saturating hydrogen bonding realized in bulk water.^[16] The experimental validation of the relation for $\vec{P}^{\#}$ and the determination of the Fernandez constant $\omega = 9.18 \times 10^{-20}$ mJ \AA^{-1} are provided elsewhere.^[15]

To obtain the electrostatic energy of the hydrogen bond $U_C(\vec{R}) = \varepsilon^{\parallel}(\vec{R}, \vec{r}_{HB})^{-1} U_{C,0}$ (\vec{r}_{HB} = spatial location of hydrogen bond proton) for the wrapping hydrophobe at position \vec{R} , we first compute the local Debye permittivity at nanoscales:^[6]

$$\begin{aligned} \varepsilon^{\parallel}(\vec{R}, \vec{r}) &= 1 + \beta(2\varepsilon_0)^{-1} \left[\left\langle \vec{p}^{\parallel}(\vec{R}, \vec{r}, 0) \cdot \vec{P}^{\parallel}(\vec{R}, \vec{r}, 0) \right\rangle \right. \\ &\quad \left. - \lim_{t \rightarrow \infty} \left\langle \vec{p}^{\parallel}(\vec{R}, \vec{r}, 0) \cdot \vec{P}^{\parallel}(\vec{R}, \vec{r}, t) \right\rangle \right] \end{aligned} \quad (2)$$

where $\vec{p}^{\parallel}(\vec{R}, \vec{r}, 0)$ denotes Debye polarization density and “ $\langle \dots \rangle$ ” indicates the average over trajectories in phase space. The permittivity ε^{\parallel} may be written in terms of the computationally accessible frustration gradient $\vec{\nabla}_{\vec{r}} \phi$. To that effect, we take into account that $\vec{P}^{\#} = \Pi_{\#} \vec{P} = -\xi \vec{\nabla}_{\vec{r}} \phi$, and hence $\vec{P}^{\parallel} = -\xi \Pi_E \Pi_{\#}^{-1} (\vec{\nabla}_{\vec{r}} \phi) = L \vec{\nabla}_{\vec{r}} \phi$, where Π_E and $\Pi_{\#}$ denote projection along the electrostatic field and orthogonal projection, respectively, and $L = -\xi \Pi_E \Pi_{\#}^{-1}$. Since $\vec{P}^{\parallel} = (\varepsilon^{\parallel} - 1) \varepsilon_0 \vec{E}$, the operator L satisfies the relation: $L \vec{\nabla}_{\vec{r}} \phi = (\frac{\beta}{2}) \sigma^2 (L \vec{\nabla}_{\vec{r}} \phi) \vec{E}$, where σ^2 denotes time correlation in the thermodynamic limit.

The water entropy content $S_w(\vec{R})$ associated with the wrapping hydrophobe at position \vec{R} is given as

$$S_w(\vec{R}) = \int k_B (\rho_0 - \rho(\vec{R}, \vec{r})) \ln \left(1 - \frac{\phi(\vec{R}, \vec{r}, \infty)}{4} \right) d\vec{r} \quad (3)$$

Equation (3) follows since the entropy differential $dS(\phi(\vec{R}, \vec{r})) = k_B \ln \left[\frac{4 - \phi(\vec{R}, \vec{r}, \infty)}{4} \right]^{(\rho_0 - \rho(\vec{R}, \vec{r}))} d\vec{r} = [k_B (\rho_0 - \rho(\vec{R}, \vec{r})) \ln (1 - \frac{\phi(\vec{R}, \vec{r}, \infty)}{4})] d\vec{r}$ corresponds to the transference of a water molecule from bulk solvent (number density $\rho_0 = 0.033 \text{\AA}^{-3}$) to a differential volume region $d\vec{r}$ located at position \vec{r} with number density $\rho(\vec{R}, \vec{r})$ and frustration $\phi(\vec{R}, \vec{r}, \infty)$.

Thus the thermodynamic cost $\Delta G(\vec{R})$ associated with unwrapping a hydrogen bond by pulling away a wrapping hydrophobe initially located at position \vec{R} is computed as

$$\Delta G(\vec{R}) = \Delta U^{\#}(\vec{R}) + \Delta U_C(\vec{R}) - T \Delta S_w(\vec{R}) \quad (4)$$

where the increment Δ is associated with the unwrapping process and refers to the difference between final wrapping hy-

drophobe location $\vec{R} \rightarrow \infty$ (in practice $\vec{R} = \vec{R}_b \approx 12 \text{\AA}$), representing embedding in bulk solvent, and initial position \vec{R} . To obtain the theoretical unwrapping free energies through Equation (4), direct numerical evaluations of the scalar fields $\phi(\vec{R}, \vec{r}, t)$, $\rho(\vec{R}, \vec{r})$ were performed within an isothermal/isobaric ensemble at $T = 298$ K. Positional restraints (force constant = 20 kJ. \AA^{-2}) were imposed for the heavy atoms of a preformed hydroxyl–oxygen hydrogen bond and on the wrapping hydrophobe (CH₄) at position \vec{R} . A free version of CHARMM, called “charmm” retaining all the features of CHARMM except for two high-performance modules was adopted with the Ewald summation scheme^[17] and TIP3P-represented solvent.^[18] Alternative more advanced models of water such as SPC/E and TIP4P yielded poorer results vis-à-vis the experimental values reported in ref. 7 ($R^2 = 0.66, 0.68$, respectively, compared with $R^2 = 0.93$ when using TIP3P). The significantly better performance of TIP3P can be attributed mainly to the fact that the force field in the CHARMM (or AMBER) molecular dynamics package was parametrized and optimized for the TIP3P model. In fairness, to properly adopt other water models would require a complete reparametrization of the force fields. Even for bulk water, it is unclear that TIP4P or SPC/E offered a significant advantage over TIP3P as evidenced by the yielded key bulk parameters (dipole moment, density, dielectric permittivity, and heat capacity) contrasted against experimental values.^[19]

Since we are required to compute the reversible work to unwrap a hydrogen bond, a special microstate sampling scheme is required. To that end, for each \vec{R} we select a representative from the set of a priori equi-probable microstates to ensure the probable lowest value of $\Delta G(\vec{R})$. Let $[\mathbf{Q}_n, \mathbf{P}_n]$ denote the microstate of the system at stage n , specified by the atomic-coordinate vector $\mathbf{Q}_n = \mathbf{Q}_n^a \oplus \mathbf{Q}_n^w$, and momentum vector $\mathbf{P}_n = \mathbf{P}_n^a \oplus \mathbf{P}_n^w$, both decomposable as direct sums of nonwater (a) and water (w) contributions. The next microstate $[\mathbf{Q}_{n+1}, \mathbf{P}_{n+1}^a \oplus \mathbf{P}_{n+1}^w]$ resulting after 5 ps of MD simulation is retained depending on a comparison of the scalar fields $\phi_n = \phi_n(\mathbf{Q}_n)$ and $\phi_{n+1} = \phi_{n+1}(\mathbf{Q}_{n+1})$. Thus, the state $[\mathbf{Q}_{n+1}, \mathbf{P}_{n+1}^a \oplus \mathbf{P}_{n+1}^w]$ is retained provided that either one of the following conditions holds:

$$\Delta G(\vec{R}, \phi_{n+1}) \leq \Delta G(\vec{R}, \phi_n),$$

$$\Delta G(\vec{R}, \phi_{n+1}) > \Delta G(\vec{R}, \phi_n) \text{ and}$$

$$r \leq \exp \left\{ -\beta \left[\Delta G(\vec{R}, \phi_{n+1}) - \Delta G(\vec{R}, \phi_n) \right] \right\}$$

with r = realization value of an evenly distributed real random variable confined to the interval [0,1] and $\beta = [k_B T]^{-1}$. On the other hand, if we get

$$\Delta G(\vec{R}, \phi_{n+1}) > \Delta G(\vec{R}, \phi_n) \text{ and}$$

$$r > \exp \left\{ -\beta \left[\Delta G(\vec{R}, \phi_{n+1}) - \Delta G(\vec{R}, \phi_n) \right] \right\}$$

we perform 5 ps MD runs on 1000 initial randomly selected a priori equi-probable microstates $[\mathbf{Q}_n, \mathbf{P}_n^a \oplus \mathbf{P}_n^{w(j)}]$, $j = 1, 2, \dots, 1000$, with randomly chosen water momenta $\mathbf{P}_n^{w(j)}$ ($\mathbf{P}_n^{w(1)} = \mathbf{P}_n^w$) subject to the constant kinetic energy constraint, and select the

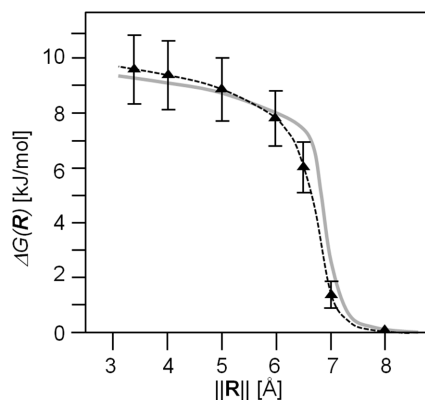


Figure 3. Experimental (dashed line) and computed (gray line) free energy cost $\Delta G(\vec{R})$ associated with unwrapping a hydrogen bond by pulling the wrapping hydrophobe from position \vec{R} to bulk solvent ($\vec{R} \rightarrow \infty$, in practice $\vec{R} = \vec{R}_b \approx 12 \text{ \AA}$). The experimental values were obtained by harvesting penetration/retraction trajectories^[7] and applying Jarzynski's equality. Error bars represent dispersion of the measured irreversible work to pull the test hydrophobe away from the hydrogen bond. Experimental datapoints from ref. [7].

microstate $[\mathbf{Q}_{n+1}, \mathbf{P}_{n+1}^a \oplus \mathbf{P}_{n+1}^w] = [\mathbf{Q}_{n+1}^*, \mathbf{P}_{n+1}^{a*} \oplus \mathbf{P}_{n+1}^{w*}]$ as the $(n + 1)$ -destiny state that realizes the lowest value $\Delta G(\vec{R}, \phi_{n+1}(\mathbf{Q}_{n+1}^{(i)}))$.

For each fixed \vec{R} and in order to minimize $\Delta G(\vec{R})$, we perform iterations of the retention/selection cycles of the scalar field, generating the sequence $\{\phi_n = \phi_n(\vec{R})\}_{n=1, \dots}$ and adopt $\Delta G(\vec{R}) = \lim_{n \rightarrow \infty} \Delta G(\vec{R}, \phi_n)$. In practice, we never observed a significant decrease (i.e., of magnitude $> 1/4 [k_B T]$) in $\Delta G(\vec{R}, \phi_n)$ for $n > 100$.

As shown in **Figure 3**, the theoretical computation of the unwrapping free energy $\Delta G(\vec{R})$ accurately reproduces the experimental values ($R^2 = 0.93$). The latter were obtained by harvesting measurements of the irreversible work of unwrapping a hydrogen bond^[7] and applying Jarzynski's equality^[13] (Section 2). In this way, we validated the nanoscale theory of dielectric response for frustrated water holding down to single-molecule contributions. Importantly, we established the nanoscale stickiness mechanically translated into a wrapping drag exerted by a preformed hydrogen bond, a many-body interaction hitherto not properly characterized.

4. Discussion and Conclusions

The dielectric response of bulk water follows continuum laws of electrostatics, a scheme often extrapolated without justification to treat confined interfacial water, where the Debye *ansatz* breaks down and discrete effects matter. A nontrivial conceptual leap is required to reconcile discrete and continuum behavior. This work takes up the challenge, identifying the stickiness of water-embedded hydrogen bonds as the kind of phenomena requiring a nanoscale treatment of dielectric response. The nanoscale stickiness or wrapping drag exerted by hydrogen bonds on nonpolar moieties has long been recognized as a crucial force steering biomolecular associations in water.^[8] The main difficulty lied in reconciling the discrete behavior of water molecules around a preformed hydrogen bond with the continuous laws governing

the dielectric response. Thus, the dielectric modulation or electrostatic enhancement brought about by expelling individual water molecules from within the hydrogen-bond environment was not readily accessible until a cogent nanoscale dielectric theory^[6] was put in place. In this work, the extant theory is put to test by computing the wrapping drag exerted by a water-embedded hydrogen bond on a test hydrophobe. This drag is regarded as the mechanical equivalent of the dehydration propensity of the hydrogen bond, and has been previously measured using a molecular force probe.^[7] The agreement found between theory and experiment validates the nanoscale treatment.

The concept is likely to impact the molecular understanding and manipulation of cellular processes and the design of materials for nanotechnology applications. Furthermore, it is probably a missing concept in first-principle approaches to the protein folding problem, as the wrapping drag promotes chain compaction beyond the nucleation events that involve intramolecular hydrogen bonding.^[8]

Conflict of Interest

The author declares no conflict of interest.

Keywords

hydrogen bonds, molecular force probes, nanoscale dielectrics, non-Debye polarization, water structure

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