

Chapter 2

Dielectric Structure of Aqueous Interfaces: From Classical Non-Debye Electrostatics to a Quantum Theory of Interfacial Tension

This chapter explores the dielectric structure of interfacial water from a classical and quantum electrostatics perspective. The protein-water interface is treated classically, while quantum effects are investigated only for simple featureless nonpolar interfaces due to the inherently higher complexities of the quantum approach. In a classical context, the most striking feature arising from the *epistructural physics* is the breakdown of the Debye *ansatz* that postulates the alignment of water polarization with the protein electrostatic field. The complexities of biological interfaces are shown to be in good measure due to this departure from the standard dielectric picture that has been historically—and incorrectly—extrapolated from a bulk interface. Accordingly, concepts like the dielectric permittivity coefficient are shown to be inadequate to describe interfacial electrostatics. The departure from bulk-like behavior is shown to enhance the physico-chemical inhomogeneity of protein surfaces and to enable the chemical functionality of the aqueous interface. Epistructural physics of the protein-water interface identifies a structural defect known as *dehydron* as causative of anomalous polarization effects causing a breakdown of the Debye standard *ansatz*. The previous chapter revealed that interfacial tension is a central thermodynamic factor driving biomolecular events and may be alternatively stored as electrostatic energy associated with the non-Debye component of water polarization. This chapter substantively supplements this picture by showing that dehydrons induce chemical basicity in interfacial water as a consequence of the breakdown of Debye dielectrics. Thus, the relevance of dehydrons as catalytic elements is highlighted. We anticipate that this discovery will prompt a re-writing of vast mechanistic chapters of biological chemistry.

A quantum mechanical approach is shown to be essential to deal with the spontaneous negative charging of aqueous nonpolar interfaces. This phenomenon has eluded quantitative first-principle prediction, in part because it steadfastly challenges the Debye dielectric picture. In this chapter we also show that quantitative prediction requires not only a substantive revision of Debye's linear dielectric

ansatz to incorporate anomalous polarization yielding interfacial tension but also the implementation of a quantum mechanical theory of interfacial tension. The latter is necessary to compute the reduction in hydrogen-bond frustration at the interface that takes place upon hydroxide anion adsorption. The quantitative predictions are validated vis-à-vis experimental measurements of hydroxide adsorption uptake obtained using sum-frequency vibrational spectroscopy.

Unfortunately, the daunting complexities prevent us from extending this quantum mechanical approach to protein-water interfaces at this time. We anticipate that ionic adsorption at packing defect sites may indeed occur and play a role in enzymatic catalysis but cannot prove it at this point in time.

2.1 Interfacial Tension Stored as Non-Debye Polarization Energy

As noted in the previous chapter, the non-Debye orthogonal component $\vec{P}^\#$ of polarization is commensurate with the distortion in the structure of water due to sub-nanoscale confinement [1]. More precisely $\vec{P}^\# = \xi \vec{\nabla} g$, yielding an equivalence between electrostatic energy of orthogonal polarization and interfacial tension:

$$U^\# = \frac{1}{2} \epsilon_0^{-1} \int \left\| \vec{P}^\# \right\|^2 d\vec{r} = \frac{1}{2} \lambda \int \left\| \vec{\nabla} g \right\|^2 d\vec{r} \quad (2.1)$$

This equation asserts the electrostatic origin of interfacial tension, prompting the question: Where do the most significant contributions to non-Debye polarization take place? Since dehydrons create the biggest drops in g -value, we may intuitively assert that dehydrons are the culprits of the breakdown of the Debye picture and a good portion of this chapter is devoted to prove this conjecture.

A measure of the local departure from the Debye scenario within the protein structure may be obtained for each residue generically denoted by n . This measure is furnished by a structure-dependent parameter ϑ_n that we term anomalous polarization fraction (APF) and define as

$$\vartheta_n = \langle U^\#_n / U_n \rangle \quad (2.2)$$

where the symbol “ $\langle \cdot \rangle$ ” denotes time average, and

$$U_n = \frac{1}{2} \epsilon_0 \int \left\| \vec{\nabla} \varphi \right\|^2 d\vec{r}, \quad U^\#_n = \frac{1}{2} \epsilon_0^{-1} \int \left\| \vec{P} - \left(\vec{P} \cdot \vec{e} \right) \vec{e} \right\|^2 d\vec{r} \quad (2.3)$$

where electrostatic potential φ is defined through the relation: $-\vec{\nabla} \varphi = \vec{E} + \epsilon_0^{-1} \vec{P}$. Integration in equations (2.3) extends over a neighborhood around residue n defined as a sphere of radius r centered at its α -carbon. To capture the environment of all side chains we adopted $r = 6$ Å, the approximate diameter of tryptophan (W), the largest side chain. We also evaluated the APF using a larger radius ($r = 8$ Å) to determine the dilution of the anomalous polarization effect as the bulk solvent region is approached. The centering of the n -residue sphere at the α -carbon is justified *a posteriori*, as shown subsequently. We expected and confirmed that anomalous polarization would be mainly related to poor packing of the protein backbone, while the packing defects are identified by introducing backbone solvation domains consisting of spheres of radius 6 Å centered at the α -carbons. Thus, by centering the residue spheres at the α -carbons we simultaneously interrogate the backbone and the side chains in search for anomalous dielectric patterns in interfacial water. A more obvious reason for choosing the α -carbon as opposed to other side chain carbon atom relates to the fact that we would otherwise need to treat glycine (G) as an exceptional case.

The parameter ϑ_n is computed at protein/water interfaces as a time average over a 5 ns-period beyond equilibration of the protein structural backbone with the solvent. Thus, water polarization for soluble natural proteins with structures reported in the Protein Data Bank (PDB) may be computed along molecular dynamics trajectories. Each 10 ns-trajectory is generated using as starting point the equilibrated structural coordinates that result after thermalization of the PDB-reported structure immersed in a pre-equilibrated solvent bath. The referenced computational details [2–9] are provided in the caption for Fig. 2.1. Simulations are performed within an isobaric/isothermal ensemble (1 atm, 298 K). The optimized systems are pre-equilibrated for 500 ps. The resulting structures become the starting point for the 5 ns-thermalization trajectories. A total of 100 interfacial solvent configurations, one every 50 ps, are used to compute the time average of ϑ_n . To this end, we recorded charge distribution $\rho(\vec{r}, t)$, internal field $\vec{E}(\vec{r}, t)$ and polarization $\vec{P}(\vec{r}, t)$ for each intermediate structure/solvent configuration.

The structure/solvent system is considered equilibrated at time t_0 if the RMSD of backbone atomic coordinates averaged over randomly chosen pairs of chain conformations within a time interval $[t_0, t_0 + \tau]$ ($\tau \approx 1$ ns) is less than 1 Å. For all nine proteins in this study (PDB entries 1SRL, 1ESR, 1A8O, 1PIT, 1QGB, 1ATA, 1Q7I, 1PI2, 2PNE), this criterion was fulfilled for $t_0 = 500$ ps. Solvent and side-chain conformations continue to vary significantly (i.e. $\text{RMSD} > 2.25$ Å) on the 1 ns timescale.

The APFs for individual residues for the natively folded SH3 domain (PDB.1SRL) are shown in Fig. 2.1. The context-dependence of APFs is evident since residues of the same type (i.e. serines S18 and S47, tryptophans W42 and W43) can have very different APFs depending on their location within the protein chain and therefore, within the structure. If we exclude the residues A12, I56 and

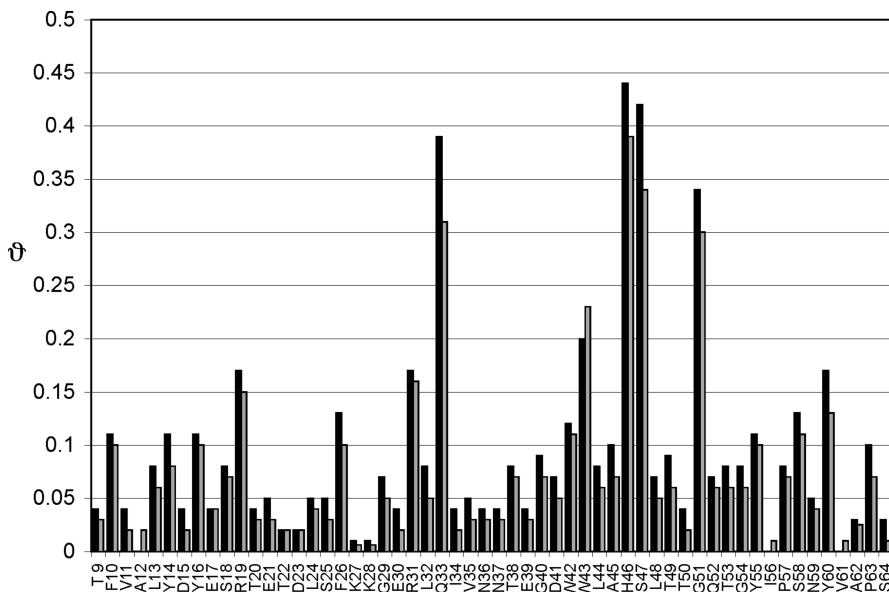


Fig. 2.1 Anomalous polarization fraction (ϑ) for each residue within the solvent-equilibrated folded protein chain for the soluble SH3 domain (PDB.1SRL). The PDB file numbering is followed in naming residues along the chain and the one-letter code for amino acids is adopted. *Black bars* represent protein surface interrogation with spheres of radius $r = 6 \text{ \AA}$ centered at α -carbons, while *grey bars* were generated using radius $r = 8 \text{ \AA}$. The all-atom trajectories used to compute the time-averaged APF values thermalize the PDB structures in contact with a pre-equilibrated solvent bath consisting in a truncated octahedral cell of TIP3P water molecules that provide at least four water layers of solvent envelope [2]. Protein atoms are described with the parm99SB force field parameterization [3]. Water molecules extended at least 12 \AA from the surface of the protein. Ewald sums [4] and an 8 \AA -distance cutoff are used for treating long-range electrostatic interactions. A Shake scheme is employed to keep bonds involving hydrogen atoms at their equilibrium length [5] which allowed us to employ a 3 fs time step for the integration of Newton's equations. Constant pressure of 1 atm and a temperature of 298 K are maintained using the Berendsen coupling scheme [6]. An AMBER package [7] was adopted for these MD simulations, with charges on the molecules assigned according to the BCC charge model using AM1 optimized geometries and potentials [8, 9]. After protein/solvent equilibration (as defined in main text), the protein backbone coordinates are partially constrained according to the Shake scheme [5] and only side chains are allowed to explore conformation space, generating a gamut of local hydration patterns. Reprinted from [Fernández Stigliano A (2013) Breakdown of the Debye polarization ansatz at protein-water interfaces. J Chem Phys 138:225103], copyright 2013 with permission from AIP Publishing LLC

V61 that are fully buried within the structure, it is clear that the positively charge lysines (K27, K28) have the lowest APFs. As described below, this is expected since the ammonium cation ($-\text{NH}_3^+$) in lysine has the highest charge concentration of all amino acids and hence it is the most capable of organizing solvent in accord with its highest hydration requirements.

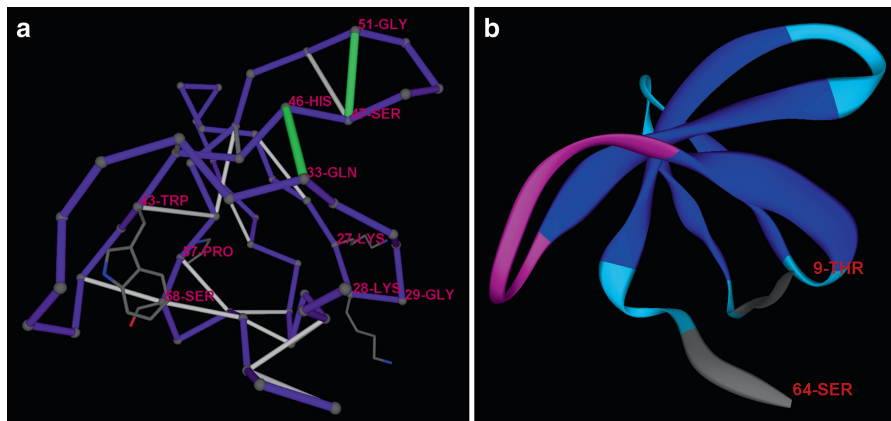


Fig. 2.2 (a) Dehydron pattern for the solvent-equilibrated structure of the soluble Src-SH3 domain (PDB. 1SRL). The backbone is represented as virtual bonds joining the α -carbons of residues along the chain, with well-shielded backbone hydrogen bonds and dehydrons shown as segments sustained between the paired residues in *grey* and *green*, respectively. Dehydrons are determined from the PDB structural coordinates following the protocol indicated in Chap. 1. In accord with this protocol, the under-wrapping of the backbone hydrogen bond due to a low number of surrounding nonpolar groups from the flanking side chains is considered to be a surrogate for the extent of solvent exposure. (b) Ribbon rendering of the structure of Src-SH3 domain

Intriguingly, a structural context becomes the determinant factor for the APF high values, superseding individual residue propensities. Thus, the residues with the highest APFs, Q33, H46, S47 and G51, are the only ones paired by *dehydrons* (marked in green in Fig. 2.2a; the ribbon rendering in Fig. 2.2b is an aid to the eye). Due to the nanoscale water confinement created by the packing defect that gives

rise to the dehydron, a significant nonvanishing component $\vec{P}^\#$ is expected for residues paired by such hydrogen bonds (cf. Chap. 1). These packing defects expose the backbone polar groups amide ($> \text{N-H}$) and carbonyl ($> \text{C=O}$) to structure-disruptive effects of backbone hydration with the net effect of steering water dipoles into orientations that are not collinear with \vec{E} . The confined water molecules relinquish some of their hydrogen bonding possibilities in order to form hydrogen bonds with the backbone polar groups. This reduction in coordination represents a departure from the bulk water structure embodied in the tetrahedral lattice and the resulting water polarization becomes statistically independent of the internal field \vec{E} . This is so since water molecules with reduced water coordination tend to preserve their hydrogen-bond pattern thereby becoming impervious to the torque

$$\vec{E}(\vec{r}) \times \vec{\mu}_P(\vec{r}) = \vec{E}(\vec{r}) \times \int (\vec{r}' - \vec{r}) \vec{\nabla} \cdot \vec{P}(\vec{r}') d\vec{r}' \quad (2.4)$$

imposed by $\vec{E}=\vec{E}(\vec{r})$ on the polarization-associated dipole with moment $\vec{\mu}_P(\vec{r}) = \int (\vec{r}' - \vec{r}) \vec{\nabla} \cdot \vec{P}(\vec{r}') d\vec{r}'$.

Thus, interfacial water polarization in this context is expected to contain and indeed contains (Fig. 2.1) a significant anomalous non-Debye contribution.

2.2 Non-Debye Dielectric Structure of the Aqueous Interface for a Soluble Protein

In Fig. 2.3a we show the individual propensities of the 20 residue types to align interfacial water along the electrostatic field by computing the APF of residues in nine PDB-reported proteins (specified in caption for Fig. 2.4). The expected APF for each residue type is obtained by averaging the APFs for that residue type in all nine proteins. The computation amounts to average over the structural contexts in the nine proteins where the particular residue type occurs. Due to the dominance of dehydrons as structural determinants of APF (Figs. 2.1 and 2.4), superseding individual propensities (Fig. 2.1), we have excluded dehydron-paired residues from the calculations in Fig. 2.3a.

As a class, the aromatic residues (H, F, W, Y) have the highest APF values due to their water-organizing power and their role as significant disruptors of the tetrahedral water structure. Their delocalized π -electron quadrupole promotes interactions with partial positive charges in vicinal interfacial water molecules. Furthermore, the side chains of such residues cannot be clathrated (surrounded without disrupting the tetrahedral water lattice) as it is the case with nonpolar aliphatic side chains (L, V, I, A). Thus, the resilient non-tetrahedral hydrogen-bond pattern of vicinal water explains the superior APF-boosting activity of aromatic residues when compared with nonpolar aliphatic ones. The sharp contrast between the lowest APF-booster lysine (K) and the highest APF-booster arginine (R), both in the same class of positively charged residues with aliphatic (methylene) linkages, is also striking, yet expected. The ammonium cation ($-\text{NH}_3^+$) in lysine has the highest charge concentration of all amino acids, therefore it strongly organizes hydration along electrostatic field lines, while the guanidinium cation ($[-\text{NH} = \text{C}(\text{NH}_2)_2]^+$) in arginine contains the most delocalized charge of all amino acids, hence the resulting local electrostatic field has the weakest water-organizing power.

From the above discussion it becomes apparent that the polarization steering power of individual residues is tightly related to the localization and concentration of their net charge. Thus, a pH dependence of the APF for an individual residue is expected in accord with the pKa of the residue within the protein structure. The titration of a residue removes a net charge and thereby increases the APF by curbing the polarization-steering capabilities of the residue. This titration effect becomes apparent as we compare the expected APFs of individual residue types at neutral pH

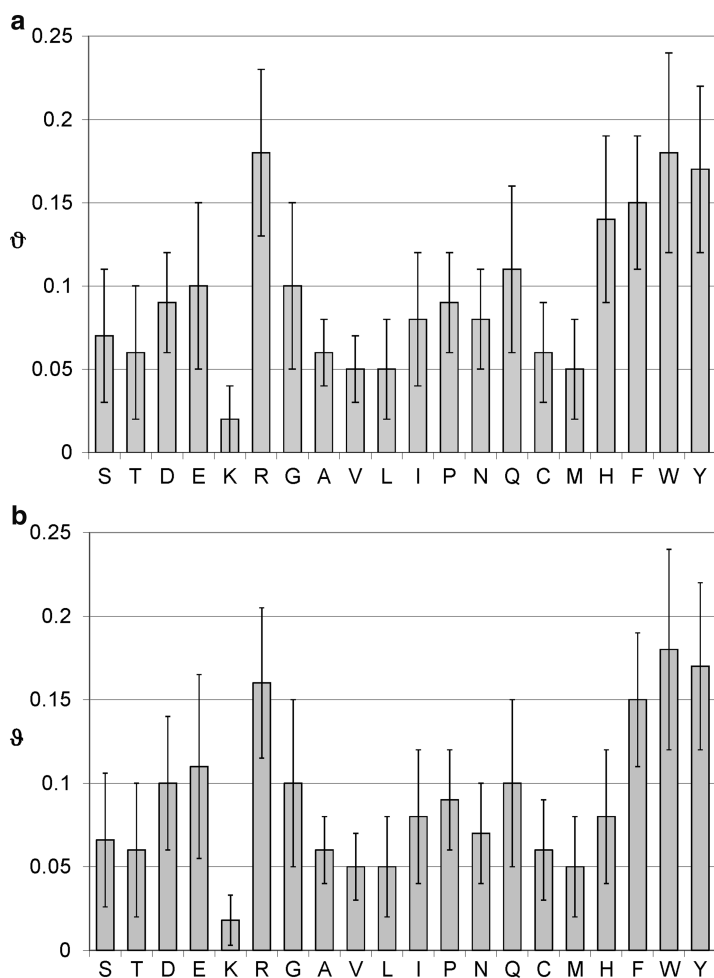


Fig. 2.3 APFs for each residue type averaged over all structural environments where the residue type occurs in 9 PDB-reported proteins described in the caption for Fig. 2.4. The radius $r = 6 \text{ \AA}$ has been adopted and the error bars represent the dispersion in ϑ -values. (a) APFs at pH 7. (b) APFs at pH 5.5. Reprinted from [Fernández Stigliano A (2013) Breakdown of the Debye polarization ansatz at protein-water interfaces. *J Chem Phys* 138:225103], copyright 2013 with permission from AIP Publishing LLC

(Fig. 2.3a) and pH 5.5 (Fig. 2.3b). The pH window 5.5–7 apparent when contrasting Fig. 2.3a, b contains only the $pK_a \approx 6.1$ of histidine (H), and hence this residue is predicted and shown to undergo the most dramatic gain in polarization steering (decrease in expected APF) as pH is decreased from 7 to 5.5. The effects of titration on other residue types could not be assessed in this study since their pK_a 's dictate extreme pH values that would introduce denaturing conditions for the proteins studied.

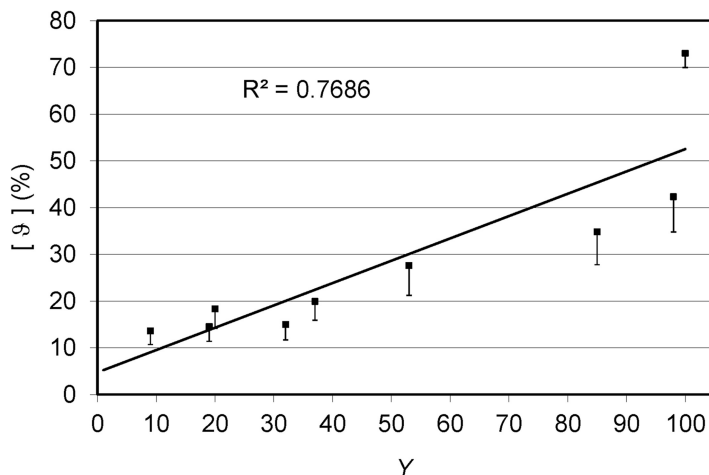


Fig. 2.4 Correlation between θ -value averaged over all residues in the protein ($[\theta]$), and the dehydron-to-backbone-hydrogen-bond ratio Y for the protein. Both parameters are given as percentages. The proteins studied identified by their respective PDB files and Y -ratios (in brackets) are: 1SRL (14.3), 1ESR (27.9), 1A8O (32.1), 1PIT (40.0), 1QGB (48.6), 1ATA (57.7), 1Q7I (70.1), 1PI2 (98.0), 2PNE (100.0). The average APF values indicated by the *filled squares* correspond to $r = 6$ Å. The low error bar indicates the net decrease in APF as bulk solvent is approached when adopting $r = 8$ Å. Reprinted from [Fernández Stigliano A (2013) Breakdown of the Debye polarization ansatz at protein-water interfaces. J Chem Phys 138:225103], copyright 2013 with permission from AIP Publishing LLC

A significant correlation is established for PDB-reported proteins (Fig. 2.4) between the average APF ($[\theta]$) over all residues in a protein and the protein ratio Y of dehydrons-to-backbone-hydrogen-bonds. This correlation validates the assertion that dehydrons are the main structural motif promoting anomalous polarization. At $Y = 100$ %, the antifreeze protein from snow flea in PDB entry 2PNE [10], with its anomalously high APF-boosting activity, is a significant outlier. This enhanced effect can be understood based on the extreme solvent exposure of its dehydrons, promoting a local backbone-hydrated state that persists on a 100 ns timescale, compared with the ~ 1 ns lifetime of the hydrated state typical of the dehydrons present in the other proteins studied. Thus, the water-organizing power of the antifreeze protein is due to nanoscale confinement and supersedes the Debye polarization tendencies, introducing a major supra-nanoscale perturbation of the water structure, in accord with its purported function as a disruptor of the ice nucleation.

This connection between disruption of ice nucleation and anomalous polarization suggests mutational studies aimed at removing dehydrons by improving the backbone protection in antifreeze proteins. We predict that the removal of dehydrons by backbone-protective valine (V) substitutions of poor backbone protector residues (S, T, G) should significantly impair the antifreeze potency of the snow flea protein reported in PDB.2PNE.

2.3 Epistructural Physics Reveals a Chemical Functionality for the Aqueous Interface

This section carries the analysis of the dielectric structure of the aqueous interface one step further by showing that dehydrons not only promote protein associations but also functionalize interfacial water by inducing basicity at the interface. These packing defects confine interfacial water molecules turning them into proton acceptors. This result has profound ramifications for bioengineering and drug design as it implies that dehydrons are actually involved in chemical events, acting as stimulators of enzymatic activity.

To make notation more agile, we introduce aligned (Γ^{\parallel}) and orthogonal ($\Gamma^{\#}$) polarization-induced charges defined as

$$-\vec{\nabla} \cdot \vec{P}^{\parallel} = \Gamma^{\parallel}; -\vec{\nabla} \cdot \vec{P}^{\#} = \Gamma^{\#}; \Gamma^{\parallel} + \Gamma^{\#} = \Gamma \quad (2.5)$$

Departures from bulk water structure (spatially measured by $\nabla g \neq 0$) induce orthogonal polarization. Given the relation $\vec{P}^{\#} = \xi \vec{\nabla} g$, the Poisson equation $\vec{\nabla} \cdot \left(\vec{P}^{\#} \right) = \rho - \vec{\nabla} \cdot \left(\epsilon_0 \vec{E} + \vec{P}^{\parallel} \right)$ may be written in terms of the curvature $\nabla^2 g$ of the scalar field g :

$$\xi \nabla^2 g = -\Gamma^{\#} \quad (2.6)$$

Equation (2.6) incorporates the nanoscale structure of water within an electrostatic relation, revealing that the curvature of the scalar field g is a measure of the departure from linear dielectrics.

We also introduce the scalar field $\phi\left(\vec{r}\right) = 4 - g\left(\vec{r}\right)$ that quantifies the extent of distortion from bulk-like water structure, with $\phi = 0$ representing no distortion. To obtain a partial differential equation for ϕ we first note that at each position \vec{r} , the quotient $\Gamma^{\#}/\Gamma$ measures the local deviation from a Debye scenario where polarization fully aligns the protein field (Fig. 2.5). Thus, we expect that a relation of the form $\frac{\Gamma^{\#}}{\Gamma} = c\phi$ must hold, where c is a proportionality constant. This relation is indeed valid with $c = 0.191$ as shown in Fig. 2.6a. The quotient $\Gamma^{\#}/\Gamma$ is computed at protein/water interfaces as a time average over a 10 ns-period beyond equilibration of the protein structure with the solvent. Thus, the epistemic polarization \vec{P} for nine soluble natural proteins with structures reported in the Protein Data Bank (Table 2.1, Protocol in legend for Fig. 2.1) is computed along thermalization molecular dynamics trajectories. Each 10 ns-trajectory is generated using as starting point the equilibrated structural coordinates that result after thermalization of the

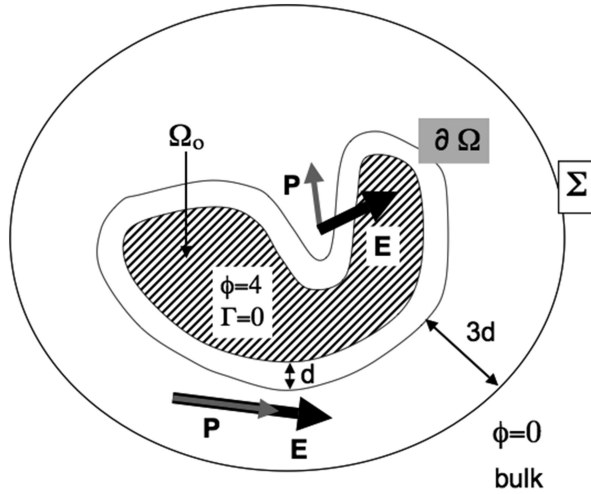


Fig. 2.5 Schematic depiction of the aqueous interface with boundary conditions that become constraints in determining the coarse-grained descriptor $\phi = 4 - g$ of water structure. The interface is defined by the water-smeared envelope $\partial\Omega$ of the solute. It is assumed that the solute surface has a topography endowed with nanoscale detail that may perturb the structure of interfacial water relative to bulk-like patterns. We get $\phi = 4$ (no water) in the “core” volume Ω_0 at distance $d = 2r = 8 \text{ \AA}$ from each point on $\partial\Omega$, while $\phi = 0$ for points at distance larger than $3d = 24 \text{ \AA}$ from $\partial\Omega$. The latter condition holds since $3d \gg 4$ water layers ($\sim 13\text{\AA}$) from the interface and hence in this region, water structure is assumed to have recovered its bulk-like pattern. The regions where the structure of interfacial water is relatively undistorted show an alignment between epistemic polarization \vec{P} and the electrostatic field \vec{E} , whereas regions of high structural distortion likely depart from the linear dielectrics picture, as reflected by a lack of alignment between the fields \vec{P} and \vec{E} . Reprinted from [1], copyright 2013 with permission from AIP Publishing LLC

PDB-reported structure immersed in a pre-equilibrated solvent bath. Simulations are performed within an isobaric/isothermal ensemble (1 atm, 298 K). A total of 100 interfacial solvent configurations, one per 100 ps along a 10 ns-thermalization trajectory, are used to compute the epistemic polarization quotient as a time average using the relation

$$\frac{\Gamma^\#}{\Gamma} = \frac{\rho - \vec{\nabla} \cdot \left[\epsilon_0 \vec{E} + \vec{P} \right]}{\rho - \vec{\nabla} \cdot \epsilon_0 \vec{E}} \quad (2.7)$$

To this end, we recorded charge distribution, internal field and polarization from the 100 snapshots that partition the 10 ns period in identical intervals.

The structure/solvent system is considered equilibrated at time t_0 if the RMSD of backbone atomic coordinates averaged over randomly chosen pairs of chain

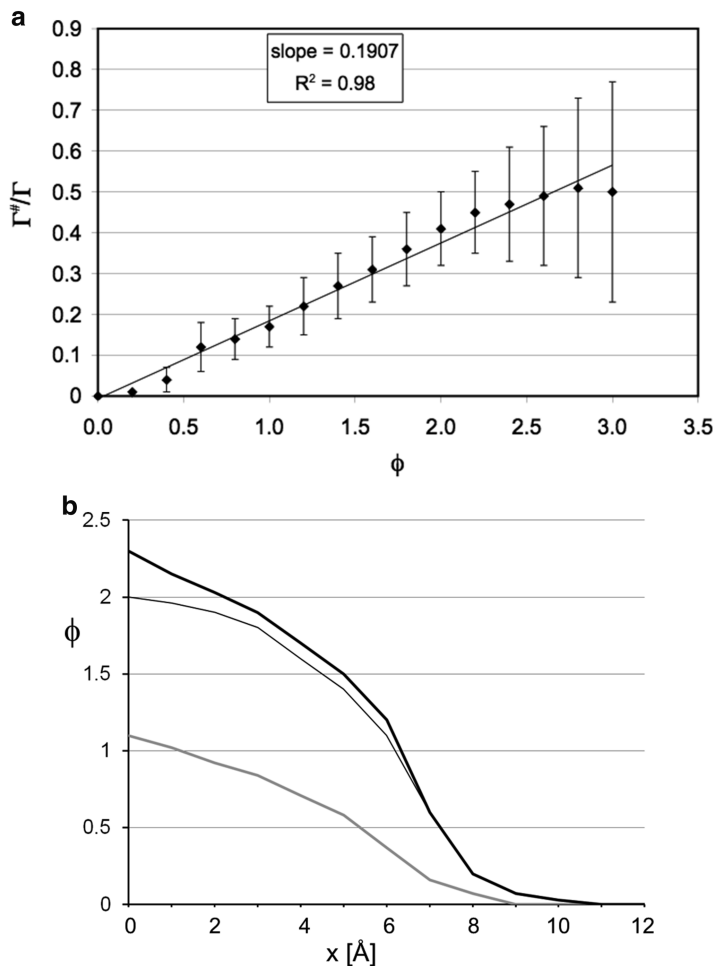


Fig. 2.6 (a) Local deviation from E -aligned polarization measured as $\Gamma^{\#}/\Gamma$ and plotted against the structural function $\phi = 4 - g$. The quotient $\Gamma^{\#}/\Gamma$ is evaluated at protein/water interfaces as a time average over an interval beyond protein structure/solvent equilibration. To determine $\Gamma^{\#}/\Gamma$, the epistemic polarization \vec{P} for nine soluble natural proteins with structures reported in the Protein Data Bank (Table 2.1) is computed and averaged for each position in space along a thermalization molecular dynamics trajectory. The region spanning a distance of $3d = 24$ Å from $\partial\Omega$ (Fig. 2.5) is exhaustively interrogated by covering it with disjoint spheres of radius 4 Å centered at points \vec{r}_n ($n = 1, 2, \dots$) to determine the set of water-structure values $\phi(\vec{r}_n)$. The trend line and correlation coefficient were obtained by linear regression. (b) Behavior of $\phi(x)$ relative to the distance x to an interface. The interface is assumed nonpolar, consisting of a concave region of fixed curvature radius θ , with $x = 0 =$ center of curvature. The flat surface represents the macroscopic limit $\theta \gg 1$ nm, and the ϕ -values are shown in the grey plot. Other perturbations were obtained for $\theta = 3$ Å (thin black plot), and $\theta = 2.5$ Å (thick black plot). Reprinted from [1], copyright 2013 with permission from AIP Publishing LLC

Table 2.1 PDB accession codes and free energy changes (ΔG) for the thermal denaturation of soluble monomeric proteins with reported structures

PDB accession code	ΔG (kJ/mol)	T (C)
1BSQ	46.46	40.00
1RTB	42.28	25.00
4LYZ	37.76	26.85
1CX1	22.52	24.85
1QG5	36.84	40.00
2AIT	28.05	25.00
3SSI	17.04	20.00
1HIC	21.01	25.00
1PMC	4.60	20.00

Fernández A (2012) Communication: Nanoscale electrostatic theory of epistuctural fields at the protein-water interface. *J Chem Phys* 137:231101, and references therein
The temperature (T) for thermal denaturation is indicated

conformations within a time interval $[t_0, t_0 + \tau]$ ($\tau \approx 1$ ns) is less than 1 Å. For all nine proteins in this study, this criterion was fulfilled for $t_0 = 500$ ps. Solvent and side-chain conformations continue to vary significantly (i.e. RMSD > 2.25 Å) on the 1 ns timescale.

By introducing the relation $\frac{\Gamma^\#}{\Gamma} = c\phi$, and defining the constant $k = c/\xi$, (2.6) becomes a linear homogeneous differential equation in $\phi(\vec{r})$ of the Schrödinger type:

$$-\nabla^2 \phi + k\Gamma \phi = 0 \quad (2.8)$$

The boundary conditions are described in Fig. 2.5 and are determined by the water-smear envelope $\partial\Omega$ of the solute-protein interface. Thus, $\phi = 4$ (no water) in the “core” volume Ω_0 at distance $d = 2r = 8$ Å from each point on $\partial\Omega$, while $\phi = 4$ for points at distance larger than $3d = 24$ Å from $\partial\Omega$. The latter condition holds since $3d \gg 4$ water layers (~ 13 Å) from the interface and hence in this region, water structure is assumed to have recovered its bulk-like tetrahedral pattern.

The behavior of $\phi = \phi(\vec{r})$ relative to the distance x to an interface is indicative of the propagation in space of the distortion of the water structure and is shown in Fig. 2.6b. The results were obtained by numerical integration of (2.8) for structural perturbations generated by confinement of water at the interface. To eliminate confounding factors, the interface was assumed nonpolar and physico-chemically featureless, consisting of a concave region of fixed curvature radius q , capable of partially confining water molecules, with $x = 0$ representing the center of curvature of the surface. The flat surface represents the macroscopic limit $\theta \gg 1$ nm, and the interfacial ϕ -value is expectedly close to 1 ($g \sim 3$), as revealed by the grey plot in Fig. 2.6b. Other perturbations were obtained for $\theta = 3$ Å (thin black plot), and $\theta = 2.5$ Å (thick black plot), generating $\phi(0)$ -values 2.01 and 2.30, respectively. In all cases, the matrix distortion decays to zero for $x > 11$ Å (less than four water

layers). No water molecule enters the cavity if doing so implies that the molecule retains on average less than 1.6 hydrogen bonds, making the range $\theta < 2.37$ Å, a forbidden region in real terms.

Equation (2.8) is the central result of this section and governs the interplay between episturctural polarization and the nanoscale structure of interfacial water.

We now compute the energy increment ΔU_ϕ associated with spanning a protein/water interface. This energy is in fact an elastic contribution stored in the distortion of water structure as shown in Chap. 1, with $\nabla\phi(\vec{r})$ measuring the local structural distortion at position \vec{r} and the elastic integrand of $\int \frac{1}{2}\lambda \left\| \vec{\nabla}\phi \right\|^2 d\vec{r}$ ($\lambda = 9.0$ mJ/m) quantifying the energetic contribution of spanning a differential region $d\vec{r}$ centered at point \vec{r} . Using Gauss' divergence theorem we obtain:

$$\Delta U_\phi = \int \frac{1}{2}\lambda \left\| \vec{\nabla}\phi \right\|^2 d\vec{r} = -\frac{1}{2}\lambda \int \phi \nabla^2 \phi d\vec{r} \quad (2.9)$$

Using (2.8), we can substitute the integrand in the r. h. s. of (2.9) obtaining the alternative expression for the interfacial elastic energy:

$$\Delta U_\phi = -\frac{1}{2}c(\lambda/\epsilon_0)^{1/2} \int \Gamma \phi^2 d\vec{r} \quad (2.10)$$

Since $\Delta U_\phi = \int \frac{1}{2}\lambda \left\| \vec{\nabla}\phi \right\|^2 d\vec{r} \geq 0$ ($\lambda > 0$), the r.h.s of (2.10) is a positive term, and thus (2.9) and (2.10) imply:

$$\int \Gamma \phi^2 d\vec{r} \leq 0. \quad (2.11)$$

Equation (2.11) implies that $\Gamma \leq 0$, that is, the polarization-induced charge is negative around dehydrons known to promote interfacial tension. This is a fundamental result of broad applicability and it establishes the following

Theorem *Interfacial water molecules tend to orient and organize in subnanoscale cavities leaving negative charges uncompensated ($\Gamma < 0$) when deprived of hydrogen-bonding opportunities ($\phi > 0$).*

This is a crucial result as it delineates the chemical basicity of dehydrons, or more properly, the dehydron-induced basicity of interfacial water. The theorem reveals that packing defects play a crucial role in enzyme catalysis and in biochemical events in general by functionalizing nucleophilic protein groups through dehydron-promoting proton accepting events. This type of catalytic stimulation requires that dehydrons be spatially close to catalytic nucleophilic groups and that the proton transfer event induced by the dehydron have a reasonable probability.

These striking aspects of the functionalization of biomolecular interfaces will be explored and ultimately established in Chap. 7.

The interfacial energy stored in the anomalous polarization or, equivalently, in the distortion of water structure, is readily evaluated using (2.10). This result has been contrasted against thermodynamic data on the spanning of aqueous interfaces with nanoscale detail. A suitable testing ground is provided by the aqueous interfaces for soluble monomeric proteins with a stable fold characterized by structural and thermodynamic information (Table 2.1) [1]. The reversible work performed on the system to span the protein-water interface is destabilizing of the native fold, thus facilitating thermal denaturation, and hence it should anticorrelate with the free energy change for protein denaturation, as it is indeed the case (cf. [1]). To compare interfacial thermodynamics with thermodynamic data on protein denaturation (Table 2.1), we introduce the entropic cost of solvent confinement at the interface $\Delta S_\phi = k_B \ln \left[\prod_{j=1}^L g_j / 4 \right]$, where k_B = Boltzmann constant, g_j = time averaged number of hydrogen bonds for the j th-water molecule (L = total number of water molecules), and the dummy index j labels molecules within $3d = 24 \text{ \AA}$ from the solvent-smeared envelope of the protein (Fig. 2.5). Note that $-T\Delta S_\phi \geq 0$ and reinforces the trend defined by ΔU_ϕ . The reversible work $\Delta G_\phi = \Delta U_\phi - T\Delta S_\phi \geq 0$ performed on the system to span the protein-water interface is destabilizing of the native fold, thus facilitating thermal denaturation.

To quantitatively assess the folding-destabilizing effects of spanning the interface of the folded protein, we examined the same soluble monomeric proteins used to generate the data in Fig. 2.6 (Table 2.1). In thermodynamic terms, protein denaturation is facilitated proportionally to the reversible work required to span the interface, attesting to the folding-destabilizing effect of interfacial tension arising from the structural distortion of surrounding water. Thus, the computed reversible work for creating the interface measures the extent to which the “protein structure is at odds with the structure of surrounding water” since it quantifies the distortion of water structure around the protein. The tight anticorrelation between interfacial free energy and the stability of the protein structure (Fig. 2.7) provides experimental support to the underlying equation (2.8) since it reveals that protein destabilization is commensurate with the thermodynamic cost of creating its interface with water, computed using (2.10). This observation prompts us to formulate the principle of minimal epistemic distortion (MED) that should govern conformational changes in the solute that generate concomitant changes in the interface. The validity of the MED principle in the context of protein folding is established in Chap. 3 that provides a semiempirical solution to the protein folding problem.

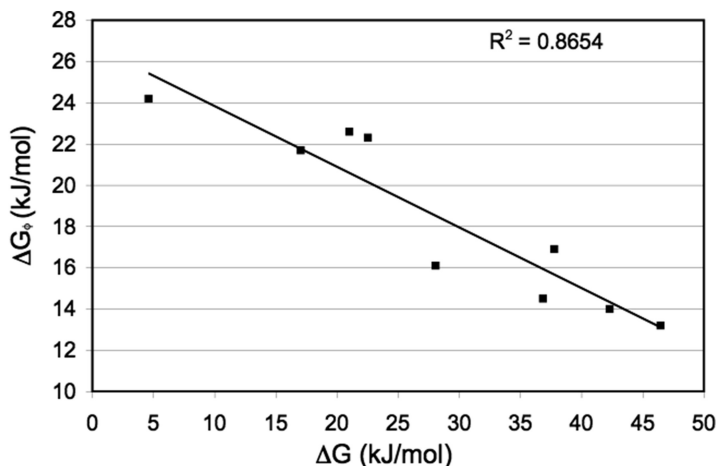


Fig. 2.7 Anticorrelation between reversible work required to span the protein-water interface, ΔG_ϕ , and free energy change for thermal denaturation, ΔG , for the nine monomeric soluble proteins indicated in Table 2.1. Reprinted from [1], copyright 2013 with permission from AIP Publishing LLC

2.4 Packing Defects as Catalytic Enablers

Enzyme catalysis is often viewed as a closed chapter where the core issues have already been dealt with [11, 12]. Yet, several open problems still stand on the way of progress in mechanistic understanding [13–15], and the design and optimization of enzyme catalysts [15–17] and drug-based enzyme inhibitors [17–19]. Especially opaque yet germane to these problems is the role of interfacial water in enzymatic reactions [13, 20, 21]. Because the protein aqueous interface is essentially sculpted by the protein structure [20, 21], the problem may be said to belong to the field of *epistructural biology*, as argued in Chap. 1. In this realm, as we have already noted, one structural feature of proteins stands out: the so-called *dehydron*, a packing defect that creates interfacial tension and thereby promotes protein associations that exclude surrounding water [21, 22]. Thus, the recently established fact that catalytic sites in enzymes are actually “decorated” with dehydrons [13] proves to be tale-telling and gives a significant spin to related biotechnologies. These observations will be properly delineated, expanded and validated in Chap. 7.

As implied by the theorem formulated in Sect. 2.3 (cf. [14]), besides promoting dehydration, dehydrons are also likely to be endowed with a biochemical role that may prove to be exquisitely complementary: they turn local interfacial water into a chemical base, a proton acceptor. Thus, if a catalytic group (hydroxyl in Ser, Thr or Tyr, thiol in Cys, amide in His) performs a nucleophilic attack on a substrate, the dehydrons nearby enhance its catalytic potential through a chemical functionalization of vicinal water that promotes deprotonation of the catalytic group.

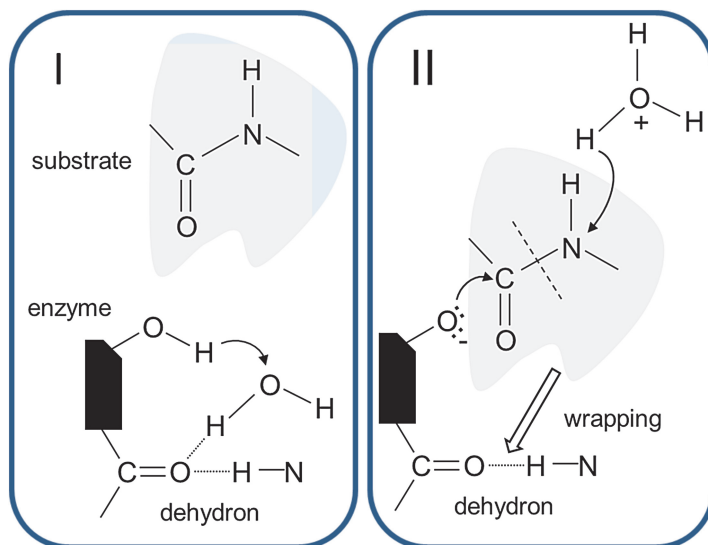


Fig. 2.8 Dehydron as two-stroke molecular engine sustaining enzyme catalysis by (a) functionalizing nano-confined water around the catalytic group and (b) promoting enzyme-substrate association, thereby correcting the nanoscale packing defect

The dual functional and interactive role of dehydrons combined with the fact that they are ubiquitously found at catalytic sites suggest a dual participation in catalysis: *first, dehydrons prepare the solvent for enzyme activity and, once the enzymatic nucleophilicity is enhanced and the solvent turns into a better leaving group (as hydronium), dehydrons promote enzyme-substrate association in consonance with their dehydration propensity.* This duality of functionality and interactivity makes dehydrons both enablers and stimulators of enzyme catalysis, an observation with paramount biotechnological implications, especially in regards to what we may term “epistucture-based enzyme design”.

In more rigorous terms, as dehydrons activate nearby catalytic groups to perform a chemical (nucleophilic) attack on the substrate, causing trans-esterification, they turn the local water into hydronium (H_3O^+ , a product of proton acceptance). In turn, the hydronium is easily removed from the active since it requires further hydration, thereby enabling enzyme-substrate association. This association process entails the exogenous “wrapping” of the dehydron, which is tantamount to the intermolecular correction of the structural defect [22]. Thus, *the dehydron may be regarded as a two-stroke molecular engine that agonizes and enables enzyme catalysis*, as described in Fig. 2.8.

This discovery heralds the advent of novel biomolecular design based on “dehydron enablers-stimulators” that may be created or removed through engineered mutations directed to fine-tune the protein structure. This finding makes it possible to activate or silence a catalytic site in a protein enzyme by respectively creating or annihilating a nearby dehydron through a change in the

chemical composition of the protein. On the other hand, novel drug-based enzyme inhibitors will emerge as dehydron enablers-agonists are targeted through engineered protein-drug associations [19], as described in Chap. 10.

The newly established participation of dehydrons in enzymatic reactions will likely invite an extensive revision of the biochemical mechanistic literature, while novel molecular designs inspired by “epistructural catalytic stimulation” are expected to herald a new era in the optimization of enzyme catalysts and pharmaceuticals.

2.5 A Quantum Theory of Interfacial Tension and Its Experimental Verification

The complexities of the protein-water interface preclude us at this time from attempting the implementation of a quantum mechanical approach to study interfacial water frustration and the polarization effects resulting thereby. Thus, we shall content ourselves with studying the simplest nonpolar aqueous interfaces using a quantum approach. Be as it may, we are keenly aware that the results may be one day extrapolated *mutatis-mutandis* to the study of realistic biological interfaces.

Interfacial water on a nonpolar surface remains a subject of intense scrutiny due to its relevance in delineating the molecular forces that steer protein folding [1] and molecular associations [20], and drive biotechnological innovation [20, 23–26]. The spontaneous negative charging of such interfaces through transference of hydroxide ions (OH^-) from bulk water has been established [27–29], yet the topic still remains a subject of contention [25, 27, 29]. No first-principle inference of spontaneous interfacial charging has so far generated quantitative predictions amenable of experimental verification [28]. In this regard, the treatment of interfacial water through molecular dynamics (MD) appears to yield contradictory results, even predictions of acidic (proton donating) interfaces [27, 30]. This controversy stimulates the fundamental approach taken in this section, which involves a substantive revision of Debye’s dielectric ansatz [24]. The latter would predict no polarization-induced charge since there is no intrinsic electrostatic field to speak of. Thus, we may state that the spontaneous charging of an aqueous nonpolar interface poses a challenge to the standard dielectric picture, demanding a reworking of the Debye ansatz starting from first principles.

In this section we argue that experimentally verifiable quantitative predictions of spontaneous negative charging of aqueous hydrophobic interfaces require a general treatment of interfacial water dielectrics that cannot be subject to the constraints imposed by Debye’s ansatz regarding polarization alignment [1, 31] but needs to be further enriched by incorporating quantum effects. The first conceptual departure is essential to include the anomalous polarization component that arises as a result of hydrogen-bond frustration at the contact region between the two bulk phases. This revision could not have been reasonably envisioned at the time Debye’s linear

dielectric ansatz was formulated since details on water hydrogen-bonding structure were unknown [24]. As shown previously in this chapter and in Chap. 1, the non-Debye contribution generates interfacial tension that is stored as electrostatic energy, a tension shown in this section to be mitigated by the adsorption of hydroxide ions. To assess the level of interfacial frustration of hydroxide ions we need to incorporate quantum effects. By frustration in this context we refer to a hydrogen bonding opportunity that cannot materialize because the proton acceptor or nucleophilic group is not physically present, as it is the case with the water layer in contact with the nonpolar surface.

As previously noted, the departure from the Debye linear dielectric *ansatz* becomes imperative because no charge $\gamma = -\nabla \cdot \mathbf{P}$ may be generated by water polarization (\mathbf{P}) if the latter is made proportional to the vanishing electrostatic field \mathbf{E} at the nonpolar surface, as it is the case with the Debye assumption $\mathbf{P} = (\epsilon - \epsilon_0)\mathbf{E}$, where ϵ, ϵ_0 denote dielectric and vacuum permittivity, respectively.

To predict quantitatively the spontaneous charging of the aqueous nonpolar interface, the more general dielectric scheme presented in Chap. 1 is required. The hydrogen-bond frustration of interfacial water, described by the scalar field $\phi = \phi(\mathbf{r})$, with $\phi(\mathbf{r}) =$ expected number of unfulfilled hydrogen bonds for a water molecule at position \mathbf{r} , generates a non-Debye polarization component $\mathbf{P}^\# = \mathbf{P} - (\mathbf{P} \cdot \mathbf{e})\mathbf{e}$ ($\mathbf{e} = \mathbf{E}/\|\mathbf{E}\|$). In turn, this “orthogonal” polarization induces a net charge $\gamma^\# = -\nabla \cdot \mathbf{P}^\#$. In previous work [1, 31] we showed that distortions from bulk structure cause $\mathbf{P}^\#$ to be proportional to the frustration gradient, according to the equilibrium relation

$$\mathbf{P}^\# = -\xi \nabla \phi \quad (2.12)$$

The generation of net charge, and thereby of polarization, arises from the hydrogen-bond frustration at the interface (2.12) that leaves partial charges on the water atoms untitrated. The lack of interfacial charge neutralization implies that interfacial tension, which by definition arises from hydrogen-bond frustration, is also stored electrostatically in the anomalous polarization [1, 24]. Equation (2.12) will be subsequently corroborated in the context of this study. From (2.12), the net charge $\gamma^\#$ induced by $\mathbf{P}^\#$ becomes $\gamma^\# = \xi \nabla^2 \phi$. By making interfacial tension proportional to electrostatic energy, the non-Debye treatment enables the computation of the net charge induced by the frustration at the water layer in contact with the nonpolar surface, and hence becomes adequate to predict quantitatively the spontaneous charging of the interface.

For simplicity, we consider water in contact with a featureless slab on nonpolar nonpolarizable material typically realized by hydrogenated graphene [29]. The frustration or net loss of hydrogen bonding opportunities at the hydrophobic interface generates interfacial tension quantifying the disruption of the bulk-like tetrahedral hydrogen-bond coordination of water, as shown in Chap. 1 [1, 20, 31]. The extent of frustration of a water molecule at a liquid/solid interface is expected to be higher than that of a hydroxide ion (OH^-) since the donated proton in

the latter chemical species is expected to have a lower electrophilicity than the water proton, and therefore is expected to be less prone to form hydrogen bonds with a nearby nucleophile [29]. This conjecture will be corroborated in this work and is based on the fact that the hydroxide oxygen is less electrophilic than the water oxygen, since the former gained an electron while the latter shares an electron pair with a hydrogen atom. The argument leads us to postulate that the interfacial tension is lowered by adsorption of hydroxide ions or, in other words, the spontaneous negative charging of the nonpolar interface is expected to result from the relief of interfacial tension.

Given the above-noted observations, a quantum parameter, ζ , will be subsumed into the MD computations scaling a term accounting for interfacial energy in order to quantify the degree of frustration of interfacial hydroxide relative to interfacial water. The relative hydrogen bond frustration is given by the quotient $\zeta = \zeta(\text{OH}^-/\text{H}_2\text{O}) = F_h/F_w = 0.021/0.179 = 0.117 \ (\pm 0.017)$, where F_h , F_w are the expected net quantum electrostatic fields (in $\text{e}/\text{\AA}^2$ units) at the position or probe site of a putative hydrogen-bonding nucleophile (lone electron pair) that acts as putative acceptor of the proton donated by OH^- and H_2O , respectively [29]. The fields were obtained by molecular projection of the delocalized quantum charges in the aqueous condensed phase [32] along the direction of proton donation for hydrogen bonding [29]. The authors in [29] implemented a Car-Parrinello molecular dynamics scheme in a Wannier representation of the condensed phase [32], thus generating a unique set of maximally localized Wannier functions that realized the solid-state equivalent of localized molecular orbitals.

Based on the estimation of the quantum parameter ζ given above, we may assert that *the extent of frustration due to an unfulfilled hydrogen bond in the hydroxide ion at a nonpolar interface is 11.7% of the hydrogen-bond frustration of water with a dangling OH group*. Conversely, $\zeta(\text{H}_3\text{O}^+/\text{H}_2\text{O}) = 1.922 \ (\pm 0.102)$, (cf. [29]) implying that hydronium adsorption would entail almost twice as much frustration than water at the interface. To summarize, mitigation of interfacial tension dictates that hydroxide ion—and not hydronium—must be preferentially adsorbed.

To predict the spontaneous charging of the hydrophobic interface, we first incorporate into the MD simulations the interfacial energy per unit volume, Δu_ζ , associated with spanning a water interface that envelops a solid non-polarizable hydrophobic slab of macroscopic dimensions. The energy-density term Δu_ζ quantifies the distortion of the water hydrogen-bond matrix and is therefore given by the elastic integral [1]:

$$\Delta u_\zeta = (1/2)\lambda \int ||\nabla\phi||^2 d\mathbf{r}, \quad (2.13)$$

where, as indicated previously, $\phi = \phi(\mathbf{r})$ denotes hydrogen-bond frustration of the solvent and the integration is carried over a spatial domain Ω large enough so that its border $\partial\Omega$ is fully contained in bulk water, where the following conditions are satisfied: $\phi(\mathbf{r}) = 0$, $\nabla\phi(\mathbf{r}) = 0 \ \forall r \in \partial\Omega$. To describe the local distortion of water

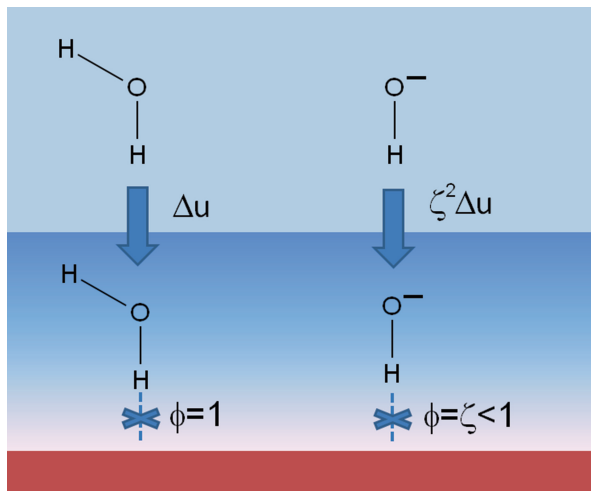


Fig. 2.9 Scheme of interfacial tension quenching due to hydroxide ion adsorption onto a nonpolar aqueous interface. The interfacial region is represented with color gradient, bulk water is in *solid blue* and the nonpolar slab is in *brown*. The frustrated hydrogen-bonding opportunity is symbolized by a *crossed dotted line*. The level of frustration at the interface with the nonpolar slab is reduced by a factor ζ (< 1) upon hydroxide ion adsorption, while the interfacial energy density change associated with transference from the bulk to the interface is reduced by a factor ζ^2 due to the square dependence on the frustration gradient of the elastic integrand in (2.13)

structure at spatial location \mathbf{r} relative to the bulk hydrogen-bond pattern, we introduce the scalar field $\phi = \phi(\mathbf{r})$ that *in this context* represents the expected frustration of a water molecule or a hydroxide ion while any of them visits a sphere of radius $r = 4 \text{ \AA}$ centered at position \mathbf{r} for a 1 ps-timespan [1, 20, 31]. If the proton donation of the hydroxide ion leading to hydrogen bonding is frustrated due the absence of a proton acceptor, we get $\phi = \zeta$ (< 1) while the frustration of water hydrogen bonding yields $\phi = 1$.

In essence, we compute the interfacial tension arising from the hydrogen bonding frustration of interfacial water, and take into account the fact that this tension is minimized by the adsorbed hydroxide ions, whose dangling donated proton is 88.3 % less frustrated than the water counterpart (Fig. 2.9). Our theoretical treatment may be benchmarked and validated, as done in this work, since progress in spectroscopic methods based on second-order nonlinear optics enable direct examination of water structure at the interface [28].

The quantum-mechanical parameter ζ is thus incorporated in the MD simulations to account for the reduction in the frustration levels on the first layer directly in contact with the nonpolar surface due to hydroxide ion adsorption.

To validate (2.12), we observe that this equation dictates that the interfacial tension is actually stored at equilibrium as the non-Debye polarization energy density since

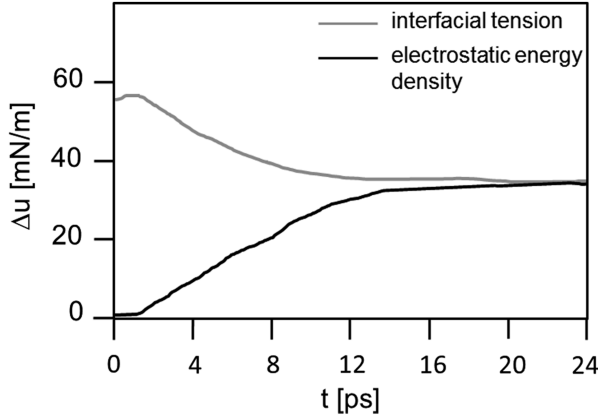


Fig. 2.10 Convergence of the interfacial tension (Eq. 2.13) and non-Debye electrostatic energy density as equilibrium is reached. The curves displayed are averages over 10 MD 24 ps-trajectories for water dynamics within an isobaric/isothermal ensemble (1 atm, 298 K) with 10,160 water molecules and a total of 160 ions, with 80 OH^- and 80 H^+ ($[\text{OH}^-] = 0.4 \text{ M}$) packed against a featureless layer of hydrogenated graphene. The MD 24 ps-trajectories for water dynamics are generated as described in [1] except for the incorporation in the potential energy of an interfacial term to account for hydrogen bond frustration at the nonpolar region of contact. The solvent bath provides at least four water layers of solvent envelope with water molecules extending 18 Å from the graphene surface. The evaluation of the interfacial tension-generating frustration incorporates a quantum mechanical parameter ζ describing the relative electrophilicity of the donated hydroxide proton relative to the water proton, a measure of the hydrogen bonding propensity of the dangling O-H bond in hydroxide relative to water

$$\Delta u_\zeta = (1/2)\lambda \int ||\nabla\phi||^2 d\mathbf{r} = (1/2)\epsilon_0^{-1} \int \mathbf{P}^{\#2} d\mathbf{r} \quad (2.14)$$

The direct MD computation of the time evolution of the electrostatic energy density and interfacial tension has been performed by incorporating the global term Δu_ζ in the potential energy stepwise evaluation. The convergence of the two quantities has been observed (Fig. 2.10), thus validating the equilibrium relation presented in (2.12).

Since the surface integral $\oint [\phi \nabla \phi] \cdot d\sigma$ over $\partial\Omega$ vanishes as per the choice of Ω , integration of (2.13) by parts yields the usual relations, now specialized for an ionic solution at the interface:

$$0 \leq \Delta u_\zeta = -(1/2)\lambda \int \phi \nabla^2 \phi d\mathbf{r} = -(1/2)(\lambda/\epsilon_0)^{1/2} \int \gamma^\# \phi d\mathbf{r} \quad (2.15)$$

Since $\phi \geq 0$, we obtain:

$$\gamma_{\text{MV}}^{\#} = -(\lambda\epsilon_0)^{1/2} \int ||\nabla\phi||^2 d\mathbf{r} / \int \phi d\mathbf{r} \leq 0, \quad (2.16)$$

where $\gamma_{\text{MV}}^{\#}$ is the mean-value induced charge per unit volume. We have then proven the following *theorem*:

An aqueous featureless nonpolar interface causes hydrogen-bond frustration which is mitigated by hydroxide anion adsorption generating a net negative charge at the interface that may be estimated at $\gamma_{\text{MV}}^{\#}$.

Thus, the net induced charge at the interface results from the adsorption of the negatively charged hydroxide ions. The net charge was estimated according to (2.16), requiring the construction of the field $\{\phi(\mathbf{r}), \nabla\phi(\mathbf{r})\}$ [1, 31] within an NPT-statistical ensemble for a featureless hydrophobic interface in the limit of infinite curvature. Thus, the calculation of $\gamma_{\text{MV}}^{\#}$ following (2.16) yields $[\text{OH}^-]^{\#} = \gamma_{\text{MV}}^{\#}/Y = 15.4 \text{ mM}$, where $[\text{OH}^-]^{\#}$ = hydroxyl molar concentration at interface and Y is the net charge per mole of hydroxyl ion. Thus, at pH 7 (neutral solution) we get the equilibrium constant

$$K = [\text{OH}^-]^{\#}/[\text{OH}^-] = 153,832 \quad (2.17)$$

Let $M = [\text{H}_2\text{O}] \gg [\text{OH}^-]$ be the molar concentration of “free adsorption sites” $\approx 55.5 \text{ mole/liter}$ at $T = 298 \text{ K}$, then $K = M^{-1} \exp(-\Delta G/RT)$, where ΔG is the free energy change associated with the transference of a hydroxide ion from bulk water to the interface. Thus, our predicted value $\Delta G = -40.02 \text{ kJ/mole}$ compares satisfactorily within experimental error with the experimental value -45 kJ/mole obtained from the Langmuir adsorption isotherm monitored by phase-sensitive sum-frequency vibrational spectroscopy [28].

The spontaneous negative charging of the aqueous interface sustained at a flat featureless nonpolar surface is a fact that challenges and hence motivates a substantive revision of Debye’s dielectric picture, whereby water polarization aligns with the intrinsic electrostatic field. This is so simply because the latter vanishes in the case under study. Thus, the hydrogen bonding frustration of water at a nonpolar interface generates a non-Debye polarization component that stores electrostatic energy as interfacial tension. As shown in this section, the reduction of the interfacial tension is caused by a net adsorption of hydroxide ions resulting in a spontaneous negative charging of the interface. The adsorption of the hydroxide ion is favored on quantum mechanical grounds as its hydrogen bond frustration at the interface is 88.3 % lower than that of water. The quantum mechanical basis for the induced interfacial effect within a non-Debye dielectric picture is quantitatively validated vis-à-vis surface measurements using second-order nonlinear optics.

The spontaneous negative charging of aqueous nonpolar interfaces has eluded quantitative first-principle prediction. We showed that quantitative prediction required a reworking of Debye’s linear dielectric *ansatz* to incorporate non-Debye electrostatic energy stored as interfacial tension. To gain interfacial stability, the minimization of interfacial tension becomes operative promoting a reduction in hydrogen-bond frustration. The latter takes place upon hydroxide ion

adsorption, as the quantum theory of interfacial tension predicts. The quantitative predictions were validated vis-à-vis experimental measurements of hydroxide adsorption uptake.

2.6 Problems

2.1. Using Gauss' divergence theorem, prove the following relation:

$$\Delta U_\phi = \int \frac{1}{2} \lambda \left\| \vec{\nabla} \phi \right\|^2 d\vec{r} = -\frac{1}{2} \lambda \int \phi \nabla^2 \phi d\vec{r}$$

2.2. Water promotes the sealing of nanoscale packing defects in proteins. This is hinted by the fact that during protein folding, a minimization of the departure from the Debye alignment is observed. How does the solvent promote the structure sealing? Assume a flexible chain that seeks to minimize interfacial free energy.

2.3. Using the protocol provided in this chapter, thermalize the monomeric soluble proteins with PDB-reported structure identified by entries 1SRL, 1ESR, 1A8O, 1PIT, 1QGB, 1ATA, 1Q7I, 1PI2, 2PNE, and validate the following empirical relation indicated in Chap. 1 concerning the density fluctuations:

$$P(N = 0) = e^{-\left[\frac{N^2}{2\sigma^2}\right]} = 1 - g/4, \sigma^2 = \left\langle (N - \langle N \rangle)^2 \right\rangle.$$

2.4. Assume you are able to monitor the extent of frustration of an interfacial water molecule at location and time t at any time along a trajectory in phase space. Provide an expression for the frequency-dependent orthogonal dielectric coefficient [15–17].

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