Nanoscale thermodynamics of biological interfacial tension

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The interfacial tension of biological water, a promoter of biomolecular interactions, is difficult to determine because of the inhomogeneous nanoscale patterns that make up the surface of biomolecules. These patterns modulate solubility in peculiar manners, enabling specific associations while preventing phase separation or precipitation. In this work, we derive de novo the nanoscale thermodynamics associated with the creation of biological interfaces and validate the results against experimentally identified complex interfaces. Interfacial tension is shown to be generated by hot spots of red-shifted dielectric relaxation. The most common spots involve hindered polar hydration. Taken collectively, these patches contribute more to the interfacial tension than the better known non-polar cavities with nanometre curvature, where our results agree with the established length-scale dependence of hydrophobicity. The thermodynamic results are validated by showing that the inferred patches of interfacial tension actually promote biomolecular associations.

Keywords: biomolecular interfaces; molecular biophysics; interfacial tension; nanoscale thermodynamics

1. Introduction

In spite of considerable progress (Gerstein & Chothia 1996; Lum et al. 1999; Despa et al. 2004; Ashbaugh & Pratt 2006; Giovambattista et al. 2008), the physical nature of biological–water interfaces remains elusive, hindering our understanding of biomolecular associations (Clackson et al. 1998; Levy et al. 2006) and function (Fenimore et al. 2004; Levy & Onuchic 2004; Frauenfelder et al. 2009). Inhomogeneous patterns of nanoscale confinement at protein–water (P–W) interfaces make it difficult to delineate statistical thermodynamics concepts, such as interfacial tension, insightful when dealing with the separation of homogeneous phases (Stillinger 1973; Rowlinson & Widom 1982). Thus, in cellular contexts, water solubility becomes a complex property, as solutes associate or even aggregate controllably to reduce interfacial tension, but they do not precipitate or create a phase separation. Therefore,

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspa.2010.0269 or via http://rspa.royalsocietypublishing.org.
A thermodynamic analysis of biological interfacial tension becomes essential to physically rationalize cellular phenomenology but requires a dielectric theory holding at nanoscale dimensions.

In this work, we determine biological interfacial tension based on a first principles treatment of nanoscale dielectric responses and curvature-dependent water fugacities. Strikingly, while hydrophobic regions with nanometre curvature (Ashbaugh & Pratt 2006) obviously contribute to the interfacial tension, our results reveal that polar groups with partially hindered polar hydration (PHPH) collectively generate the most significant contribution. In fact, the highest interfacial tension over all soluble proteins with the reported structure is shown to result exclusively from hindered polar hydration and is actually generated by neurotoxins with sub-picomolar affinity for potassium channels (MacKinnon et al. 1998). Finally, our results are validated by showing that patches of interfacial tension between protein and water actually promote protein associations.

2. Nanoscale thermodynamics of the biological interface

As shown below, the polarization response and fugacity of interfacial water are key nanoscale parameters needed to determine, respectively, the enthalpic and entropic contribution to interfacial tension. The enthalpy term can be estimated by introducing a measure of interfacial water confinement given by a position \( \mathbf{r} \)-dependent parameter \( \Gamma = \Gamma(\mathbf{r}) \), the expected number of hydrogen bond partnerships of a water molecule at position \( \mathbf{r} \). Thus, the solvent-accessible envelope of the protein surface (Street & Mayo 1998; Zhang et al. 2004) may be covered by a minimal set \( \mathcal{W} \) of water-confining osculating (first-order contact) spheres \( D_j \), \( j \in \mathcal{W} \), each with its average value \( \Gamma_j \), which depends on the radius \( \theta = \theta_j \) of \( D_j \) (the curvature radius of the protein envelope) and the physico-chemical nature of protein group(s) in the contact region (figure 1).

To determine the entropic cost of creating nanoscale biological interfaces, a fugacity \( f_j \) is introduced and defined as \( f_j = N_j / N_j(b) \), where \( N_j \) is the expected number of water molecules in \( D_j \) at equilibrium and \( N_j(b) \) is the number associated with the same volume of bulk solvent. Thus, the chemical potential \( \mu_j \) of water in \( D_j \) becomes \( \mu_j = k_B T \ln[N_j/N_j(b)] \) (where \( k_B \) is the Boltzmann constant). The \( \theta \)-dependence of \( \Gamma \) and \( f \)-values (figure 1) are obtained at equilibrium within a particle number–pressure–temperature ensemble, as described in the caption to figure 1. The computations start with the protein structure embedded in a pre-equilibrated cell of water molecules. A non-redundant exhaustive set of 2661 reported structures of monomeric proteins (see the electronic supplementary material) was used to generate the data.

To determine the \( \Gamma \)-dependence of water polarization \( \mathbf{P} = \mathbf{P}(\mathbf{r}) \), we adopt the Fourier-conjugate frequency space (\( \nu \)-space) and represent the dipole correlation kernel \( K_p(\boldsymbol{\nu}) \) and the electrostatic field \( \mathbf{E} = \mathbf{E}(\mathbf{r}) \) in this space to enable a treatment valid at all length scales. In contrast with other treatments (Schutz & Warshel 2001), we note that \( \mathbf{P} \) and \( \mathbf{E} \) are indeed proportional, but the proportionality constant is actually \( \nu \)-dependent (Debye 1929; Scott et al. 2004). Thus, in \( \nu \)-space, we get

\[
F(\mathbf{P})(\boldsymbol{\nu}) = K_p(\boldsymbol{\nu})F(\mathbf{E})(\boldsymbol{\nu}), \tag{2.1}
\]

Figure 1. Scheme and plots illustrating the dependence of nanoscale confinement parameters $\Gamma$ and $f$ on the curvature radius $\theta$ of the osculating sphere at the P–W interface. First-order contacts with a polar (exposed backbone HB) or non-polar patch on the protein surface are treated individually. Negative $\theta$-values indicate convex surfaces, while positive values indicate solvent cavities. Flat interfaces correspond to $\theta = \infty$. The $\Gamma$ and $f$ values plotted, grey contour and solid black line, respectively, are equilibrium averages obtained from an exhaustive dataset of 2661 monomeric proteins with Protein Data Bank (PDB)-reported structure (see the electronic supplementary material). To determine the dependence of nanoscale parameters $\Gamma$ and $f$ on the local curvature radius $\theta$ of the solvent-accessible envelope of the protein surface (Lum et al. 1999; Ashbaugh & Pratt 2006), the latter was covered by a minimal set $W$ of disjoint water-confining osculating (first-order contact) spheres $D_j, j \in W$. The dynamics and equilibrium parameters for local hydration patterns within each osculating sphere were obtained from classical trajectories generated by molecular dynamics (MD). The computations started with the PDB structure embedded in a pre-equilibrated cell of explicitly represented water molecules and counterions (Jorgensen et al. 1983; Rizzo & Jorgensen 1999). The PDB structures for an exhaustive set of 2661 monomeric uncomplexed proteins (see the electronic supplementary material) lacking prosthetic groups were used in the computations. The MD trajectories were generated by adopting an integration time step of 2 fs in a particle number–pressure–temperature ensemble with box size $10^3$ nm$^3$ and periodic boundary conditions (Lindahl et al. 2001). The box size was calibrated so that the solvation shell extended at least 10 Å from the protein surface at all times. The long-range electrostatics were treated using the particle-mesh Ewald summation method (Darden et al. 1993). A Nose–Hoover thermostat (Hoover 1985) was used to maintain the temperature at 300 K, and a TIP3P water model with optimized potential for liquid simulations force field was adopted (Rizzo & Jorgensen 1999). A barostat scheme was maintained through a dedicated routine with the pressure held constant at 1 atm using a weak-coupling algorithm (Berendsen et al. 1984). After 300 ns equilibration, average water hydrogen bond partnerships and fugacity were determined in each osculating sphere for each of the 2661 proteins in the database. The $\theta$-dependence of $\Gamma$ and $f$ was established by computing average values $[\Gamma]_\theta$, $[f]_\theta$ of the nanoscale parameters over all osculating spheres with fixed curvature radius (at 1/2 Å resolution). Low dispersions in the parameters were obtained for $\theta$-values ($\Delta\Gamma < 0.12[\Gamma]_\theta$, $\Delta f < 0.10[\Gamma]_\theta$ for polar moieties and $\Delta\Gamma < 0.15[\Gamma]_\theta$, $\Delta f < 0.12[\Gamma]_\theta$ for non-polar moieties), justifying average estimations.
where $F$ denotes three-dimensional Fourier transform $F(g)(\mathbf{v}) = (2\pi)^{-3/2} \int e^{i\mathbf{v} \cdot \mathbf{r}} g(\mathbf{r}) d\mathbf{r}$ and the kernel $K_p(\mathbf{v})$ is the Lorentzian $K_p(\mathbf{v}) = (\epsilon_b - \epsilon_0)/(1 + (\tau c)^2|\mathbf{v}|^2)$, with the dielectric relaxation scale $\tau_c \approx 3$ cm for $\tau = \tau_b \approx 100$ ps, $c$ the speed of light, $\epsilon_b$ the bulk permittivity and $\epsilon_0$ the vacuum permittivity. Since $P(\mathbf{r})$ satisfies the Debye relation $\nabla \cdot (\epsilon_0 \mathbf{E} + \mathbf{P})(\mathbf{r}) = \rho(\mathbf{r})$ (where $\rho(\mathbf{r})$ is the charge density), equation (2.1) yields the following equation in $\mathbf{r}$-space:

$$\nabla \cdot \left[ F^{-1}(K)(\mathbf{r} - \mathbf{r}') \mathbf{E}(\mathbf{r}') d\mathbf{r}' \right] = \rho(\mathbf{r}),$$

(2.2)

with $K(\mathbf{v}) = \epsilon_0 + K_p(\mathbf{v})$ and $F^{-1}(K)(\mathbf{r}) = (2\pi)^{-3/2} \int e^{-i\mathbf{v} \cdot \mathbf{r}} K(\mathbf{v}) d\mathbf{v}$. The convolution $\int F^{-1}(K)(\mathbf{r} - \mathbf{r}') \mathbf{E}(\mathbf{r}') d\mathbf{r}'$ captures the correlation of the dipoles with the electrostatic field. Note that equation (2.2) is not the Poisson–Boltzmann equation, which requires a proportionality between the fields $\mathbf{E}$ and $\mathbf{P}$ under the ad hoc assumption $K(\mathbf{v}) \equiv \text{const.}$ (Schutz & Warshel 2001).

Upon water confinement, the dielectric relaxation undergoes a frequency red shift arising from the reduction in hydrogen bond partnerships that translates in a reduction in dipole orientation possibilities. Thus, $\tau = \tau_b \exp(B(\Gamma)/k_B T)$, where the kinetic barrier $B(\Gamma) = -k_B T \ln(\Gamma/4)$ yields $\tau = \tau_b (\Gamma/4)^{-1}$. To obtain the polarization as a $\Gamma(\mathbf{r})$-dependent parameter, we assume a generic charge density distribution

$$\rho(\mathbf{r}) = \sum_{j \in W} \sum_{n \in M(j)} 4\pi q_n \delta(\mathbf{r} - \mathbf{r}_n),$$

(2.3)

where $M(j)$ denotes the set of charges labelled by index $n$, localized on the protein surface and interfacing with $D_j$, and $\mathbf{r}_n$ is the position vector for charge $n$. Then, the polarization may be obtained by integrating equation (2.2) (see the electronic supplementary material), yielding

$$P(\mathbf{r}) = \int F^{-1}(K_p)(\mathbf{r} - \mathbf{r}') \mathbf{E}(\mathbf{r}') d\mathbf{r}'$$

$$= (2\pi)^{-3} \sum_{j \in W} \sum_{n \in M(j)} \left[ d\mathbf{r}' F^{-1}(K_p(j))(\mathbf{r} - \mathbf{r}') \nabla_{\mathbf{r}'} \int d\mathbf{v} e^{-i\mathbf{v} \cdot (\mathbf{r}' - \mathbf{r}_n)} 4\pi q_n |\mathbf{v}|^2 K(j)(\mathbf{v}) \right]^{-1},$$

(2.4)

with $K(j)(\mathbf{v}) = \epsilon_0 + K_p(j)(\mathbf{v}) = \epsilon_0 + (\epsilon_b - \epsilon_0)/(1 + (\tau_j c)^2|\mathbf{v}|^2)$, $\tau_j = \tau_b (\Gamma_j/4)^{-1}$ and $F^{-1}(K_p(j))(\mathbf{r}) = \int d\mathbf{v} e^{-i\mathbf{v} \cdot \mathbf{r}} (\epsilon_b - \epsilon_0)/(1 + (\tau_j c)^2|\mathbf{v}|^2)$.

Thus, the free-energy change $\Delta G_{ij} = \sum_{j \in W} \Delta H_j - T \Delta S_j$ associated with creating the $W$–$\bar{W}$ interface is

$$\Delta G_{ij} = \sum_{j \in W} \left[ -\frac{1}{2} \int |P(j)(\mathbf{r})|^2 d\mathbf{r} - T \Delta S_j \right]$$

$$= -\left(\frac{1}{2}\right) \sum_{j \in W} \int d\mathbf{r} \left(2\pi)^{-3} \sum_{n \in M(j)} \left[ d\mathbf{r}' F^{-1}(K_p(j))(\mathbf{r} - \mathbf{r}') \nabla_{\mathbf{r}'} \right. \right.$$

$$\times \left. \int d\mathbf{v} e^{-i\mathbf{v} \cdot (\mathbf{r}' - \mathbf{r}_n)} 4\pi q_n |\mathbf{v}|^2 K(j)(\mathbf{v}) \right]^{-1} \left. - \sum_{j \in W} k_B T C_b N_j (4/3) \pi \theta_j^3 \ln (\Gamma_j/4) \right].$$

(2.5)
Since \( N_j = c_b N f_j (4/3) \pi \theta_j^3 \) (\( c_b = 55.5 \text{ mol}/(10^{-3} \text{ m}^3) \) = bulk water concentration and \( N = 6.023 \times 10^{23} \text{ mol}^{-1} = \text{Avogadro’s number} \)), the last term in equation (2.5) represents the sum of entropic contributions (\(- \sum_{j \in W} T \Delta S_j\)), where the \( j^{th} \) term in the sum is associated with transferring \( N_j \) water molecules from bulk solvent (\( \Gamma = 4 \)) to the interfacial osculating sphere \( D_j \) (with \( \Gamma = \Gamma_j \)). Thus, we get the relation

\[-T \Delta S_j = -k_B T c_b N f_j \frac{4}{3} \pi \theta_j^3 \ln \frac{\Gamma_j}{4} \geq 0.\]

We now take into account the asymptotic behaviour for a non-polar interface in the macroscopic limit \( q/1 \text{ nm} \to \infty \) at \( T = 298 \text{ K} \) (figure 1): \( G \to 3 \exp(-l/\theta) \to 3(\lambda = 1.56 \text{ nm}) \) and \( f \to 1 \). This limit behaviour yields for a non-polar interfacial patch

\[\Delta G_{ij} \approx -T \Delta S = -[55.5 \text{ mol}/(10^{-3} \text{ m}^3)] \times (2.47 \times 10^6 \text{ mJ mol}^{-1}) \left[ \frac{4}{3} \pi \theta^3 \right] \left[ \left(-\frac{1.56 \times 10^{-9} \text{ m}}{\theta} \right) - 0.288 \right] = (71.9 \text{ mJ m}^{-2})(4\pi \theta^2) + O[\theta^3] \approx \gamma(4\pi \theta^2) + O[\theta^3],\]

(2.6)

where \( \gamma \) is the macroscopic surface tension of water at \( T = 298 \text{ K} \). Thus, our treatment of the interfacial tension for non-polar interfacial patches (\( P = 0 \)) is consistent with alternative treatments of length scale-dependent hydrophobicity (Lum et al. 1999; Ashbaugh & Pratt 2006).

3. Hot spots of biological interfacial tension

We now unravel the nature of interfacial hot spots. Interfacial tension arises in \( D_j \) when \( \Delta G_j > 0 \). The most common patches generating interfacial tension involve PHPH, that is, backbone hydrogen bonds of the protein that are partially exposed to water so that the amide and carbonyl are hydrated by low-density water (figure 2). Thus, the most common osculating spheres contact polar patches arising from incomplete shielding of the backbone. These spheres constitute approximately 70 per cent of the interfacial spheres that fulfill \( \Delta G_j > 0 \) in the database. They are described by the ranges \( \theta_j = 2.3 \pm 0.4 \text{ Å}, \Gamma_j = 1.2 \pm 0.2 \) and \( f_j = 0.75 \pm 0.1 \) (figure 1), yielding a red-shifted dipole relaxation \( \tau_j = (3.42 \pm 0.58)\tau_b \), with \(-T \Delta S_j = (2.48 \pm 0.45)k_B T \) and \( \Delta G_j = (1.54 \pm 0.40)k_B T \).

Since \( \Gamma \) and \( f \) remain invariant for \( \theta \geq 7 \text{ Å} \), interfacial polar regions with curvature radius \( \theta > 7 \text{ Å} \) are covered with disjoint spheres with \( \theta_j = 7 \text{ Å} \) (figure 1). At \( \theta_j = 7 \text{ Å} \), we get \( \Gamma_j = 4(\Delta S_j = 0) \); hence, these osculating spheres generate no interfacial tension. By contrast, non-polar interfacial regions with \( \theta \geq 7 \text{ Å} \) may be also covered with disjoint spheres of radius \( \theta_j = 7 \text{ Å} \) (the corresponding values \( \Gamma = 3 \) and \( f = 1 \) remain invariant for \( \theta_j \geq 7 \text{ Å} \), but, in this case, these regions yield the highest interfacial tension at \( \Delta G_j \approx -T \Delta S_j = -k_B T c_b N f(4/3)\pi \theta^3 \times 10^{-30} \text{ m}^3 \ln(3/4) = 13.17k_B T \). The same contribution is associated with each disjoint \( \theta = 7 \text{ Å} \) sphere covering convex regions (\( \theta < -2.7 \text{ Å} \)). By contrast, the
Figure 2. Illustration of the poor hydration of a polar group, a common contributor to P–W interfacial tension. Poor hydration of a solvent-exposed carbonyl (greater than C=0) from an incompletely water-sequestered backbone hydrogen bond pairing residues Arg277 and Arg280 (Arg = arginine) in the p53 DNA-binding domain (PDB.2GEQ). The interfacial water molecule hydrogen bonding the carbonyl has $\Gamma = 3$. The backbone is displayed in ribbon representation and the side chains for Arg277 and Arg280 are shown.

‘clathrate’ range $0 \geq \theta \geq -2.7$ Å, where non-polar moieties can be hydrated while preserving the tetrahedral hydrogen bond lattice ($\Gamma = 4$), does not generate tension, $\Delta S = 0$, as expected.

The monomeric protein with the reported structure generating the highest interfacial tension ($\gamma = 9.05$ mJ m$^{-2}$; see the electronic supplementary material) is the neurotoxin with PDB accession 1QUZ, a protein with sub-picomolar affinity for the Kv1.3 potassium channel (MacKinnon et al. 1998). Strikingly, this protein does not present hydrophobic patches, and its surface tension results exclusively from PHPP, with 100 per cent of its backbone hydrogen bonds partially exposed to water (figure 3).

To validate our results, we examined 28 protein complexes (table 1) with defined contact topologies (Levy et al. 2006). The total area of the protein–protein (P–P) interfaces for each complex was computed (Street & Mayo 1998; Zhang et al. 2004) after identification of the residues in each subunit that are engaged in intermolecular contacts. On the other hand, the P–W interfacial tension of each free subunit was computed by numerical integration of equation (2.5) with charge and atomic radii assigned using the program PDB.2PQR (Dolinsky et al. 2004). We defined filtered sets $W_n = \{ j \in W : \Delta G_j \geq nk_BT \}$ ($n = 1, 2, \ldots$) of contributors to the P–W interfacial tension in the free subunits ($W_n \subset W_{n'}$ for $n > n'$), and $S_n$, the total P–W interfaces associated with $W_n$. In all 28 complexes examined, the total P–P interfaces distributed among the subunits within the complex was 100 per cent contained in $S_2$, and it was only $60 \pm 7\%$ contained in $S_3$. 

Figure 3. PHPH spots in proteins. (a) Protein backbone of human ubiquitin (PDB.1UBI) with buried backbone hydrogen bonds represented as grey segments joining the α-carbons of the paired residues and solvent-exposed backbone hydrogen bonds producing PHPH spots shown in green. (b) Ribbon representation of human ubiquitin. (c) PHPH spots for the potassium channel neurotoxin with PDB.1QUZ, amounting to 100% of its backbone hydrogen bonds. (d) Ribbon rendering of the neurotoxin, the highest producer of interfacial tension of all monomeric proteins with reported structure (see the electronic supplementary material). (b) $\gamma = 4.02 \text{ mJ m}^{-2}; \Delta G = 77.10 \text{ kJ mol}^{-1}$. (d) $\gamma = 9.05 \text{ mJ m}^{-2}; \Delta G = 113.84 \text{ kJ mol}^{-1}$.

These results suggest that interfacial solvent cavities spanned at a thermodynamic cost $\Delta G \geq 2k_B T$ are the promoters of protein associations. This conclusion is confirmed by the tight correlation ($R^2 = 0.83$) between $A_2$, the surface area of $S_2$, and the total P–P interfacial area of each complex (figure 4). This correlation becomes weaker for $S_1$ with area $A_1$ ($R^2 = 0.60$) and nearly vanishes when we consider (as control) the total solvent-exposed surface area of free subunits.

4. Discussion

Whether ephemeral or obligatory, biomolecular associations are recognized as central events in the recruitment of molecular machinery required to carry out cellular processes. Since such associations take place in aqueous media, they depend pivotally on the interfacial tension generated by the constitutive subunits. This thermodynamic attribute is most difficult to determine in a biological
Figure 4. Biological interfacial tension promotes biomolecular associations. Total area of the surfaces that generate P–W interfacial tension in the free subunits (uncomplexed state) plotted against the total P–P interfacial area for 28 protein complexes (table 1). Filled diamonds, $A_1$; filled triangles, $A_2$; open squares, solvent-exposed surface area.

Table 1. PDB entries for 28 protein complexes with representative topologies of inter-subunit contacts. The dataset is used to unravel the role of P–W interfacial tension patches as promoters of protein associations. In the topological representation of a complex, a ball represents a protein subunit and a stick between two balls represents a P–P interface. Numbers in brackets denote per cent decrease in overall interfacial tension upon complexation starting with free subunits. A representative set from the 2661 monomeric proteins (single-dot topology) used to generate figure 1 (see the electronic supplementary material) is also included.
context owing to the inhomogeneous nanoscale patterns that modulate the solubility of biomolecules. Thus, interfacial tension arises in biological solutes and promotes specific associations or even controlled aggregation, but prevents precipitation or phase separation.

This work introduces a nanoscale thermodynamic treatment of biological interfacial tension, relying pivotally on a de novo dielectric theory that holds for water confined at nanoscale and sub-nanoscale dimensions. This confined interfacial water is appropriately endowed with a curvature-dependent fugacity and our results are consistent with the length scale-dependent hydrophobicities independently obtained beyond the nanometre limit.

Experimental identification of the interfaces of protein complexes validated the nanoscale thermodynamics derivation of interfacial tension and upheld the view that this attribute is a selective promoter of biomolecular associations.

References


