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Communication: Chemical functionality of interfacial water enveloping nanoscale structural defects in proteins

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Building upon a non-Debye multiscale treatment of water dielectrics, this work reveals the biochemical role of interfacial water enveloping nanoscale structural defects in soluble proteins, asserting its role as a chemical base. This quasi-reactant status is already implied by the significant concentration of structural defects in the vicinity of an enzymatically active site, delineating their role as promoters or enhancers of catalytic activity. © *2014 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4882895]

The dielectric behavior of *episteric* ("around the solid") water in soluble proteins and in other chemically complex solutes endowed with nanometer-level detail is a subject of intense scrutiny.[1–4] This is mostly due to its pivotal importance in nanotechnologies[5,6] and in protein interactions understood in the broadest sense.[7] The understanding and manipulation of such interfaces require a multiscale approach to water dielectrics, an unsettled matter at this time.[2,4,8,9] As recently argued, such an approach demands a conceptual departure from the Debye ansatz,[2] that in this context has been taken to mean that water polarization $\mathbf{P}$ aligns with the internal electrostatic field $\mathbf{E}$ of the solute: $\mathbf{P} = \mathbf{P}^{\|}$, $\mathbf{P}^{\|}$ = Debye polarization component. In contrast with the bulk hydrogen-bond pattern, water molecules under nanoscale confinement are forced to relinquish hydrogen bonding opportunities to fit in the nanometer cavity, generating a net polarization component $\mathbf{P}^{\#}$ that is orthogonal and statistically independent of $\mathbf{E}$.[2] While the Debye polarization introduces the well-known rescaling (screening) of the inherent solute charge,[10] a consequence of the extreme resilience of water structure to variations in the electrostatic field,[5] the orthogonal polarization component induces an $\mathbf{E}$-independent net charge $\gamma^{\#} = -\nabla.\mathbf{P}^{\#}$. The latter component is of paramount importance in assessing chemical functionalities related to topographical features of protein interfaces as it generates charges that are not accounted for by the protein chemical composition. As shown in this work, this net induced charge endows nano-confined water with a chemical functionality in accord with the sign of $\gamma^{\#}$, i.e., proton donor if $\gamma^{\#} > 0$ and proton acceptor if $\gamma^{\#} < 0$. Thus, the inequality $\gamma^{\#} > 0$ indicates that at least one water hydroxyl proton is on average forced by the interface geometry to relinquish its hydrogen-bonding capability, while $\gamma^{\#} < 0$ implies that at least one water oxygen atom remains on average unpaired to a hydroxyl proton.

Building on a non-Debye treatment of the water interface,[2] we identify the episteric chemical functionalities arising from nanoscale structural defects in soluble proteins and other topographically/chemically complex solutes and establish their role as promoters or enhancers of catalytic activity.

To identify the chemical role of interfacial water subject to partial nanoscale confinement, we first define the physical framework of relevance. Thus, we introduce a scalar field $\phi = \phi(\mathbf{r})$ that quantifies on average the level of local distortion of water structure at spatial location $\mathbf{r}$ relative to the bulk hydrogen-bond pattern.[2] The field $\phi(\mathbf{r})$ is defined as $\phi(\mathbf{r}) = 4 - \mathrm{g}(\mathbf{r})$, where $\mathrm{g}(\mathbf{r}) \leq 4$ is the time-averaged number of hydrogen bonds (coordination number) that a water molecule sustains while it visits a sphere of radius r = 4 Å centered at position $\mathbf{r}$ for a minimum timespan $\tau = 1$ ns. The choice of temporal and spatial averaging parameters has been justified by the need to operate with a smooth scalar field $\phi = \phi(\mathbf{r})$.[2] Distortions from bulk-like structure generate a non-Debye polarization component $\mathbf{P}^{\#}$ proportional to the distortion gradient, according to the relation $\mathbf{P}^{\#} = -\xi\nabla\phi$, where $\xi = (\lambda\varepsilon_o)^{1/2}$ and $\lambda = 9.0$ mJ/m at 298 K.[2] The net charge $\gamma^{\#}$ induced by $\mathbf{P}^{\#}$ is then $\gamma^{\#} = -\nabla.\mathbf{P}^{\#} = \xi\nabla^2\phi$.

Within this framework, interfacial tension arises wherever $\phi > 0$ and the interfacial energy, $\Delta \mathrm{U}_\phi$, associated with spanning the solute-water interface is given by the elastic integral[2]

$$\Delta \mathrm{U}_\phi = (1/2)\lambda \int \|\nabla\phi\|^2 \, d\mathbf{r}, \tag{1}$$

where the integration is carried over a spatial domain Ω large enough so that its border $\partial\Omega$ is fully contained in bulk water. Thus, Ω is subject to the condition: $\{\phi(\mathbf{r}) = 0$ and $\nabla\phi(\mathbf{r}) = 0\}$ $\forall \mathbf{r} \in \partial\Omega$.

Integration of the elastic integral (Eq. (1)) by parts yields

$$0 \leq \Delta \mathrm{U}_\phi = -(1/2)\lambda \int \phi\nabla^2\phi d\mathbf{r}$$

$$= -(1/2)(\lambda/\varepsilon_o)^{1/2} \int \gamma^{\#}\phi d\mathbf{r} \tag{2}$$

since the close surface integral $\oiint[\phi\nabla\phi]\cdot d\boldsymbol{\sigma}$ vanishes on $\partial\Omega$. Since $\phi \geq 0$, the mean value theorem of integral calculus yields

$$\gamma^{\#}{}_{\mathrm{MV}} = -(\lambda\varepsilon_o)^{1/2} \int \|\nabla\phi\|^2 d\mathbf{r} / \int \phi d\mathbf{r} \leq 0, \tag{3}$$

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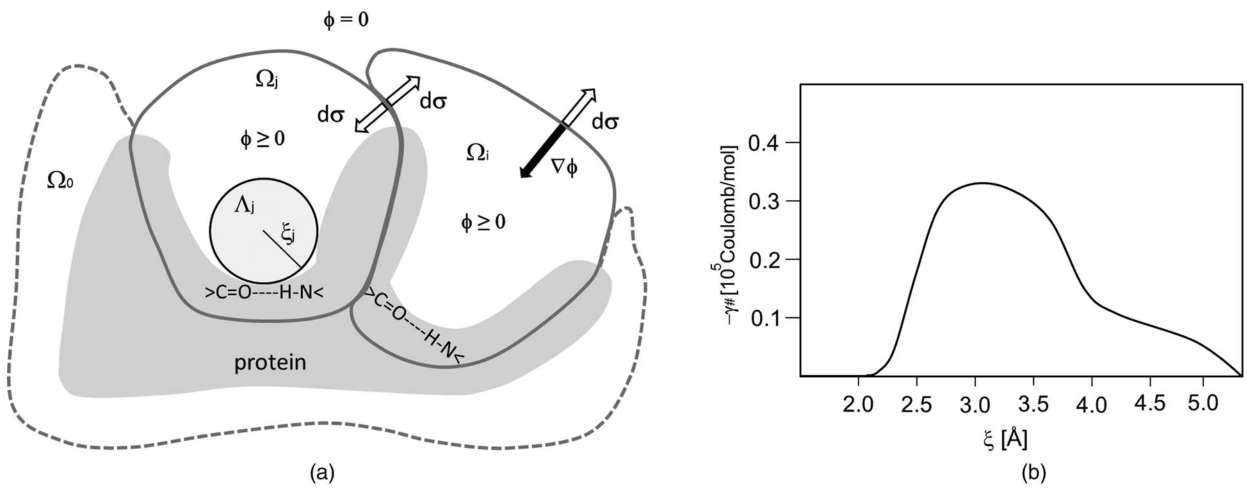

FIG. 1. (a) Schematic representation of the partition of domain Ω into subdomains $\{\Omega_n\}_{n=0,1,\ldots}$ where a single subdomain with $n > 0$ is required to contain each ($\phi > 0$)-dehydron in the protein structure and Ω_0 = closure of $[\Omega/\bigcup_{n=1,\ldots}\Omega_n]$. The generic subdomain Ω_j contains the amide-carbonyl dehydron *j* and the set $\Lambda_j = \Lambda_j(\xi_j)$, the first-order contact (osculating) sphere of the water-smeared envelope of the protein surface at dehydron *j* with local curvature radius ξ_j. (b) Net non-Debye polarization-induced charge $\gamma^{\#}$ as a function of the local curvature radius ξ at a dehydron site. The charge units for $\gamma^{\#}$ are scaled by the proton charge 1.6×10^{-19} C or $\sim 10^5$ C/mol.

where $\gamma^{\#}{}_{MV}$ is the mean-value constant yielding: $\gamma^{\#}{}_{MV}\int\phi d\mathbf{r} = \int\gamma^{\#}\phi d\mathbf{r}$.

We have then proven the following theorem: *Interfacial water under local nanoscale confinement with* $\phi \geq 0$ *yields a non-Debye polarization-induced charge* $\gamma^{\#}{}_{MV} \leq 0$. *The net negative charge begets a proton-acceptor role compatible with unfulfilled hydrogen-bond coordination of water oxygen (where partial negative charge is located), turning the local interfacial water into a chemical base*.

As previously noted, the interfacial tension in proteins is generated by nanoscale structural defects known as *dehydrons*, consisting of backbone amide-carbonyl hydrogen bonds partially exposed to the solvent.[7,11] While the physicochemical properties of dehydrons have been characterized in previous work,[2,7,11] their chemical role has not been assessed so far. To determine the chemical functionality of individual dehydrons in the protein structure, or rather of the interfacial water enveloping them, we consider a covering of the spatial domain Ω made up of closed convex subsets Ω_j j = 0,1,2,... fulfilling the following conditions (cf. Fig. 1(a)):

(1) $\Omega = \bigcup_{j=0,1,\ldots}\Omega_j$
(2) For $i \neq j$, $\Omega_i \cap \Omega_j = \partial\Omega_i \cap \partial\Omega_j$ (closed subsets only overlap along their borders).
(3) Let the dummy index j = 1,... label the dehydrons in the protein structure, then the subset Ω_j j = 1,2,... contains dehydron *j* and also satisfies $\Lambda_j = \Lambda_j(\xi_j) \subset \Omega_j$, where the set Λ_j is the osculating (first-order contact) sphere of the water-smeared envelope of the protein surface at dehydron *j* with local curvature ξ_j.[12]
(4) $\forall j = 0,1,\ldots$ and $\forall\mathbf{r} \in \partial\Omega_j$, one of the two conditions is fulfilled:
 a. $\{\phi(\mathbf{r}) = 0$ and $\nabla\phi(\mathbf{r}) = 0\}$ or
 b. if $\{\phi(\mathbf{r}) \neq 0$ or $\nabla\phi(\mathbf{r}) \neq 0\}$ $\exists\Omega_i$: $\mathbf{r} \in \partial\Omega_i \cap \partial\Omega_j$, and the respective differential area normal vectors $d\boldsymbol{\sigma}(\mathbf{r})$ at $\mathbf{r}$ on the two subsets cancel each other out (cf. Fig. 1(a)).

Given conditions 1–4, the vanishing surface integral $\oiint[\phi\nabla\phi]\cdot d\boldsymbol{\sigma}$ extending over $\partial\Omega$ may be written as

$$0 = \oiint[\phi\nabla\phi]\cdot d\boldsymbol{\sigma} = \sum\nolimits_{j=0,1,\ldots}\oiint[\phi\nabla\phi]\cdot d\boldsymbol{\sigma}_j, \quad (4)$$

where $\oiint[\phi\nabla\phi]\cdot d\boldsymbol{\sigma}_j$ denotes the surface integral extended over $\partial\Omega_j$. Thus, we obtain

$$0 \leq \Delta U_\phi = (1/2)\sum\nolimits_{j=1,\ldots}\left\{\lambda\oiint[\phi\nabla\phi]\cdot d\boldsymbol{\sigma}_j - (\lambda/\varepsilon_o)^{1/2}\gamma^{\#}{}_{MV}(j)\int\phi d\mathbf{r}_j\right\}, \quad (5)$$

where the integral $\int\phi d\mathbf{r}_j \geq 0$ extends over Ω_j and the mean value constant $\gamma^{\#}{}_{MV}(j)$ satisfies the relation: $\gamma^{\#}{}_{MV}(j)\int\phi d\mathbf{r}_j = \int\gamma^{\#}\phi d\mathbf{r}_j$. Thus, the individual contribution of dehydron *j* to the protein interfacial tension is

$$0 \leq (1/2)\lambda\oiint[\phi\nabla\phi]\cdot d\boldsymbol{\sigma}_j - (1/2)(\lambda/\varepsilon_o)^{1/2}\gamma^{\#}{}_{MV}(j)\int\phi d\mathbf{r}_j. \quad (6)$$

Since either $\nabla\phi(\mathbf{r}) = 0$ or the vectors $\nabla\phi(\mathbf{r})$ and $d\boldsymbol{\sigma}_j(\mathbf{r})$ point in opposite directions for $\mathbf{r} \in \partial\Omega_j$ (cf. Fig. 1(a)), the following inequality holds for all j's:

$$\oiint[\phi\nabla\phi]\cdot d\boldsymbol{\sigma}_j \leq 0. \quad (7)$$

Thus, combining Eqs. (6) and (7), it follows that $\gamma^{\#}{}_{MV}(j) \leq 0$. Thus, we have proven the following result:

As a generator of interfacial tension, a dehydron yields a negative non-Debye polarization-induced charge. Thus, interfacial water enveloping a dehydron constitutes a chemical base.

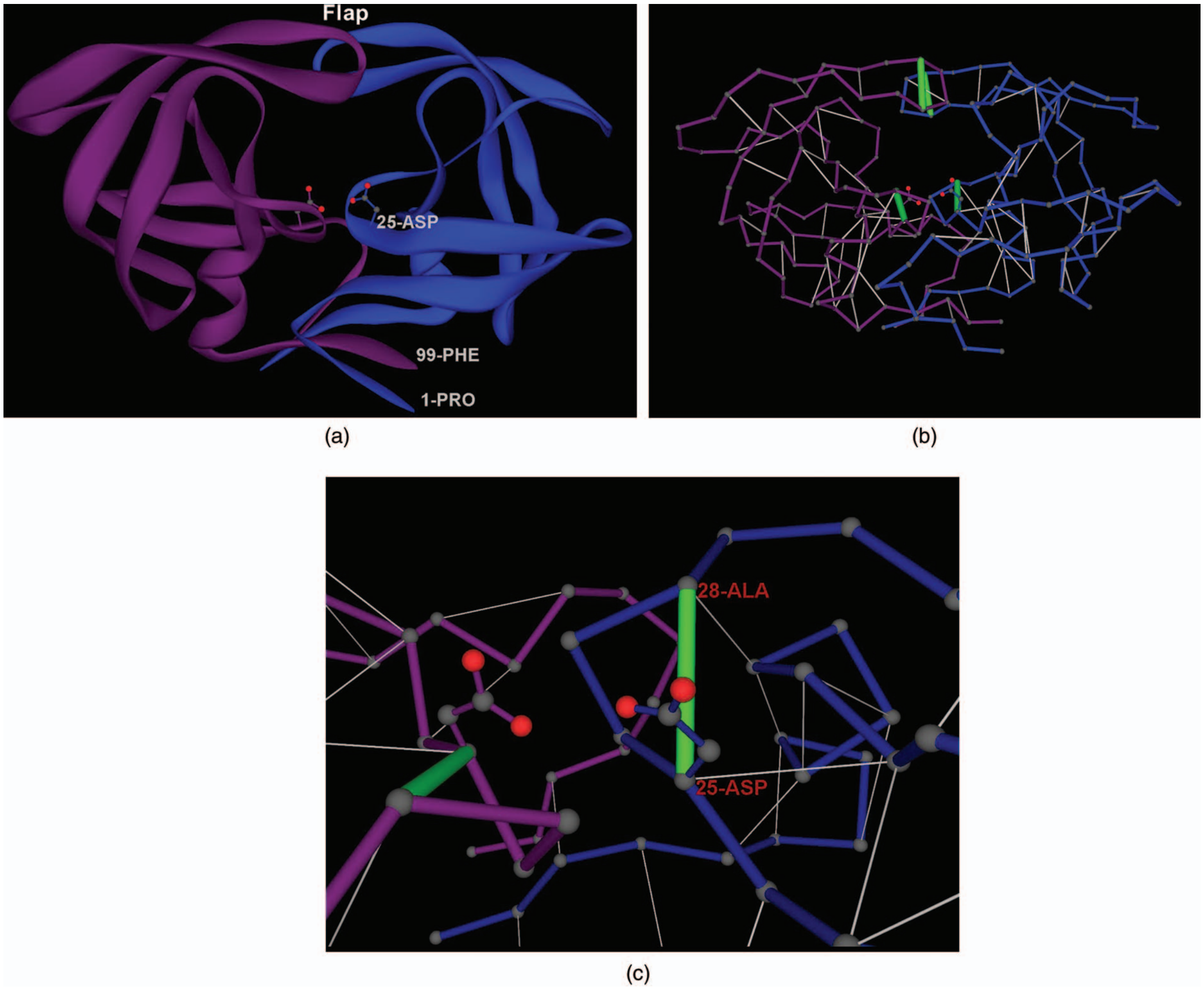

FIG. 2. (a) Ribbon representation of the functional homodimeric HIV-1 protease (PDB.4DJP), with active site Asp25 with displayed side chain. The monomeric chains in the complex are depicted in magenta and blue, respectively. (b) Dehydron distribution in HIV-protease. Dehydrons are shown as green segments joining the α-carbons of the paired residues, while solvent-shielded (well wrapped) backbone hydrogen bonds are shown in grey. (c) The ($\phi > 0$)-dehydron Asp25-Ala28 flanking the active site Asp25 in the dimeric HIV-1 protease.

From Eq. (4) it follows that the net contribution to interfacial energy from dehydron j is simply: $(1/2)\lambda\int\|\nabla\phi\|^2 d\mathbf{r}_j = -(1/2)(\lambda/\varepsilon_o)^{1/2}\gamma^{\#}{}_{MV}(j)\int\phi d\mathbf{r}_j$, yielding the net non-Debye polarization-induced charge

$$\gamma^{\#}{}_{MV}(j) = -(\lambda\varepsilon_o)^{1/2}\int\|\nabla\phi\|^2\, d\mathbf{r}_j \Big/ \int\phi d\mathbf{r}_j. \qquad (8)$$

The disruption of bulk water structure quantified by the field $\phi(\mathbf{r})$, $\nabla\phi(\mathbf{r})$ is computed using the molecular dynamics protocol previously described for dehydron cavities of variable curvature radius $\xi = 1-6$ Å.[2,12] The results are integrated according to Eq. (8) to yield a net non-Debye polarization-induced charge $\gamma^{\#}{}_{MV}(j)$ shown in Fig. 1(b).

The basicity of dehydrons, or rather of their enveloping aqueous interface, is in consonance with their abundance near catalytically active side chains involved in nucleophilic attacks in enzymatic reactions, as hereby shown. We may assert that the concentration of ($\phi > 0$)-dehydrons around side chains involved in intermolecular transesterification is indicative of their proton-acceptor role as promoters or enhancers of active-site nucleophilicity. Dehydrons vicinal to the catalytic site stabilize the polarized deprotonated state that empowers nucleophilic activity.

To become acquainted with this chemical picture, we first examine the dehydron distribution of the aspartic (Asp) protease from HIV-1 virus (PDB.4DJP),[13] a functionally competent homodimer. Each monomer within the complex has two dehydrons located in the vicinity of the active site (Asp25) and at the highly flexible flap (Fig. 2), with a single ($\phi > 0$)-dehydron involving the pair Asp25-Ala28. "Vicinal" is hereby defined as being within 6 Å of the α-carbon (desolvation domain of a dehydron-paired residue)[7,11] of the catalytically active residue. Examination of an exhaustive nonredundant set of 198 Asp proteases with PDB representation at resolution better than 1.5 Å and relational Uniprot[14] sequence annotation reveals the same localization pattern of ($\phi > 0$)-dehydrons found exclusively in the vicinity of the active site.

A similar localized concentration of ($\phi > 0$)-dehydrons is identified at phosphorylation sites that involve the participation of a nucleophilic group (usually hydroxyl and less frequently histidine amide) from a side chain (Ser, Thr, Tyr,

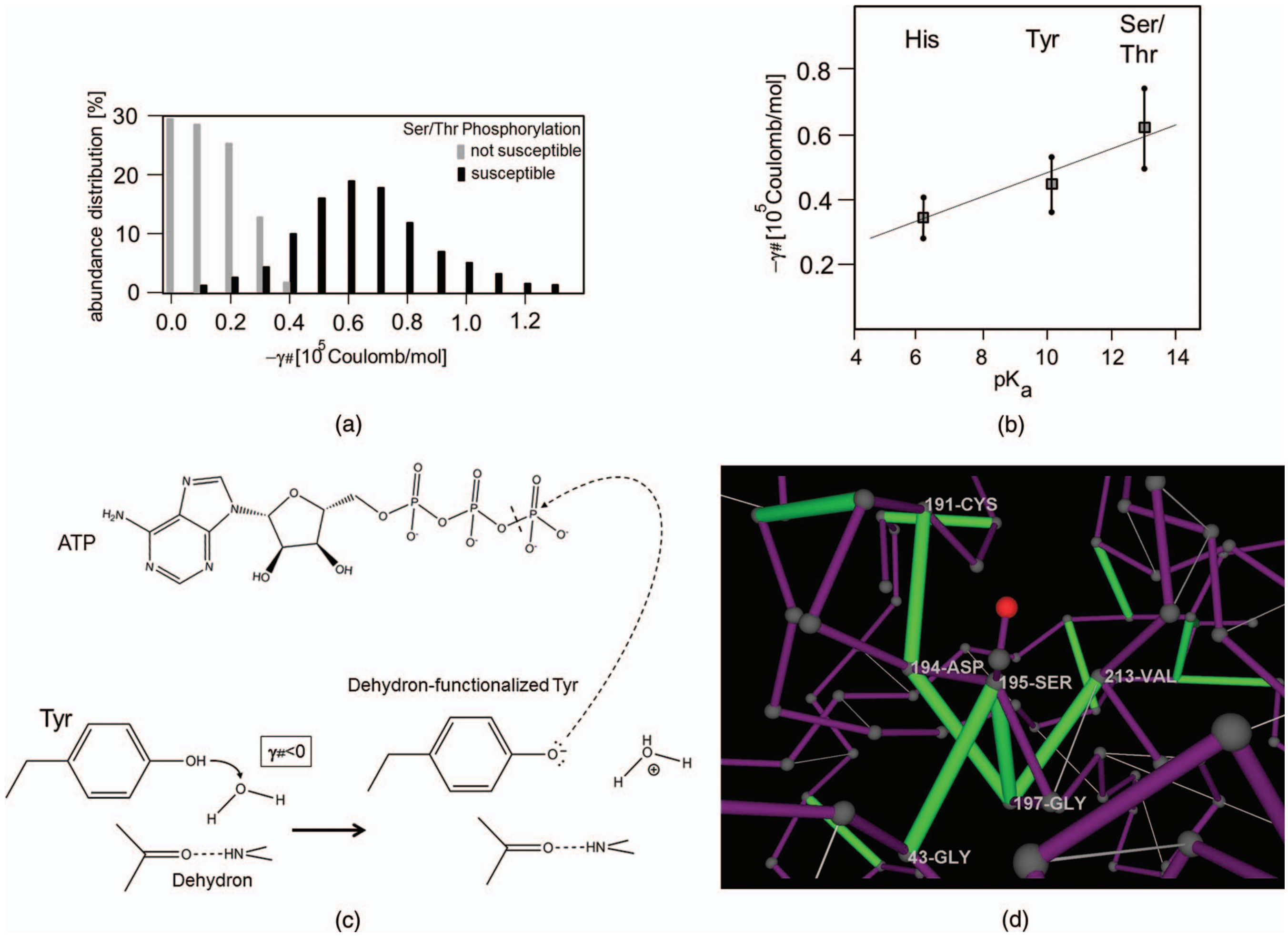

FIG. 3. (a) Abundance distribution of phosphorylation-susceptible Ser/Thr residues according to their vicinal non-Debye polarization-induced charge $\gamma^{\#}$. The $\gamma^{\#}$-distribution for phosphorylation-impervious Ser/Thr residues is displayed as control. (b) Dispersion ranges (error bars) of $\gamma^{\#}$-values as a function of bulk pK$_a$ of the phosphorylation-susceptible side-chain group. (c) Chemical reaction of trans-phosphoesterification involving a nucleophilic attack on the terminal phosphoester linkage of ATP by a dehydron-functionalized Tyr (the phosphorylation-susceptible residue). The nucleophilicity of the Tyr phenolic hydroxyl is significantly enhanced through polarization induced by the proton-acceptor water molecule that envelops the vicinal dehydron with $\gamma^{\#} < 0$. (d) Cluster of five ($\phi > 0$)-dehydrons (maximum concentration) vicinal to the catalytic residue Ser195 in bovine trypsin (PDB.4I8G). The structure is rendered following the convention in Fig. 2 with backbone in magenta. The chemical basicity of these structural defects enables the proton relay mechanism from Ser to His in the Ser-His-Asp catalytic triad of this enzyme, which would otherwise be impaired due to the significant gap in pK$_a$ value (~7 log units) between adjacent side chains Ser and His.

His) in a trans-phosphoesterification of the ligand ATP.[15] The reaction promotes the cleavage of the terminal (γ) phosphoryl group from ATP and its covalent attachment to the protein through a phospho-ester linkage. The net non-Debye polarization-induced charge at such sites is significantly larger in magnitude than that for a control residue of the same type but not susceptible to phosphorylation (Figs. 3(a) and 3(b)). This assertion has been validated by examining phosphorylation sites with reported local structure[16] in exhaustive nonredundant sets of PDB-reported kinases at resolution better than 1.5 Å with relevant sequence annotation on phosphorylation sites obtained from Uniprot. Thus, we examined 307 Ser/Thr kinases, 214 Tyr kinases, and 19 His kinases. The tight correlation ($R^2 = 0.82$) between $\gamma^{\#}$ and bulk pK$_a$ of the phosphorylation-susceptible residue is indicative of the deprotonation requirements of the side-chain group to enhance its nucleophilicity and thereby its susceptibility to phosphorylation. Thus, the basicity of the vicinal ($\phi > 0$)-dehydrons as measured by net non-Debye polarization-induced charge functionalizes the residue group implicated in the nucleophilic attack on the ATP terminal phospho-ester linkage (Fig. 3(c)).

Due to the extremely high pK$_a$ (≈13), the functionalization of Ser/Thr as nucleophile requires a very large vicinal $\gamma^{\#}$ value, which in turn typically requires a substantial dehydron concentration. This situation is illustrated in the active Ser195 of the serine protease trypsin (PDB.4I8G[17]) that contains 5 vicinal dehydrons (Fig. 3(d)), the maximum concentration found in PDB.[18] Since dehydron clusters are unstable and expose the backbone to hydration, they become disruptors of protein structure and so an extreme concentration (>5) of vicinal dehydrons yields a natively disordered region.[18] Such disordered regions are often found around nucleophilically functional and extreme base-demanding Ser/Thr and may be regarded as "ephemerally basic" since they visit conformations so rich in dehydrons that can only be temporarily sustained in water.[11,18] Yet, while the dehydrons are present in a transient conformation they act as proton acceptors and functionalize the Ser/Thr by stabilizing the polarized state, switching on their nucleophilic nature.

In the case of serine proteases,[19] the high concentration of serine-vicinal ($\phi > 0$)-dehydrons becomes adjuvant to the inherent Ser-His-Asp proton-relay mechanism of such enzymes, facilitating proton transfer from serine to the nearby histidine. It should be noted that such transference is unlikely to occur without the assistance of vicinal basic dehydrons due to the large gap in pK_a (approximately 7 log units) between serine and the nearby histidine in the catalytic triad.

This work reveals the chemical functionality of interfacial water enveloping nanoscale structural defects in soluble proteins or other nano-materials and asserts its role as a quasi-reactant in biochemical reactions. Many such reactions require the activation of protein groups that perform or promote a nucleophilic attack leading to transesterification. In this work, we show that interfacial water enveloping a dehydron under nanoscale confinement acts as a chemical-base effector, enhancing the nucleophilicity of the adjacent active site. The same effect is not to be expected from interfacial water confined by a concave purely hydrophobic surface of sub-nanometer curvature. In the latter case, the interface becomes orientationally disordered, a tendency that gets exacerbated with the decrease in curvature radius.[20] On the other hand, bulk hydrophobic interfaces have been reported to be basic.[21]

The concept of functionalized episteric water is introduced in this work and the results invite a revision of the purported elementary steps in biochemical reactions. On the other hand, novel biomolecular engineering is also likely to emerge from the physico-chemical foundations delineated, as dehydron-based enzymatic effectors may be created or removed though site-directed mutation altering side-chain packing.

The hereby established biochemical role of dehydrons as promoters of basicity actually complements their own hydrophobicity or dehydration propensity[7,11] in the context of enzymatic mechanisms. Thus, water enveloping a dehydron becomes a better leaving molecule (hydronium seeking full hydration) as it functionalizes the nucleophilic moiety of the enzyme and the dehydration propensity of the dehydron induces the expulsion of the hydronium as it promotes the binding of the enzyme substrate. This migration of dehydron-enveloping hydronium is entropically favored due to a gain in translational and conformational freedom as the hydronium is transferred to the bulk, and is also enthalpically favored, as the transference enables the fulfillment of the hydration demands of the hydronium. Thus, the thermodynamic cost of transferring the proton from the catalytic group to the dehydron-functionalized water molecule is defrayed by the subsequent stabilization of the dehydron that results from its wrapping or shielding upon substrate association and by the free-energy gain associated with transferring confined ionized water to the bulk region. This role of the dehydron as a two-step molecular engine assisting enzyme catalysis will be the subject of forthcoming investigations.

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