JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Nonlinear Scaling of Surface Water Diffusion with Bulk Water Viscosity of Crowded Solutions

John M. Franck, John A. Scott, and Songi Han*

Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106, United States

Supporting Information

ABSTRACT: The translational hydration dynamics within 0.5−1.5 nm of the surface of a DPPC liposome, a model biomacromolecular surface, is analyzed by the recently developed Overhauser dynamic nuclear polarization (ODNP) technique. We find that dramatic changes to the bulk solvent cause only weak changes in the surface hydration dynamics. Specifically, both a >10-fold increase in bulk viscosity and the restriction of diffusion by confinement on a multiple nm length-scale change the local translational diffusion coefficient of the surface water surrounding the lipid bilayer by <2.5-fold. By contrast, previous ODNP studies have shown that changes to the biomacromolecular surface induced by folding, binding, or aggregation can cause local hydration dynamics to vary by factors of up to 30.[1,2] We suggest that the surface topology and chemistry at the ≤1.5 nm scale, rather than the characteristics of the solvent, nearly exclusively determine the macromolecule's surface hydration dynamics.

Most structural biology and biochemistry studies analyze biomolecules dissolved in simple and dilute buffers. However, in nature, a complex mixture of macromolecules and small molecular constituents crowd the cytoplasm.[3] The crowded environment dramatically alters not only the kinetics of biomolecular function but also the thermodynamic activity of various conformational states, as many studies have demonstrated.[4−7] The compartmentalization and nanoscale confinement of the cytoplasm by lipid membranes play a similarly important role in biology.[8−10]

Curiously, biochemical studies in dilute buffer solutions still serve as good and representative models of many biologically important processes that occur in the crowded cell. Even moreso, proteins tolerate a broad range of perturbations to the bulk solvent; many proteins can recover near complete function with the addition of only a 40% weight ratio of water.[11,12] This broad tolerance would seem to imply an intimate link between the properties and function of the macromolecule itself and the macromolecule's surface hydration water. Therefore, we seek to compare the properties of this surface hydration water in the highly viscous and sometimes opaque conditions implied by crowding to that in a dilute solution environment.

Here, we successfully utilize Overhauser effect dynamic nuclear polarization (ODNP), an emerging and novel magnetic resonance technique, to compare the surface hydration dynamics of high-viscosity solutions to those of low-viscosity solutions. The ODNP tool has now been used in several studies[1,2,13−17] to perform highly localized measurements of translational diffusivity. It is a hybrid of ESR and NMR that reads out the self- and cross-relaxivities of water molecules near a specifically attached nitroxide radical moiety, which functions as a "spin label" that can be attached to biomacromolecular surfaces. The ODNP measurement and subsequent analysis approximate a correlation time, τ_c, which gives the time scale it takes for the proton spin of the water to pass within the magnetic field generated by the spin label, which extends outward for 0.5−1.5 nm.[14] The value of τ_c is inversely proportional to the diffusivity of the water.[14] We denote this as the "local" water diffusivity, since it is specific to water molecules passing through the magnetic field generated by the spin label, i.e., within a 0.5−1.5 nm distance of the spin label. Here, we compare this value of τ_c, e.g., against the reference value for a small nitroxide molecule freely dissolved in water, which we denote as $\tau_{c,w}$. The value of $\tau_{c,w}$, which picks out the diffusivity of the relatively unperturbed[18] bulk water (2.3×10^{-9} m^2 s^{-1}) around the small spin label, is 33.3 ps.[19] We calculate the retardation factor, $\tau_c/\tau_{c,w}$, of the diffusion dynamics of the local surface water relative to that of the bulk water. In this study, we examine such retardation factors in order to directly analyze the impact of confinement and macromolecular crowding on the local translational diffusivity of the surface hydration water.

This model system, a 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) lipid vesicle bilayer, displays a low (3 mol %) concentration of covalently attached spin labels ∼0.6−0.8 nm above the phosphate level. Thus, ODNP probes the surface dynamics of a few water layers well above the DPPC headgroup, broadly termed "biological" or "hydration" water.[17]

Our experimental results indicate that the diffusion of hydration water near the lipid membrane surface is largely decoupled from the diffusion of the crowded bulk solution, i.e., the bulk viscosity. We suggest that biomacromolecules can preserve similar structure, dynamics, and function in an exceedingly crowded cytoplasmic environment because the characteristic and crucial hydration shell remains relatively decoupled from its bulk environment and is preserved.

The DPPC bilayer system displays several desirable properties for ODNP analysis. The dynamics of water associated with lipid vesicle surfaces have been shown by field cycling relaxometry (FCR) to adhere well to the force-free hard sphere[22] model (i.e., assuming translational diffusion as the main contributor to cross relaxation) employed in the ODNP analysis.[14,23,24] Furthermore, we prepared the vesicles to generate both 200 nm diameter

Received: November 16, 2012
Published: January 24, 2013

   dx.doi.org/10.1021/ja3112912 | J. Am. Chem. Soc. 2013, 135, 4175−4178

Table 1. Experimental Hydration Dynamics Data from ODNP and NMR Relaxation Measurements[a]

	composition	η/η_{H_2O}	$k_\sigma s_{max}/s^{-1}$ M^{-1}	k_{low}/s^{-1} M^{-1}	$\xi/0.01$	$\tau_c/\tau_{c,w}$	$\tau_c/\tau_{c,DPPC}$
LUV	DPPC	1	18.1 ± 1.2	320 ± 120	9.2 ± 2.2	5.8 ± 1.2	–
	DPPC + Ficoll	10	21.9 ± 0.7	490 ± 260	8.6 ± 4.2	6.1 ± 2.4	1.05 ± 0.41
	DPPC + sucrose	10	14 ± 5	1600 ± 1600	3.9 ± 3.0	10.9 ± 5.7	1.9 ± 1.0
MLV	DPPC	1	15.7 ± 2.9	330 ± 100	7.7 ± 1.9	6.7 ± 1.3	1.15 ± 0.23
	DPPC + Ficoll	10	17.9 ± 6.2	420 ± 220	8.6 ± 4.5	6.1 ± 2.6	1.07 ± 0.45
	DPPC + sucrose	10	17.7 ± 1.6	1170 ± 470	2.9 ± 1.3	13.2 ± 3.9	2.26 ± 0.68

[a]The various experiments control for increases in viscosity due to confinement (MLV vs LUV) as well as crowding induced viscosity, η. The values $k_\sigma s_{max}$, k_{low}, and ξ are derived from fundamental ODNP relaxation rates, as further described in the SI. τ_c, refers to the translational correlation time of water at the surface of the aqueous DPPC sample listed in a particular row, while $\tau_{c,DPPC}$ refers specifically to τ_c at the surface of DPPC in pure buffer (no viscogens), and $\tau_{c,w}$ refers to τ_c near a small nitroxide in pure water, which is 33.3 ps, as measured elsewhere.[19−21] Thus, the final column calculates the slowdown of the water relative to the surface dynamics of the DPPC LUV system, and the last two columns clearly present the nonlinear scaling of the diffusion dynamics of surface hydration vs bulk water.

unilamellar vesicles (LUVs), where nanoscale confinement should not affect the hydration dynamics, as well as multilamellar vesicles (MLVs), which could exhibit additional retardation due to confinement within the inter bilayer volume.

An analysis of the ODNP data shows that the water near the surface of the DPPC lipid bilayer vesicles translates ∼5.8× slower than it translates in the bulk (see Table 1). Previous studies of DPPC lipid vesicle surfaces[2] reported a retardation factor of the same order, yielding a retardation factor ($\tau_{c,DPPC}/\tau_{c,w}$) of 7.4. Both results show that the hydration dynamics at the surface of the lipid bilayer is significantly slowed.

Interestingly, the additional slowdown of the hydration dynamics on MLV surfaces, which are predominantly sandwiched between bilayers, vs on LUV surfaces, which are entirely exposed to bulk water, is surprisingly small. This is a particularly unintuitive result, given that in DPPC MLV systems water diffuses within a confined space encompassing an inter bilayer distance of only 1.44 nm.[25] Despite this dramatic confinement, the hydration dynamics on the LUV surface is only 20% faster than on the MLV surface (Table 1). Next, we investigate the effect of controlled crowding.

As has been thoroughly discussed in the literature, Ficoll and sucrose do not substantially interact with the surface of proteins. Timosheff et al. have shown that sucrose is actively excluded from the vicinity of proteins, thus stabilizing protein structures,[26,27] i.e., acting as a "protecting osmolyte".[6,28,29] Ficoll, a branched sucrose polymer, has been shown to similarly avoid interactions with proteins.[30,31] Pielak et al. have shown that proteins will engage in nonspecific, but clearly measurable, interactions with protein-based crowding agents, while they exhibit little to no interaction with Ficoll.[32,33] For this reason, both Ficoll and sucrose are commonly used as viscogens that facilitate ESR line shape analysis by slowing down the overall tumbling of the biomolecule in order to highlight the local spin label dynamics and environment without introducing any substantial interactions with the biomolecular surfaces.[5,34−36]

As described in the SI, both Ficoll and sucrose have been added to increase the bulk viscosity of the solution by 10-fold. If a molecule undergoes nonanomalous Brownian diffusion, the Stokes−Einstein relationship allows one to relate the viscosity to the diffusion constant as follows:[33,37−40]

$$D = \frac{k_B T}{6\pi\eta R_H} \tag{1}$$

where k_B is Boltzmann's constant, T the absolute temperature, R_H the effective radius of the diffusing particle (here, a water molecule), and η the viscosity of the bulk solution. It is worth noting that certain studies have claimed that the diffusion of small tracer molecules (e.g., fluorophores) in the presence of crowding agents or viscogens, such as Ficoll or sucrose, is entirely nonanomalous, following Gaussian Brownian motion,[40] while other studies have presented anomalous diffusion of biomolecules (e.g., proteins) when either synthetic polymers or protein-based crowding agents are present in the solution.[32,33] However, even under conditions where the diffusion is anomalous, we can define an "effective" local surface viscosity, η, based on the Stokes−Einstein relationship with the local diffusivity, D, determined from ODNP. The correlation time for translational diffusion is inversely proportional to the local diffusivity of the water, i.e., where we designate values for two different samples as primed and unprimed:

$$\frac{\tau_c'}{\tau_c} \approx \frac{D}{D'} \tag{2}$$

The relative retardation factor, the ratio of the correlation times under different conditions, should then reflect the ratio of the effective local viscosities (from eqs 1 and 2):

$$\frac{\tau_c'}{\tau_c} \approx \frac{\eta'}{\eta} \tag{3}$$

Thus far we have discussed the retardation factor of the local environment relative to bulk water, i.e., $\tau_c'/\tau_c = \tau_c/\tau_{c,w}$. We can now use ODNP to examine whether this effective local viscosity couples to the viscosity of the bulk solvent, i.e., whether the viscosity at the LUV surface remains unchanged at 5.8× greater than the bulk viscosity or whether it is further retarded as the bulk viscosity increases. In other words we can analyze the retardation relative to the uncrowded DPPC surface, i.e., $\tau_c'/\tau_c = \tau_c/\tau_{c,DPPC}$.

We make the surprising observation (Table 1) that the coupling between the dynamics at the surface of our DPPC LUV model system and the bulk viscosity is extremely weak. While the crowding agents induce a 10-fold increase in the bulk viscosity, they slow the translational dynamics of the hydration water by a factor of <2.

The relatively small change of the local dynamics is not an artifact of the ODNP analysis or experiment, which can and does see very large modulation in the local dynamics due to interactions and structural rearrangements. For instance, a previous study analyzed various sites of apomyoglobin in the folded and unfolded form with ODNP to find values of τ_c from 178 to >1000 ps, corresponding to retardation factors of between

dx.doi.org/10.1021/ja3112912 | J. Am. Chem. Soc. 2013, 135, 4175−4178

5.3 and 30. On transitioning from an unfolded to native folded conformation, the translational hydration dynamics of apomyoglobin slow down by factors in the range of 4.15 ± 2.9 (here, the ±2.9 indicates the SD across different residues).[1] Similarly, tau187, a short amyloid peptide, exhibits retardation factors of 4.3 and 15.3 before and after aggregation, respectively, exhibiting a 3.5-fold change in local hydration dynamics upon aggregation.[2] Based on these studies, we designate a slowdown by a factor of 3.5−4.5 as typical for the retardation of hydration dynamics that accompanies structural changes in biomacromolecular systems.

Table 1 also presents that crowding with a viscous sucrose solution reduces the water mobility at the DPPC surface measurably more than a viscous solution of Ficoll of the same nominal bulk viscosity. Sucrose has a thermodynamic activity much greater than the activity of an isoviscous solution of Ficoll 400, as it exhibits an osmolality about an order of magnitude greater.[7] The activity of a sucrose solution should similarly (though to a slightly lesser extent) exceed the activity of an isoviscous solution of Ficoll 70. On the most rudimentary level, the smaller activities needed to achieve the same level of viscosity express the fact that Ficoll is larger than the sucrose (see Figure 1). However, on a deeper level, the larger activity coefficient

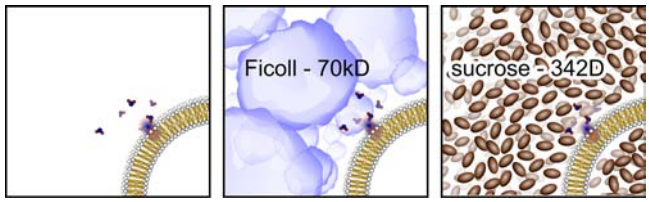

Figure 1. A schematic of the three LUV samples employed in this study. Plain DPPC (left), DPPC + Ficoll 70 (center), DPPC + sucrose (right) are all dissolved in PBS buffer. Ficoll 70 has a radius of 5.5 nm (and at these concentrations, the different Ficoll molecules likely interact with each other).[41] Sucrose exhibits an effective hydrodynamic radius of 0.44−0.52 nm, depending on the method of determination.[42]

required of the sucrose states that a sucrose−water solution needs to release a greater free energy before the solution viscosity will increase appreciably. Therefore, the slightly greater perturbation of the surface hydration dynamics exerted by sucrose may be an indication of this, part of which may be due to interactions between the sucrose and the water.

Not surprisingly, when the effects of confinement inside the MLV layers and the effects of crowding induced by sucrose act together, they lead to the largest retardation of translational water mobility observed here. Likely, this arises from intercalation of sucrose into the interlamellar space of the MLV, which could create obstructions preventing the free passage of water on nanometer length scales. This hypothesis is supported by the fact that k_{low} changes more dramatically than for the other samples, where k_{low} is a parameter that increases along with the population of "bound" water molecules that remain stationary on a timescale of ∼6 ns (see SI). Still, even in the sucrose-crowded and MLV-confined interface, the local hydration dynamics decreases only by an additional factor of 2.3.

This is the first direct observation of site-localized translational hydration dynamics in crowded environments of different viscosities. In fact, the hydration water probed here represents a very small fraction of the total water content of the solutions analyzed. Reanalysis of previous ODNP results and comparison to results acquired by other methods find that the decoupling of the surface and bulk water dynamics is compatible with previous observations in the literature. One crowding agent that has been studied by ODNP is PEG. After the addition of 20% PEG, the surface dynamics of a DOPC lipid has been shown to slow down by a factor of only 1.7,[2] even though the viscosity of a 20% PEG 8000 solution (i.e., the same molecular weight used in ref 2; private communication C.Y.C.) has a viscosity 20-fold greater than water.[43] We can infer from this that any interaction between PEG polymers and the surface of the lipid vesicle is limited. In a different study, Robinson et al.[44] have presented ESR data showing that the rotational diffusion of small nitroxide molecules in glucose follows a power law dependence on the bulk viscosity, rather than obeying the Stokes−Einstein relationship. This again likely reflects effects of volume exclusion due to size or the existence of specific interactions between the nitroxide probe and glucose.

At first, the variations we present might seem odd. The dynamics at the surface of the DPPC bilayer are decoupled from the bulk dynamics but by no means immobilized; water at the surface only moves at ∼20% of the typical diffusion rate and exhibits insensitivity to changes in the bulk viscosity. Interestingly, the same 1 nm length scale that the ODNP approach probes corresponds to the correlation length of water, which determines the length scale of structural and dynamic changes of the fluid.[45,46] This suggests that the DPPC surface itself controls the dynamics of local water molecules within 1 nm of the lipid vesicle surface, while Ficoll or sucrose controls the dynamics of water molecules located a few nm away from the DPPC surface, in the bulk solvent.

The results presented here show that even dramatic (10-fold) changes in the bulk viscosity can lead to <2-fold change in the local dynamics observed by ODNP. Therefore, a crucially important consequence of this discussion is that when ODNP measures modulation in hydration dynamics that exceed a factor of 2.5, this clearly implies either a change in the local structure or the local chemistry within 1−2 nm of the spin label or genuinely cooperative effects affecting the hydrogen-bonding interaction between the macromolecular surface and the hydration water. These observations also emphasize the importance and the potential impact of comparing ODNP measurements to studies based on other experimental methodologies that might be able to quantify the length scale of the crossover between bulk and surface dynamics. For instance, the dynamic surface force apparatus has measured viscous forces near both the hydrophilic and hydrophobic surfaces of DPPC mono- and bilayers.[47] While the typical analysis assigns a location to the hydrodynamic no-slip boundary condition, results as presented here and elsewhere encourage reconsidering such results as a means for extracting the transitions between variable rates of diffusion near the surface. Furthermore, measurements of the ζ-potential under different salt conditions give an estimate of the slipping plane, which might allow insight into how far these viscosity effects penetrate into the bulk solvent.[48] Finally, observations made with quasi-elastic neutron scattering[49,50] observe a sharp increase in translational water mobility on moving away from the surface of a model peptide: The retardation of the water's translational diffusion decreases from 3.1 to 1.8 to 1.4 (i.e., diffusion coefficients of 0.75, 1.26, 1.65 × 10^{-9} m^2 s^{-1}) as the concentration of the model peptide, NALMA, in water scales from 2.0 M (corresponds to about one hydration shell) to 1 to 0.5 M, respectively.[50]

As a concluding note, the decoupling of the surface and bulk dynamics may prove important to the function of biomolecular systems in cellular environments. Proteins and cell membranes

dx.doi.org/10.1021/ja3112912 | J. Am. Chem. Soc. 2013, 135, 4175−4178

can gather a soft shell of hydrating water molecules. They provide a microenvironment that remains relatively unperturbed by the surrounding crowded environment and is likely crucial for maintaining structure and function. Even in the incredibly crowded cytoplasm, where macromolecules occupy 20−30% of the volume,[4] these results imply that water at the surface of the macromolecule will display relatively unperturbed properties, unless interactions occur that penetrate into and perturb this microenvironment at the nanometer scale. This perspective could explain a variety of phenomena, most importantly the concept that the first few layers of water molecules appear sufficient, yet also indispensible, for ensuring the proper function of a folded protein,[11,12] the concept that crowding and confinement might allow compartmentalization of the cell without impacting the fundamental properties of proteins,[4] and the concept that the properties of the hydration water might be tied to the functions of proteins on a fundamental level.[51]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Includes experimental procedures and ODNP analysis details. This material is available free of charge via the Internet at http://pubs.acs.org/.

■ AUTHOR INFORMATION

Corresponding Author

songi@chem.ucsb.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Dr. C. Cheng for useful scientific discussion and Mr. G. Wylde (preliminary experiments). This work was supported by the UCSB NSF-MRSEC Program (DMR-1121053), the NSF IDBR (DBI-1152244), and the 2011 NIH Innovator award awarded to S.H. J.M.F. acknowledges support by the CNSI Elings Prize Postdoctoral Fellowship. This project made use of the UCSB MRL Shared Experimental Facilities, which are supported by the NSF-MRSEC Program (DMR 1121053); a member of the NSF-funded Materials Research Facilities Network.

■ REFERENCES

(1) Armstrong, B. D.; Choi, J.; López, C. J.; Wesener, D. A.; Hubbell, W.; Cavagnero, S.; Han, S. *J. Am. Chem. Soc.* **2011**, *133*, 5987.
(2) Ortony, J. H.; Cheng, C.-Y.; Franck, J. M.; Kausik, R.; Pavlova, A.; Hunt, J.; Han, S. *New J. Phys.* **2011**, *13*, 015006.
(3) Zimmerman, S. B.; Trach, S. O. *J. Mol. Biol.* **1991**, *222*, 599.
(4) Ellis, R. *Curr. Opin. Struct. Biol.* **2001**, *11*, 114.
(5) López, C. J.; Fleissner, M. R.; Guo, Z.; Kusnetzow, A. K.; Hubbell, W. L. *Protein Sci.* **2009**, *18*, 1637.
(6) Arakawa, T.; Timasheff, S. N. *Biophys. J.* **1985**, *47*, 411.
(7) Galiano, L.; Blackburn, M. E.; Veloro, A. M.; Bonora, M.; Fanucci, G. E. *J. Phys. Chem. B* **2009**, *113*, 1673.
(8) Lizana, L.; Konkoli, Z.; Bauer, B.; Jesorka, A.; Orwar, O. *Annu. Rev. Phys. Chem.* **2009**, *60*, 449.
(9) Luby-Phelps, K.; Lanni, F.; Taylor, D. L. *Annu. Rev. Biophys. Biophys. Chem.* **1988**, *17*, 369.
(10) Bangham, A. D. *Annu. Rev. Biochem.* **1972**, *41*, 753.
(11) Ball, P. *Chem. Rev.* **2008**, *108*, 74.
(12) Rupley, J. A.; Careri, G. In Protein Hydration and Function. *Advances in Protein Chemistry*; Anfinsen, C. B., Frederic, M., Richards, J. T. E., Eisenberg, D. S., Eds.; Academic Press: San Diego, CA, 1991; Vol. *41*, pp 37.
(13) Armstrong, B. D.; Han, S. *J. Chem. Phys.* **2007**, *127*, 104508.
(14) Armstrong, B. D.; Han, S. *J. Am. Chem. Soc.* **2009**, *131*, 4641.
(15) Cheng, C.-Y.; Wang, J.-Y.; Kausik, R.; Lee, K. Y. C.; Han, S. *Biomacromolecules* **2012**, *13*, 2624.
(16) Kausik, R.; Han, S. *J. Am. Chem. Soc.* **2009**, *131*, 18254.
(17) Kausik, R.; Han, S. *Phys. Chem. Chem. Phys.* **2011**, *13*, 7732.
(18) Armstrong, B. D.; Soto, P.; Shea, J.-E.; Han, S. *J. Magn. Reson.* **2009**, *200*, 137.
(19) Türke, M.-T.; Bennati, M. *Phys. Chem. Chem. Phys.* **2011**, *13*, 3630.
(20) Türke, M.-T.; Bennati, M. *Phys. Chem. Chem. Phys.* **2010**, *12*, 5893.
(21) Franck, J. M.; Pavlova, A.; Han, S. arxiv:1206.0510 2012, 25.
(22) Hwang, L.; Freed, J. *J. Chem. Phys.* **1975**, *63*, 4017.
(23) Hodges, M. W.; Cafiso, D. S.; Polnaszek, C. F.; Lester, C. C.; Bryant, R. G. *Biophys. J.* **1997**, *73*, 2575.
(24) Polnaszek, C. F.; Bryant, R. G. *J. Chem. Phys.* **1984**, *81*, 4038.
(25) Kiselev, M.; Lesieur, P.; Kisselev, A.; Grabielle-Madelmond, C.; Ollivon, M. *J. Alloys Compd.* **1999**, *286*, 195.
(26) Lee, J. C.; Gekko, K.; Timasheff, S. N. *Methods Enzymol.* **1979**, *61*, 26.
(27) Lee, J. C.; Timasheff, S. N. J. *Biol. Chem.* **1981**, *256*, 7193.
(28) Yancey, P. H. *J. Exp. Biol.* **2005**, *208*, 2819.
(29) Yancey, P. H.; Clark, M. E.; Hand, S. C.; Bowlus, R. D.; Somero, G. N. *Science* **1982**, *217*, 1214.
(30) Luby-Phelps, K.; Castle, P. E.; Taylor, D. L.; Lanni, F. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4910.
(31) Zhou, H.-X.; Rivas, G.; Minton, A. P. *Annu. Rev. Biophys.* **2008**, *37*, 375.
(32) Miklos, A. C.; Li, C.; Sharaf, N. G.; Pielak, G. J. *Biochemistry* **2010**, *49*, 6984.
(33) Wang, Y.; Li, C.; Pielak, G. J. *J. Am. Chem. Soc.* **2010**, *132*, 9392.
(34) Popova, A. M.; Qin, P. Z. *Biophys. J.* **2010**, *99*, 2180.
(35) Popova, A. M.; Kálai, T.; Hideg, K.; Qin, P. Z. *Biochemistry* **2009**, *48*, 8540.
(36) Zhang, Z.; Fleissner, M. R.; Tipikin, D. S.; Liang, Z.; Moscicki, J. K.; Earle, K. A.; Hubbell, W. L.; Freed, J. H. *J. Phys. Chem. B* **2010**, *114*, 5503.
(37) Debye, P. J. W. *Polar molecules*; Dover: New York, 1929; Vol. *172*.
(38) Einstein, A. *Investigations on the Theory of the Brownian Movement*; Dover: New York, 1956.
(39) Einstein, A. *Ann. Phys.* **1906**, *324*, 371.
(40) Dix, J. A.; Verkman, A. S. *Annu. Rev. Biophys.* **2008**, *37*, 247.
(41) van den Berg, B.; Wain, R.; Dobson, C. M.; Ellis, R. J. *EMBO J.* **2000**, *19*, 3870.
(42) Schultz, S. G. *J. Gen. Physiol.* **1961**, *44*, 1189.
(43) Gonzalez-Tello, P.; Camacho, F.; Blazquez, G. *J. Chem. Eng. Data* **1994**, *39*, 611.
(44) Robinson, B. H.; Mailer, C.; Reese, A. W. *J. Magn. Reson.* **1999**, *138*, 210.
(45) Chandler, D. *Nature* **2005**, *437*, 640.
(46) Widom, B. *Annu. Rev. Phys. Chem.* **2011**, *62*, 1.
(47) Cross, B.; Steinberger, A.; Cottin-Bizonne, C.; Rieu, J.-P.; Charlaix, E. *Europhys. Lett.* **2006**, *73*, 390.
(48) Bardonnet, P.-L.; Faivre, V.; Pirot, F.; Boullanger, P.; Falson, F. *Biochem. Biophys. Res. Commun.* **2005**, *329*, 1186.
(49) Russo, D.; Murarka, R. K.; Copley, J. R. D.; Head-Gordon, T. *J. Phys. Chem. B* **2005**, *109*, 12966.
(50) Russo, D.; Hura, G.; Head-Gordon, T. *Biophys. J.* **2004**, *86*, 1852.
(51) Frauenfelder, H.; Chen, G.; Berendzen, J.; Fenimore, P.; Jansson, H.; McMahon, B.; Stroe, I.; Swenson, J.; Young, R. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 5129.