Probing the hydration water diffusion of macromolecular surfaces and interfaces

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Abstract. We probe the translational dynamics of the hydration water surrounding the macromolecular surfaces of selected polyelectrolytes, lipid vesicles and intrinsically disordered proteins with site specificity in aqueous solutions. These measurements are made possible by the recent development of a new instrumental and methodological approach based on Overhauser dynamic nuclear polarization (DNP)-enhanced nuclear magnetic resonance (NMR) spectroscopy. This technique selectively amplifies ¹H NMR signals of hydration water around a spin label that is attached to a molecular site of interest. The selective ¹H NMR amplification within molecular length scales of a spin label is achieved by utilizing short-distance range (∼r⁻³) magnetic dipolar interactions between the ¹H spin of water and the electron spin of a nitroxide radical-based label. Key features include the fact that only minute quantities (<10 µl) and dilute (≥100 µM) sample concentrations are needed. There is no size limit on the macromolecule or molecular assembly to be analyzed. Hydration water with translational correlation times between 10 and 800 ps is measured within ∼10 Å distance of the spin label, encompassing the typical thickness of a hydration layer with three water molecules across. The

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hydration water moving within this time scale has significant implications, as this is what is modulated whenever macromolecules or molecular assemblies undergo interactions, binding or conformational changes. We demonstrate, with the examples of polymer complexation, protein aggregation and lipid–polymer interaction, that the measurements of interfacial hydration dynamics can sensitively and site specifically probe macromolecular interactions.

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1. Introduction

All biological processes take place in the presence of bulk water, where water has been long recognized as much more than a passive medium in which biochemical interactions occur. Instead, water is thought to play an active role in mediating biological function, e.g. by slaving protein dynamics [1, 2], facilitating protein folding [3, 4] or mediating hydrophobic interactions that drive the assembly of biological constituents [5]. While many types of water (e.g. structural or bulk) with dynamics of vastly different time scales (ps to ms) play their unique roles, what is termed hydration water typically extends up to three to four water layers, and is critical in mediating the dynamics and interactions of biological constituents. When two macromolecules interact, what is fundamentally occurring at the interface is the perturbation of the interfacial dynamics or even the expulsion of the hydration water layer that is cushioning the macromolecule, depending on the strength of the binding interaction. Similarly, when macromolecules undergo conformational changes, such as in the folding of proteins, the hydration water dynamics of particular sites get hindered or seized as the sites become buried in a hydrophobic configuration. Changes of surface hydration dynamics of lipid vesicles in solutions have also been proposed to underlie membrane fusion, as well as the function of lipid-active polymers that affect membrane permeability or stability. Therefore, the translational diffusion dynamics of the hydration water layer is an exquisite tool for probing molecular interactions and conformational changes. The translational correlation times of this disordered, bulk-like, water lie in the 10–1000 ps regime; such time scales are most sensitively modulated by these dynamic and/or transient interaction processes. However, probing techniques based on mapping out hydration dynamics are not yet among the standard toolkits for interrogating biochemical interactions because of two key challenges: (i) the difficulty of quantifying
the diffusivity of surface or interfacial water that represents a nearly insignificant number of molecules relative to the bulk solvent, especially given indistinguishable spectroscopic signatures between hydration and bulk water, and (ii) the need to achieve site-specific probing of the local hydration water dynamics of macromolecular sites of interest. We present \(^1\)H Overhauser dynamic nuclear polarization (DNP) of water, in conjunction with electron spin resonance spectroscopy (ESR), as a viable approach to probing the diffusivity of hydration water on the surface of macromolecules or molecular assemblies, as well as at the interface of molecular interactions \([6]–[8]\). The Overhauser DNP method overcomes both challenges by selectively amplifying the hydration water’s \(^1\)H NMR signature by orders of magnitude within 10 Å of a nitroxide radical-based spin label that is placed at the specific site of interest on the surface of macromolecules or assemblies \([7, 9]\). Recombinant proteins allow for site-directed mutagenesis to introduce single cysteine residues and subsequent spin labeling to achieve amino acid residue-specific labeling \([10]\). For synthetic polymers and lipid molecules, spin labels are incorporated using covalent functionalizing schemes, appropriately adapted for the molecule or functional group that is targeted.

Here, we present the measurement of the diffusion coefficients of the surface hydration water layer surrounding a number of systems: synthetic polyelectrolytes, lipid vesicles and intrinsically disordered proteins. We find that the translational diffusivity of the hydration water layer of all solvent-exposed surfaces is about 2–6 times slower than that of bulk water, unless the investigated site is buried from bulk solvent in a local restricted structure. This result is in agreement with previous findings by computation and experiments that surface hydration water is slowed by a factor of 2–6 compared to bulk water \([11]–[14]\). We further demonstrate the sensitivity of hydration water diffusivity to probing molecular interactions on selected examples, e.g. the complex coacervation of oppositely charged polyelectrolytes \([6, 15]\), the interaction between lipid vesicle surfaces and polyethylene glycol (PEG) and the aggregation of tau-187 proteins \([8]\). DNP is a unique experimental approach to measuring the surface hydration water diffusivity of macromolecules or assemblies that are fully immersed in bulk water and in dilute concentrations (e.g. <10 \(\mu\)l sample volumes and \(\geq 100 \mu\)M spin label concentration). This sensitivity makes DNP broadly applicable to biochemical analysis as it allows access to a large sample space of biological relevance and for probing their interactions.

2. Theory

The detailed description of Overhauser DNP can be found in several works in the literature \([16]–[19]\). Here, we only provide a brief summary of the theory relevant to this work. \(^1\)H Overhauser DNP relies on the polarization transfer from the electron spin of nitroxide-free radicals to the \(^1\)H of water via cross relaxation. The maximum \(^1\)H NMR signal enhancement is given by \([17]\)

\[
E_{\text{max}} = 1 - \rho f s_{\text{max}} \frac{|\gamma_S|}{\gamma_I},
\]

where \(E_{\text{max}}\) is the DNP enhancement factor extrapolated to infinite microwave power, \(\rho\) is the coupling factor describing the dipolar and/or scalar interactions between the electron and proton, \(f\) is the leakage factor describing how efficiently the electron spin relaxes the proton spin, \(s_{\text{max}}\) is the maximum electron spin saturation factor, and \(\gamma_S\) and \(\gamma_I\) are the gyromagnetic ratios of the electron and proton spins, respectively, which is given by \(|\gamma_S|/\gamma_I = 658\). The

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electron–proton coupling factor $\rho$ varies between 0.5 for pure dipolar coupling and $-1$ for pure scalar coupling and is strongly field dependent [17]. In the case where nitroxide-free radicals are fully solvated in water, the coupling between the $^1$H of water and the electron spin of radicals has been shown to be dominated by dipolar interaction. In essence, $\rho$ carries information about the translational dynamics of $^1$H-bearing molecules in solutions with respect to the electron spin label and is thus the key parameter to be determined. By measuring the longitudinal relaxation times in the presence ($T_1$) and absence ($T_{10}$) of the spin labels, the leakage factor can be determined following $f = 1 - T_1/T_{10}$. The leakage factor, unlike the coupling and saturation factor, critically depends on the spin label concentrations. Therefore, variations in concentration of the spin-labeled species can be accounted for by the measurement of the leakage factor. An important characteristic to take into account, when employing nitroxide-based spin labels, is the presence of three ESR lines due to the hyperfine coupling of the electron spin to the $^{14}$N nuclei of the nitroxide radical. In order to experimentally determine the saturation factor, full saturation of all ESR transitions, and thus complete exchange of the hyperfine lines, has to be achieved [9, 16]. Measuring the DNP enhancements as a function of spin label concentration, and then extrapolating to infinite concentrations, enables us to approach the maximum saturation factor of 1. Thus, the coupling factor can be determined by measuring $E_{\text{max}}$ of equation (1) as a function of spin label concentration and extrapolating it to infinite concentrations [16]. On the other hand, tethered spin labels on macromolecules, such as lipid vesicle surfaces, polymers or proteins, are subject to slow rotational tumbling [20]. The resulting decrease in the $T_1$ relaxation time of $^{14}$N nuclei of nitroxides leads to an efficient mixing of the three nitroxide hyperfine energy levels and thereby facilitates their complete saturation. Thus, even in the absence of Heisenberg spin exchange due to the very low spin label concentrations used, the saturation factor approaches 1 at extrapolated high microwave power [16]. In such cases, the analysis does not require the measurements of DNP enhancements at a series of concentrations in order to approach the limit of saturation factor of 1. We experimentally tested the validity of this assumption by comparing enhancements measured with varying degrees of spin label concentrations (1–10 mol%) distributed on lipid headgroups, whereby the degree of electron spin label dipolar coupling and thus the ESR linewidth is varied. Our finding shows that the coupling factor remains constant as a function of spin label concentration, providing strong evidence that all hyperfine states in these sample systems are well mixed [9, 16].

The fluctuations in the proton–electron dipolar interaction due to the solvent dynamics can be described in terms of a single correlation time that lies in the tens of picoseconds to sub-nanosecond time scale, and this enables the use of a single spectral density function ($J$) to describe the interaction [21]. With this assumption, $\rho$ is given by

$$\rho = \frac{6J(\omega_S + \omega_I, \tau) - J(\omega_S - \omega_I, \tau)}{6J(\omega_S + \omega_I, \tau) + 3J(\omega_I, \tau) + J(\omega_S - \omega_I, \tau)},$$

where $\omega_S$ is the electron spin Larmor frequency, $\omega_I$ the nuclear spin Larmor frequency and $\tau$ the translational correlation time between the electron and nuclear spins. This relationship bears the strong magnetic field dependence of the coupling factor, $\rho$, dominated by the electron Larmor frequency, $\omega_S$. All measurements presented in this paper are carried out at 0.35 T, where $\omega_S$ is of the order of 10 GHz and $\omega_I$ of the order of 15 MHz. In this regime, the closer $\tau$ is to $1/(10 \text{ GHz})$, the more sensitively the variation in motion (of hydration water) is to affecting the quantity $\rho$, while $\tau$ values between 10 and 800 ps and in some cases up to 1000 ps (depending on the electron spin concentration) will lead to measurable modulation of $\rho$ and thus DNP.

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signal enhancements. Typically, \( \tau \) of this magnitude is caused by free, as well as restricted, translational diffusive motion of bulk-like or at least disordered water in solutions. Here, once the coupling factor is obtained by DNP through the measurement of \(^1\)H NMR enhancements and \( f \), the translational correlation time \( \tau \) of the interacting species can be obtained using the appropriate spectral density function and for a given distance of closest approach between the coupling electron and the nuclear spin. In the spectral density function \( J \) shown in equation (2), we used the force-free hard-sphere (FFHS) dynamic model \([22]\), which can adequately describe the surface relaxation in spin-labeled soft matter systems whose interfacial hydration dynamics are mediated by translational diffusion. In this case, the spectral density function can be expressed by

\[
J(\omega, \tau) = \frac{1 + 5\sqrt{2}/8(\omega\tau)^{1/2} + \omega/4}{1 + (2\omega\tau)^{1/2} + (\omega\tau)^{3/2} + 16/81(\omega\tau)^2 + 4\sqrt{2}/81(\omega\tau)^{5/2} + (\omega\tau)^3/81}.
\]  

(3)

Although there are variations of this model including the effects of off-center rotational contributions of spin labels and spin-labeled molecules, they have been shown to provide similar fit parameters \([9]\). The translational correlation times obtained from the spectral density function are related to the diffusion coefficients by

\[
\tau = \frac{d^2}{D_I + D_S},
\]  

(4)

where \( D_I \) and \( D_S \) are the diffusion coefficients of the proton and electron spin-bearing molecules, here water and the nitroxide spin label, and \( d \) their distance of closest approach. For systems where \( d \) is known or assumed, one can express the total diffusivity in terms of solvent diffusivity \( D_I \) alone in the limit \( D_I \gg D_S \), which is valid for tethered spin labels. For spin labels freely dissolved in bulk water, \( \tau \) quantified by DNP using our setup was calibrated to be 42.5 ps, and the diffusion coefficients \( D_S \) and \( D_I \) have been accurately determined by pulsed-field gradient NMR measurements. This yielded a calibrated distance of closest approach of 3.4 Å based on equation (4) \([9]\). Therefore, the diffusion coefficients of the hydration water, \( D_I \), can be determined by DNP, using the same instrumental setup, for a number of representative macromolecules and molecular assemblies. Note that previous publications reported on a longer correlation time for bulk water of 76 ps and a greater distance of closest approach of 4.5 Å \([6]–[9], [16]\). As will be discussed in greater detail in our forthcoming papers, DNP instrumental settings, e.g. the quality factor of the microwave cavity, can alter the maximum enhancements measured, leading to the measurement of translational correlation times at different distances of closest approach, \( d \), between the electron spin of the label and water protons. Although corrections to previous published absolute \( \rho \) values for bulk water need to be made, note that relative \( \rho \) values (and thus translational hydration dynamics, \( \tau \)) to bulk water dynamics measured with the unchanged instrumental and experimental setting remain correct. Therefore, for example, the previous finding that the translational diffusion coefficient of hydration water on a given lipid membrane surface is two to four fold impeded compared to that of bulk water remains a valid finding \([7]\), even though different absolute numbers for \( \rho \) and \( \tau \) are reported here for hydration water. The confidence in these new values for bulk water is supported by the agreement between the DNP versus NMR relaxation dispersion measurements \([23]\) for bulk water dynamics.
Figure 1. Chemical structures and their corresponding graphical representations of the various synthetic (a–e) and protein (f, g) polyelectrolytes, as well as lipid vesicles with lipid constituents (h–j). Spin labels were covalently attached to polyelectrolytes by chemical reactions. For synthetic polyelectrolytes (a, b, d, e), typically 1–2% spin labels were randomly distributed over the polymer chain. For (c), each disaccharide was functionalized with a spin label. A single spin label was incorporated in a site-directed fashion into the proteins, between the segments mfp1 and mfp5 for mfp151 (f), and at the 322-position for tau-187 [8, 25] (g). The proteins mfp151 and tau-187 are presented schematically, while details of their sequence, structure and function can be found in the literature [8, 24–26]. For vesicles, lipid molecules spin labeled off the headgroup (k) were mixed into the lipid mixture (for details, see section 3). PEG polymers (l) were mixed into the lipid vesicle solutions for the purpose of studying the lipid membrane–PEG interactions.

3. Materials and methods

3.1. Polymer synthesis and functionalization

3.1.1. Preparation of spin-labeled triblock copolymers. The cysteamine hydrochloride functionalized P(AGE-b-EG-b-AGE) (poly-allylglycidylether-b-ethyleneglycol-allylglycidylether) (see figure 1(a)) was spin labeled by coupling the amine functional groups to 4-carboxy-2,2,6,6-tetramethylpiperidine-1-oxyl (carboxy-TEMPO) with crosslinker, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). An aqueous solution of 150 mg EDC and a suspension of 30 mg carboxy-TEMPO in water were added dropwise to a 150 mg solution of aqueous a. The reaction mixture was stirred overnight at room temperature (RT). The product was purified by dialysis and isolated by lyophilization. Triblock copolymer b also consists of a functionalized P(AGE-b-EG-b-AGE) backbone, but is functionalized with thioglycolic acid to yield pendant groups terminated by carboxylates. The carboxylate groups on the polymer were

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coupled to 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (amino-TEMPO) by EDC coupling with the same stoichiometry as above. Purification and isolation were carried out as described for \(a\).

### 3.1.2. Preparation of spin-labeled hyaluronic acid.

35 kDa sodium hyaluronate (HA), \(c\), was purchased from Lifecore Biomedical (Chaska, MN). Each disaccharide contains one carboxylate functionality that is coupled to amino-TEMPO with EDC. An aqueous solution of 70 mg EDC and 125 mg amino-TEMPO was added dropwise to 50 mg aqueous HA. The reaction mixture was stirred overnight at RT before purification and isolation, as described for \(a\).

### 3.1.3. Preparation of poly(aspartic acid) and poly(N-vinylimidazole).

Synthesis and spin labeling of \(d\), poly(aspartic acid) (PAsp), and \(e\), poly(N-vinylimidazole) (PVIm), were carried out as described previously \([6]\).

### 3.1.4. Preparation of mfp151.

*Mytilus* foot protein mfp151, \(f\), was generously donated by Professor H J Cha (POSTECH). The sequence was previously reported \([24]\) and consists of an mfp5 sequence sandwiched between two domains, each consisting of six repeats of the decapeptide AKPSYPPTYK from mfp1. The only cysteine residue, located in between the first mfp1 sequence and mfp5, was used for spin labeling. To remove impurities on mfp151, the protein is redissolved in 5% acetic acid, and polished by C8 reverse-phase HPLC using an acetonitrile gradient in water with 0.1% trifluoroacetic acid. Following HPLC, the proteins were lyophilized and stored at \(-80^\circ\)C. Spin labeling was carried out in sodium acetate buffer (NaAc) at pH 5. To the solution, the spin label \(S-(2,2,5,5\text{-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl})\text{methyl methanesulfonothioate (MTSL)}\) was added in \(10\times\) molar excess. The reaction mixture was stirred overnight at RT and then dialysed in HPLC-grade water. Spin-labeled mfp151 was obtained as a slightly off-white powder after lyophilization.

### 3.1.5. Preparation of tau-187 protein.

Preparation and spin labeling of tau-187, \(g\), were carried out as previously reported \([8, 25]\). All studies were carried out in solutions of tau-187, in which every protein molecule is spin labeled at site 322 \([8]\). Aggregation was induced by the addition of heparin (18 kDa, Sigma) in a molar ratio of 8 : 1, \(\text{tau-187} : \text{heparin}\).

### 3.1.6. Preparation of lipid vesicles.

The lipid samples \(h, i\) and \(j\), as well as phospholipid spin-label probe PC-TEMPO, \(k\), were purchased from Avanti Polar Lipids (Alabaster, AL). Large unilamellar vesicles (LUV) were prepared according to the conventional thin-film hydration method. Generally, a stock lipid was prepared in a chloroform : methanol (9 : 1, v/v) mixture. The desired amount of stock lipid was transferred to a glass vial. The solvent was then dried under flowing N\(_2\), and the formed thin film was thoroughly dried in a desiccator connected with a mechanical vacuum pump. The dry lipid was hydrated by Millipore water for 1 h, followed by vortexing for 1 h. The extrusion was performed by passing the lipid through a polycarbonate membrane with 200 nm pore size above the lipid transition temperature. The same batch of fresh vesicle sample was used for each set of experiments. Polyethylene glycol, \(l\), (average molecule weight \(= 8000\)) was mixed into the lipid vesicle solutions immediately before the measurements were carried out.
3.2. Sample preparation

Measurements were carried out at the following sample concentrations: triblock copolymers a and b, 250 mg ml$^{-1}$; HA, c, 100 mg ml$^{-1}$; PAsp, d, 20 mg ml$^{-1}$; PAsp/PVIm-TEMPO, d + e, 50 mM; mfp151, f, 200 µM; tau-187, g, 500 µM. For the vesicle samples, the final lipid concentration was 25 mg ml$^{-1}$. The concentration of PC-TEMPO spin label was 2.5wt% per lipid.

All samples were injected into capillary tubes for DNP and ESR measurements. 4 µl of a, b, c and f were injected into PTFE tubes (0.76 mm I.D.) and sealed at each end with Critoseal. For all other solutions, 4 µl was injected into 0.6 mm I.D. quartz tubes and sealed at each end with wax.

3.3. Electron spin resonance (ESR)

ESR was measured on a Bruker EMX X-band spectrometer equipped with a rectangular and/or dielectric cavity. Samples were irradiated with a microwave frequency of 9.8 GHz, while the center field was held at 0.35 T. The field was modulated at <0.2 times the linewidth of the center peak. All spectra were obtained at 298 K with 14 L min$^{-1}$ flow of N$_2$ through the cavity.

3.4. Dynamic nuclear polarization (DNP)

The field was set to that of the ESR peak center, and DNP spectroscopy was carried out in the same instrument as was used for ESR. The samples were inserted into a home-made U-shaped NMR coil tuned to ~14.8 MHz and connected to a broadband channel of a Bruker Avance NMR spectrometer. A typical inversion-recovery pulse sequence was used for $T_1$ relaxation measurements. The radical electrons were irradiated continuously with a power range between 0.1 mW and 1.5 W using a home-built X-band microwave amplifier while the NMR signal was recorded [27].

4. Results and discussions

Overhauser DNP experiments were carried out to determine the coupling factor ($\rho$), translational correlation time ($\tau$) and diffusion coefficient ($D_I$) of the hydration water for selected polyelectrolytes, intrinsically disordered proteins and lipid vesicles in aqueous solution, according to the analysis laid out in section 2. All of the experimental DNP parameters of hydration water are listed in table 1, and the corresponding ESR spectra are displayed in figure 2. The chemical structure and the corresponding graphical representations of the molecules and assemblies that are presented here are depicted in figure 1. The polyelectrolytes included in this study are the unbranched polycations, PVIm, and the polyanions, PAsp and the polydisaccharide hyaluronic acid (HA; 35 kDa). The charged moieties in the synthetic polyelectrolytes are equally distributed over the macromolecular backbone. In addition, two different polyelectrolyte triblock copolymers were studied. In contrast to the polyelectrolytes with evenly distributed charges, these triblocks contain a neutral PEG midblock and endblocks with charged pendent groups, whose detailed studies of assembly formation will be discussed separately in the future. Two variations of the triblock copolymer were studied: one cationic species (formed from a
Table 1. Translational diffusion coefficient ($D_I$) of surface hydration water and its associated DNP parameters in various synthetic polyelectrolytes, protein polyelectrolytes, lipid vesicles, as well as some complexes formed from the components.

| Sample                              | $E_{max}$ | $T_1$ (s) | $T_{10}$ (s) | $f$  | $\rho$ | $\tau$ (ps) | $D_I \times 10^{-9}$ m$^2$ s$^{-1}$ |
|-------------------------------------|-----------|-----------|--------------|------|-------|-------------|--------------------------------------|
| Synthetic polyelectrolytes          |           |           |              |      |       |             |                                      |
| $a$ Carboxylate triblock            | $-82.3$   | $0.09$    | $1.16$       | $0.922$ | $0.137$ | $134$       | $0.863$ (d = 3.4 Å)                  |
| $b$ Amine triblock                  | $-86.4$   | $0.10$    | $1.23$       | $0.918$ | $0.141$ | $130$       | $0.889$                              |
| $c$1 HA fully spin labeled          | $-136$    | $0.26$    | $1.64$       | $0.843$ | $0.246$ | $63$        | $1.835$                              |
| $c$2 HA 0.5% spin labeled           | $-4.30$   | $1.52$    | $1.63$       | $0.067$ | $0.117$ | $156$       | $0.741$                              |
| $d$ PASp                            | $-10.4$   | $1.36$    | $1.54$       | $0.117$ | $0.148$ | $123$       | $0.940$                              |
| $d+e$ PASp/PVIm-TEMPO not coacervated| $-10.2$   | $2.30$    | $2.60$       | $0.117$ | $0.145$ | $126$       | $0.917$                              |
| Protein polyelectrolytes            |           |           |              |      |       |             |                                      |
| $f$ mfp151                          | $-1.80$   | $1.79$    | $2.09$       | $0.113$ | $0.037$ | $375$       | $0.308$                              |
| Lipid vesicles                      |           |           |              |      |       |             |                                      |
| $g$ tau-187                         | $-14.6$   | $1.95$    | $2.42$       | $0.19$  | $0.127$ | $144$       | $0.803$                              |
| $h$ DOPC                            | $-15.9$   | $1.69$    | $2.36$       | $0.284$ | $0.091$ | $195$       | $0.593$                              |
| $h+i$ DOPC:DPPC (1 : 1)             | $-6.40$   | $1.67$    | $2.17$       | $0.227$ | $0.077$ | $223$       | $0.518$                              |
| $h+j$ DOPC:DOPG (9 : 1)             | $-14.9$   | $1.54$    | $2.02$       | $0.236$ | $0.103$ | $175$       | $0.661$                              |
| $h+l$ DOPC/5% PEG                   | $-9.00$   | $1.76$    | $2.21$       | $0.202$ | $0.075$ | $226$       | $0.512$                              |
| $h+l$ DOPC/20% PEG                  | $-5.30$   | $1.57$    | $2.00$       | $0.216$ | $0.044$ | $331$       | $0.349$                              |

protonated amine) and an anionic species (formed from a deprotonated carboxylate). The two disordered and net positively charged proteins included in this study are tau-187, which is N-terminally truncated from the longest human tau isoform and includes the C-terminus and all four microtubule-binding regions [25], and a variant of the Mytilus edulis (mussel) foot protein, mfp151 [24]. mfp151 is a recombinant hybrid protein constructed from two types of independent proteins, mfp1 and mfp5, which are found in the adhesive plaque that anchors mussels to underwater surfaces [15, 24, 28]. mfp1 has been shown to have an extended form in solution and it exhibits no secondary structure. From this observation, it follows that in the hybrid structure with mfp5, mfp151 too should be extended in solution [29]. Both proteins, tau-187 and mfp151, qualify as protein-based polyelectrolytes because of their unfolded, unstructured nature and their high net positive charge, which is distributed over the entire protein backbone. The ESR spectra of all non-complexed and non-aggregated macromolecules present overall high spin label and polymer segment dynamics, although differences can be recognized; for example, spin labels on the triblock polyelectrolytes show more restricted dynamics compared to that on mfp151 or tau-187 proteins (figure 2). The results for the hydration water dynamics measured with DNP, as presented in table 1, are striking in that all non-complexed and non-aggregated
polypelectrolyte surfaces, except for the *mfp151* protein and maximally spin-labeled HA, display translational diffusion with correlation times of the order of 120–160 ps, despite their vastly different chemical structure, composition and charge density. These values are about two to four fold impeded compared to the diffusivity of bulk water ($\tau = 42.5$ ps), and these relationships have also been found by molecular dynamics simulation studies and later confirmed by experimental studies of various surfaces, including peptide or lipid vesicles [7], [30]–[34]. However, experimental values for hydration water dynamics in the literature are limited to specialized systems, such as highly concentrated peptide solutions with all embedded water molecules belonging to the peptide’s hydration layer [30], or selected lipid vesicle systems [34]. The lack of experimental data on a wider sample space can be attributed to the lack of analysis tools for probing hydration dynamics with site specificity and detection sensitivity, as discussed earlier. These literature findings as well as our results are consistent with the idea that the translational diffusivity of hydration water molecules are slowed down by two to six fold as a result of the physical presence of a polar molecular surface that presents a perturbation to water’s hydrogen bonding network. However, we also observe that hydration water dynamics on surfaces with varying chemistry or charges are reproducibly diversified, and can even distinctly vary from one protein site to another along the same disordered, unfolded, protein backbone. This shows that the local chemical and electrostatic environment influences the fine print for the translational dynamics of local and surface hydration water. This is consistent with results obtained for the intrinsically disordered *tau-187* proteins, where different sites display distinctly different local hydration dynamics, but whose values fall roughly within this 120–160 ps range. If a site, e.g. site 322 of *tau-187*, is probed that becomes part of the inter-*tau* binding interface and eventually gets buried in the hydrophobic core during the aggregation process, a gradual decrease in hydration dynamics with time around that particular site is observed by DNP. The result for the local water dynamics around a specific site, site 322, of monomeric *tau-187* before aggregation with $\tau = 144$ ps and after $>12$ h of aggregation with $\tau = 511$ ps is listed in table 1. This distinct increase in translational correlation time confirms significant burial of the particular site that is studied.
The unbranched, synthetic polyelectrolytes with uniform charge distributions, PAsp and HA, present surface hydration dynamics in the expected range. However, surprisingly high hydration dynamics with $\tau = 63$ ps are observed for the solution of HA in which every single HA chain is highly spin labeled on every carboxylate functionality along the HA chain. We believe that complete spin labeling of HA, as expected, has entirely modified the polymer characteristics, likely by creating a significantly more hydrophobic surface compared to that of native HA, on which surface higher translational diffusion dynamics for hydration water are expected [30, 35]. For this reason, authentic probing experiments are typically performed with 1–2% level spin labels included in the mixture or with only one spin label implemented per macromolecule (e.g. for tau-187 or mfp151). Anyhow, mixing in a small fraction (0.5%) of this highly spin-labeled HA into a solution of native HA (corresponding to sample c2 in table 1) provides the characteristic surface hydration dynamics of solvent-exposed (polar) macromolecular surfaces with $\tau = 156$ ps, as discussed above. This observation leads us to conclude that the spin labels on the highly spin-labeled HA chain (acting as a probe molecule) are not mainly probing the surface hydration dynamics of its own host chain whose chemical property and surface hydration dynamics are vastly modified, but that of neighboring, abundant, HA molecules that are not spin labeled and thus have the expected surface hydration dynamics of unstructured, typical, polyelectrolytes. This result is not observed for other polyelectrolytes and implies that HA molecules must be intertwined and more strongly interacting with each other in solution compared to other types of polyelectrolytes.

All of these (and other) polyelectrolytes can undergo a fluid–fluid phase separation called complex coacervation that results in polyelectrolyte-rich fluid droplets of tens of nm to $\mu$m diameter dispersed in dilute aqueous solutions, when oppositely charged natural or synthetic polyelectrolytes are mixed in solution at a pH where they neutralize one another [15, 26, 36]. When coacervated, PVIm and PAsp form dense and rather hydrophobic fluidic droplets that are significantly dehydrated (according to polarity parameters obtained from the ESR spectra), and present internal hydration water with measurably restricted dynamics [6]. Six fold arrested water dynamics from $\tau = 126$ ps on the surface of solvated polyelectrolytes to $\tau = 766$ ps in complex coacervates suggests that water is retained in nm-scale pores in which translational dynamics are restricted, but still display quasi bulk-like dynamics.

$mfp151$, spin labeled between the $mfp1$ and $mfp5$ protein segments, displays the slowest hydration of any non-complexed, extended polyelectrolyte with $\tau = 375$ ps. This value suggests that this site is somewhat buried in a (possibly transient) local structure of this protein segment, e.g. a kink, loop or other conformation that results in some degree of shielding from the bulk solvent. The presentation of significant dispersion of local hydration dynamics is, in fact, a key feature of proteins whose surface displays a rugged landscape, and whose heterogeneity and dispersion dramatically increase from an unfolded and partially folded to a structured protein. It is remarkable, although not unexpected from theory studies, that both intrinsically disordered proteins, tau-187 and mfp151, display a measurably heterogeneous landscape for surface hydration dynamics. This may suggest the existence of local or transient secondary structures and/or the influence of the chemical heterogeneity of the protein backbone on the local hydration dynamics.

Lipid vesicles present important biochemical entities, whose interactions with small molecules, proteins and polymers are well suited to mimic the interactions of the biologically relevant constituents with cell membranes. Numerous biochemical processes, such as receptor-regulated signal transduction and immunoglobulin recognition, take place on the surface of
cell membranes and thus the surface of lipid vesicles presents the most relevant platform for mediating these interactions. Similar to what has been discussed for protein aggregation or polymer complexation, the translational water dynamics of the hydration layer on lipid vesicle surfaces also present an exquisite probe for reporting on interactions between membranes and macromolecules. The diffusivity of the surface hydration layer in unilamellar 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DPPG), as well as their binary lipid vesicles of ∼200 nm diameter, was measured using 1–2 mol% of spin-label probe made of 1,2-dioleoyl-sn-glycero-3-phospho(tempo)choline lipids (PC-TEMPO), spin labeled off (above) the lipid headgroup. We found the translational correlation time for lipid surface hydration to be τ = 195 ps for DOPC, τ = 248 ps for DPPC, τ = 223 ps for DOPC : DPPC (1 : 1) and τ = 175 ps for DOPC : DOPG (9 : 1) surfaces, all of these values lying in the range between τ = 175 and 250 ps. These dynamics are again impeded, to a somewhat greater extent by approximately two to four fold, compared to bulk water diffusion. We can also observe that the lipid surface hydration dynamics are measurably slower compared to that of solvent-exposed polyelectrolyte surfaces, which may be attributed to the larger perturbation to hydration dynamics caused by the larger and two-dimensional (2D)-like physical surface presented by lipid vesicles compared to flexible, single-chain and extended, polyelectrolytes. However, the ESR spectra of surface-labeled lipid vesicles compared to the unstructured polyelectrolytes present spin-labeled dynamics that are slowed to a significantly greater degree (figure 2), compared to changes observed with surface hydration dynamics measurements. This confirms that the spin label dynamics do not necessarily correlate with hydration dynamics, as expected.

Finally, we studied the surface hydration dynamics of DOPC lipid vesicles with the addition of PEG and found that the surface hydration dynamics become measurably slowed from τ = 195 ps to τ = 226 ps when adding 5% PEG, and further to τ = 331 ps when adding 20% PEG. PEG has been widely used as an agent to induce cell fusion and aggregation, to stabilize bio-relevant nanoparticles, and to control the vesicle functions for drug delivery [37]–[39]. However, the mechanism of PEG’s membrane-assisting function has not been elucidated. As an example, it has been speculated that PEG’s membrane-assisting function is the result of the dehydration of lipid membrane surfaces. It has been reported before that the adsorption of PEG on the phospholipid headgroups could result in not only vesicle shrinkage [40] but also surface dehydration, and that discussion is still ongoing [41]. While we do not strictly measure the effects of ‘dehydration’, our experimental results confirm that the surface hydration dynamics of lipid vesicles in the presence of PEG are measurably decreased by 14–41%, depending on the PEG concentration, in agreement with expected characteristics upon (partial) solvent depletion at the interface. It should be noted, however, that the decrease in hydration dynamics is not necessarily equivalent to dehydration effects. We noted that in the literature the term dehydration is often used for systems where a decrease in hydration dynamics would be a more appropriate explanation, e.g. to describe the modulation of hydration dynamics at lipid vesicle surfaces that are fully exposed to bulk water. This can be attributed to the lack of tools available with which to measure the direct effect of hydration dynamics, especially of molecular systems fully dispersed in bulk water. The most compelling observation that supports the probing of local hydration dynamics as an unusually sensitive approach for mapping molecular interactions is that the ESR spectral line-shapes of surface-labeled lipid vesicles showed no measurable changes when adding 5% PEG (see figure 2, h2) and only very slight changes when adding 20% PEG (see figure 2, h3). The packing of headgroups of lipid bilayers only increases slightly when adding
massive quantities of PEG of the order of 40%, according to ESR [42, 43] and zeta-potential studies [44]. Quantitative dehydration of lipid surfaces must be correlated to the modulation of headgroup dynamics and/or packing, which effect only became barely noticeable with high 40% PEG addition, while DNP has been shown to sensitively detect weak and transient polymer–membrane interactions in the presence of as little as <5% PEG through significant changes in surface hydration dynamics at the interaction interface.

5. Conclusions

We present the measurements of the translational correlation times and diffusion coefficients of local surface and interfacial hydration water on a series of polyelectrolytes, proteins and lipid vesicle systems dispersed in aqueous solutions using a recently introduced technique named $^1$H Overhauser DNP. We found that the translational dynamics of the hydration water of a non-complexed, non-interacting, hydrophilic and solvent-exposed surface are about two to six fold impeded compared to that of bulk water. We observed deviations from these trends for protein surfaces that may display transient secondary structures and for maximally spin-labeled HA whose molecular surface is dramatically altered, possibly with increased hydrophobicity. In addition, we found a two to four fold arrest of hydration dynamics on the surface of lipid vesicles as compared to that of bulk water. We demonstrate that probing the modulation of the solvent-exposed hydration dynamics near these surfaces is an exquisite parameter for revealing the nature of molecular interactions. For example, the following phenomena are all concomitant with a reduction in hydration dynamics at the interacting surface or interface: the complex coacervation of polyelectrolytes, aggregation of proteins or addition of PEG polymers to lipid vesicles. Complementary to the trends in hydration dynamics in these systems are the ESR spectra that clearly show significantly slowed (side chain or segment) mobility of polyelectrolytes upon complex coacervation and of tau-187 upon aggregation. In contrast to these processes, ESR spectra reveal that lipid molecular dynamics in vesicles remain unaltered up to the addition of 20–30% PEG to the solution. This is in stark contrast to the significant slowing of translational hydration dynamics by up to 41% caused by lipid vesicle–PEG interactions with 20% PEG concentration, measured with the $^1$H Overhauser DNP. These findings suggest that lipid–PEG interactions are strong enough to impede interfacial hydration dynamics, but sufficiently weak to not alter the dynamics of the spin-labeled lipid segments.

Key strengths of the Overhauser DNP technique include the high selectivity for mapping local hydration dynamics within 10 Å of spin labels, allowing for the site-specific mapping of molecular interfaces, and the high detection sensitivity provided by the amplification of $^1$H NMR signal by orders of magnitude, facilitating systematic analysis of a large sample space because only minute sample quantities are needed. Also important is the possibility of concurrent electron spin resonance analysis for measuring the polarity and chain rotational dynamics of the same samples.

While our experiments have been carried out at 0.35 T and are thus sensitive to motion in the picoseconds to sub-nanosecond time scale, the technique could be extended to cover other time scales as well by carrying out DNP at different field strengths. Overhauser DNP, for example, will capture tens of nanoseconds hydration dynamics at 0.04 T or sub- to several picoseconds dynamics at 7 T. The capability of probing the translational hydration dynamics of macromolecular surfaces and their interaction interfaces, as provided by Overhauser DNP, has very important and wide-ranging implications for the study of polymer complexation, protein folding and aggregation, as well as membrane fusion, transport or stability.
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References

[1] Fenimore P W, Frauenfelder H, McMahon B H and Young R D 2004 Proc. Natl Acad. Sci. USA 101 14408–13
[2] Frauenfelder H, Chen G, Berendzen J, Fenimore P W, Jansson H, McMahon B H, Stroe I R, Swenson J and Young R D 2009 Proc. Natl Acad. Sci. USA 106 5129–34
[3] Cheung M S, Garcia A E and Onuchic J N 2002 Proc. Natl Acad. Sci. USA 99 685–90
[4] Dill K A 1990 Biochemistry 29 7133–55
[5] Chandler D 2005 Nature 437 640–7
[6] Kausik R, Srivastava A, Korevaar P A, Stucky G, Waite J H and Han S 2009 Macromolecules 42 7404–12
[7] Kausik R and Han S 2009 J. Am. Chem. Soc. 131 18254–56
[8] Pavlova A, McCarney E R, Peterson D W, Dahlquist F W, Lew J and Han S 2009 Phys. Chem. Chem. Phys. 11 6833–9
[9] Armstrong B D and Han S 2009 J. Am. Chem. Soc. 131 4641–7
[10] Hubbell W L, Cafiso D S and Altenbach C 2000 Nat. Struct. Biol. 7 735–9
[11] Fitter J, Lechner R E and Dencher N A 1999 J. Phys. Chem. B 103 8036–50
[12] Absheer R, Schreiber H and Steinhauser O 1996 Proteins 25 366–78
[13] Steinhoff H J, Kramm B, Hess G, Owerdieck C and Redhardt A 1993 Biophys. J. 65 1486–95
[14] Polnaszek C F and Bryant R G 1984 J. Chem. Phys. 81 4038–5
[15] Hwang D S, Waite J H and Tirrell M 2010 Biomaterials 31 1080–4
[16] Armstrong B D and Han S 2007 J. Chem. Phys. 127 104508–10
[17] Hauser K H and Strehl D 1968 Adv. Magn. Reson. 3 79–139
[18] Overhauser A W 1953 Phys. Rev. 92 4115
[19] Potenza J 1972 Adv. Mol. Relax. Proc. 4 229–354
[20] Robinson B H, Haas D A and Mailer C 1994 Science 263 490–9
[21] Abragam A 1961 The Principles of Nuclear Magnetism (Oxford: Clarendon)
[22] Hwang L P and Freed J H 1975 J. Chem. Phys. 63 4017–25
[23] Bennati M, Luchinat C, Parigi G and Turke M T 2010 Phys. Chem. Chem. Phys. 12 5902–10
[24] Hwang D S, Kim Y, Yoo H J and Cha H J 2007 Biomaterials 28 3560–8
[25] Peterson D W, Zhou H J, Dahlquist F W and Lew J 2008 Biochemistry 47 7393–404
[26] Hwang D S, Zeng H B, Srivastava A, Krogstad D V, Tirrell M, Israelachvili J N and Waite J H 2010 Soft Matter 6 3232–6
[27] McCarney E R, Armstrong B D, Kausik R and Han S 2008 Langmuir 24 10062–72
[28] Hwang D S, Sim S B and Cha H J 2007 Biomaterials 28 4039–46
[29] Deacon M P, Davis S S, Waite J H and Harding S E 1998 Biochemistry 37 14108–12
[30] Russo D, Hura G and Head-Gordon T 2004 Biophys. J. 86 1852–62
[31] Qvist J, Persson E, Mattea C and Halle B 2009 Faraday Discuss. 141 131–44
[32] Makarov V A, Feig M, Andrews B K and Pettitt B M 1998 Biophys. J. 75 150–8
[33] Jansson H, Kargl F, Fernandez-Alonso F and Swenson J 2009 J. Chem. Phys. 130 205101–13
[34] Hodges M W, Cafiso D S, Polnaszek C F, Lester C C and Bryant R G 1997 Biophys. J. 73 2575–9
[35] Sorenson J M, Hura G, Soper A K, Pertsemidis A and Head-Gordon T 1999 J. Phys. Chem. B 103 5413–26
[36] Srivastava A, Waite J H, Stucky G D and Mikhailovsky A 2009 Macromolecules 42 2168–76
[37] Woodle M C and Lasic D D 1992 Biochim. Biophys. Acta 1113 171–199
[38] Schifferlers R M, Ansari A, Xu J, Zhou Q, Tang Q Q, Storm G, Molema G, Lu P Y, Scaria P V and Woodle M C 2004 Nucl. Acids Res. 32 10
[39] Boni L T, Hah J S, Hui S W, Mukherjee P, Ho J T and Jung C Y 1984 Biochim. Biophys. Acta 775 409–18
[40] Ito T, Yamazaki M and Ohnishi S 1989 Biochemistry 28 5626–90
[41] Lehtonen J Y A and Kinnunen P K J 1994 Biophys. J. 66 1981–90
[42] Herrmann A, Pratsch L, Arnold K and Lassmann G 1983 Biochim. Biophys. Acta 733 87–94
[43] Bartucci R, Montesano G and Sportelli L 1996 Colloid Surf. A 115 63–71
[44] Arnold K, Zschoernig O, Barthel D and Herold W 1999 Biochim. Biophys. Acta 1022 303–10