Electronic Supporting Information

Water Proton NMR—A Sensitive Probe of Solute Association

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Materials and Methods:

General Information:

BSA (bovine serum albumin), γ-globulin and sodium octanoate (NaC_8) were purchased from Sigma Aldrich; sodium perfluorooctanoate ($NaFC_8$) was purchased from Alfa Aesar. All compounds were used without further purification.

NMR experiments were carried out at 9.4 T using a Varian INOVA spectrometer equipped with a broad band probe with Z-gradient; and at 0.47 T using a UNIQ PMR benchtop spectrometer with a 10-mm proton probe.

Sample Preparation:

1. BSA solutions

BSA was dissolved in phosphate-buffered saline (PBS, 50 sodium phosphate, 100 sodium chloride, pH 7.4) of different concentrations (0, 2, 5, 7, 10, 15, 20, 25 and 30 mg/mL). Aggregation was induced by heating BSA solutions to 55 or 60 °C for 2 – 30 min. The heated solutions were then cooled down to room temperature for NMR or DLS measurements. For the determination of the correlation between the water proton R_2 and the average molecular weight of the aggregate, BSA concentration was fixed at 15 mg/mL.

2. γ-Globulin solutions

γ-Globulin was dissolved in PBS at 15 mg/mL. Aggregation was induced by heating in the same way as BSA.

3. Sodium octanoate (NaC_8)

15 samples of different concentrations of NaC_8 (0.009, 0.014, 0.019, 0.038, 0.075, 0.15, 0.30, 0.60, 0.90, 1.20, 1.50, 1.80, 2.00, 2.20 and 2.40 M) were prepared using volumetric serial dilution from a stock solution of 2.40 M of NaC_8 in water, which was prepared by gradually adding DI-water to NaC_8 in a 50-mL centrifuge tube and nutated slowly overnight at room

temperature. Afterwards, the solutions were let to equilibrate at room temperature for 4-6 weeks before measurements.

Previous works on NaC_8 were mostly reported in molality.[S1] To convert the literature reported CMC values from molality (mol/kg) to molarity (mol/L), the specific density ρ (in g/mL) of each solution was measured at 22.5°C using a density meter (DMA 5000 from Anton Paar).

4. Sodium perfluorooctanoate ($NaFC_8$)

16 samples of different concentration (1.7, 2.5, 3.9, 5.8, 8.7, 13.0, 19.5, 29.3, 36.6, 43.9, 65.8, 98.8, 148.2, 222.2, 333.3 and 500 mM) were prepared by serial dilution from a stock solution of 500 mM $NaFC_8$ in DI water.

NMR experiments:

In all NMR experiments, the CPMG (Carr-Purcell-Meiboom-Gill) sequence[S2] was used to measure the water proton transverse relaxation time (T_2). The relaxation delay (*d1*) was larger than $5\times T_1$ in all cases. The water proton T_2 value can be extracted from the following equation:

$$I(t) = I_0 \times \exp(-t/T_2) \quad (1)$$

where $I(t)$ is the water proton signal intensity at time t; I_0 is the initial signal intensity when $t = 0$; and t is the T_2 delay time. By increasing t, a signal intensity decay curve can be obtained. The relaxation constant T_2 was extracted by fitting the $I(t)$ vs. t curve to Eqn. 1.

For all NMR experiments carried out at 9.4 T, the sample was loaded into a 3-mm NMR tube, which was then inserted into a 5-mm NMR tube that was preloaded with D_2O and trace amount of trimethylsilyl propionate (TSP) to provide the deuterium lock signal and chemical shift reference. At 9.4 T, a small flip-angle excitation pulse (about 10°) was used to avoid overflowing the receiver by the water proton signal.

1. BSA solutions

For the CPMG experiments at 9.4 T, the interval between the 180° pulses (2τ) was 120 μs, and 4 transients (nt=4) were collected. Ten different values of t, the T_2 delay time, were used ($t = 2n\tau$, n is the number of 180° pulses. The t value was varied by varying n while τ was fixed at 120 μs). To get good fitting results, these parameters were optimized based on the specific T_2 value of each sample.

For the CPMG experiments at 0.47 T, the interval between the 180° pulses (2τ) was 2 ms. Instead of using the peak area as the signal intensity, the amplitude of the FID of each CPMG echo was used as $I(t)$ in Eqn. 1. Based on the specific T_2 value of each sample, the echo numbers were optimized (400-2000) to get the proper signal decay curve.

2. γ-Globulin solutions

For γ-globulin solutions, the NMR experiment parameter settings were same as that of BSA.

3. NaC_8

Chemical shifts of NaC_8 hydrocarbon protons were obtained from 1D pre-saturation experiments. 5 s recovery delay ($d1$), 30° excitation pulse was used and 32 transients (nt=32) were collected. The chemical shift of the C^2 methylene protons and that of the C^3 methyl protons were used for plotting Figure 2a.

For the water proton R_2 measurements at 9.4 T, $nt = 4$, $\tau = 1.0$ ms, and the values of $2n\tau$ were optimized based on the R_2 value of each sample. For instance, for the 0.009 M sample, $2n\tau$ increased from 0.1 to 5 s in 13 steps; while for the 2.40 M sample, $2n\tau$ increased from 0.1 to 2 s in 9 steps.

For the water proton R_2 measurements in NaC_8 solutions at 0.47 T, the NMR parameters were the same as those for BSA.

4. $NaFC_8$

For $NaFC_8$ solutions, the parameter settings for the water proton R_2 measurements were same as those of NaC_8.

Dynamic Light Scattering (DLS) Experiments:

For DLS measurements, BSA and γ-globulin samples were the same as used in NMR studies. 1 mL of each sample was loaded into a cylindrical glass vial (6 mm in diameter). Data collection at 90° scattering angle started after complete equilibration at 25 °C (± 0.1 °C) in the cavity of the light scattering setup. DLS experiments were performed with a PhotoCor Instruments instrument,[S3] and the software *DynaLS* (SoftScientific, Inc.) was used to process the scattering data. For a single-exponentially decaying relaxation process, the intensity autocorrelation function $g_2(t)$ (obtained in the homodyning mode) is given as[S4, S5]

$$g_2(t) - 1 = A\exp[-2\frac{t}{\tau}] \tag{2}$$

where A is the amplitude of the relaxation process, t is the "lag" (or "delay") time of photon correlation, and τ is the characteristic relaxation time of the polarization fluctuation which essentially gives rise to light scattering. For a diffusive relaxation process, the decay (relaxation) time τ reflects the average time of the particle travels within the laser spot of the instrument and, thus, is related to particle mobility, and, hence, the collective diffusion coefficient D_c as[S4, S5]

$$\tau = \frac{1}{D_c q^2} \tag{3}$$

where q is the difference in the wave vectors between the incident and scattered light beams,

$$q = \frac{4\pi n \sin(\frac{\theta}{2})}{\lambda} \tag{4}$$

n is the refractive index of the solvent (1.33245095 for water), λ is the wavelength of the incident light in vacuum (λ = 633 nm for a He−Ne laser), and θ is the scattering angle (90°). Thus, q = 0.0187 nm^{-1}. For mono-disperse, non-interacting, spherical Brownian particles, the hydrodynamic radius R_h can be calculated with the Stokes-Einstein relation[S4, S5]

$$R_h = \frac{k_B T}{6\pi\eta D_c} \tag{5}$$

where k_B is Boltzmann's constant (1.381×10^{-23} J/K), T is the absolute temperature (298 Kelvin), and η is the viscosity of the solvent ($8.93904021 \times 10^{-4}$ Pa·s for water at 25°C). The mean values R_h of the observed size distributions of the aggregates were used to obtain the average molecular weight of the aggregates based on the known relationship[S6] of the R_h and number average molecular weight of the polymer $\overline{\text{M.W.}}$:

$$R_h \sim \overline{\text{M.W.}}^{0.5} \tag{6}$$

Figure S1. ^{1}H spectrum of 15 mg/ml BSA (Fig. S1a and S1c) and γ-globulin solutions (Fig. S1b and S1d). (a) and (b), without water suppression: the water signal are sharp and very easy to be detected (only 1 scan), while the protein signal are invisible at the same condition. (c) and (d), with pre-saturation water suppression: even with 100 scans, the protein proton signal are still weak and complex.

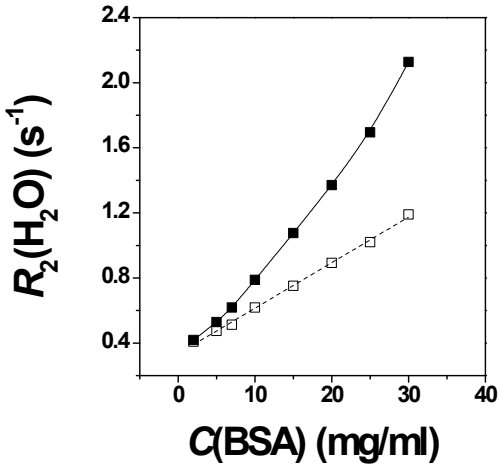

Figure S2. Water proton R_2 vs. BSA concentration without (open squares) and with (solid squares) heat-induced aggregation. In the absence of aggregation, a linear relationship between the water proton R_2 and BSA is observed. At a given BSA concentration, aggregation caused a jump of the water proton R_2.

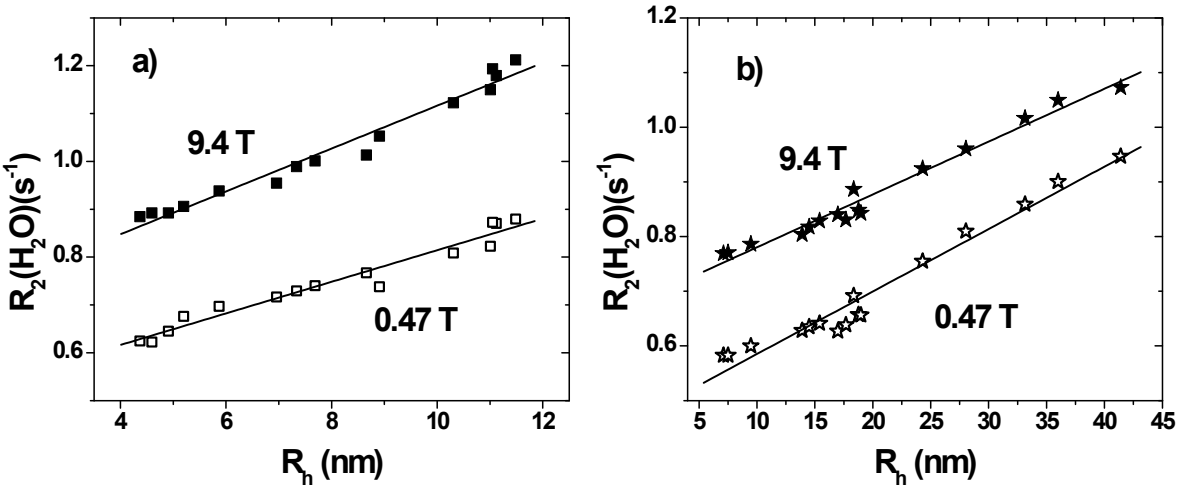

Figure S3. Water proton R_2 vs R_h of a) BSA and b) γ-globulin detected at 9.4 T and 0.47 T.

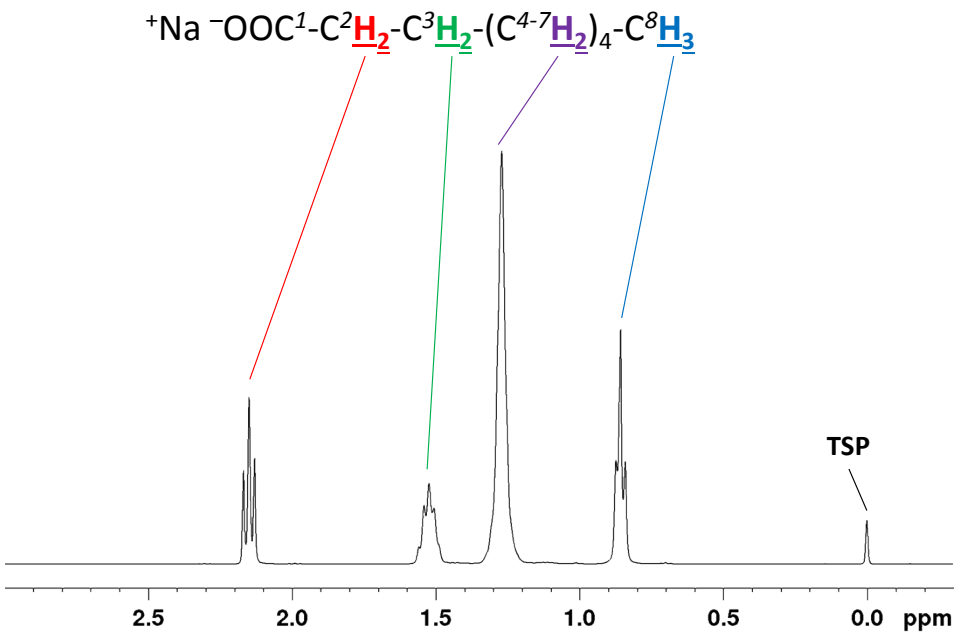

Figure S4. Structure of NaC_8 and its ^1H spectrum. TSP is the chemical shift reference.

Figure S5. Protein aggregation is not always visually obvious. Vial #0: phosphate buffered saline (PBS), Vial #1: bovine serum albumin (BSA, 4.5×10^{-4} M) in PBS; Vial #2: BSA (2.3×10^{-4} M) after 30 min heating to 60°C; Vial #3: BSA (1.5×10^{-4} M) after 30 min heating to 60°C; Vial #4: BSA (7.5×10^{-5} M) after 30 min heating to 60°C. Aggregates are absent in Vials 0, 1; and aggregates are present in Vials 2, 3, 4.

Figure S6. A sealed vial of a pharmaceutical product can be loaded into a benchtop NMR tube for water proton R_2 measurement.

Table S1. Diamagnetic Susceptibility of Compounds

Compound	χ_v ($\times 10^{-6}$)	$\Delta\chi_V$ ($\times 10^{-6}$)
H_2O	-9.05	0
Partially fluorinated		
$C_6H_5CH_3$(toluene)	-7.75	1.29
$C_6H_5CF_3$	-7.84	1.21
2-CH_3-phenol (m-cresol)	-8.67	0.38
2-CF_3-phenol	-8.66	0.39
C_6H_6	-7.73	1.32
C_6H_5F	-7.82	1.23
CH_3CH_2OH	-7.25	1.80
CHF_2CH_2OH	-8.14	0.90
Perfluorinated		
n-C_6H_{14}	-7.14	1.91
n-C_6F_{14}	-8.36	0.69
c-C_6H_{10}	-7.20	1.85
c-C_6F_{10}	-8.07	0.98
CH_3COOH	-12.94	-3.90
CF_3COOH	-7.29	1.76
C_2H_5COOH	-7.24	1.81
C_2F_5COOH	-7.30	1.75
C_3H_7COOH	-7.50	1.55
C_3F_7COOH	-7.83	1.22

Notes:

1. For each pair of compound, the hydrogenated one is in black while the fluorinated one is in blue. In each pair, the fluorinated compound has smaller diamagnetic susceptibility contrast $\Delta\chi_v$ (= χ_v(compound) - χ_v(water)) with water than the hydrogenated one.
2. The volume diamagnetic susceptibility χ_v of a compound was calculated from the molar diamagnetic susceptibility χ_m, the density and the molecular weight of that compound listed in reference,[S7, S8] and converted to the SI unit. Unfortunately, there is no published data on χ_v of $NaFC_8$. But the three perfluorinated carboxylic acids in this Table all have χ_v values closer to water than their hydrogenated counterparts.

References:

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S8 R. Perceau, *Compt. Rend.*, 1954, **239**, 170-171.