Internal mobility limits the accuracy of NMR1 structures:2 NOEs are quenched, and conformational and/or chemical exchange broaden resonances, thus impeding extraction of conformational constraints. A shift of temperature, T, may move such processes into regimes of very fast or slow exchange on the chemical shift time scale. While a large increase of T is limited by macromolecular stability and excitation of yet additional motions, a decrease well below 0 °C is attainable in supercooled water.3 This promises more accurate NMR structures, a means to freeze out conformations and novel insights into biomolecular dynamics, hydration, and cold denaturation. NMR of small carbohydrates allowed observation of hydroxyl protons,4 but multidimensional spectra of macromolecules have not been reported. Here we show the feasibility of NMR-based structural biology in supercooled water.

NMR in supercooled water is hampered by high viscosity, η, yielding long overall rotational correlation times, τc, and line broadening; an exponential, η(T), was fitted to published values5 (Figure 1a). Hybrid dynamic theory6 predicts for rigid spherical proteins that τc = 4πη(μT)τR/3kT (eq 1). τR is the effective radius with ρR = 3Mw/4πNh2/3 + rw (eq 2), where V = 0.73 cm3/g, M, Nw, and rw are the protein’s specific volume and molecular weight, Avogadro’s number, and the added radius of a monolayer of water, respectively. With rw = 3.2 Å, eq 2 yields τR = 17.2 Å for 9.4 kDa recombinant ubiquitin.6 To verify that theory applies at ~0 °C; we determined τc between 25 and −15 °C from 1H T1/T2 ratios7,8 (Figure 1b; Table S1). With η(T) of Figure 1a, a fit of eq 1 to τc yields τR = 17.2 ± 1.0 Å and allows prediction of τc below −15 °C (Figure 1b). The very good agreement

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(1) Abbreviations used: NMR, nuclear magnetic resonance; 1D, 2D, 3D, one-, two-, three-dimensional; HCNA, NMR experiment correlating polypeptide backbone1H,15N, and 13C chemical shifts; HSQC, heteronuclear single quantum correlation; NOE, nuclear Overhauser effect; T1l, longitudinal nuclear spin relaxation time; T2l, transverse nuclear spin relaxation time in the rotating frame; TROSY, transverse relaxation-optimized spectroscopy; 1H, 2D; deoxyguanosine-5-triphosphate; dTTP, 2D; deoxythymidine-5-triphosphate; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.


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Figure 1. Overall rotational tumbling of globular proteins in supercooled water. The freezing point of water (273 K) is indicated. (a) Viscosity, η, of water as a function of T. The dots represent published values. The fitted curve represents the indicated exponential function. (b) Rotational correlation time, τc, of ubiquitin7 versus T. Experimental values8 are represented by dots, and the middle curve (asterisk) was obtained from a fit of eq 1 yielding τR = 17.2 Å. The upper (τR = 18.2 Å) and the lower curve (τR = 16.6 Å) enclosing the experimental values shown at higher resolution in the insert. Fits were performed with SigmaPlot 4.0. Between theory and experiment suggests that theory, in general, allows estimation of τc of macromolecules in supercooled water.

Here we present the first multidimensional NMR spectra acquired9 for a protein (ubiquitin) in supercooled water. The good quality of 1H NMR spectra (Figures 2a, S3, S4) shows that structure determinations of small proteins (<10 kDa) pursued below −10 °C will profit from homonuclear 1H NMR. High-quality 2D [13C,1H]-HSQC (Figure 2b) at −15 °C and 3D HCNA at −11 °C (Figure 2c) show that heteronuclear resolved NMR determinations serve well to obtain assignments. TROSY10 is tailored for long range 2D [15N, 1H]-TROSY (Figure 2d) shows that such spectroscopy is well suited below 0 °C (pronounced differential line broadening was observed in 15N-ωH-J15N-coupled HSQC at −15 °C, Figure 3b). For structure determinations in supercooled water, measurements of residual dipolar couplings11 are attractive12 since large τc may require deuteration.13 Since bicele systems are restricted to ambient T, we explored the Phe phage system.14 1% (0.5%) solutions in capillaries4 can be cooled to −8 °C (−15 °C), i.e., at −0.5 °C the impact of capillaries4 is reduced. Moreover,