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A Facile Oxygen-17 NMR Method to Determine Effective Viscosity in Dilute, Molecularly Crowded and Confined Aqueous Media

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EXPERIMENTAL SECTION

Materials

Ficoll 70 and 1,1,1,3,3,3-Hexafluoro-2-propanol (HFiP) were obtained from Sigma-Aldrich. Sucrose was from Merck and agarose from Invitrogen. Deionized Milli-Q water was used for all aqueous solutions.

The Ile-Phe dipeptide was purchased from Bachem (product number 4001668.0001) and used without further purification. The peptide was dissolved in HFiP at 200 mg/mL concentration.[1] For gel formation, the stock solution of the peptide was diluted to 30 mg/mL by adding heated water containing 10% D_2O.[1, 2] The sample was then transferred to a 3-mm NMR tube before it formed the gel.

The FG-based peptide (sequence: Acetyl-GGGGGGLFGGNNNQQTNPTA-Amid) capped at both N- and C-termini, was obtained from Peptide Specialty Laboratory (PSL, Heidelberg, Germany). For gel formation, peptide solutions in 50 mM potassium phosphate (pH 6.8) were prepared at three concentrations, 1.6, 8 and 20 mM, and immediately transferred to 3-mm NMR tubes. The gel formation was evident in the samples leftover in the Eppendorf tube.

^{15}N,^{13}C-labeled ubiquitin and heterotrimeric G protein beta subunit 3 (GB3) were prepared recombinantly, as described in references[3, 4].

^{17}O NMR

^{17}O nucleus has spin quantum number *I* of 5/2 and natural abundance of 0.037%. The quadrupole moment (Q) of ^{17}O is -2.63 fm^2, which interacts with the electric field gradient present at the site of ^{17}O nuclei.[5] The quadrupolar interaction is anisotropic and therefore in the absence of rotational averaging leads to broad powder patterns in NMR spectra.[6] In solution, the traceless quadrupolar interaction is entirely averaged out by fast unrestrained molecular reorientations and only indirectly contributes to relaxation processes.[7] As the dominant relaxation mechanism of ^{17}O, the quadrupolar interaction in general case leads to a three-exponential relaxation process.[8] However, in the extreme narrowing regime, the terms related to two of these relaxation processes are cancelled and consequently a single-exponential relaxation is recovered.[7, 8]

^{17}O NMR experiments were performed at a Bruker spectrometer with proton Larmor frequency of 400.13 MHz. The spectrometer was equipped with a room-temperature triple resonance broadband (TBO) probe, where for the ^{17}O-detected experiments the inner coil of the probe was tuned and matched at ^{17}O Larmor frequency of ~ 54.24 MHz. The temperature was controlled to ±0.05 K using the Bruker VT unit calibrated using a standardized thermocouple. The NMR samples contained H_2O/D_2O at a ratio of 90%/10% (v/v), unless specified otherwise, and the deuteron signal was used for frequency locking. The NMR samples were prepared using deionized Millipore water and were degassed under N_2 gas for ~ 10

minutes before NMR measurements. To alleviate the problem of baseline distortions potentially caused by the transient response of the NMR probe, a relatively long pre-acquisition delay of 18 μs was used. The ^{17}O spectra are shown using the frequency of H^2DO lock signal as the chemical shift reference (4.700 ppm).

The ^{17}O longitudinal relaxation rates (R_1) were measured through standard inversion-recovery (d_1-180°-t-90°-acq) pulse sequence, where duration of recovery time, t, varied from 0.25 to 30 ms. A total of 21 recovery data points were collected. A recycle delay (d_1) of 0.5 s was used. The data were fitted to a three-parameter single-exponential recovery function, as follows:

$$I = I_\infty(1 - 2be^{-R_1 t}) \text{ (eq. S1)}$$

where I_∞ represents signal intensity after complete recovery, R_1 is the longitudinal relaxation rate, and parameter b takes care of slight imperfections in the inversion pulse.

The rotational correlation time of water molecules (τ_{rot}) were calculated from ^{17}O R_1 rates through equation S2:

$$R_1 = \frac{3\pi^2}{10}\left(\frac{2I+3}{I^2(2I-1)}\right)\chi^2\left(1+\frac{\eta^2}{3}\right)\tau_{rot} \quad \text{(eq. S2)}$$

where I=5/2 is the spin quantum number of ^{17}O, $\chi = \frac{e^2 Q q_{zz}}{h}$ is quadrupole coupling constant (C_Q) and η is the asymmetry parameter, describing the deviation of electric field gradient tensor, q, from axial symmetry. This equation relies on the assumption of the extreme-narrowing regime, where $J(0)=J(\omega)=J(2\omega)$.[7] This is a good approximation, when rotational correlation times are shorter than $1/(20\omega_O)$, i.e. ~ 150 ps, a condition which holds for bulk water ($H_2{}^{17}O$ or $D_2{}^{17}O$) molecules in all our experimental conditions. It is notable that the obtained τ_{rot} corresponds to the rotational correlation time of the principal axis of the electric field gradient tensor at the ^{17}O nucleus of water molecules, which is an out-of-plane vector orthogonal to O-H bonds, and therefore does not necessarily represent rotational correlation times around other axes of water molecules.[9]

To find an empirical relation between the ^{17}O R_1 rates of water, which in the extreme narrowing regime are proportional to the τ_{rot} of water molecules, and solution viscosity in glycerol-water mixtures, polynomial equations of degrees 1, 2 and 3 were tried. At 298 K, the fit to a quadratic equation was significantly better than a linear equation (p-value < 0.001), but a cubic equation did not show a significant improvement in the fit when compared to a quadratic equation (p-value ~ 0.744). It is however important to note that the effective viscosity experienced by relatively large biomolecules is a collective property determined through a complex function of the properties of individual water molecules, e.g. their τ_{rot}. Consequently, the quadratic equation (equation 1 in the main text) should be seen only as an empirical relation without implying any particular physical model or interpretation for the coefficients.

^{15}N relaxation

^{15}N relaxation experiments were performed at a Bruker spectrometer with a proton Larmor frequency of 599.9 MHz equipped with a cryogenic QCI probe. NMR samples contained 4 mM uniformly labelled ubiquitin or 3 mM GB3, buffered with 50 mM sodium phosphate and 100 mM sodium chloride at pH 6.5. The ^{15}N/^{15}N-^{1}H (CSA/DD) cross-correlated relaxation (*CCR*) rates were measured at 298 K, following a standard pulse sequence [10], using relaxation delays of 40, 60, 80, 100 and 120 ms. The obtained *CCR* rates closely matched the rates measured through another pulse sequence,[11] where the use of four complementary experiments enabled cancelling errors due to pulse miscalibrations and uncontrolled attenuation factors. The residue-specific rotational correlation times (τ_c) were estimated from the *CCR* rates, using a ^{15}N CSA magnitude of -160 ppm and an angle of 17° between the principal axis of the ^{15}N CSA tensor and internuclear N-H vectors.[12]

The ^{15}N longitudinal (R_1) and transverse (R_2) relaxation rates of 0.37 mM ubiquitin in dilute and crowded solutions (200 mg/mL Ficoll 70 or 200 mg/mL sucrose) solutions were obtained from reference [13]. The residue-specific τ_c values were calculated from R_2/R_1 ratios,[14] using an in-house MATLAB script (see below, page S14).

Hydrodynamic calculations

To predict viscosity in highly concentrated protein solutions, hydrodynamic calculations were performed using HYDROPRO 10, [15] which provide hydrodynamic parameters of proteins at infinite dilution using a bead model of their known atomistic structures as the starting point. The PDB entries of 1UBQ (ubiquitin) and 1P7F (GB3) were used for hydrodynamic calculations.[16, 17] The bead models were constructed using an atomic effective radius (AER) of 2.5 to 3.0 Å.[15] The errors in hydrodynamic calculations were estimated through standard deviation of the obtained results over the range of AER values. Taking the intrinsic viscosity-related radii from HYDROPRO 10 results, the volume occupancy (ϕ) was calculated, then, the relative viscosity (η/η_0) was predicted using the Guth-Gold-Simha equation,[18]

$$\frac{\eta}{\eta_0} = 1 + 2.5\phi + 14.1\phi^2 \quad \text{(eq. S3)}$$

where η_0 is the bulk viscosity. Equation 3 reduces to the Einstein equation,[19]

$$\frac{\eta}{\eta_0} = 1 + 2.5\phi \quad \text{(eq. S4)}$$

at sufficiently small ϕ values. The calculated ϕ values were 5.2±0.2% in 4 mM ubiquitin and 2.7±0.1% in 3 mM GB3 solutions. Using the Guth-Gold-Simha equation (eq. S3), the increase in solution viscosity was predicted to be 17±1 % for 4 mM ubiquitin and ~ 8% for 3 mM GB3 protein.

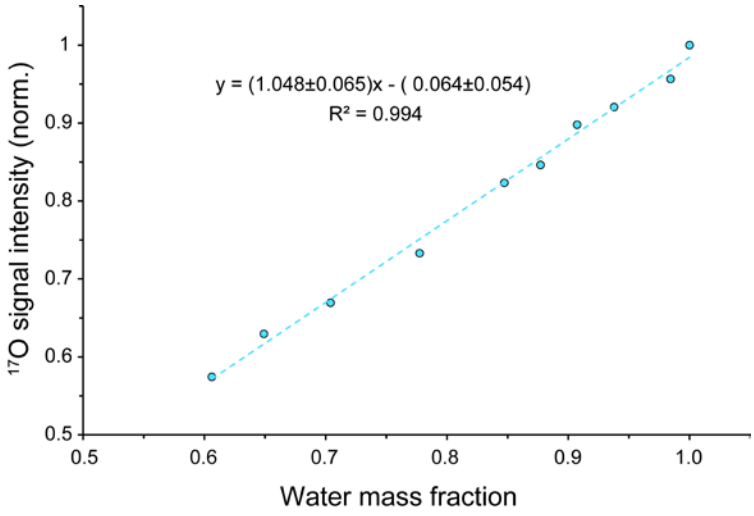

Figure S1. In 1D ^{17}O NMR spectra of water-glycerol mixtures, obtained through pulse-acquire experiments, the integrated intensity of the $H_2{}^{17}O$ signals changes in line with water content of the measured samples. The signal intensities are normalized to the value obtained at 0% glycerol (i.e. 100% water). A good linear relation with a slope of 1.048 (95% CI: 0.983-1.113) is observed.

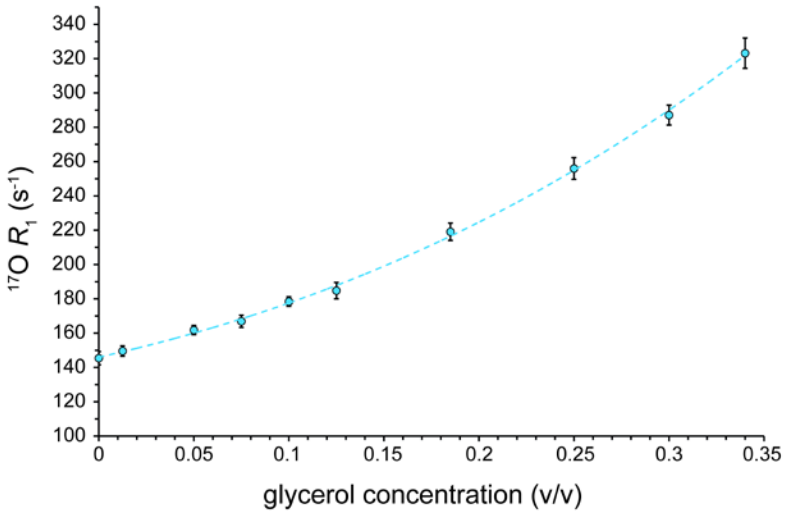

Figure S2. ^{17}O longitudinal relaxation rate (R_1) of water in water-glycerol mixtures, measured through inversion-recovery experiments, as a function of glycerol (v/v) concentration.

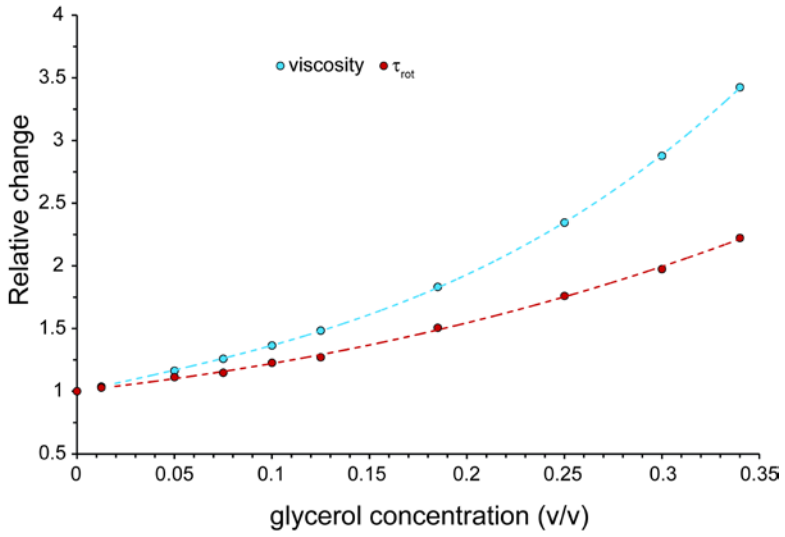

Figure S3. Upon increasing glycerol concentration, relative changes in solution viscosity and rotational correlation time (τ_{rot}) of water molecules calculated from ^{17}O R_1 rates are compared. The solution viscosity is affected by glycerol addition more prominently than the τ_{rot} of water molecules.

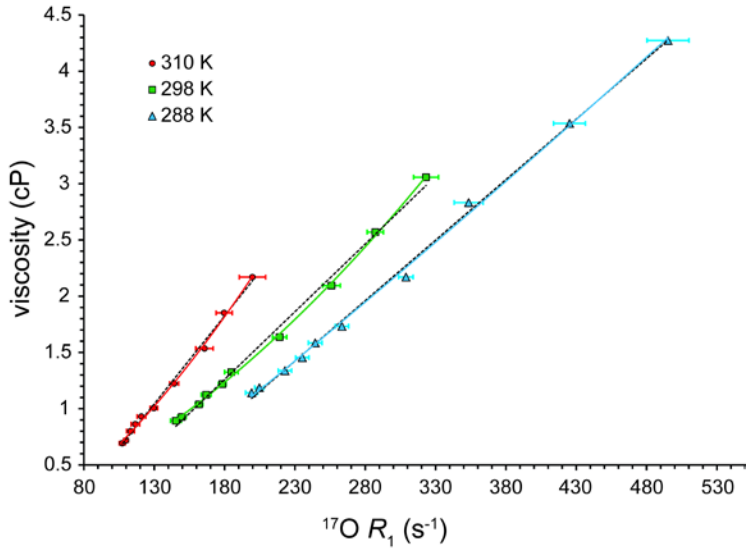

Figure S4. Relation between viscosity of water-glycerol mixtures and the ^{17}O longitudinal relaxation rate (R_1) of water, obtained at three temperatures of 288, 298 and 310 K. The linear (black dashed lines) and quadratic (solid lines) fits are shown.

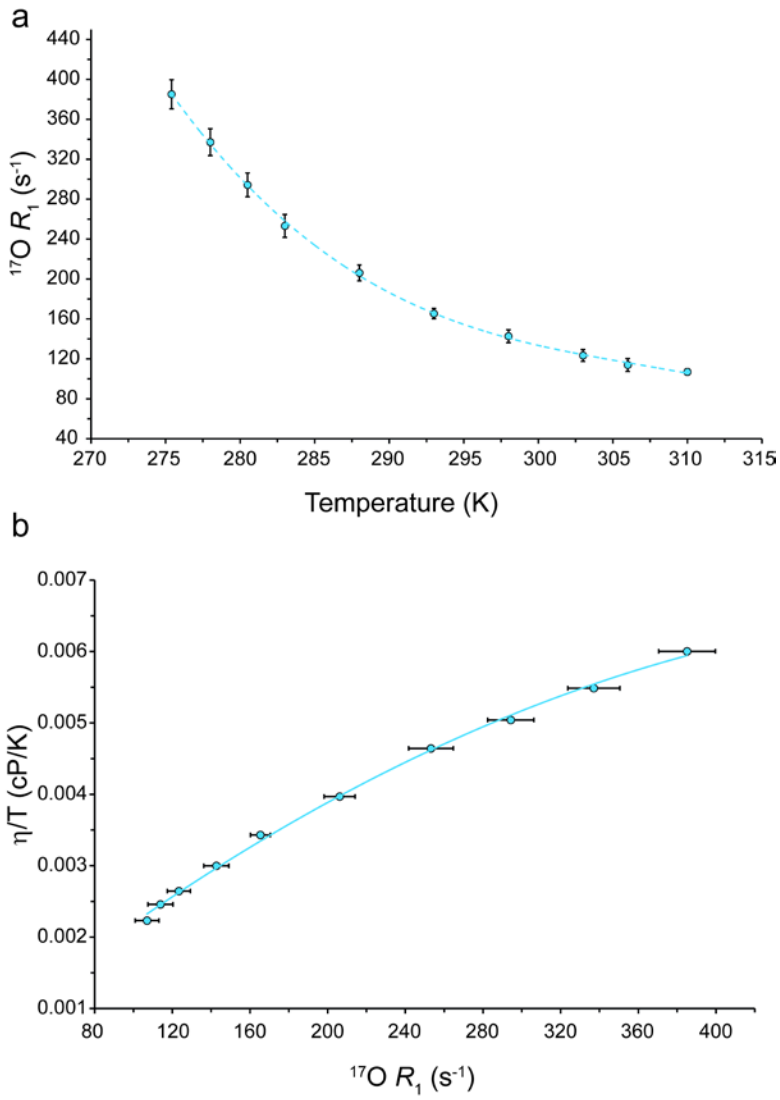

Figure S5. (a) Temperature-dependence of ^{17}O longitudinal relaxation rate (R_1) of water, showing the expected decrease of ^{17}O R_1 of water upon increasing temperature from 275.5 to 310 K. (b) The relation between viscosity/temperature ratio (η/T) and ^{17}O R_1 of water exhibits a deviation from linearity. The quadratic fit corresponding to equation, $\frac{\eta}{T}\left(\frac{cP}{K}\right) = -2.037 * 10^{-8} * (R_1)^2 + 2.303 * 10^{-5} * R_1 + 9.305 * 10^{-5}$, is shown as solid line.

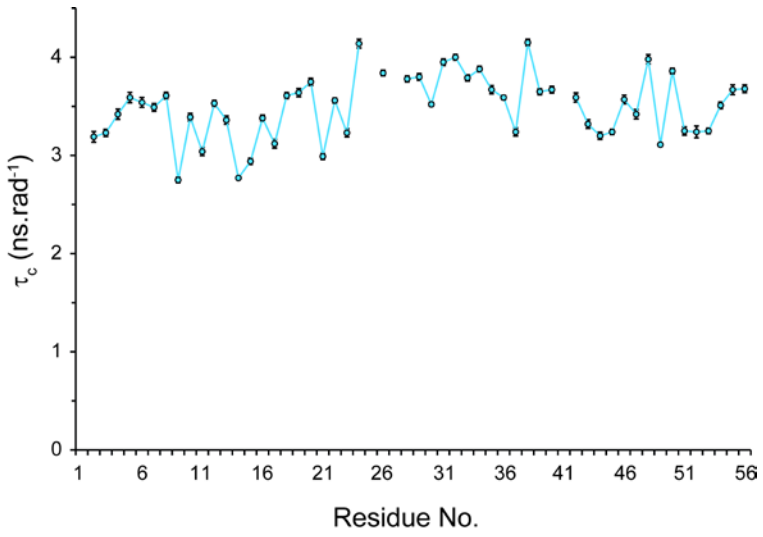

Figure S6. Residue-specific rotational correlation times (τ_c) of GB3 at 3 mM concentration, derived from ^{15}N/^{15}N-^{1}H CSA/DD *CCR* rates measured at 298 K. For the *CCR*-based calculation of τ_c, the Lipari-Szabo's N-H squared order parameters (S^2) of GB3 were taken from literature.[20] Little variation (less than 0.02 ns.rad^{-1}) was observed when rotational correlation time of internal motion (τ_i) ranged between 40 and 100 ps.

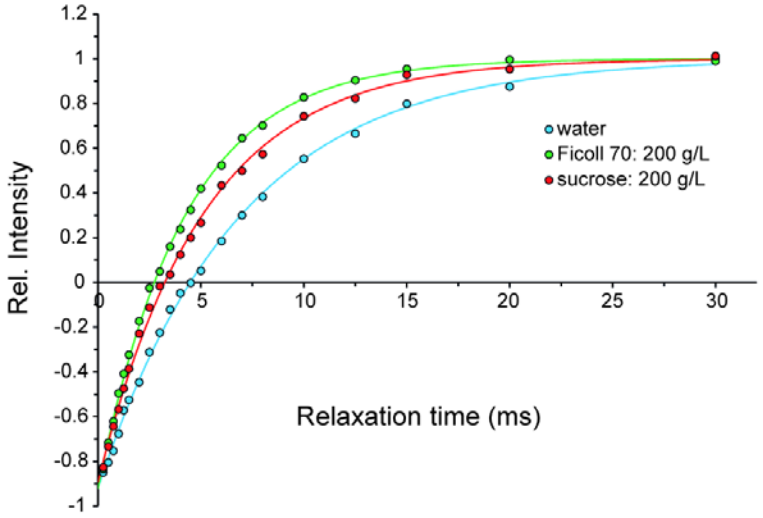

Figure S7. ^{17}O longitudinal relaxation rate (R_1) of water in dilute and crowded (200 g/L Ficoll 70 or sucrose) media, measured through inversion-recovery experiments. Relative intensities of water ^{17}O signals are shown as a function of recovery time. Faster recovery is observed in sucrose and particularly Ficoll solutions, compared to water.

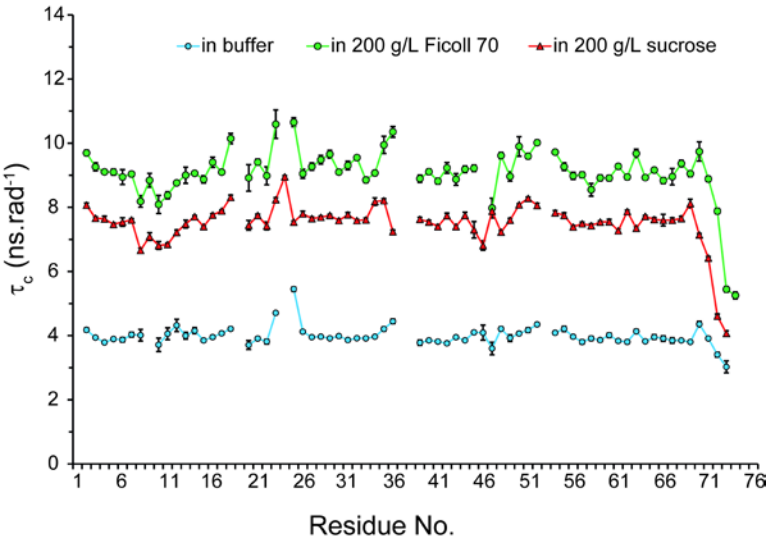

Figure S8. Residue-specific rotational correlation times (τ_c) of ubiquitin, derived from ^{15}N R_2/R_1 ratios. The ^{15}N R_1 and R_2 rates are taken from reference [13]. The τ_c values are shown for 0.37 mM ubiquitin in buffer, in 200 g/L sucrose and 200 g/L Ficoll 70 concentrations.

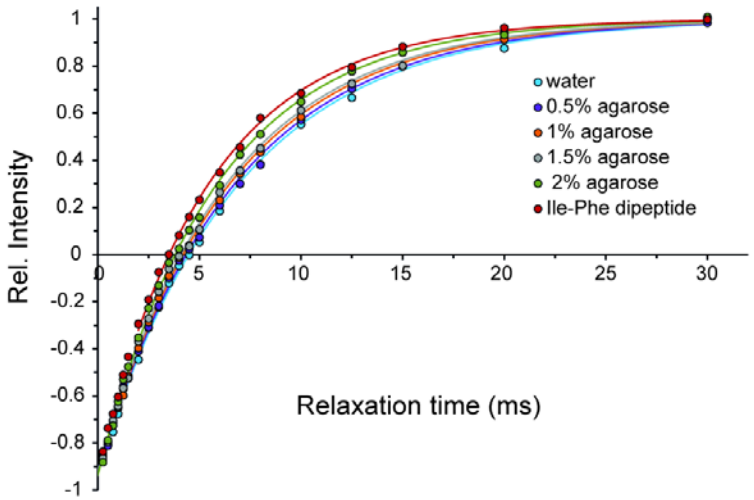

Figure S9. Water dynamics in the confined media of biological hydrogels, as probed by ^{17}O longitudinal relaxation rate (R_1) of water. ^{17}O R_1 of water in agarose and Ile-Phe hydrogels were measured through inversion-recovery experiments. Relative intensities of water ^{17}O signals are shown as a function of recovery time. Intensity recovery becomes faster when agarose concentration is increased.

```
clc
clear all

%% INTRODUCTION
% disp('AUTHOR: N. Rezaei-Ghaleh (Hessam): nare@nmr.mpibpc.mpg.de')
  disp('This program is to derive tauc from 15N R2/R1 ratio')
% disp ('Based on Biochemistry 1989, 28(23): 8972-8979')
% disp(' ')
  disp('Check field (Bh), rhn and csan parameters and the input .xlsx file ')
  disp('Set n as the number of residues you have in the input file ')
% disp ('Results will be written in the output.txt file; There will be 8 tau-c solutions per
residue, take the physically meaningful values.')


%% INPUTS %%
n = 73 ;   % No. of residues
Bh = 600.13 ; % (MHz)
rhn = 1.02*10^-10 ; % (* m *)
% rhc = 1.106*10^-10 ; % (* m *)
csan = -160*10^-6; % CSA of 15N
R = xlsread ('input.xlsx','B3:B75'); % R2/R1 ratio taken from the input .xlsx file, column B
%----------------------%

Gn = -2.712*10^7 ; % (* rad/(T s) *)
Gh = 2.6752*10^8 ; % (* rad/(T s) *)
%Gc = 6.728*10^7  ; % (* rad/(T s) *)
h=6.62606896*10^-34 ; % J.s
u0=4*pi*10^-7 ; % (* uo = Bo/H; H = A/m; uo = T m/A *)
B0=Bh*10^6*2*pi/Gh ;  % (in Tesla)
dd= (u0/4/pi)^2*(h/2/pi)^2*Gn^2*Gh^2/((rhn)^6) /(10^9);% dipolar coupling prefactor, scaled down
cc= csan^2*B0^2*Gn^2/3 /(10^9); % csa prefactor, scaled down
Wh=Bh*10^6*2*pi ; % angular freq. of 1H
Wn=Wh*Gn/Gh ; % angular freq. of 15N


for j=1:1:n ;

%% OPENING the file to write down the results
fid=fopen('output.txt','a+') ;

%% Solve the nonlinear equation f(t)=0
% Define x (tauc)
syms x
f=((dd/8)*(4*x+(x/(1+((Wh-
Wn)*x)^2))+3*(x/(1+((Wn)*x)^2))+6*(x/(1+((Wh)*x)^2))+6*(x/(1+((Wh+Wn)*x)^2)))+(cc/6)*(3*(x/(1+((W
n)*x)^2))+4*(x)))/((dd/4)*((x/(1+((Wh-
Wn)*x)^2))+3*(x/(1+((Wn)*x)^2))+6*(x/(1+((Wh+Wn)*x)^2)))+(cc)*((x/(1+(Wn*x)^2)))) - R(j) ;


%% DISPLAYING INPUTS

disp('INPUTS')

func=['  The equation to be solved is ' char(f), '=0'];
disp(func)
disp('  ')

% solving the equation
soln=solve(f,x);
solnvalue=double(soln);

disp('OUTPUTS')

fprintf(fid, '\nThe residue# %f ', j) ;
for i=1:1:length(solnvalue)
fprintf('\nThe solution# %g is %g', i,solnvalue(i))
fprintf(fid, '\nThe solution# %g is %g', i,solnvalue(i));
end

end
fclose(fid) ;
disp('  ')
```

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