Biomolecular phase separation through the lens of sodium-23 NMR

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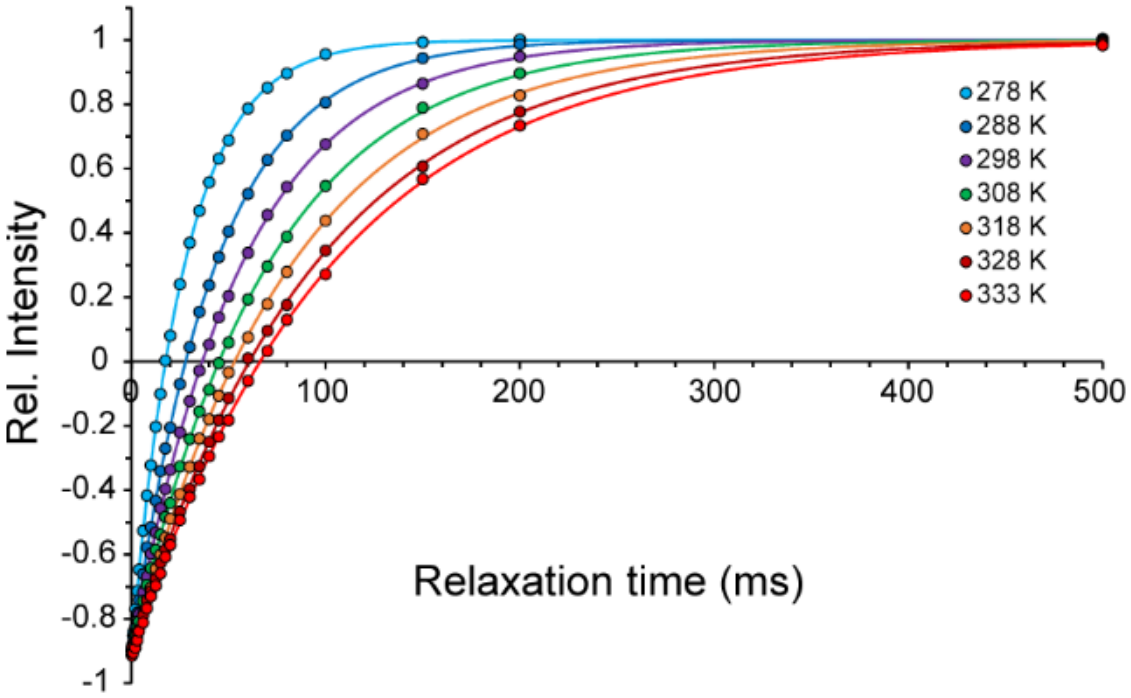

Figure S1. Temperature dependence of ^{23}Na longitudinal relaxation (R_1) rates in 100 mM NaCl solution. Recovery of ^{23}Na signal intensity following 180-degree inversion is shown at different temperatures ranging from 278 K to 333 K. The ^{23}Na signal intensities follow single-exponential recovery curves. More rapid recoveries are observed at lower temperatures.

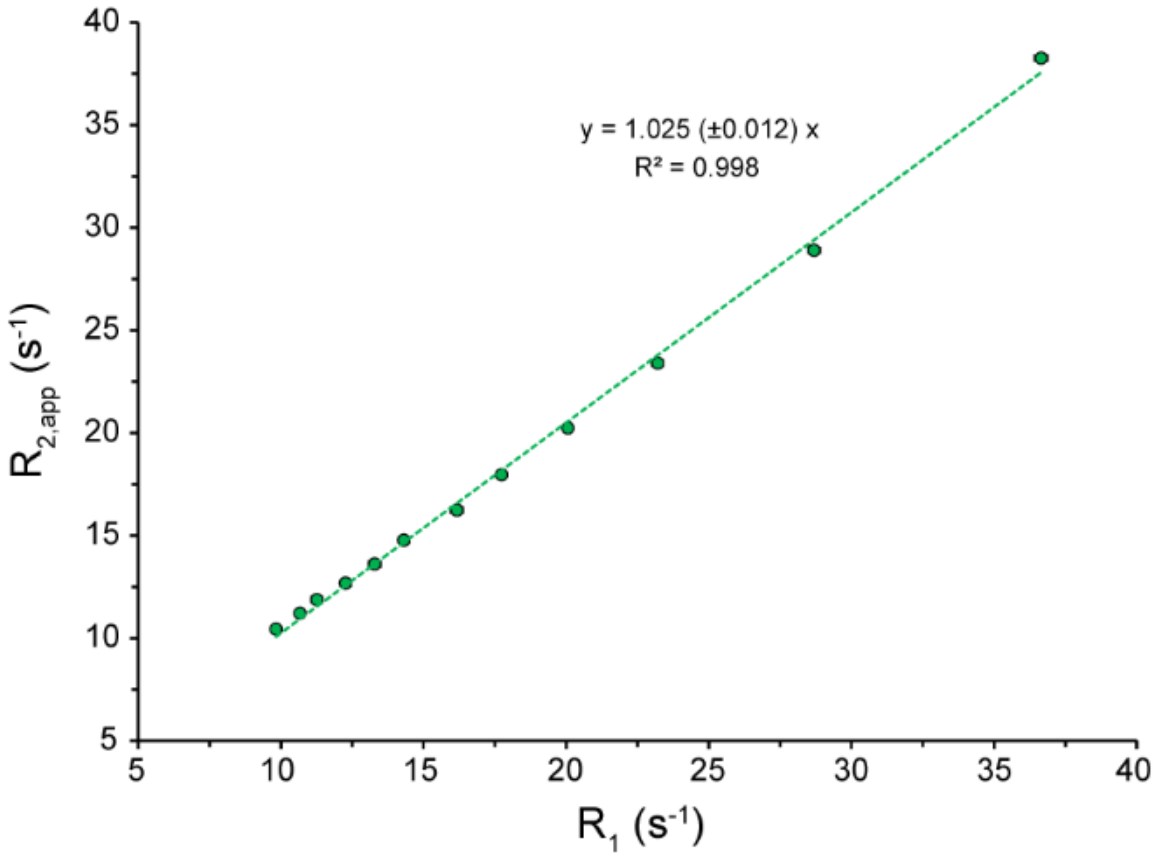

Figure S2. The ^{23}NaCl longitudinal relaxation (R_1) and linewidth-based apparent transverse relaxation ($R_{2,app}$) rates measured in the temperature range 278-333 K show strong correlation and a nice fit to a line with a slope close to 1. The error bars are smaller than the symbol size.

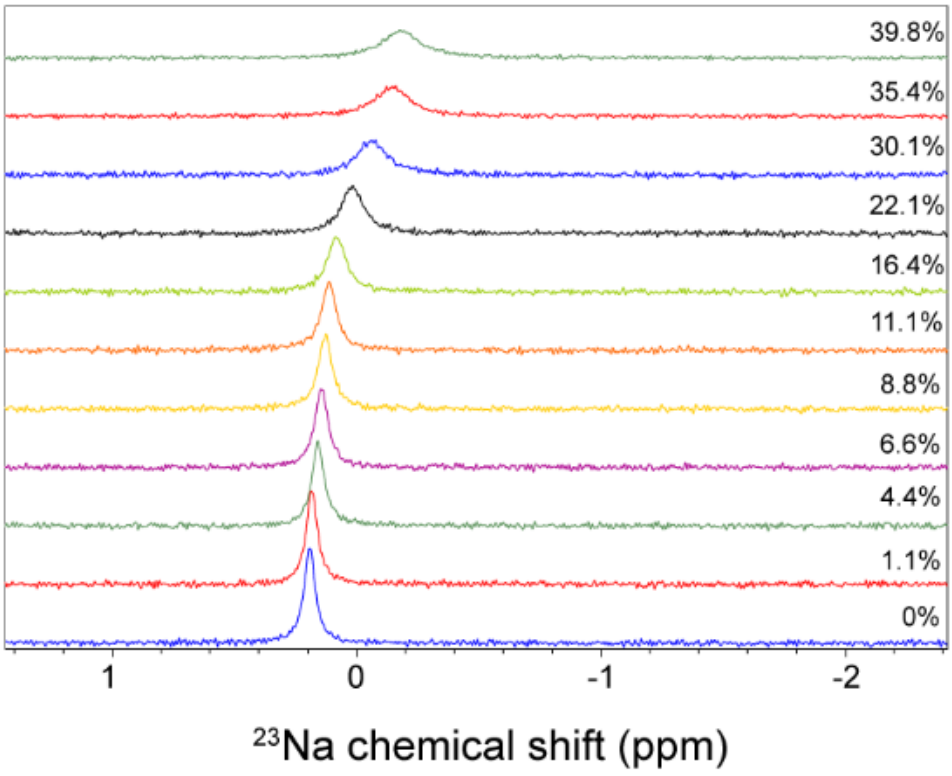

Figure S3. Viscosity dependence of ^{23}NaCl NMR in glycerol-water mixtures. 1D ^{23}Na NMR spectra show gradual peak displacement towards lower chemical shifts and broadening in dependence of glycerol (v/v, %) concentration.

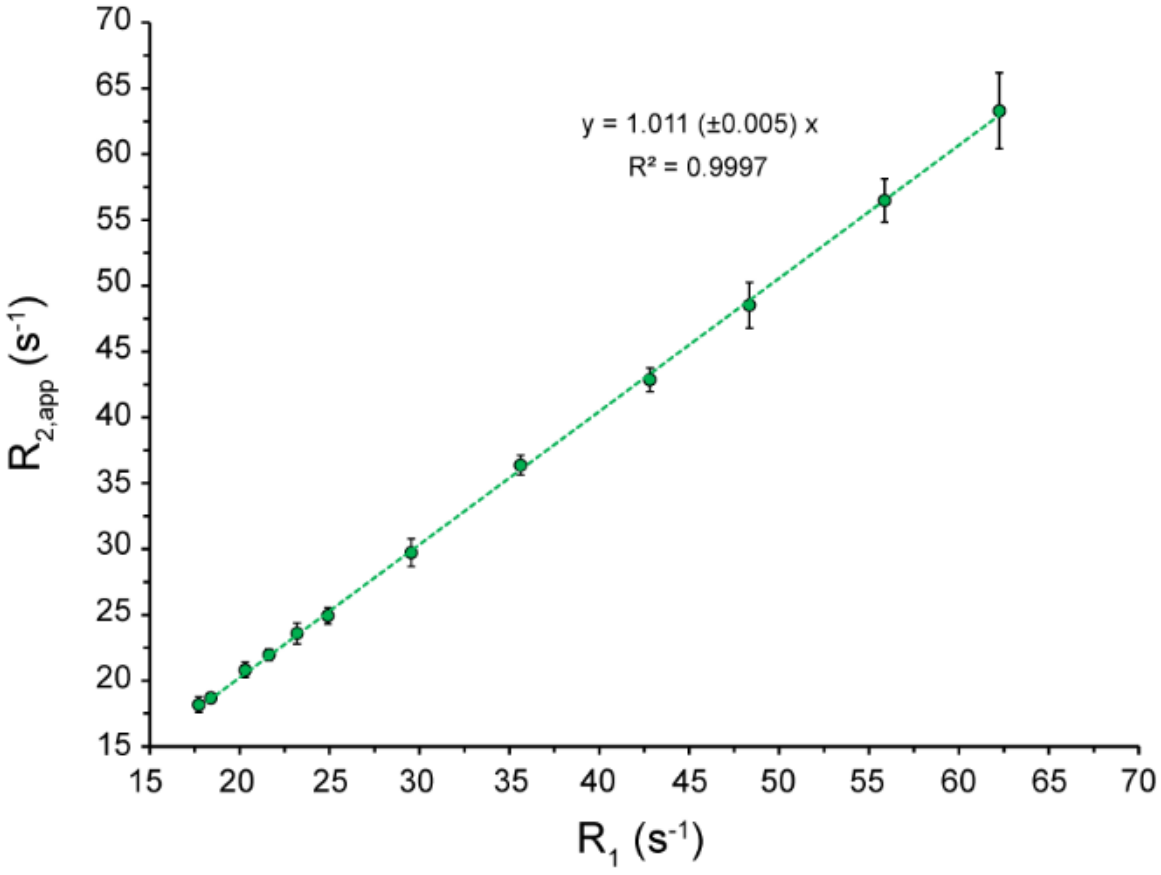

Figure S4. The ^{23}NaCl longitudinal relaxation (R_1) and linewidth-based apparent transverse relaxation ($R_{2,app}$) rates measured in the glycerol-water mixtures from 0 to ca. 40% (v/v) glycerol concentration. Strong correlation and a nice fit to a line with a slope close to 1 is observed. The error bars for $R_{2,app}$ represent the standard deviation of multiple (at least five) measurements of linewidth. The error bars for R_1 rates represent the fitting error and are smaller than the symbol size.

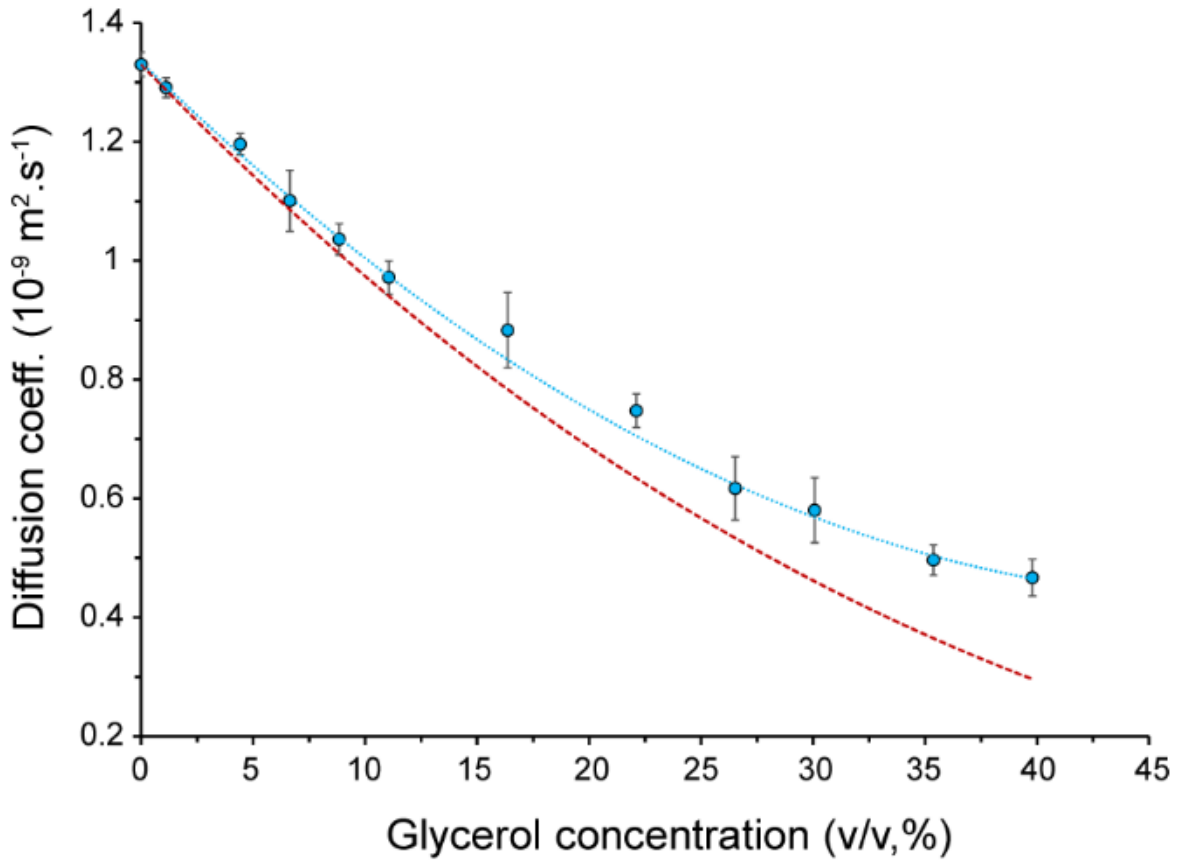

Figure S5. ^{23}NaCl translational diffusion coefficient (D_{tr}) as a function of glycerol concentration in glycerol-water mixtures. The decrease in D_{tr} is less pronounced than the glycerol concentration-dependent decrease in $1/\eta$ (shown as dashed line in dark red). The error bars represent the fitting errors for diffusion coefficients.

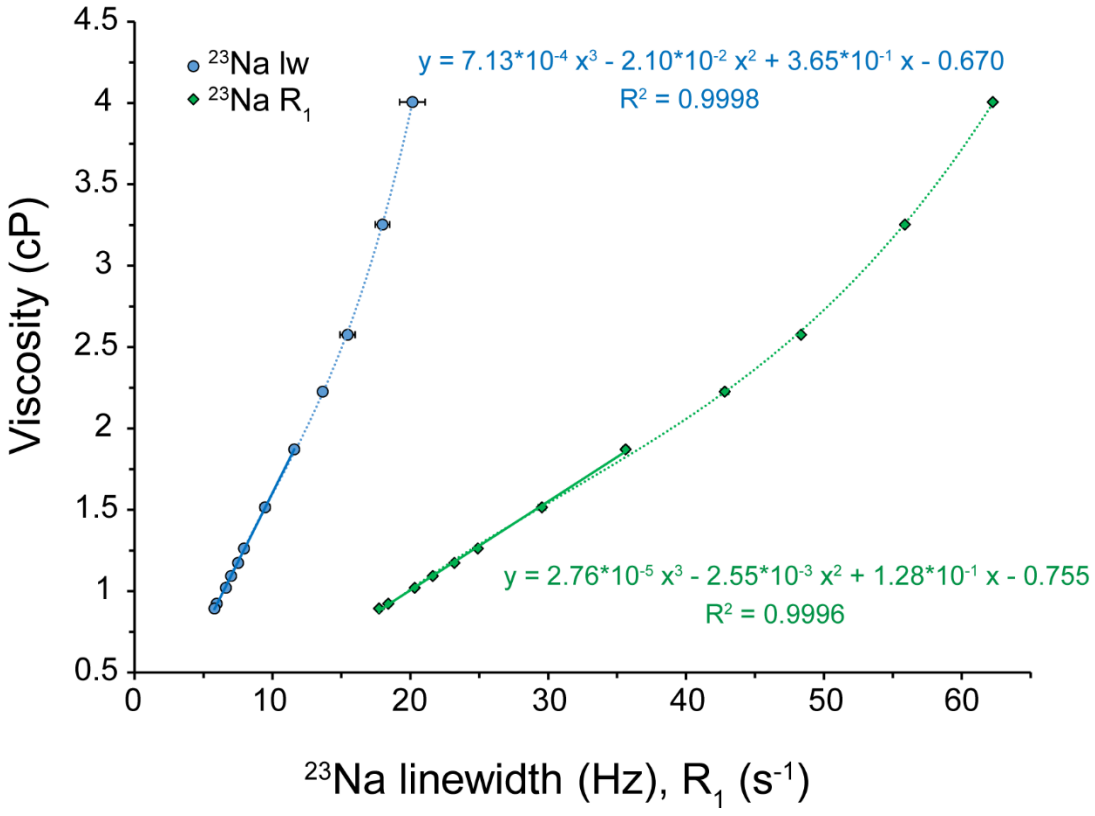

Figure S6. Viscosity determination based on ^{23}Na NMR measurements in glycerol-water mixtures (0-40%, v/v). Viscosity variation by ^{23}Na linewidth and longitudinal relaxation (R_1) rates are fitted to cubic equations. The initial variation of viscosity by ^{23}Na NMR linewidth or R_1 rates follows linear trends (solid lines). The error bars for ^{23}Na R_1 rates are smaller than the symbol size.

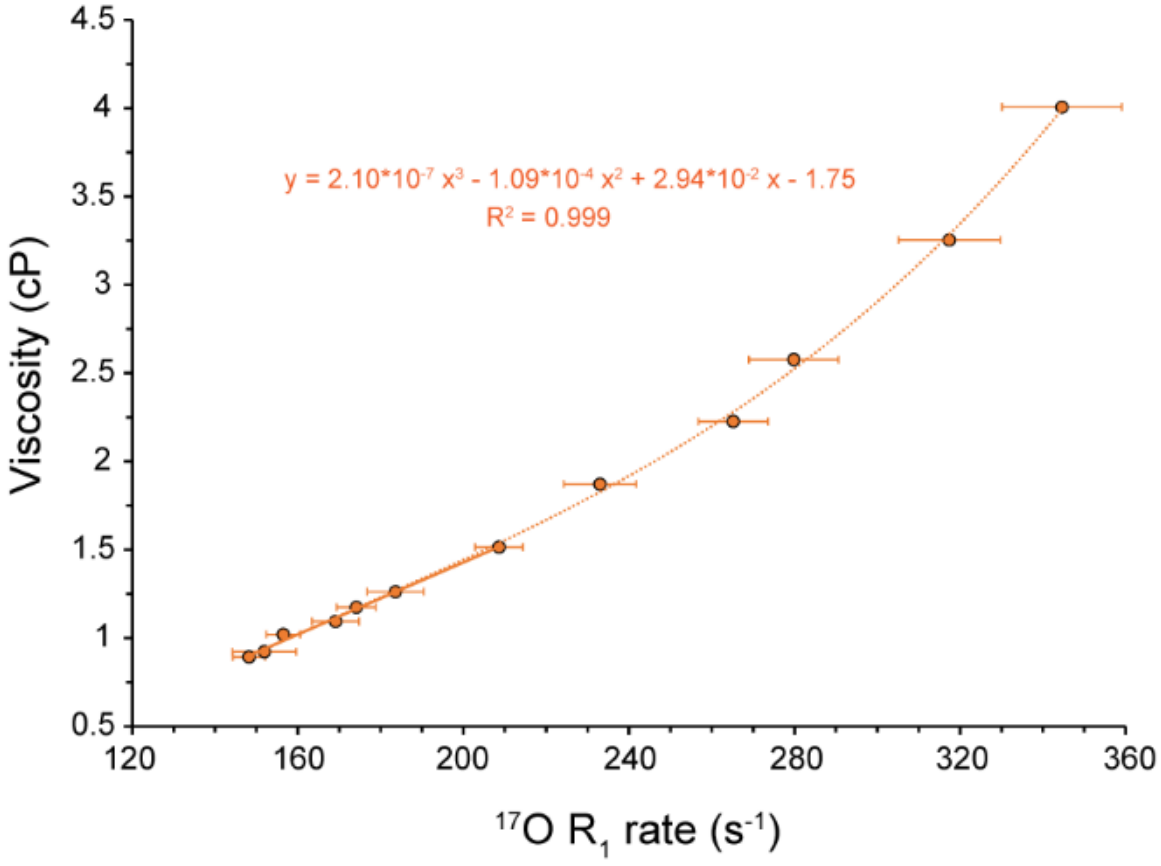

Figure S7. Viscosity determination based on ^{17}O NMR measurements in glycerol-water mixtures (0-40%, v/v). Viscosity variation by ^{17}O R_1 rates of water is fitted to a cubic relation in the whole region, however the initial variation obeys a linear relation (solid line). The error bars represent the fitting errors for ^{17}O R_1 rates.

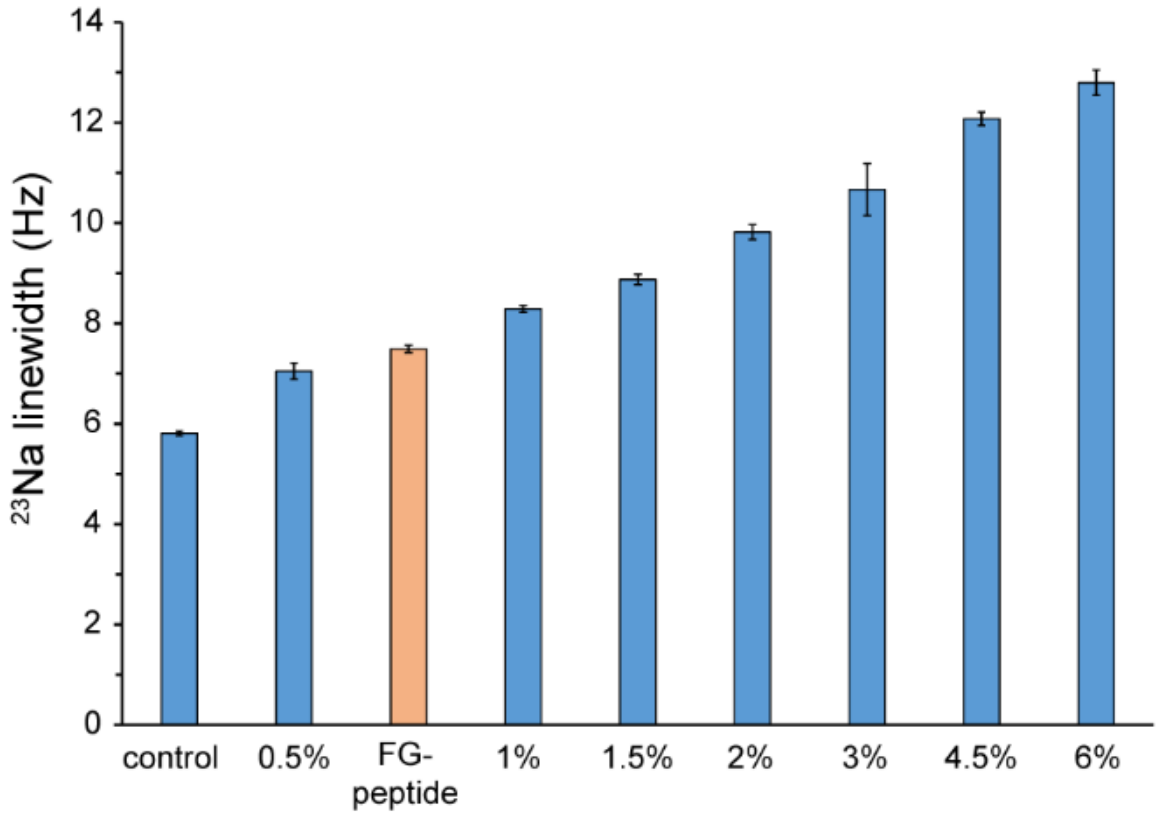

Figure S8. ^{23}NaCl NMR probes of confinement level in biological hydrogels. The ^{23}Na linewidth captures the agarose concentration-dependent restriction in rotational mobility of sodium ions. The degree of sodium ion (rotational) mobility inside FG-peptide hydrogel (3 mM) is revealed by its measured ^{23}NaCl linewidth, in comparison with the agarose gels.

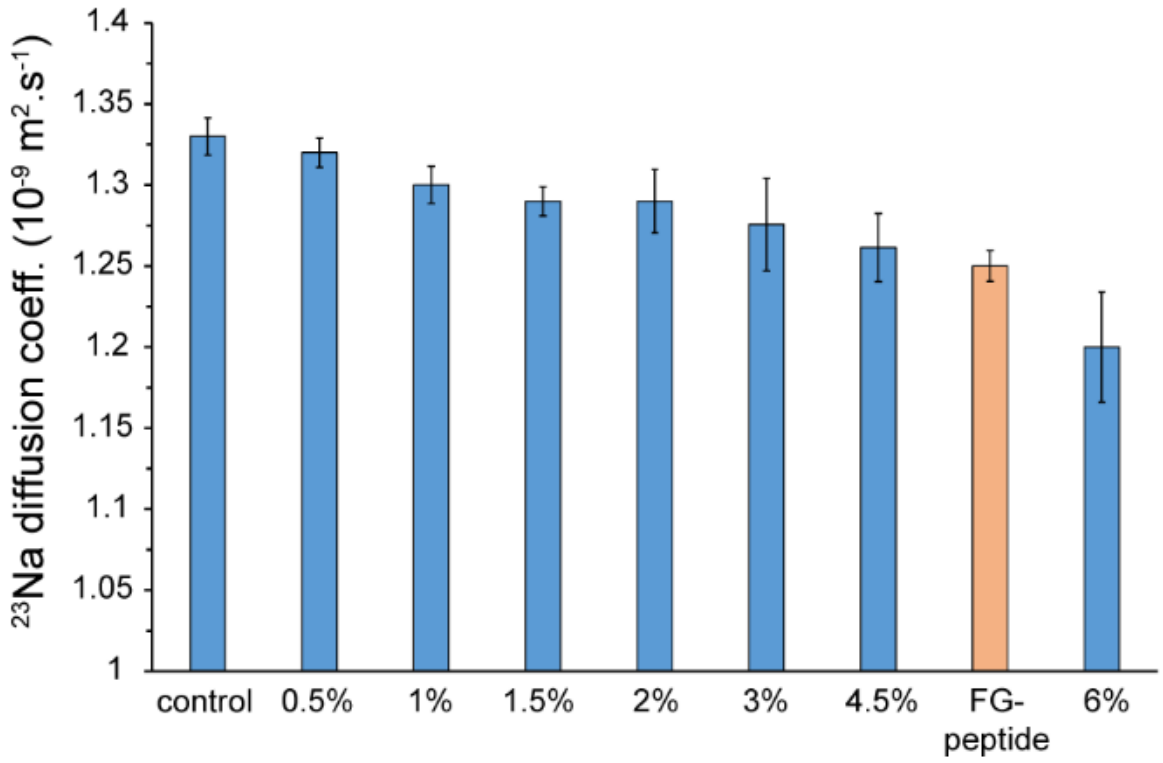

Figure S9. ^{23}NaCl translational diffusion coefficient (D_{tr}) slightly decreases in dependence of agarose gel concentration, reflecting considerable albeit small restriction in translational mobility of sodium ions. The degree of sodium ion (translational) mobility inside FG-peptide hydrogel is revealed by its measured ^{23}NaCl diffusion coefficient, in comparison with the agarose gels.

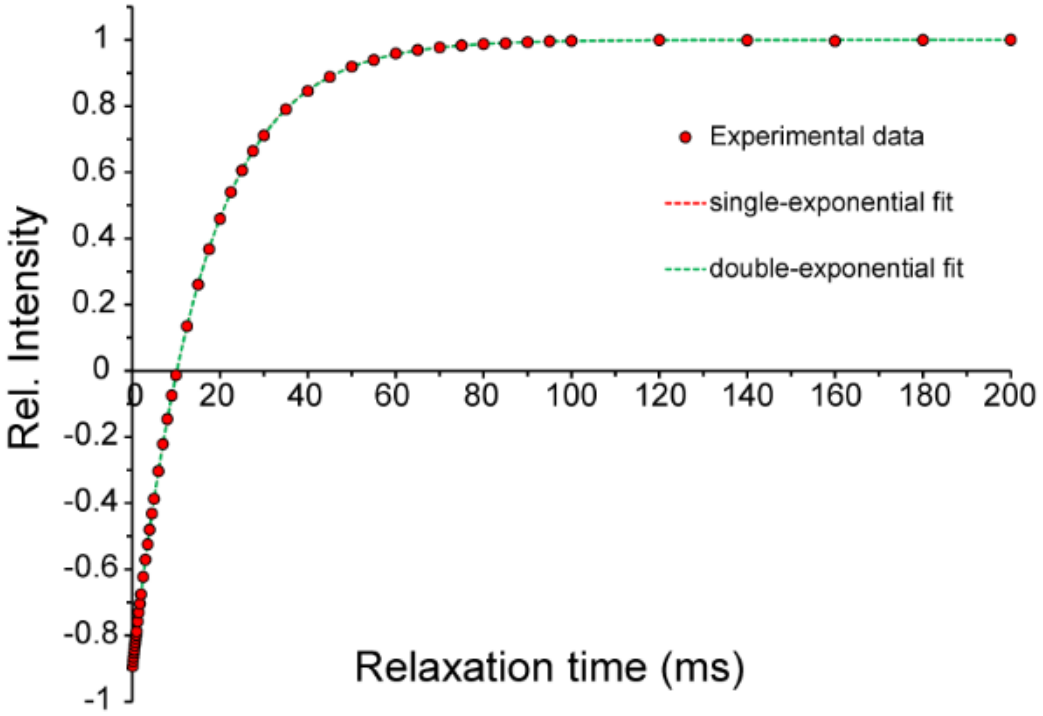

Figure S10. ^{23}Na signal intensity recovery following 180-degree inversion in a 39.8% glycerol solution. The single- (red dashed line) and double-exponential (green dashed line) fits are indistinguishable, indicating that even at this sample exhibiting the largest ^{23}Na R_1 rate in our study the ^{23}Na NMR relaxation remains effectively single-exponential (as in fast "extreme-narrowing" regime).

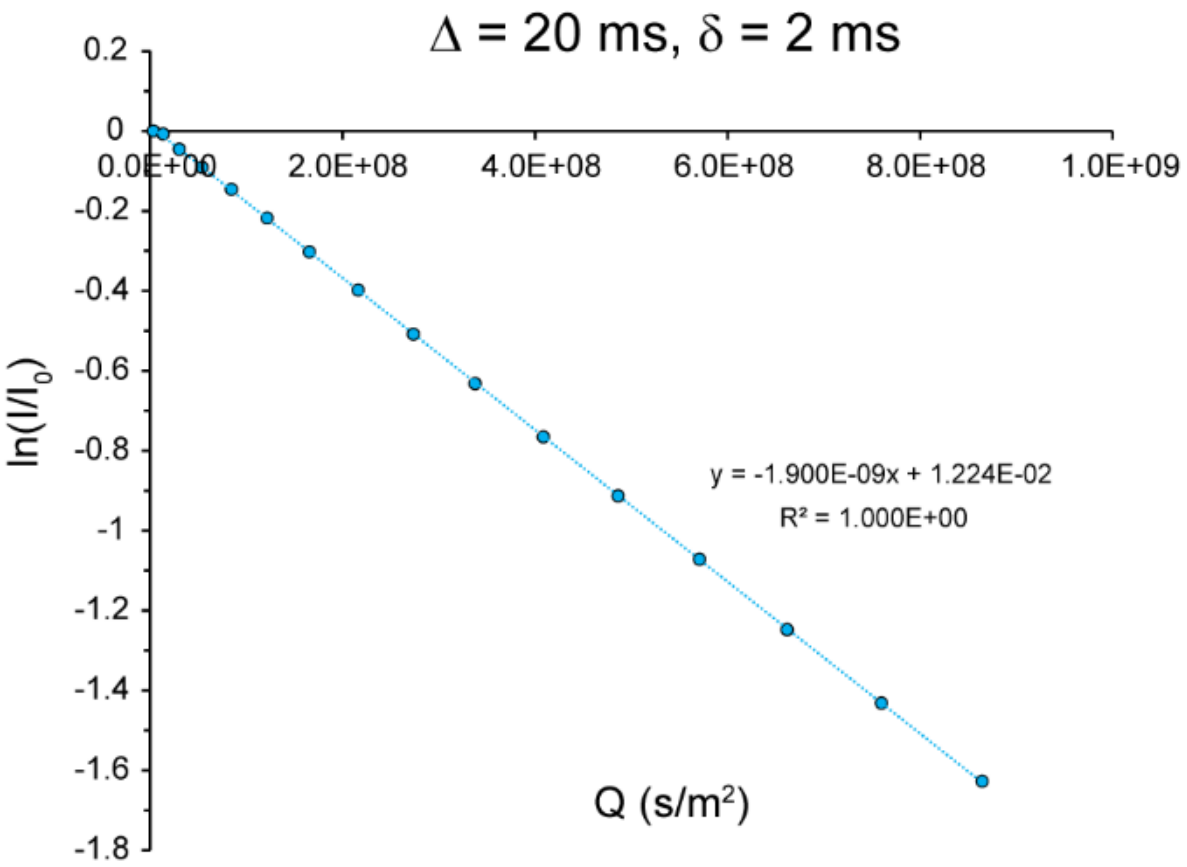

Figure S11. Gradient calibration through PFG-NMR measurement of residual HDO in 99.8% D_2O at 298.14 K. The values of experimental diffusion times (big and little delta) are shown at top. The known value of HDO diffusion coefficient ($1.9*10^{-9}$ $m^2.s^{-1}$) was obtained after scaling nominal gradient values by 1.026.