Supplementary Information for

Mapping the per-residue surface electrostatic potential of CAPRIN1 along its phase separation trajectory

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Supplementary Table 1

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Supplementary Table 1. Diffusion constants of PROXYL derivatives

Compound	Diffusion constant ($cm^2\ s^{-1}$)
3-Aminomethyl-PROXYL	$(7.03 \pm 0.07) \times 10^{-6}$
3-Carbamoyl-PROXYL	$(7.30 \pm 0.05) \times 10^{-6}$
3-Carboxy-PROXYL	$(7.05 \pm 0.03) \times 10^{-6}$

The diffusion constants were measured using samples containing 25 mM MES-NaOH, 10 mM ascorbic acid, 5 mM PROXYL derivative, 3% D_2O/97% H_2O at pH 5.5, 25 °C, 600 MHz.

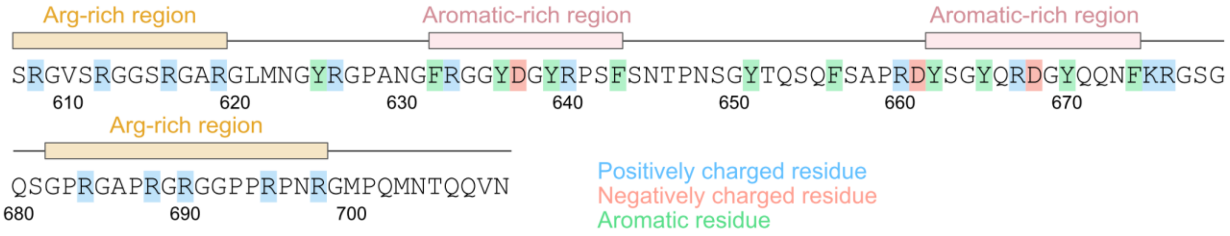

Figure S1. Amino acid sequence of the C-terminal low complexity region of CAPRIN1. Positively and negatively charged amino acids are colored blue and red, respectively, and aromatic amino acids are colored green. The Arg-rich and aromatic-rich regions are indicated on top. As mentioned in the main text, a double mutant of CAPRIN1 (N623T/N630T) was used in this study.

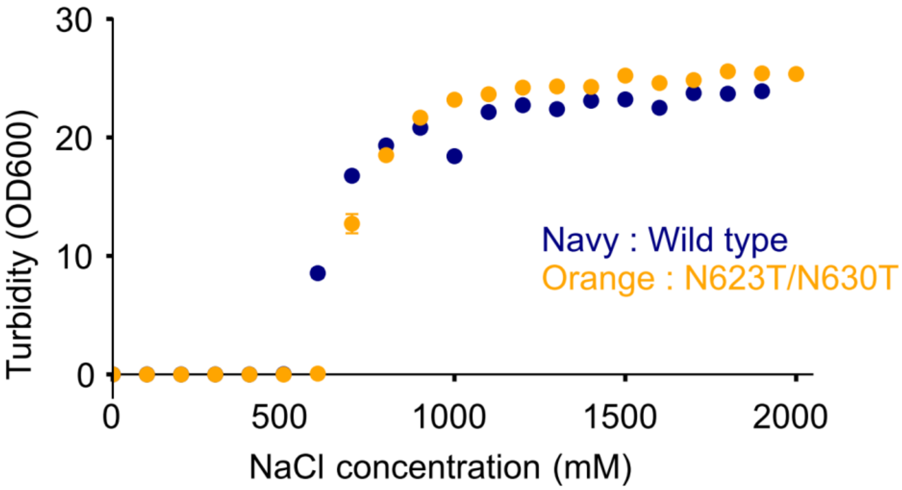

Figure S2. Turbidity assays of wild-type and mutant CAPRIN1. Turbidity assays measuring phase separation propensities of 300 µM samples of wild-type (navy) and N623T/N630T double mutant (orange) CAPRIN1 as a function of NaCl in 25mM MES-NaOH buffer pH 5.5 at room temperature. Error bars (smaller than the data points) were obtained from triplicate measurements.

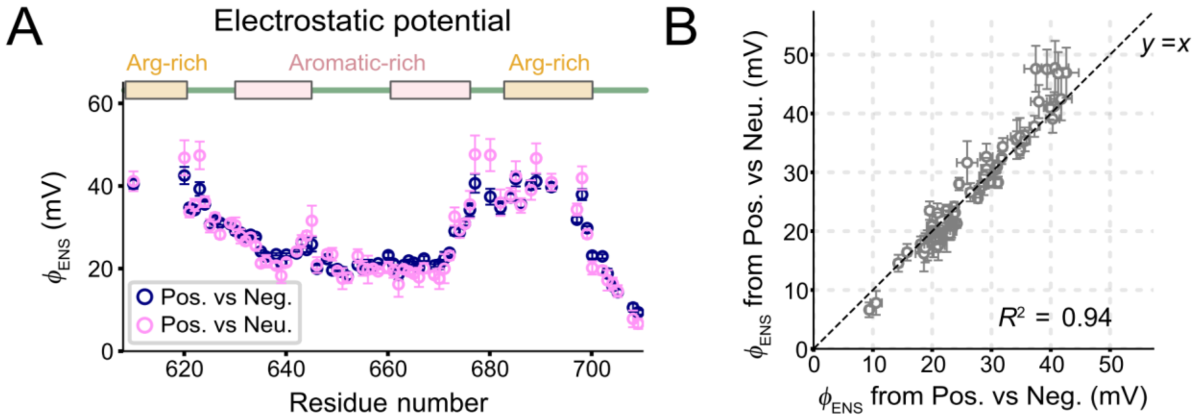

Figure S3. Comparison of electrostatic potentials of CAPRIN1 obtained from different pairs of PROXYL derivatives. (A) Plots of ϕ_{ENS} potential values of CAPRIN1 determined by using either 3-carboxy-PROXYL and 3-aminomethyl-PROXYL (navy) or 3-aminomethyl-PROXYL and 3-carbamoyl-PROXYL (pink) co-solutes are shown. (B) Correlation plot of the electrostatic potentials obtained by two different pairs of PROXYL derivatives as shown in (A). R^2 is Pearson's correlation coefficient squared. NMR data were acquired using 300 µM samples of CAPRIN1, 25 mM MES-NaOH, 3% D_2O/97% H_2O, pH 5.5, 25 °C at 1 GHz. Error bars were obtained by propagating the error in R_2.

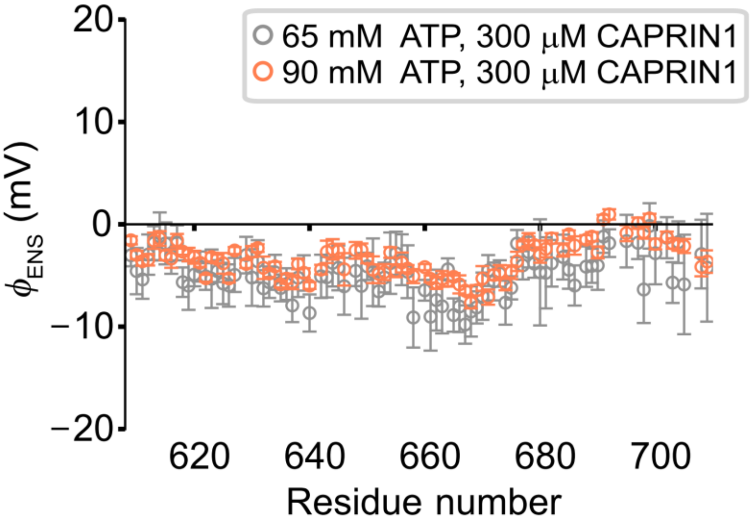

Figure S4. CAPRIN1 ϕ_{ENS} potentials in the re-entrant mixed phase. Plots of ϕ_{ENS} values measured with 65 mM (gray) and 90 mM (orange) ATP using 300 μM samples of CAPRIN1, 25 mM MES-NaOH, 3% D_2O/97% H_2O, pH 5.5, 25 °C, 1 GHz. ϕ_{ENS} potentials were determined by using 3-carboxy-PROXYL and 3-aminomethyl-PROXYL derivatives. In the 65 mM ATP dataset the fraction of ^{13}C, ^{15}N-labeled CAPRIN1 was reduced to 25% of the protein, with the remaining 75% unlabeled, accounting for the larger errors. Errors were obtained by propagating uncertainties in R_2 rates.

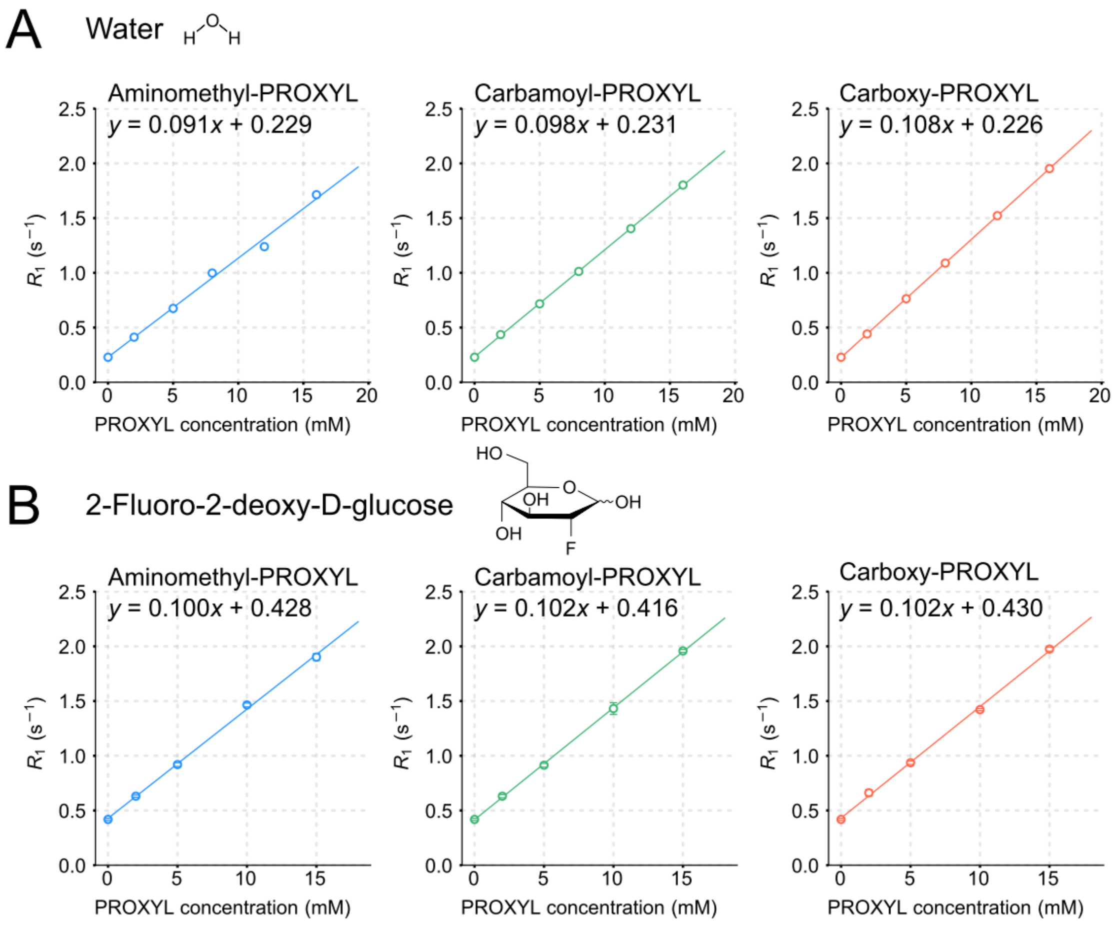

Figure S5. R_1 PREs of standard molecules in buffer solution. Plots of (A) water ^{1}H or (B) 2-fluoro-2-deoxy-D-glucose (FDG) ^{19}F R_1 rates measured in the presence of various concentrations of PROXYL derivatives (*left*: 3-aminomethyl-PROXYL, *center*: 3-carbamoyl-PROXYL, *right*: 3-carboxy-PROXYL). The chemical structures of each molecule are shown on top. The results of the linear regression analyses are shown on top of each panel, where the slopes correspond to the k values in Eqs. 3 and 4 in the main text used to correct measured R_2 PRE ratios in demixed state samples (see *Materials and Methods*). The measurements in panel A were performed at 600 MHz, 40 °C and those in panel B were performed at 500 MHz, 40 °C, in buffer containing 25 mM MES-NaOH (pH 5.5), 3% D_2O. The concentration of FDG used was 1 mM. Note that very similar ratios of slope values from the three co-solutes were also obtained at 25 °C or when mixed-state samples were used with $\approx$6 mM CAPRIN1. Errors in panel A were computed from the standard error of the fit (smaller than data points), and those in panel B were obtained from triplicate measurements.