Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Impact of Macromolecular Crowding on RNA/Spermine Complex Coacervation and Oligonucleotide Compartmentalization

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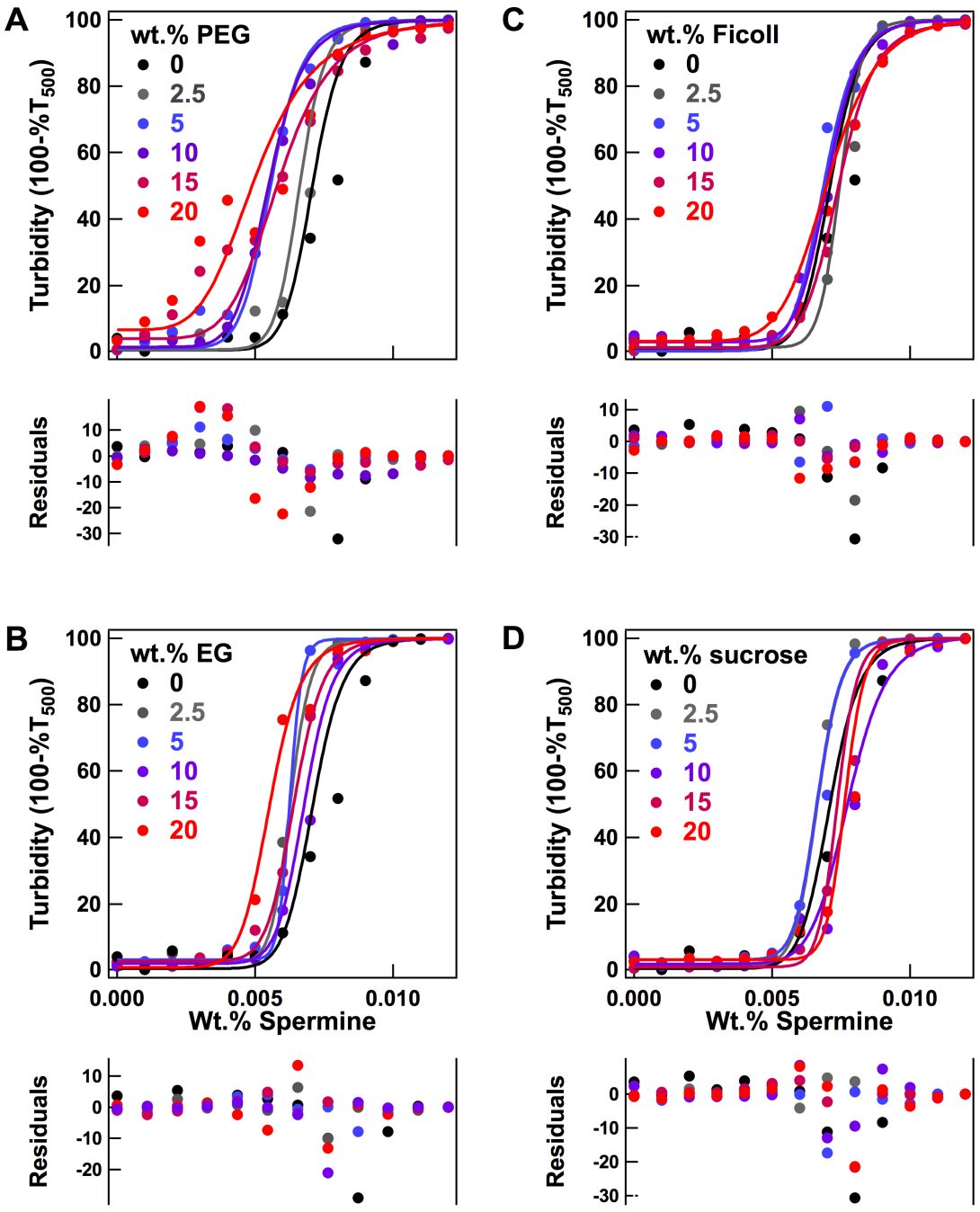

Figure S1: Effect of crowders and their small molecule analogs on the amount of spermine required to induce coacervation. Fitting of turbidity (to eq 1) as a function of wt.% spermine for 0.05 wt.% polyU, parametric in wt.% **(A)** PEG, **(B)** EG, **(C)** Ficoll, and **(D)** sucrose. Data points represent the average of triplicate measurements. Residuals represent error in fit and are weighted by the standard deviation between triplicate measurements. Although the residuals in regions of transition are large, this error is taken into account in standard deviations in Figure 2E.

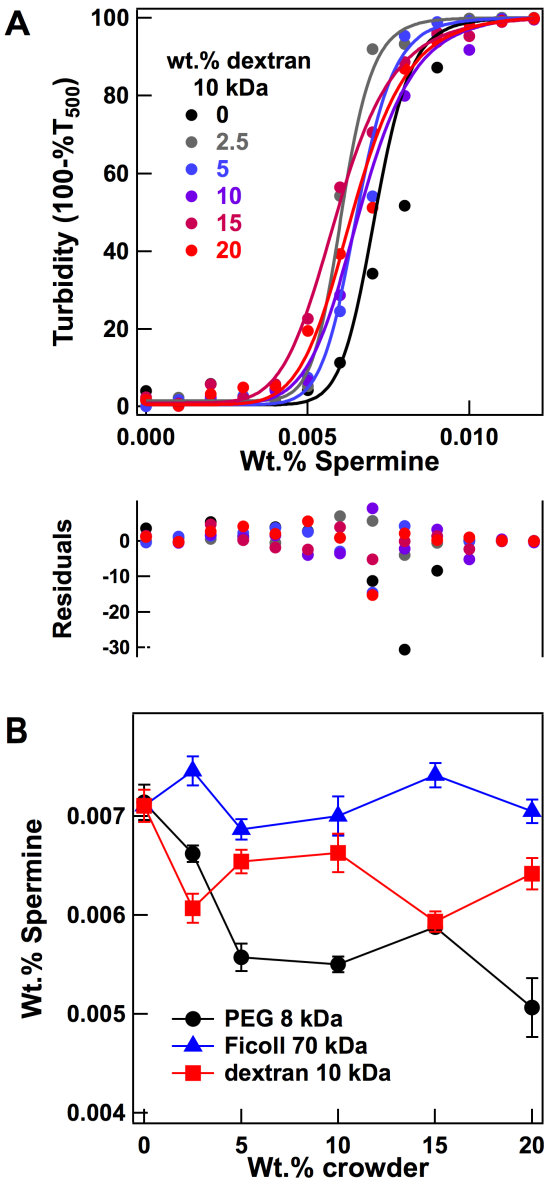

Figure S2: (A) Fitting of turbidity (to eq 1) as a function of wt.% spermine for 0.05 wt.% polyU parametric in wt.% dextran 10 kDa. Varying the amount of dextran does not impact the amount of spermine required for coacervation as dramatically as PEG 8 kDa, despite their similar size. Data points represent the average of triplicate measurements. Residuals represent error in fit and are weighted by the standard deviation between triplicate measurements. Although the residuals in regions of transition are large, this error is taken into account in standard deviations in **(B)**. **(B)** Spermine required to induce coacervation as a function of wt.% probe molecule. Turbidity midpoints were calculated from fits as described in Methods. Error bars represent standard deviation calculated from fits in **(A)**.

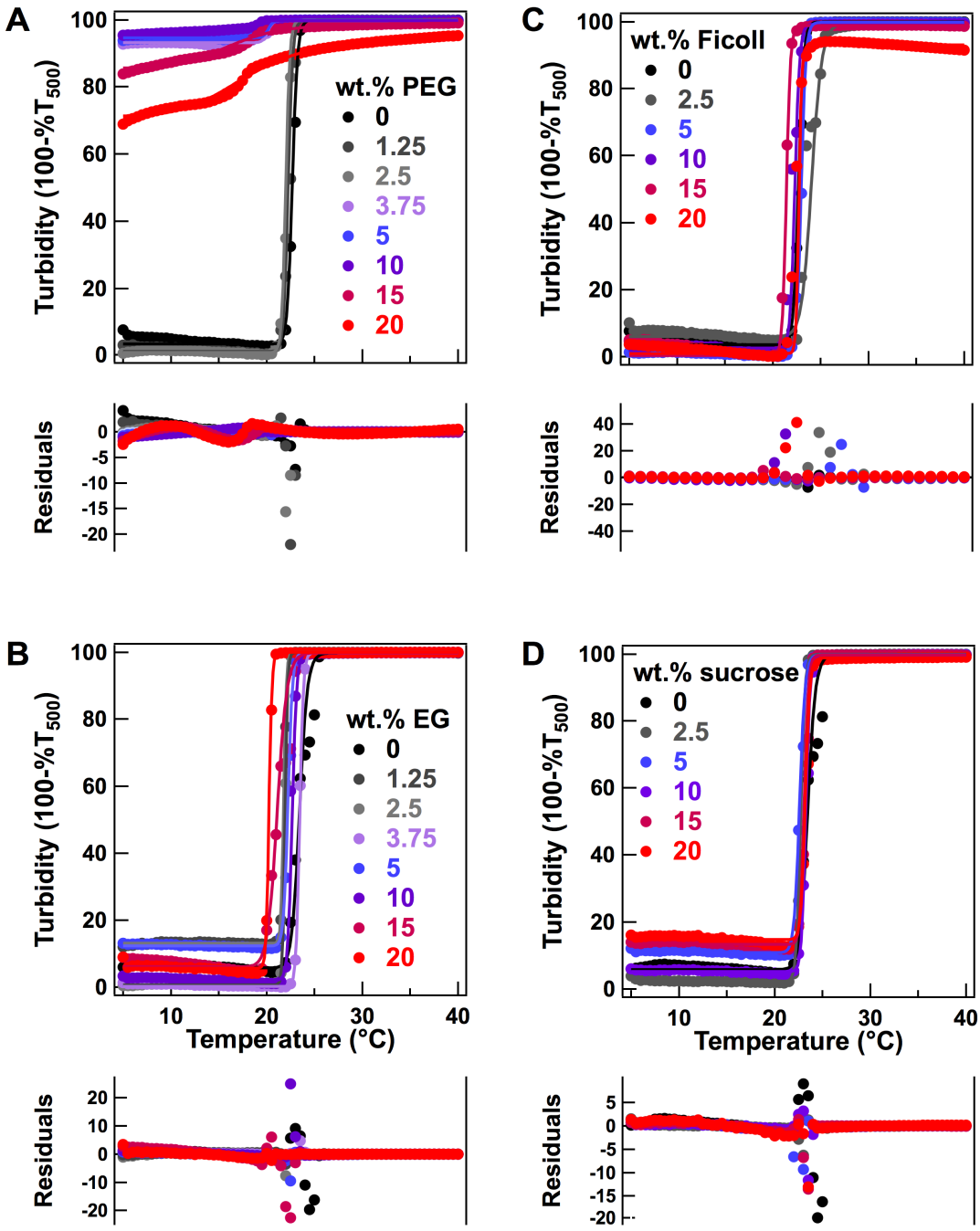

Figure S3: Effect of crowders and their small molecule analogs on the coacervation temperature (T_C) of polyU/spermine. Fitting of turbidity (to eq 1) as a function of temperature for 0.05 wt.% polyU/0.01 wt.% spermine, parametric in wt.% **(A)** PEG, **(B)** EG, **(C)** Ficoll, and **(D)** sucrose. Data points represent the average of triplicate measurements. Residuals represent error in fit and are weighted by the standard deviation between triplicate measurements. Although the residuals in regions of transition are large, this error is taken into account in standard deviations of Figure 3E.

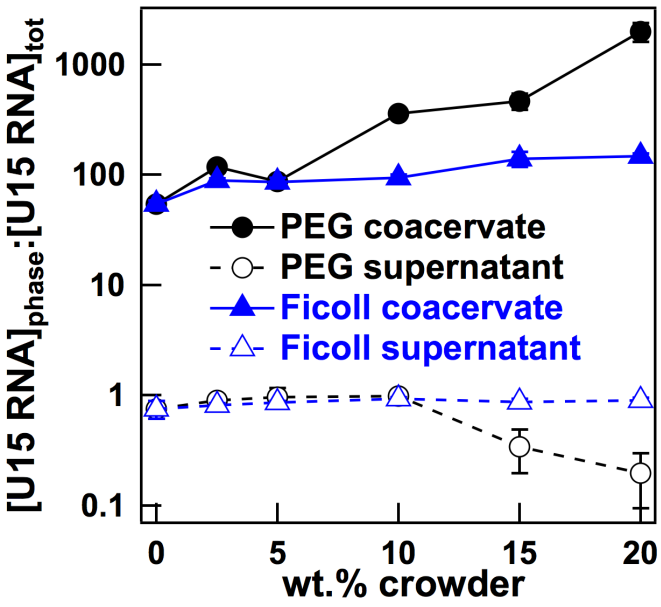

Figure S4: Concentrations of Alexa 647-U15 RNA in the coacervate and supernatant phases of a 0.05 wt.% polyU/0.5 wt.% spermine coacervate system relative to the total concentration of Alexa 647-U15 RNA added as a function of wt.% crowder. The concentration of Alexa 647-U15 RNA in the coacervate phase increases with an increase of crowder, and the impact is much more significant for PEG crowding than Ficoll crowding. Concentration of Alexa 647-U15 RNA added to each sample was 0.2 µM with the exception of 15 and 20 wt.% PEG, which had 0.04 µM added. Coacervate phase concentrations were measured using confocal fluorescence microscopy and calibration curves of Alexa 647-U15 RNA in 5 mM HEPES (pH 7.4), 1 mM $MgCl_2$ buffer. Error bars represent standard deviation between 10 measurements. Supernatant phase concentrations were measured using bulk fluorescence measurements and calibration curves of Alexa 647-U15 RNA in 5 mM HEPES (pH 7.4), 1 mM $MgCl_2$ buffer. Error bars represent standard deviation between triplicate measurements.

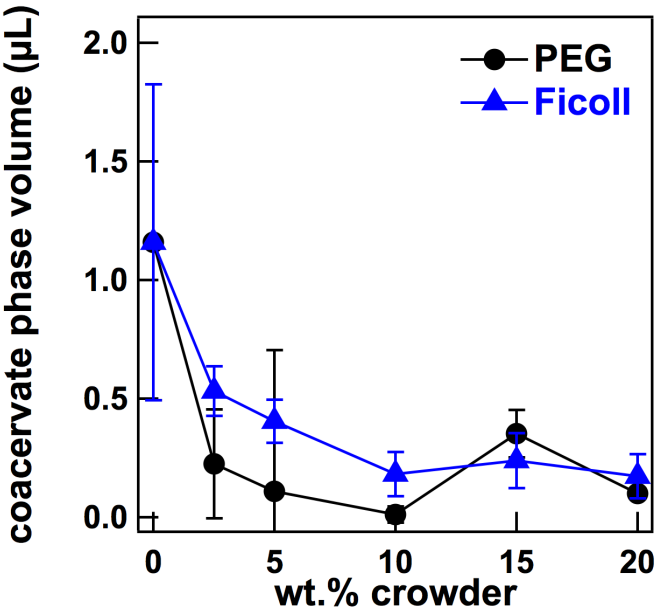

Figure S5: Calculated coacervate phase volumes (eq S1) as a function of wt.% crowder. Standard deviations take into account error from 10 measurements of coacervate phase concentration and triplicate measurements of supernatant phase concentration of Alexa 647-U15 RNA.

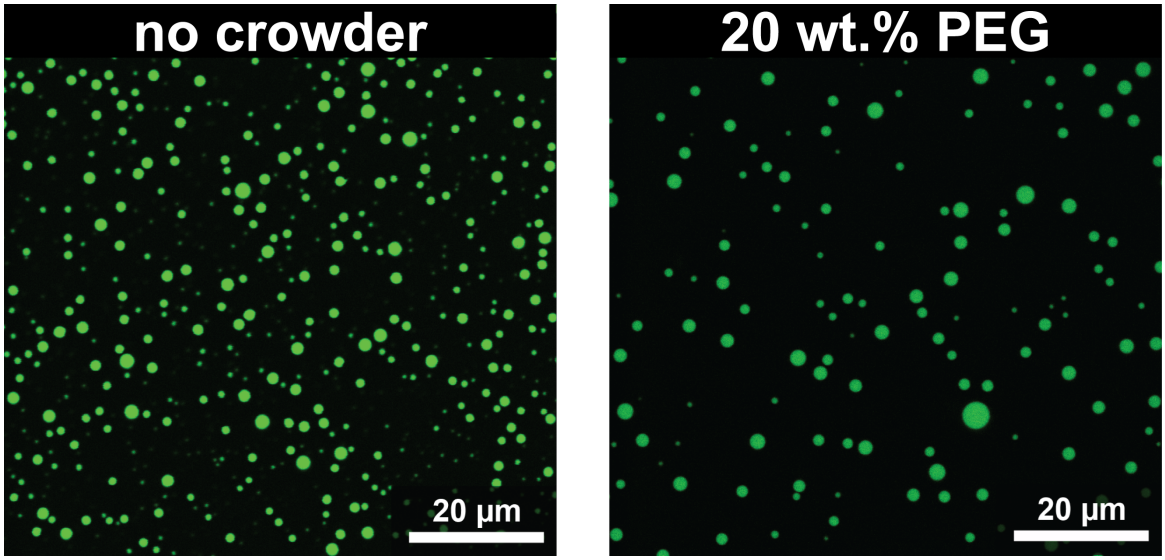

Figure S6: Confocal fluorescence images of fluorescein isothiocyanate (FITC) partitioning into 0.05 wt.% polyU/0.5 wt.% spermine coacervate droplets with **(left)** no crowder and **(right)** 20 wt.% PEG 8 kDa. FITC is accumulated within the coacervate droplets. Images were false-colored and their brightness adjusted (by the same degree for both images) to aid in visualization.

Equation S1A:

$$\left([\text{U15 RNA}]_{\text{coacervate}}\right)\left(\text{volume}_{\text{coacervate}}\right)+\left([\text{U15 RNA}]_{\text{supernatant}}\right)\left(\text{volume}_{\text{supernatant}}\right)=\text{total moles of U15 RNA added}$$

where [U15 RNA] in the coacervate and supernatant phases are expressed in mol/L (M) and phase volumes are expressed in L. $5\text{x}10^{-11}$ moles of polyU15 were added to all samples except 15 and 20 wt.% PEG, which had $1\text{x}10^{-11}$ total moles of U15 RNA added.

Equation S1B:

$$\text{total sample volume} = \text{volume}_{\text{coacervate}} + \text{volume}_{\text{supernatant}}$$

The total sample volume for all samples was 250 µL or $2.5\text{x}10^{-4}$ L.

Equation S1C:

$$\text{Volume}_{\text{coacervate}} = \frac{\text{total moles of U15 RNA added} - \left([\text{U15 RNA}]_{\text{supernatant}}\right)\left(\text{total sample volume}\right)}{[\text{U15 RNA}]_{\text{coacervate}} - [\text{U15 RNA}]_{\text{supernatant}}}$$