Supplemental Information

Viscoelasticity of globular protein-based biomolecular condensates

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Methods

No unexpected or unusually high safety hazards were encountered.

Protein Expression and Purification. Tag12-GFP plasmid was purchased from Twist and transformed into NiCo21 (DE3) cells, tag6-GFP(-12) and iso-GFP(-12) were previously cloned in the Obermeyer lab and stored as glycerol stocks. Plasmids are available from Addgene.

GFPs were expressed in NiCo21 (DE3) cells in LB media. Cells were induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at an OD_{600} of between 0.8-1, grown overnight at 25 °C and harvested by centrifugation (4000 rpm for 20 min). The resulting cell pellet was flash frozen and stored at -80 °C. Cell pellets were defrosted and resuspended in lysis buffer (50 mM sodium phosphate, 300 mM sodium chloride, pH 8) followed by 10 min of sonication (33% duty cycle). Soluble and insoluble fractions were separated by centrifugation at 10,000 xg for 30 min. The resulting supernatant was incubated with Ni-NTA beads equilibrated with Buffer 1 (50 mM sodium phosphate, 300 mM NaCl, and 35 mM imidazole, pH 8.0) for 1 h at 4 °C. Weakly bound protein was removed by washing with Buffer 1. GFP was eluted with Buffer 2 (50 mM sodium phosphate, 300 mM NaCl, and 250 mM imidazole, pH 8). Purified GFP was concentrated by centrifugal ultrafiltration (molecular weight cutoff, MWCO, of 10 kDa) and buffer exchanged into 10 mM Tris (pH 7.4) by dialysis (MWCO of 3.5 kDa). GFP was stored in solution at 4 °C for short term use or mixed with glycerol (10%), frozen in liquid nitrogen, and stored at -80 °C. GFP concentration was determined by measuring absorbance at 488 nm (ε = 83000 M^{-1} cm^{-1}) using a Cary 60 UV-Vis spectrophotometer.

Turbidity Assay. GFP and polycation solutions were combined in tissue culture-treated polystyrene 384-well plates (ThermoFisher) and mixed by pipetting the mixture several times. Turbidity measurements were performed on a Tecan Infinite M200 Pro plate reader within 15 min of sample preparation. After orbital shaking for 30 s, samples were allowed to sit for 30 s prior to an absorbance measurement at λ = 600 nm. Percent transmittance (%T) was calculated from absorbance (A) using the formula A = 2 − log(%T). Measurements were performed in triplicate across several days.

Coacervate imaging. Coacervates were prepared in a tissue culture-treated polystyrene 384-well plates treated with a 1% pluronics solution (PLF 127). Tris (10 mM, pH 7.4) was added first followed by 3 M NaCl in 10 mM Tris to a final concentration of either 0, 50, 100, 150 or 200 mM NaCl, then GFP and finally polyK were added. A GFP concentration of 40 µM was used, the concentration of polyK was varied: 2, 5, 10, 20, 40, 80, 148, 500 or 1000 mM resulting in charge fraction of 0.11, 0.23, 0.38, 0.55, 0.71, 0.83, 0.90, 0.97 and 0.98 respectively. Samples were imaged on an EVOS™ FL Imaging System equipped with ×60/0.75 numerical aperture (NA) long-working distance Plan fluorite objective. GFP was excited with a 470 +/- 22 nm LED. Fluorescence images were acquired with a CCD camera and brightfield with a CMOS camera controlled by EVOS software in manual mode. ImageJ was used to further format and process images. As standard, images were collected 24 h post coacervate formation.

Particle Tracking Microrheology. Coacervates were prepared in glass bottom 384-well plates (Cellvis) by mixing polyK, 500 nm PEG-functionalized microspheres, and GFP in that order, all measurements were performed at 40 µM GFP in tris (10 mM, pH7.4) with 100 mM NaCl. All measurements were performed within 2 h of sample preparation. Carboxy-functionalized microspheres (FluoSpheres, Life Technologies Corporation, 2% solution) were modified with poly(ethylene glycol) (PEG) by addition of an equal volume of PEG-amine (Mr 2 kDa, 5 mg/mL) in MES buffer (50 mM, pH 6) to the microspheres. The solution was incubated for 15 min after which time 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) was added (final concentration, 25 mM). The pH was adjusted to 6.5 using 1 M NaOH. The solution was incubated overnight. The reaction was then quenched by addition of glycine (final concentration 100 mM). Microspheres were centrifuged at 14,000 g for 15 min, sonicated, and washed with phosphate buffer (50 mM). This was repeated four times, for a total of 5 wash steps.

Imaging was performed using a Nikon Eclipse Ti inverted microscope, equipped with a ×60/1.4 numerical aperture (NA) Plan Apo objective (Nikon) and Perfect Focus System to minimize drift. Red fluorescent beads were excited with the 555 nm line from a LIDA solid-state laser (Lumencor) and collected with a 552 nm edge BrightLine (Semrock) and a FF01 brightline emission filter 572/28 (Semrock). Images were acquired with a sCMOS Andor Zyla VSC-04733 camera controlled using Nikon Elements AR software. 1000 images were collected at 500 ms time intervals. These were exported as tiffs using ImageJ and further analyzed in MatLab using code from the Elbaum-Garfinkle lab, available on online at https://zenodo.org/record/6818910#.YsxnrnbMI2x

From calculated trajectories mean squared displacement (MSD) was calculated as:

$$MSD(\tau) = \langle \big(r(\tau + t) - r(t)\big)^2 \rangle$$

Where r is position, t is time, τ is lag time.

Plotting MSD as a function of time yielded plots with a slope approximately equal to one, indicating that beads were displaying Brownian motion (Figure 3a). This indicates that at the time resolution of these measurements, the coacervates were behaving as Newtonian fluids. Consequently, the diffusion coefficient (D) can be calculated as

$MSD = 2dD$t

Where d is number of dimensions, in this case 2.

From this, condensate viscosity (η) can then be calculated using the Stokes-Einstein relation:

$$D = \frac{k_B T}{6\pi\eta R}$$

Where k_B is the Boltzman constant, T is temperature, and R is bead radius.

Dynamic light scattering (DLS). DLS rheology measurements were performed as described by Cai et al[1] and analysed using their open access method (Version: 0.0.21) which can be found at https://dlsur.readthedocs.io/ and on GitHub at https://github.com/PamCai/DLSuR.

All measurements were performed in a Malvern low-volume quartz cuvette (ZEN2112). Coacervate samples (2 mL) were prepared by adding buffer, polycation, beads, and then GFP in that order directly to

the cuvette followed by pipetting to mix. The cuvette was then sealed and left to stand for 30 min, before being placed into a falcon tube and centrifuged at (4000 xg) for 10 min to allow the condensed phase to settle to a layer at the bottom of the cuvette (SI Fig 12). Measurements were performed in this condensed phase without removal of the dilute phase so as to preserve equilibrium. Measurements were performed within 2 hours of samples being prepared.

Beads that had been surface modified with PEG readily partitioned into GFP condensates. These same surface passivated beads did not readily partition into condensates of polyK-polyD. For example, if beads were injected into the condensates they would migrate to the interface between the dense and dilute phases on a timescale comparable to the DLS measurement length. Unmodified carboxy-beads were consequently used to measure polyK-polyD condensates. Both types of beads were used to measure a condensate with iso-GFP (f^+ = 0.71) and the results were comparable (SI Fig 13).

Autocorrelation curves were collected using a Malvern Zetasizer Nano ZS (633 nm laser) running version 7.12 Zetasizer software and operated in 173° non-invasive backscatter detection mode. The raw intensity autocorrelation function was collected at 4.2 mm, using automatic attenuator selection, for 1000 s. This was followed by fifteen 30 second measurements of the derived photon count rate at a series of positions, used to determine the scattering intensity. Data was exported and MSDs and complex moduli calculated as described in Cai et al[1]. Fitting data to extract cross over points and plateau moduli was performed in Matlab or python.

To perform these measurements, beads were embedded within a large coacervate phase (>12 µL) (SI Fig 12). These beads scatter incident light, the scattering intensity is then measured as a function of time (where τ is lag time), and from this the intensity autocorrelation function $g^2(\tau)$ is calculated. Mean squared displacement can be calculated from $g^2(\tau)$ via the intermediate scattering function $g^1(\tau)$ where:

$$g^1(\tau) = exp\left(\frac{-q^2\Delta r^2(\tau)}{6}\right)$$

q is the scattering wave vector, calculated as 4πn sin(Θ/2)/λ where n is refractive index (assumed to be that of water), Θ is back scatter angle of the detector and λ is wavelength of the laser. The frequency dependent linear viscoelastic shear modulus ($G*(\omega)$) can then be calculated as:

$$G*(\omega) = \frac{k_B T}{\pi R i\omega \mathcal{F}\{\langle\Delta r^2(\tau)\rangle\}}$$

Where $\mathcal{F}\{\langle\Delta r^2(t)\rangle\}$ is the unilateral Fourier transform of mean square displacement. Using this technique, we were able to measure the rheology across a frequency range of 10^{-6} to 10 s in one measurement without the need for time temperature/salt superposition.

Previous condensate rheology results have been fit using a simple Maxwell model[2]. The Maxwell model is one of the most fundamental models to describe viscoelastic materials, it describes an exponential decay, with the relaxation time τ where relaxation originates from relaxation of segments between bonds or from motion of whole polymers flowing.

$$G(\omega) = G_0 exp(\frac{-t}{\tau})$$

$$G'(\omega) = G_0 \frac{\omega^2\tau^2}{1+\omega^2\tau^2} \text{ and } G''(t) = G_0 \frac{\omega\tau}{1+\omega^2\tau^2}$$

As the timescale for sticky interactions are significantly longer than the timescale for polymer chain relaxation, the stress relaxation can be split into two contributions. A slow component originating from sticker interactions and a fast relaxation due to Rouse motion of the polymer between sticky bonds.

$$G(t) = G_{slow/sticky}(t) + G_{fast/Rouse}(t)$$

Each of these components can be described by a Maxwell relaxation.

$$G'(\omega) = G_0\omega^a + \sum_{i=1}^{n} g_{s,i} \frac{\omega^2\tau_{s,i}^2}{1+\omega^2\tau_{s,i}^2} + \sum_{i=1}^{n} g_{f,i} \frac{\omega^2\tau_{f,i}^2}{1+\omega^2\tau_{f,i}^2}$$

$$G''(\omega) = G_0\omega^a + \sum_{i=1}^{n} g_{s,i} \frac{\omega^2\tau_{s,i}}{1+\omega^2\tau_{s,i}^2} + \sum_{i=1}^{n} g_{f,i} \frac{\omega^2\tau_{f,i}}{1+\omega^2\tau_{f,i}^2}$$

Fitting with this two component Maxwell type model we see reasonable agreement (Figure 4,b), as expected addition of further components representative of the slightly different types of interactions the protein and polymer can have leads to a better fit.

Dilute Phase Concentration Determination. Dilute phase concentration was determined by first preparing 50 µL coacervate samples in a 384 well plate as described above. Samples were sealed with parafilm and left to settle overnight. Plates were then centrifuged at 4000 xg for 10 min to remove any remaining coacervates from the dilute phase. 40 µL of the dilute phase was carefully pipetted into a new well. Absorbance (A) was measured at 488 nm (ε = 83000 M^{-1} cm^{-1}) using a Tecan Infinite M200 Pro plate reader. 40 µL of GFP at a known concentration was used to calculate pathlength (l) and concentrations were calculated using the Beer-Lambert Law, A=cεl. Measurements were performed in triplicate across several days.

Dense Phase Concentration Determination

PolyK30 and polyD30 were labelled using with Alexa Fluor™ 594 NHS Ester (ThermoFisher #A20004) and Alexa Fluor™ 488 NHS Ester (ThermoFisher #A20000) respectively per manufacturer instructions. Two NAP columns were run in series to remove unreacted dye. Labelled polymers were then lyophilized overnight before resuspension in tris buffer.

Dense phase concentrations were determined by preparing 50 µL coacervate samples in a 384 well plate as described above. Samples were sealed with parafilm and left to settle overnight. Plates were then centrifuged at 4000 xg for 10 min to remove any remaining coacervates from the dilute phase. The dilute phase was removed by gentle vacuum and the remaining dense phase was resuspended in 40 µL of 3 M NaCl. Fluorescence intensity at 488 and 594 nm was measured using a Tecan Infinite M200 Pro plate reader and used to calculate concentrations relative to a standard. Standard curves were prepared by preparing dilution series of polyK-AF594, polyD-AF488 and GFP Measurements were performed in triplicate.

Confocal microscopy

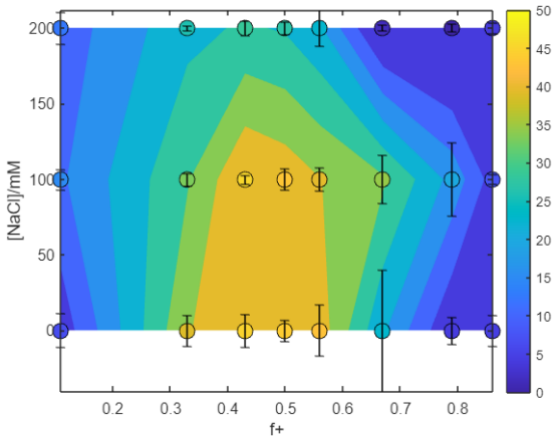
Fluorescence imaging was conducted using an inverted A1R laser scanning confocal microscope (Nikon) with a ×60/1.4 NA Plan Apo oil objective (Nikon) and Perfect Focus System 4 to minimize focus drift. AF-

488 and GFP were excited with the 488 nm line and AF-594 with the 561 line from a LU-NV solid-state laser system (Nikon) and collected with a 405/488/561/640 dichroic and 525/50 nm or 595/50 nm emission filters respectively. Images were acquired with a DU4 detector unit controlled using Nikon Elements AR software. 9 tiled images were collected and stitched using Nikon Elements AR software. These were exported and further analyzed using python to determine the average fluorescence intensity within the droplets. To calculate the concentration of polyK in the droplets, a standard curve with known polyK-AF594 concentration was used as a reference.

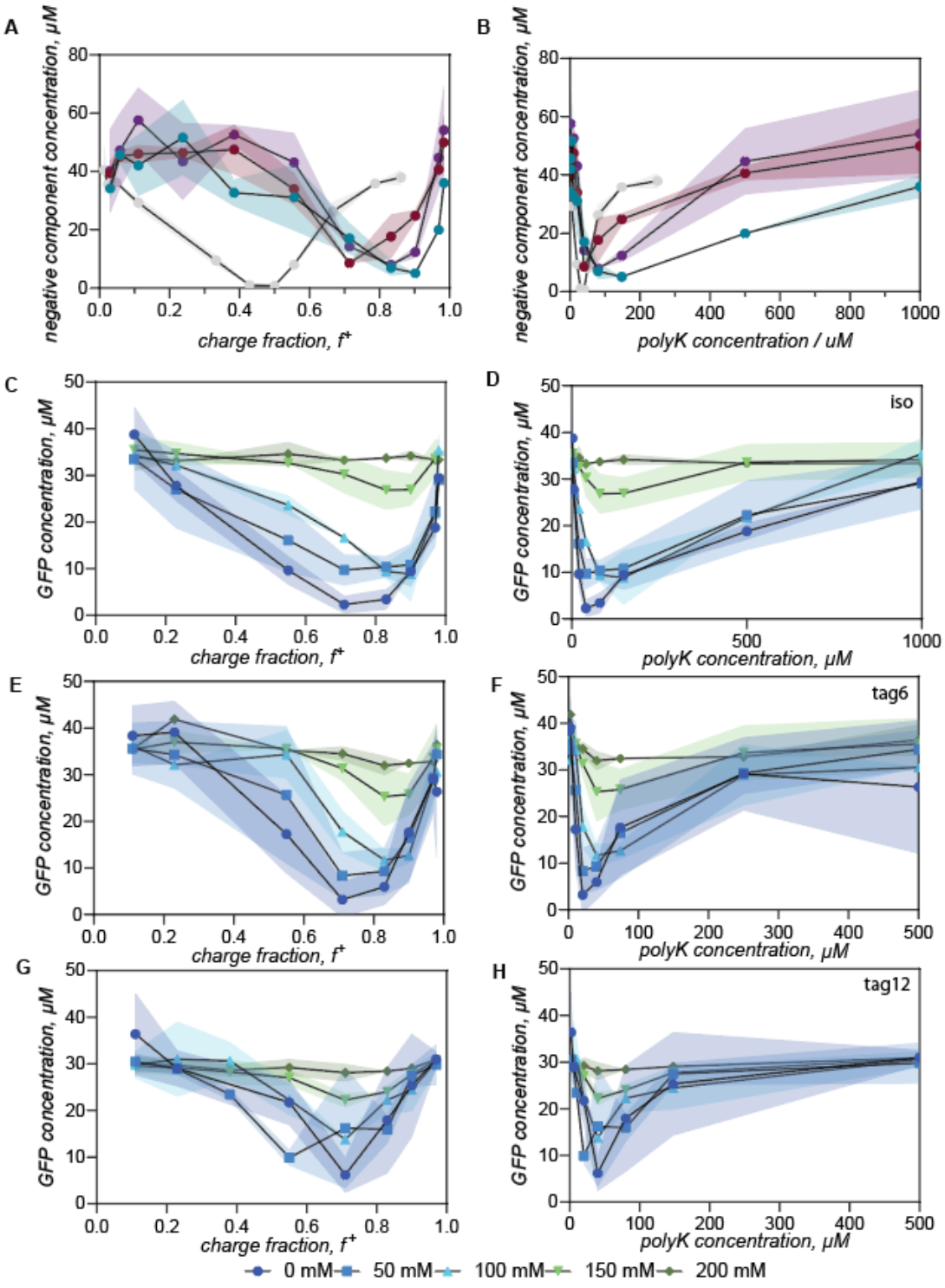
Supplementary Table 1. Protein sequences

Protein name	Sequence
Iso-GFP	MGHHHHHHGGASKGE**E**LFTGVVPILVELDGDVNGHKFSVRGEGEGDAT**E**GKLTLKFICTTGKLPVPWPTLVT TLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKG**I**DFKEDG NILGHKLEYNFNSHNVYITADKQ**E**NGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLP**D**NHYLSTQSA LSKDP**N**E**D**RDHMVLLEFVTAAGITHGMDE**L**YK
Tag6-GFP	MGHHHHHHGGASKGE**E**LFTGVVPILVELDGDVNGHKFSVRGEGEGDAT**N**GKLTLKFICTTGKLPVPWPTLV TTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKG**I**DFKEDG NILGHKLEYNFNSHNVYITADKQ**K**NGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLP**D**NHYLSTQSA LSKDP**N**E**K**RDHMVLLEFVTAAGITHGMDE**L**YK**DEEEDD**
Tag12-GFP	MGHHHHHHGGASKGE**R**LFTGVVPILVELDGDVNGHKFSVRGEGEGDAT**N**GKLTLKFICTTGKLPVPWPTLV TTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKG**R**DFKED GNILGHKLEYNFNSHNVYITADKQ**K**NGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLP**R**NHYLSTQS ALSKDP**K**E**K**RDHMVLLEFVTAAGITHGMDE**R**YK**DEEEDDDEEEDD**

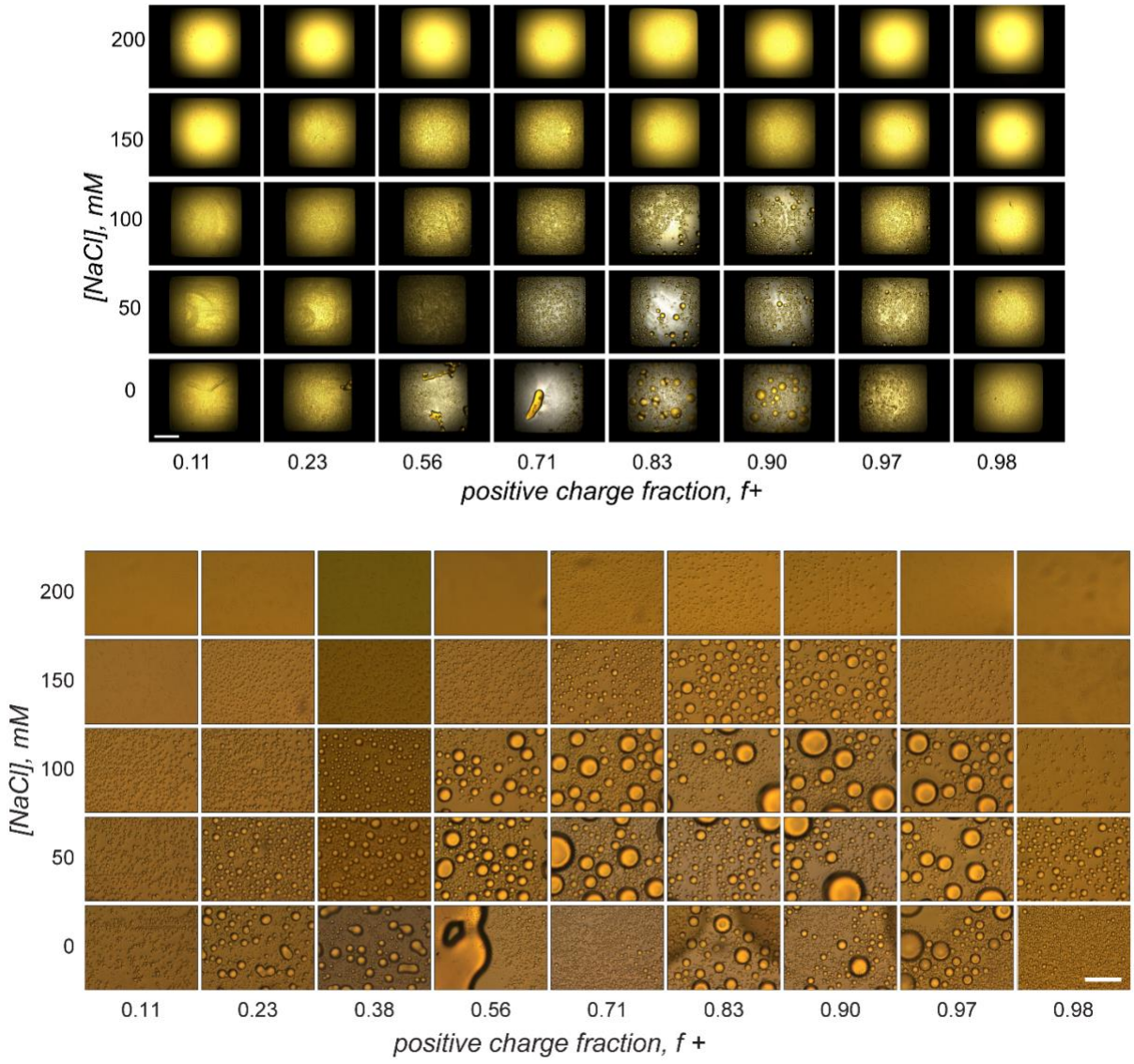
*__bold__ residues indicate differences between the protein variants



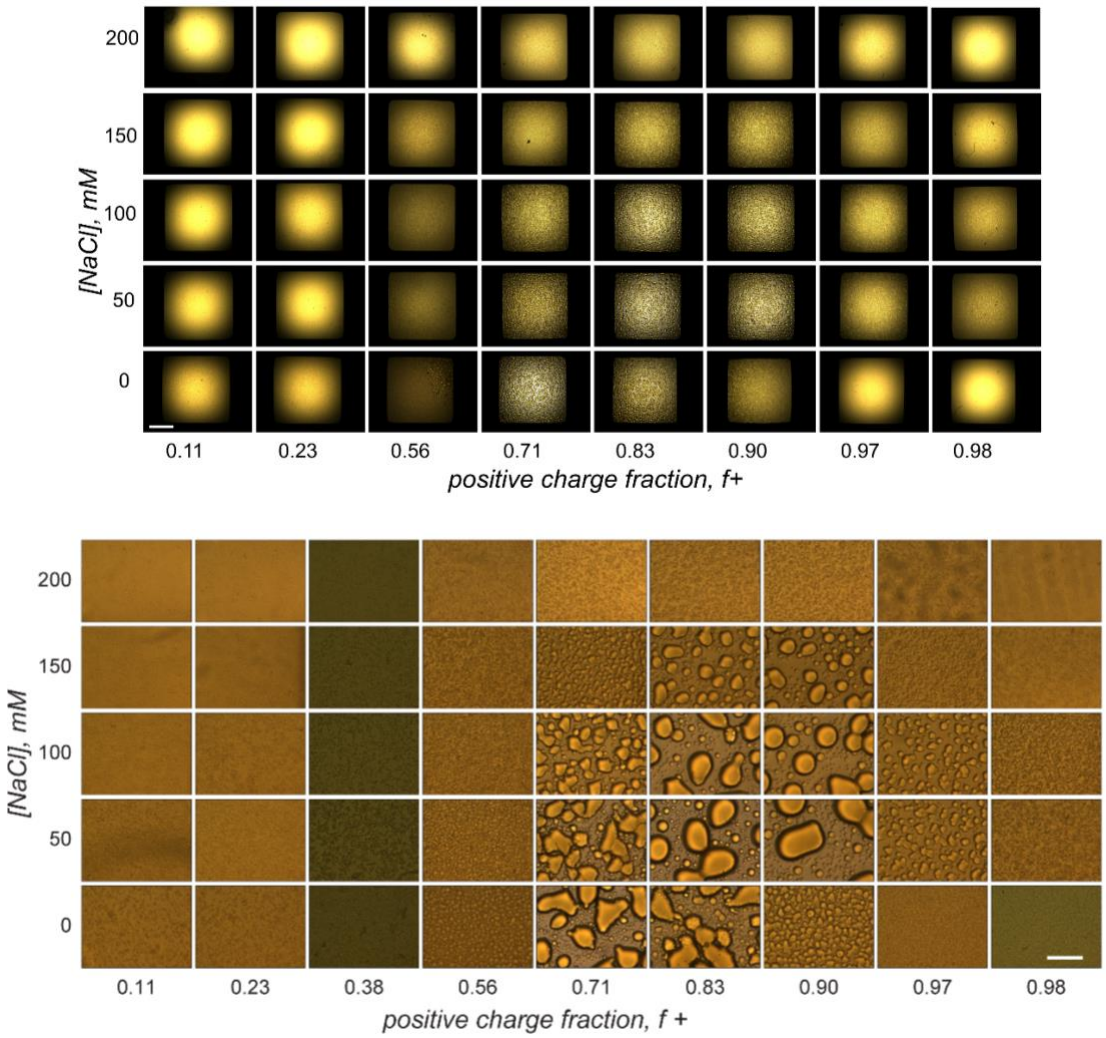
Supplementary Figure 1. Turbidity as a function of charge fraction (f^+) calculated as $M^+/(M^+ + M^-)$ where M^+ and M^- are charge per polyK and polyD polymer respectively measured at 0, 100 and 200 mM NaCl. To prepare coacervates the polyD concentration was held constant at 40 µM in tris (10 mM, pH 7.4) and varying concentration of polyK in tris (10 mM, pH 7.4) was added (5-250 µM).



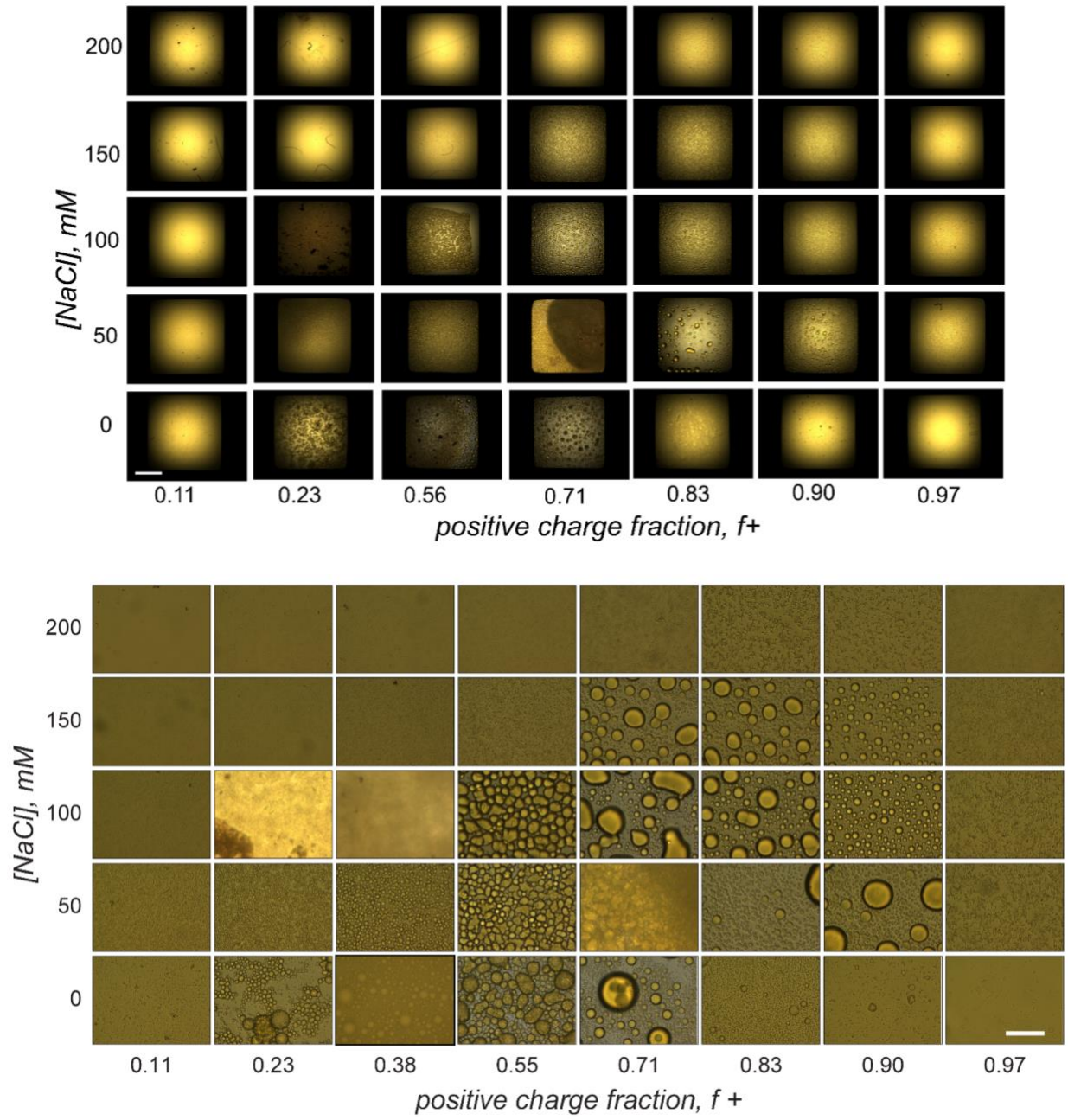
Supplementary Figure 2. (A,B) Concentration of polyD30-AF488 or GFP in the dilute phase as determined by fluorescence spectroscopy: iso-GFP (blue), tag6-GFP (purple), tag12-GFP (maroon) and polyD (grey) in coacervates. Concentration of (C,D) iso-GFP, (E,F) tag6-GFP, and (G,H) tag12-GFP in the dilute phase, as measured at 488 nm. GFP (40 μM) with increasing polyK concentration at 0, 50, 100, 150 and 200 mM NaCl (in 10 mM tris buffer pH 7.4). Data plotted as either charge fraction (A,C,E,G) or concentration (B,D,F,H)



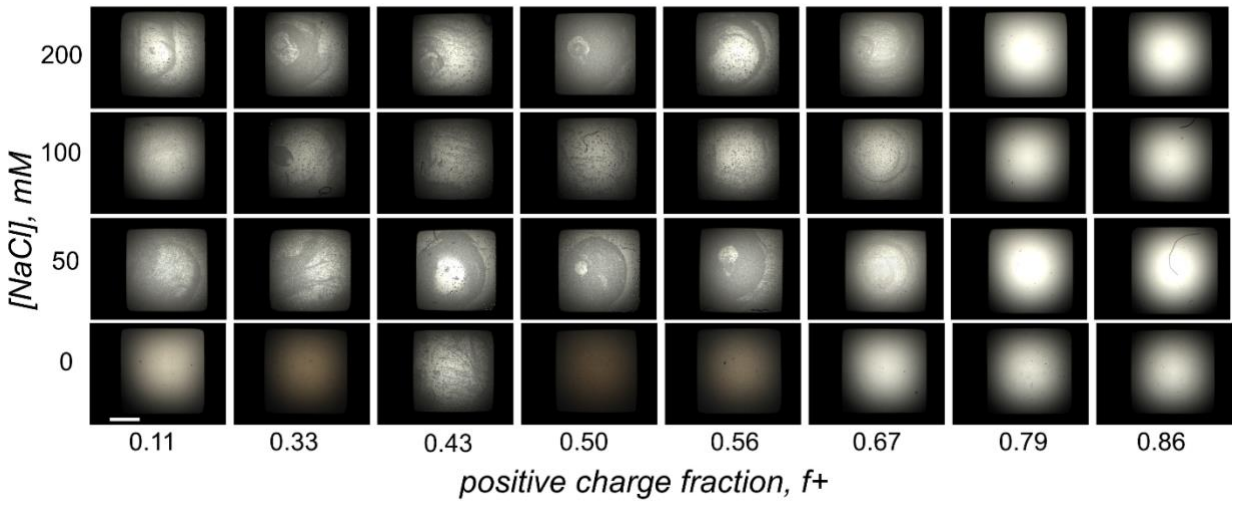
Supplementary Figure 3. Brightfield images of iso-GFP (40 µM) with increasing polyK (f^+ = 0.11 – 0.98) at 0, 50, 100, 150 and 200 mM NaCl (in 10 mM tris buffer pH 7.4). Top panel is 4x magnification and shows the entire sample, scale bar 1000 µm. Bottom panel is 60x magnification and more clearly shows the difference between liquid and solid-like condensed phases, scale bar 10 µm.



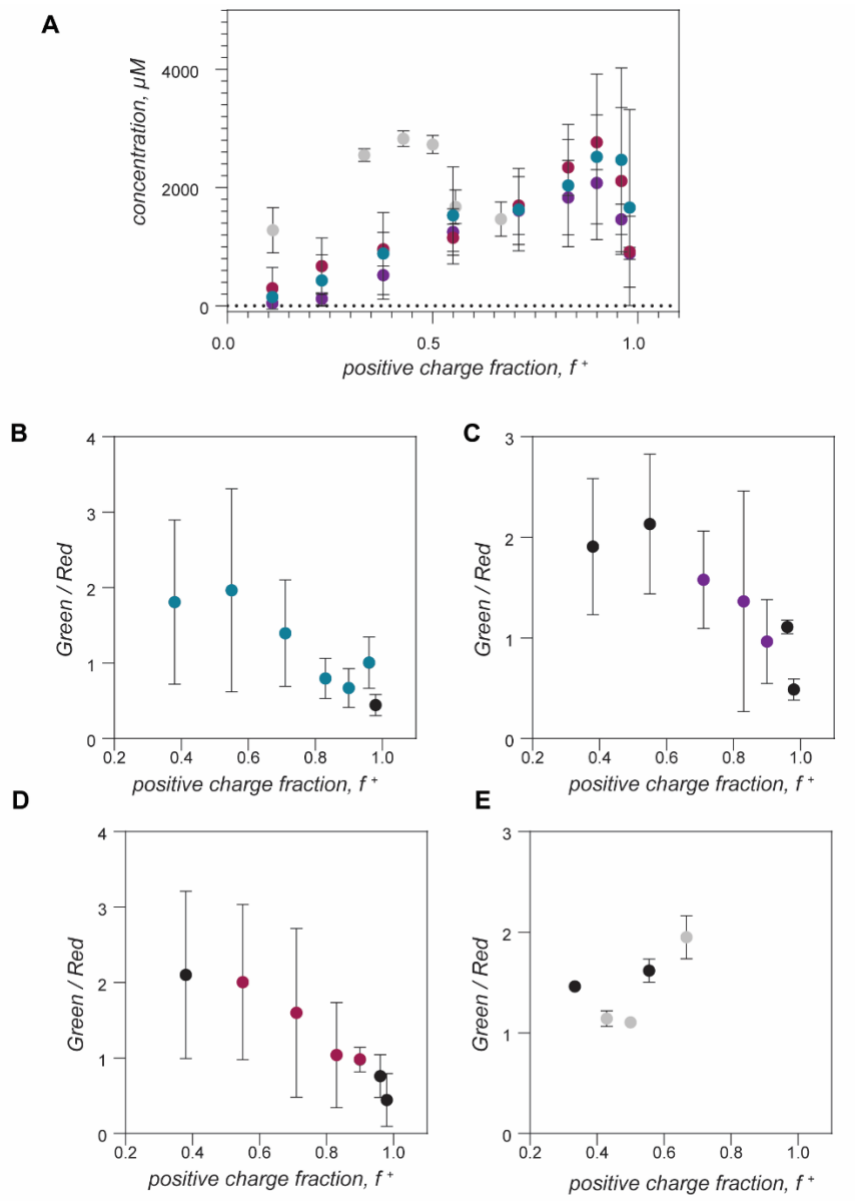
Supplementary Figure 4. Brightfield images of tag-6-GFP (40 µM) with increasing polyK (f^+ = 0.11 – 0.98) at 0, 50, 100, 150 and 200 mM NaCl (in 10 mM tris buffer pH 7.4). Top panel is 4x magnification and shows the entire sample, scale bar 1000 µm. Bottom panel is 60x magnification and more clearly shows the difference between liquid and solid-like condensed phases, scale bar 10 µm.



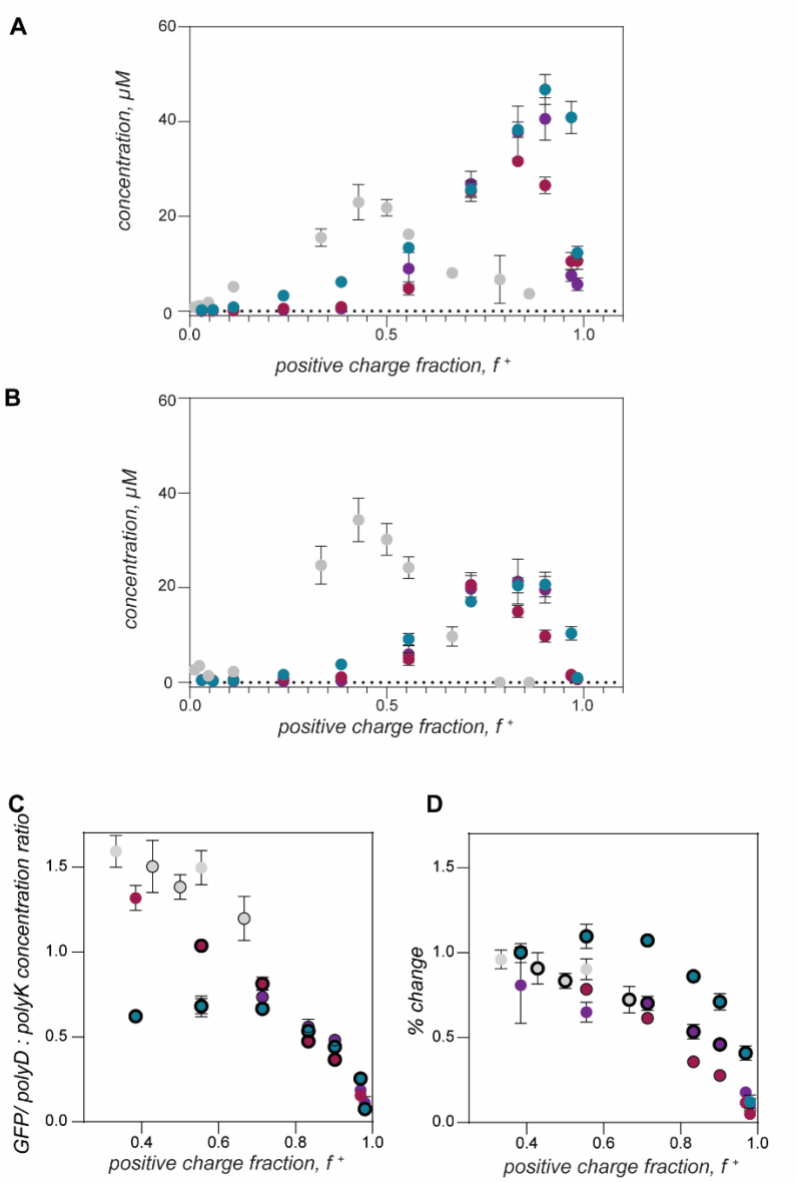
Supplementary Figure 5. Brightfield images of tag-12-GFP (40 µM) with increasing polyK (f^+ = 0.11 – 0.98) at 0, 50, 100, 150 and 200 mM NaCl (in 10 mM tris buffer pH 7.4). Top panel is 4x magnification and shows the entire sample, scale bar 1000 µm. Bottom panel is 60x magnification and more clearly shows the difference between liquid and solid-like condensed phases, scale bar 10 µm.



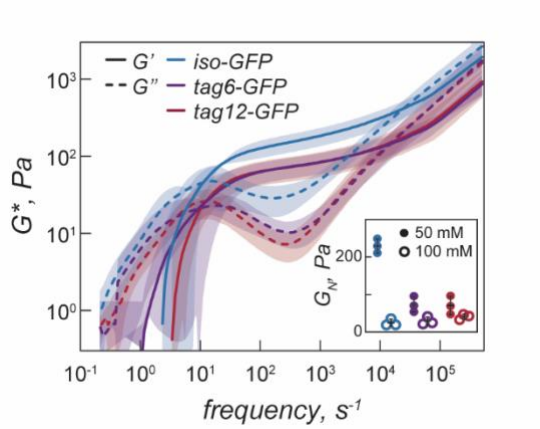
Supplementary Figure 6. Brightfield images of polyD (40 µM) with increasing polyK (f^+ = 0.11 – 0.86) at 0, 50, 100 and 200 mM NaCl (in tris buffer pH 7.4). The panel shows the entire sample (4x magnification), scale bar 1000 µm.



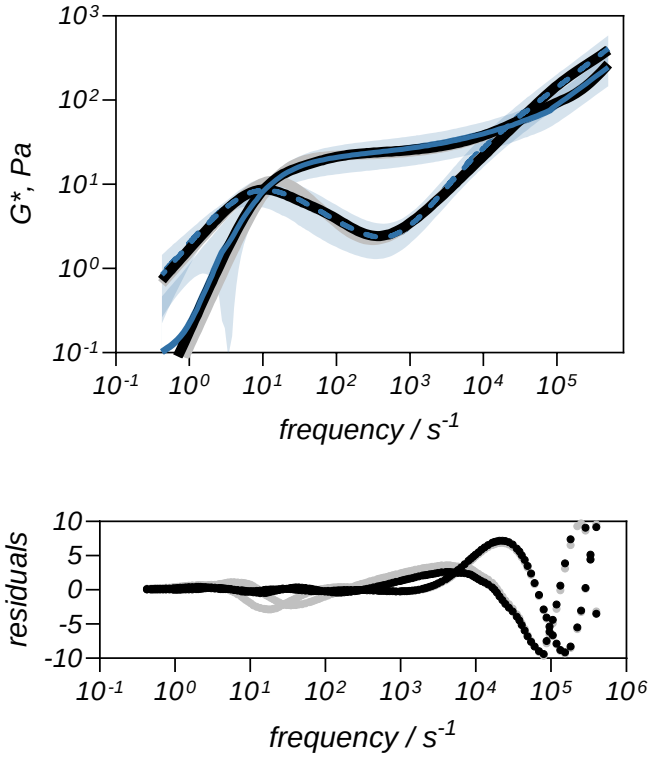
Supplementary Figure 7. (A) Concentration of polyK30 in droplets composed of iso-GFP (blue), tag6-GFP (purple), tag12-GFP (maroon) and polyD (grey) in coacervates as determined by confocal microscopy. GFP (40 μM) with increasing polyK (f^+ = 0.11 – 0.98) at 100 mM NaCl (in 10 mM tris buffer pH 7.4). (B) Ratio of fluorescence intensity of iso-GFP / fluorescence intensity of polyK30-AF594 within coacervates, as determined by confocal microscopy. Blue circles represent conditions under which VPT microrheology was performed. (C) Ratio of fluorescence intensity of tag6-GFP / fluorescence intensity of polyK30-AF594 within coacervates, as determined by confocal microscopy. Purple circles represent conditions under which rheology was performed. (D) Ratio of fluorescence intensity of tag12-GFP / fluorescence intensity of polyK30-AF594 within coacervates, as determined by confocal microscopy. Maroon circles represent conditions under which rheology was performed. (E) Ratio of fluorescence intensity of polyD30-AF488 / fluorescence intensity of polyK30-AF594 within coacervates, as determined by confocal microscopy. Grey circles represent conditions under which rheology was performed.



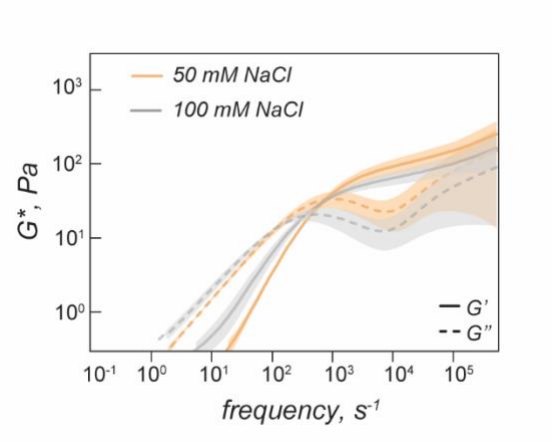
Supplementary Figure 8. (A) Concentration of polyK30 in droplets composed of iso-GFP (blue), tag6-GFP (purple), tag12-GFP (maroon) and polyD (grey) in coacervates as determined by redissolving the dense phase with 3 M NaCl. Condensates made at conditions: GFP or polyD (40 μM) with increasing polyK (f^+ = 0.11 – 0.98) at 100 mM NaCl (in 10 mM tris buffer pH 7.4). Note that the concentration reported is that measured in the resuspended volume (40 μL) and not the estimated concentration in the dense phase itself (~0-1 μL). (B) Concentration of polyD30 or GFP in droplets composed of polyK30 with iso-GFP (blue), tag6-GFP (purple), tag12-GFP (maroon) and polyD (grey) in coacervates as determined by redissolving the dense phase in 3 M NaCl. Condensates made at conditions: GFP or polyD (40 μM) with increasing polyK (f^+ = 0.11 – 0.98) at 100 mM NaCl (in 10 mM tris buffer pH 7.4). (C) Ratio of GFP or polyD to polyK30 in droplets composed of iso-GFP (blue), tag6-GFP (purple), tag12-GFP (maroon) and polyD (grey) in coacervates as determined by redissolving the dense phase in 3 M NaCl. Points with a black outline represent conditions under which rheology was performed. (D) Results from (C) normalized to concentrations at f^+ = 0.33 for polyD droplets and 0.38 for GFP containing droplets.



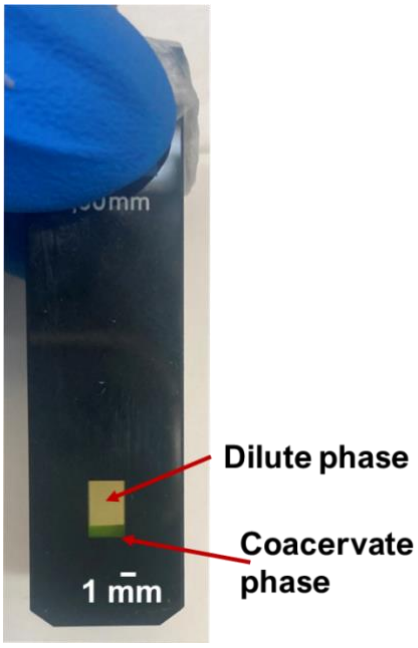
Supplementary Figure 9. Complex modulus of iso-GFP (blue), tag6-GFP (purple) and tag12-GFP (red) (40 µM) with polyK (40 µM, f+ = 0.71) at a NaCl concentration of 50 mM. Inset shows plateau modulus (G_N) at 50 mM NaCl concentration (solid circles) and 100 mM NaCl (open circles).



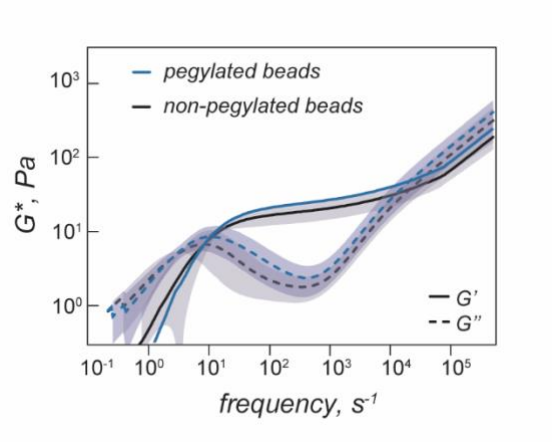
Supplementary Figure 10. (A) Complex modulus of iso-GFP (40 µM, polyK 40 µM, 100 mM NaCl in 10 mM tris buffer pH 7.4) (blue). Grey line represents 2 component Maxwell Fit (as shown in Figure 4B), black line represents 4 component Maxwell fit. (B) corresponding residuals for 2 and 4 component fit.



Supplementary Figure 11. Complex modulus of polyD30 with polyK (40 µM, f+ = 0.50) at a NaCl concentration of 100 mM (grey) and 50 mM (orange).



Supplementary Figure 12. Quartz micro-cuvette with condensed phase at the bottom of cuvette and dilute phase filling the full 2 mL volume of the cuvette.



Supplementary Figure 13. Complex modulus of iso-GFP with polyK (40 µM, f+ = 0.71) at a NaCl concentration of 100 mM collected using pegylated 500 nm beads (blue) and non-peg treated beads (black).

References

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