Ionic polypeptide tags for protein phase separation

*Rachel A. Kapelner, Allie C. Obermeyer**

Department of Chemical Engineering, Columbia University, New York, NY 10027

Supporting Information

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S.1 Materials and Methods

Unless otherwise noted, all primers were purchased from IDT, all enzymes and competent cells were purchased from New England Biolabs. DNA purification kits (miniprep, PCR purification and gel purification) were purchased from Qiagen. All media components and chemicals were purchased from Sigma Aldrich or Fisher Scientific and were used as received.

S.2 Cloning

The sfGFP plasmid was a gift from the Banta Group. The GFP(0), GFP(-12), GFP(-18), and GFP(-24) plasmids were obtained from GenScript. Both contained an N-terminal 6xHis tag (sequence: MGHHHHHHGG).

Primers to introduce ionic tags were purchased from IDT. Primer sequences and template plasmids used to prepare the mutated genes are included below.
Forward primer (all tag mutants): 5' – GATATACCATGGGTCATCACCACCACC – 3'

tag-GFP(-7):
template: iso-GFP(0)
Reverse primer: 5' – GAATGGACGAACGCTACAAGGATGAGGAAGAGGACGATTAATAAC TCGAGCTCAG – 3'

tag-GFP(-12):
template: iso-GFP(-7)
Reverse primer: 5' – GAATGGACGAGCTGTACAAGGATGAGGAAGAGGACGATTAATAAC TCGAGCTCAG – 3'

tag-GFP(-18):
template: iso-GFP(-7)
Reverse primer: 5' – GAATGGACGAGCTGTACAAGGATGAGGAAGAGGACGATGATGAGG AAGAGGACGATTAATAACTCGAGCTCAG – 3'

tag-GFP(-24):
template: tag-GFP(-18)
Reverse primer: 5' – ATGATGAGGAAGAGGACGATGACGAAGAGGAAGATGACTAATAAC TCGAGCTCAG – 3'

Primer stocks (10 μM) were prepared in Milli-Q water. Each PCR reaction was performed using 200 μM of dNTPs, 0.5 μM each forward and reverse primer, 50 ng of template DNA, 1 μL of Phusion DNA polymerase, 10 μL of 5X Phusion HF Buffer, and Milli-Q water, for a total reaction volume of 50 μL. The denaturation, annealing and extension temperatures were 98 °C, 52 °C, and 72 °C respectively. PCR was done for a total of 35 cycles.

Dpn1 digestion of the template was performed by adding 1 μL of Dpn1 to each PCR reaction and incubating at 37 °C for 1 hour. PCR products were purified using the PCR purification kit following DpnI digestion.

PCR amplified inserts were digested with NcoI and XhoI. The digestion reaction contained 1 μL of each enzyme (20,000 units mL^{-1}), 5 μL of 10X reaction buffer, 1 μg of DNA and Milli-Q water to give a total reaction volume of 50 μL. The digested DNA was purified using the Qiagen PCR purification kit. The

sfGFP or GFP(0) vector DNA was digested using the same protocol and was purified via agarose gel electrophoresis followed by extraction and purification using a gel purification kit.

Ligation was performed using a 5:1 molar ratio of the insert to the vector, 2 µL Ligation Buffer, 1 µL of DNA ligase and Milli-Q water for a total reaction volume of 10 µL. The ligated DNA (2 µL) was transformed into NEB5α cells. The mutated sequence was confirmed by Sanger sequencing (Genewiz).

The *cat* gene was amplified from a custom plasmid containing the resistance gene (generously donated by the Dickinson Lab). An N-terminal 6xHis tag was appended using the following primers:

Forward primer: 5' – ACTTTAAGAAGGAGATATACATGGGTCATCACCACCACCATCACGGTG GCAGCATGGAGAAAAAAATCACTGGATATACCAC – 3'
Reverse primer: 5' – CAGCGGTTTCTTTACCAGACTTATTACGCCCCGCCCTG – 3'

Primer stocks (10 µM) were prepared in Milli-Q water. Each PCR reaction was performed using 200 µM of dNTPs, 0.5 µM each forward and reverse primer, 50 ng of template DNA, 1 µL of Phusion DNA polymerase, 10 µL of 5X Phusion HF Buffer, and Milli-Q water, for a total reaction volume of 50 µL. The denaturation, annealing and extension temperatures were 95 °C, 52 °C, and 70 °C respectively. PCR was done for a total of 30 cycles.

PCR products were purified using the Qiagen PCR purification kit. PCR amplified inserts were assembled using NEBuilder HiFi DNA Assembly Master Mix with sfGFP vector DNA that had been digested using NcoI and XhoI following the protocol described above. The fragments (14 ng insert; 50 ng vector) were annealed at 50 °C for 15 min. The assembled DNA (2 µL) was transformed into NEB5α cells. The mutated sequence was confirmed by Sanger sequencing (Genewiz).

The anionic tag sequence was appended to the C-terminus using the following primers:
Forward primer (vector): 5' – GATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAGAGAG G – 3'
Reverse primer (vector): 5' – CGTCCTCTTCCTCATCCGCCCCGCCCTGCCACTC – 3'

Forward primer (insert): 5' – GGGCGGGGCGGATGAGGAAGAGGACGATGATGAGGAAGAGGAC GATTAATAAGTCTGGTAAAGAAACC – 3'
Reverse primer (insert): 5' – TACCCCCATGAACAGAAATCCCCCTTAC – 3'

Primer stocks (10 µM) were prepared in Milli-Q water. Each PCR reaction was performed using 200 µM of dNTPs, 0.5 µM each forward and reverse primer, 50 ng of template DNA, 1 µL of Phusion DNA polymerase, 10 µL of 5X Phusion HF Buffer, and Milli-Q water, for a total reaction volume of 50 µL. For the amplification of the insert, the denaturation, annealing and extension temperatures were 95 °C, 56°C, and 72 °C respectively. For the amplification of the vector, the denaturation, annealing and extension temperatures were 95 °C, 69 °C, and 72 °C respectively. PCR was done for a total of 35 cycles.

PCR products were purified using the Qiagen PCR purification kit. PCR amplified inserts were subsequently assembled using NEBuilder HiFi DNA Assembly Master Mix. The fragments (50 ng insert; 110 ng vector) were annealed at 50 °C for 15 min. The assembled DNA (2 µL) was transformed into NEB5α cells. The mutated sequence was confirmed by Sanger sequencing (Genewiz).

Amino Acid Sequences of isotropic mutants:
Mutations from sfGFP are **bold and underlined**.

GFP(0)
M G H H H H H H G G A S K G E **R** L F T G V V P I L V E L D G D V N G H K F S V R G E G E G
D A T N G K L T L K F I C T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H
D F F K S A M P E G Y V Q E R T I S F K D D G T Y K T R A E V K F E G D T L V N R I E L K G
R D F K E D G N I L G H K L E Y N F N S H N V Y I T A D K Q K N G I K A N F K I R H N V E D
G S V Q L A D H Y Q Q N T P I G D G P V L L P **R** N H Y L S T Q S A L S K D P **K** E K R D
H M V L L E F V T A A G I T H G M D E **R** Y K

Iso-GFP(-7) (sfGFP)
M G H H H H H H G G A S K G E E L F T G V V P I L V E L D G D V N G H K F S V R G E G E G
D A T N G K L T L K F I C T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H
D F F K S A M P E G Y V Q E R T I S F K D D G T Y K T R A E V K F E G D T L V N R I E L K G I
D F K E D G N I L G H K L E Y N F N S H N V Y I T A D K Q K N G I K A N F K I R H N V E D G
S V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L
L E F V T A A G I T H G M D E L Y K

Iso-GFP(-12)
M G H H H H H H G G A S K G E E L F T G V V P I L V E L D G D V N G H K F S V R G E G E G
D A T **E** G K L T L K F I C T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H
D F F K S A M P E G Y V Q E R T I S F K D D G T Y K T R A E V K F E G D T L V N R I E L K G I
D F K E D G N I L G H K L E Y N F N S H N V Y I T A D K Q **E** N G I K A N F K I R H N V E D G
S V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D P N E **D** R D H M V L
L E F V T A A G I T H G M D E L Y K

Iso-GFP(-18)
M G H H H H H H G G A S K G E E L F T G V V P I L V E L D G D V N G H K F S V R G E G E G
D A T **E** G K L T L K F I C T T G **E** L P V P W P T L V T T L T Y G V Q C F S R Y P D H M **D** Q H
D F F K S A M P E G Y V Q E R T I S F K D D G T Y K T R A E V K F E G D T L V N R I E L K G I
D F K E D G N I L G H K L E Y N F N S H **D** V Y I T A D K Q **E** N G I K A N F K I R H N V E D G
S V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T **E** S A L S K D P N E **D** R D H M V L
L E F V T A A G I T H G M D E L Y K

Iso-GFP(-24)
M G H H H H H H G G A S K G E E L F T G V V P I L V E L D G D V N G H **E** F S V R G E G E G
D A T **E** G K L T L K F I C T T G **E** L P V P W P T L V T T L T Y G V Q C F S **D** Y P D H M **D** Q H
D F F K S A M P E G Y V Q E R T I S F K D D G T Y K T R A E V K F E G D T L V N R I E L K G I
D F K E D G N I L G H K L E Y N F N S H **D** V Y I T A D K Q **E** N G I K A **E** F K I R H N V E D G
S V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T **E** S A L S K D P N E **D** R D H M V L
L E F V T A A G I **D** H G M D E L Y K

Amino Acid Sequences of tagged mutants:

Tag-CAT(-18)
M G H H H H H H G G S M E K K I T G Y T T V D I S Q W H R K E H F E A F Q S V A Q C T Y N
Q T V Q L D I T A F L K T V K K N K H K F Y P A F I H I L A R L M N A H P E F R M A M K D G
E L V I W D S V H P C Y T V F H E Q T E T F S S L W S E Y H D D F R Q F L H I Y S Q D V A C
Y G E N L A Y F P K G F I E N M F F V S A N P W V S F T S F D L N V A N M D N F F A P V F T
M G K Y Y T Q G D K V L M P L A I Q V H H A V C D G F H V G R M L N E L Q Q Y C D E W Q
G G A D E E E D D D E E E D D

Mutant	Globular Domain	Tag Sequence
tag-GFP(-7)	iso-GFP(0)	DEEEDD
tag-GFP(-12)	iso-GFP(-7)	DEEEDD
tag-GFP(-18)	iso-GFP(-7)	DEEEDDDEEEDD
tag-GFP(-24)	iso-GFP(-7)	DEEEDDDEEEDDDEEEDD
Tag-CAT(-18)	CAT	DEEEDDDEEEDD

S.3 Protein, expression, purification, and preparation

Protein Expression. All GFP mutants were expressed in NiCo21(DE3) cells in 1 L cultures of LB media supplemented with 100 μg/mL ampicillin. Cultures were incubated at 37 °C, with shaking at 250 rpm. Cultures were grown to an OD_{600} of ca. 0.8-1.0 and were subsequently induced by addition of 1 mL of 1 M isopropyl β-D-1-thiogalactopyranoside (IPTG). Isotropic and tagged GFP(-7) and GFP(-12) were incubated at 37 °C after induction and isotropic and tagged GFP(-18) and GFP(-24) were incubated at 25 °C after induction. Cultures were incubated for 16-18 h after induction.

Tag-CAT(-18) was expressed in NiCo21(DE3) cells in 1 L cultures of LB media supplemented with 100 μg/mL ampicillin. Cultures were incubated at 37 °C, with shaking at 250 rpm. Cultures were grown to an OD_{600} of ca. 1.0 and were subsequently induced by addition of 1 mL of 1 M IPTG. Cultures were incubated at 37 °C after induction for an additional 16 h.

Protein Purification. Cells were harvested by centrifugation (4000 rpm for 15 min) and cell pellets were resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0; 15 mL buffer per L of culture). The cells were lysed by sonication and cell debris was removed by centrifugation at 10,000 rpm for 45 min. The protein was purified using Ni-NTA metal affinity chromatography, according to the manufacturer's instructions with the following modifications: (i) 6 mL of resin was used per L of culture, (ii) imidazole was eliminated from the lysis buffer, (iii) the imidazole concentration in the wash buffer was increased to 50 mM, (iv) the volume of wash and elution buffer was optimized to maximize protein yield and purity. The flow through, washes, and elutions were analyzed by SDS-PAGE and pure fractions were combined and concentrated via centrifugal utrafiltration with a 10 kDa molecular weight cutoff (MWCO) filter. The mass of the purified proteins was confirmed by MALDI-TOF mass spectrometry (Figure S2). Samples were prepared by performing a 10-fold dilution of the 1 mg mL^{-1} stock protein solution (Sample preparation) into MilliQ water. Sinapinic acid was diluted into 7:3 water:acetonitrile (10 mg mL^{-1}). Final samples comprised of 60% matrix solution and 40% protein solution.

Sample preparation. Poly(4-vinyl *N*-methylpyridinium iodide) (sample # P231-4VPQ) was purchased from Polymer Source. Poly-L-lysine hydrochloride (P2658, 15-30 kDa) and polyethylenimine hydrochloride (764965, 20 kDa) were purchased from Sigma Aldrich. The polymer was dissolved in 10 mM tris buffer, pH 7.4 at 1 mg mL^{-1}. The pH was adjusted to 7.4 after polymer dissolution using a minimal volume of 6 M HCl.

Protein solutions were prepared by dialyzing the purified protein against 10 mM tris buffer, pH 7.4 (at 4 °C, in the dark) using a cellulose dialysis membrane with a 3.5 kDa MWCO. At least seven buffer changes were performed over a minimum of 21 hours to ensure complete buffer exchange. The concentration of all GFP variants was determined by absorbance. An extinction coefficient for each variant at 488 nm was determined using the superfolder GFP as a reference ($\varepsilon = 83{,}300\ M^{-1}cm^{-1}$). As the negative charge on the engineered proteins increased, a second peak (λ_{max} = 395 nm) became more prominent. The second peak corresponds to the absorbance of the protonated GFP chromophore.[1] The concentrations of tag-GFP(-7), iso-GFP(-7), tag-GFP(-12), iso-GFP(-12), tag-GFP(-18) and tag-GFP(-24) was determined using the absorbance at 488 nm as they did not display a second peak at 395 nm (Figure S3). The concentration of iso-GFP(-18) and iso-GFP(-24) was determined by using the absorbance at 280 nm to account for protein both with and without the protonated chromophore. The protein solution volume was adjusted with additional buffer to create a stock solution of 2 mg mL^{-1} protein. The concentration of tag-CAT(-18) was determined using the extinction coefficient ($\varepsilon = 44{,}140\ M^{-1}cm^{-1}$) at 280 nm, which was calculated by ExPASy using the primary amino acid sequence. The protein and polymer solutions were stored at 4 °C until use.

S.4 Evaluation of protein complex coacervation

Turbidimetric titrations. Protein and polymer solutions were prepared at 1 mg mL^{-1}. Solution turbidity as a function of macromolecule charge stoichiometry was measured by mixing the protein and polymer samples at mass ratios varying from 100% protein to 100% polymer in 4% increments. Samples were prepared in triplicate in a 96 well half area cell culture treated plate (Corning). The absorbance was measured at 600 nm in a Tecan M200 Pro plate reader. The measured absorbance values were converted to turbidity values using the equation: $turbidity = 100 - 10^{2-A}$.

Encapsulation Efficiency. Protein and polymer samples were prepared at 1 mg mL^{-1} in 10 mM tris buffer, pH 7.4. The proteins and polymer were mixed at 5 different ratios determined from the turbidity screens (Table S1 and S2). Each sample had a final volume of 100 µL and samples were prepared in triplicate. The samples were mixed by pipetting and were then centrifuged for 10 min at 10,000 rpm to facilitate the separation of the two phases. 55 µL of the dilute phase was removed and diluted 2-fold and 20-fold with 10 mM Tris, pH 7.4 (tag-GFP(-7), iso-GFP(-7), tag-GFP(-12), iso-GFP(-12), tag-GFP(-18) and tag-GFP(-24)) or 5 M NaCl, 10 mM Tris, pH 7.4 (iso-GFP(-18) and iso-GFP(-24)). The absorbance (λ = 488 nm) and fluorescence (λ_{ex} = 488 nm, λ_{em} = 530 nm) of the dilute phase was measured in a Tecan M200 Pro plate reader. The concentration of the protein in the dilute phase was determined using a calibration curve previously calculated from serial dilutions of each GFP variant (1.0 µM to 0.01 µM) in 10 mM Tris, pH 7.4 or 5 M NaCl, 10 mM Tris, pH 7.4. The concentration in the second phase was calculated by subtracting the protein concentration in the dilute phase from the total protein concentration in the sample.

Ionic strength dependence of phase separation. *Complex dissociation studies.* Protein and polymer samples were prepared at 1 mg mL^{-1} in 10 mM tris, pH 7.4. The proteins and polymer were mixed based on the optimal ratio determined from the turbidity screens (Table S1 and S2). Each sample had a final volume of 1 mL. The sample was stirred continuously at 500 rpm and the absorbance was measured at 600 nm with a Cary Ultraviolet/visible spectrophotometer. Sodium chloride (5 M in 10 mM tris buffer, pH 7.4) was added in 1 µL increments until the turbidity of the solution was less than 10%.

Complex formation studies. Protein and polymer samples were prepared at 0.97 mg mL^{-1} in 150 mM NaCl, 10 mM tris, pH 7.4. The proteins and polymer were mixed based on the optimal ratio determined from the turbidity screen (Table S1 and S2). Each sample had a final volume of 1 mL. The absorbance was

measured at 600 nm in a Cary 60 Ultraviolet/visible spectrophotometer. Phase separation was also confirmed by optical microscopy.

Calculation of the mean minimum distance between negatively charged residues. The PDB file for sfGFP (PDB ID: 2B3P) and CAT (PDB ID:1PD5) was used as a template. For the isotropic variants, mutations were made using PyMol (v. 2.0.6). For the ionic tag variants, the polypeptide solution structure was minimized using the PEP-FOLD 3 web-server. This peptide structure was then appended to the C-terminus of sfGFP and CAT in PyMol. For tag-CAT(-18), the pdb file of the trimer was used. The .pdb file for each engineered GFP was generated to get the coordinates for each atom in every amino acid in the protein. The .pdb file was filtered to get the x, y and z coordinates for the nitrogen atom (atom 1, N) for each aspartic acid and glutamic acid (D+E). Given the PDB files for the engineered proteins were approximated, the backbone nitrogen was selected for distance calculations to minimize error due to uncertain conformations of the side chains. MATLAB was used to calculate the straight-line distance between each of these nitrogens, resulting in a square matrix (# D+E x # D+E) with the straight-line distance (in angstroms) between all the negatively charged amino acids. The minimum distance between two negatively charged amino acids was determined for all D+E residues by determining the minimum value (> 0 angstroms) in each column (resulting in a 1 x # D+E). The mean minimum distance between two negatively charged residues was determined by calculating the mean of this matrix.

pH dependence of phase separation. Protein and polymer samples were prepared at 1 mg mL^{-1} in 10 mM tris, pH 7.4 and filtered with a 0.2 µm syringe filter. The protein and polymer samples were independently adjusted to pH 3 with 6 M HCl. The proteins and polymer were mixed based on the optimal ratio determined from the turbidity screens (Table S1 and S2). Each sample had a final volume of 1 mL. The sample was continuously stirred at 500 rpm, the absorbance was measured at 600 nm with a Cary 60 Ultraviolet/visible spectrophotometer, and the pH was monitored simultaneously using a pH micro electrode (Mettler Toledo). A solution of 0.1 or 0.01 M NaOH was added in 1 µL increments until the macromolecule solution was greater than pH 7.4.

Optical Microscopy. Microscopy images were taken using an Evos FL Auto 2 optical microscope. Samples were prepared at 1 mg mL^{-1} in a CultureWell multiwall chambered coverslip. The total volume of each sample was 5 µL. Samples were prepared at a protein/polymer ratio determined by the turbidity screen. Images were taken using a 20X, long working distance objective (NA 0.4) with illumination by brightfield and GFP (Ex: 470/22; Em: 525/50) LED light cubes.

S.5 Supporting references

1R. Y. Tsien, *Annual Review of Biochemistry*, 1998, **67**, 509–544.

S.6 Summary of protein and phase separation parameters

Table 1: Summary of isotropic mutants

	protein pI	mean min distance between negative residues	f+	protein mass fraction	% turbidity	fraction encapsulated	Critical [NaCl] (mM)	Critical pH
iso-GFP(-7)	5.94	5.8927	0.422	0.96	50.73	0.375	-	-
			0.792	0.82	85.61	0.915	-	-
			0.882	**0.7**	83.24	0.980	79	5.8
			0.927	0.6	44.28	0.921	-	-
			0.957	0.44	10.02	0.731	-	-
iso-GFP(-12)	5.20	5.6495	0.573	0.88	40.25	0.185	-	-
			0.793	0.72	87.41	0.862	-	-
			0.877	**0.58**	60.11	0.995	112	5.06
			0.937	0.4	29.68	0.929	-	-
			0.962	0.28	8.46	0.570	-	-
iso-GFP(-18)	4.86	5.5111	0.51	0.86	42.06	0.402	-	-
			0.644	0.78	63.13	0.691	-	-
			0.734	**0.7**	83.17	0.929	135	4.5
			0.784	0.64	68.49	1.002	-	-
			0.823	0.58	47.26	1.005	-	-
iso-GFP(-24)	4.38	5.5844	0.478	0.84	39.10	0.129	-	-
			0.627	0.74	53.87	0.517	-	-
			0.73	**0.64**	87.56	0.801	174	4.43
			0.776	0.58	65.76	0.813	-	-
			0.816	0.52	44.41	0.918	-	-

***Bold** macromolecule ratios were used for optical microscopy, salt and pH studies

Table 2: Summary of tagged mutants

	protein pI	mean min distance between negative residues	f+	protein mass fraction	% turbidity	fraction encapsulated	Critical [NaCl] (mM)	Critical pH
tag-GFP(-7)	6.33	5.8188	0.470	0.96	73.76	0.692	-	-
			0.802	0.84	87.72	0.981	-	-
			0.892	**0.72**	79.34	0.921	98	6.16
			0.934	0.60	35.83	0.800	-	-
			0.959	0.48	4.21	0.556	-	-
tag-GFP(-12)	5.29	5.4760	0.557	0.88	2.22	0.929	-	-
			0.697	0.80	89.12	0.999	-	-
			0.782	**0.72**	87.90	0.967	139	5.08
			0.838	0.64	86.71	0.894	-	-
			0.879	0.56	60.85	0.829	-	-
tag-GFP(-18)	4.82	5.1357	0.608	0.80	8.14	0.300	-	-
			0.726	0.70	90.13	1.000	-	-
			0.791	**0.62**	88.84	0.984	183	5.41
			0.841	0.54	78.85	0.917	-	-
			0.888	0.44	53.65	0.703	-	-
tag-GFP(-24)	4.61	4.8559	0.645	0.74	10.30	0.241	-	-
			0.740	0.64	90.90	0.999	-	-
			0.812	**0.48**	86.86	0.986	257	5.53
			0.865	0.44	44.01	0.812	-	-
			0.908	0.34	5.64	0.372	-	-
tag-CAT(-18)	4.99**	5.1170	0.605	0.8	38.35	-	287	-

*Bold macromolecule ratios were used for optical microscopy, salt and pH studies
**Calculated using protcalc.sourceforge.net

S.7 Electrophoresis analysis of engineered proteins

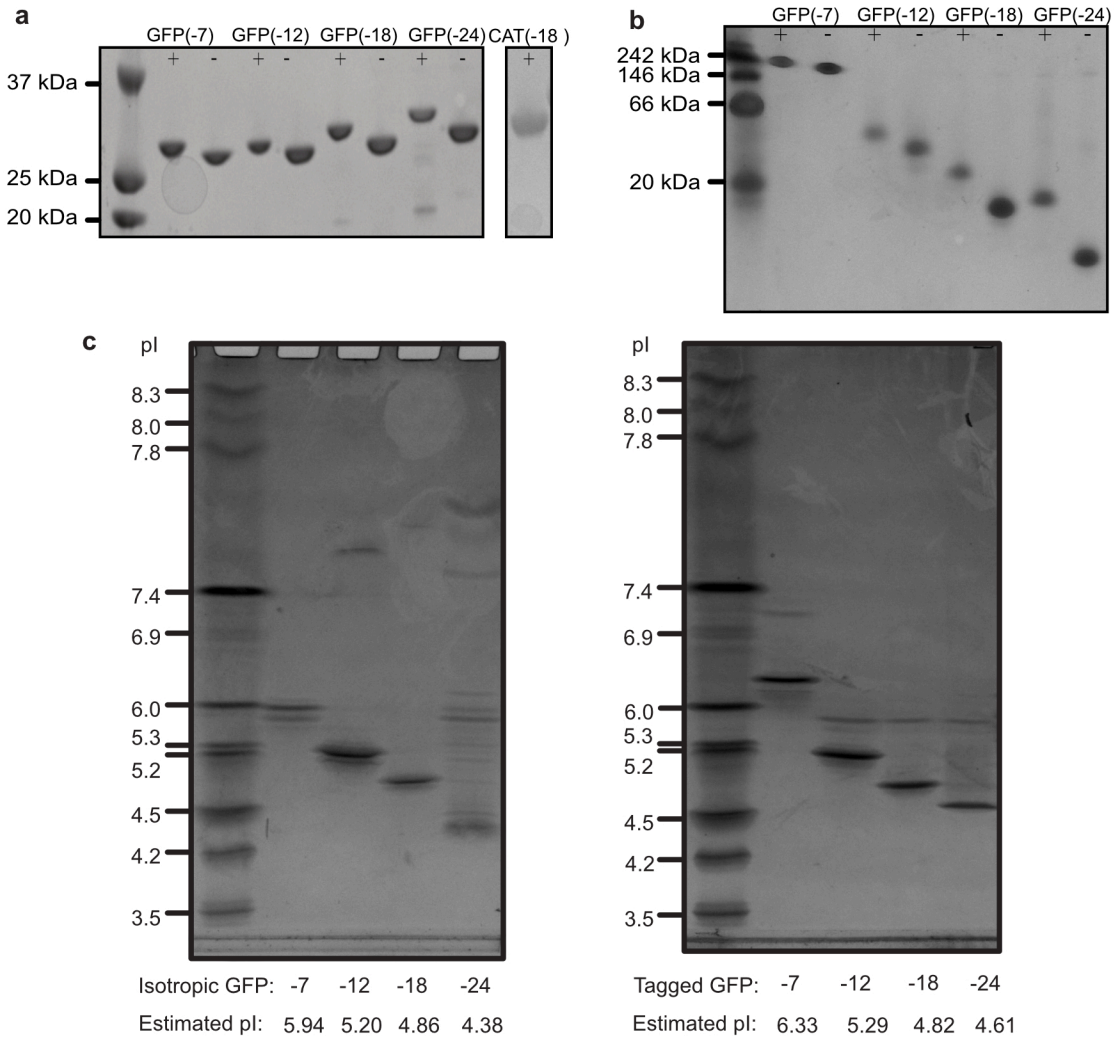

Figure S1. a) SDS-PAGE analysis of GFP mutants and CAT mutant for (+) tagged mutants and (-) isotropic mutants. **b)** Native-PAGE analysis of GFP mutants for (+) tagged mutants and (-) isotropic mutants. **c)** Isoelectric focusing gels of isotropic and tagged mutants. IEF gels and buffers were from Invitrogen. GFP Samples were prepared with 0.25 mg mL^{-1} protein and IEF loading buffer. Gels were run using the IEF cathode and anode buffers chilled to 4℃. Gels were run at 100 V for 1 h, 200 V for 1 h and 500 V for 30 min. Gels were fixed using 125 mL of 12% TCA for 5 minutes and stained using SimplyBlue Safe Stain.

S.8 MALDI-TOF MS of engineered proteins

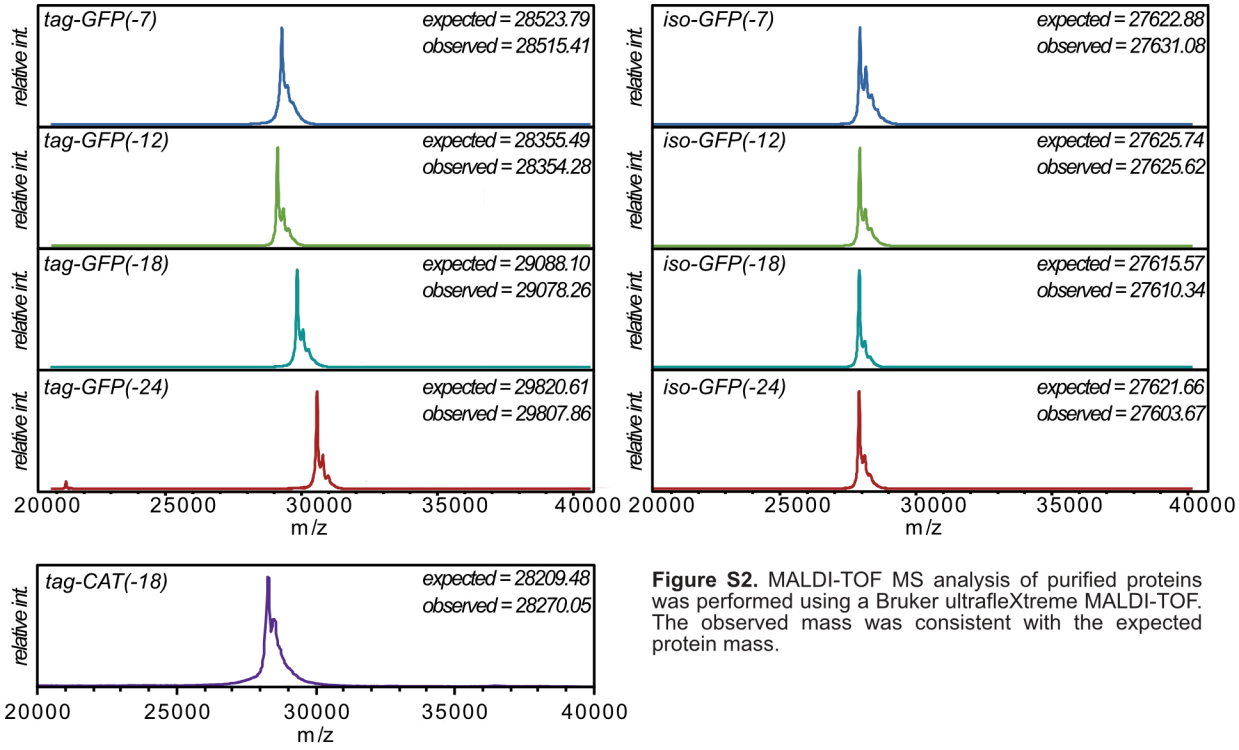

Figure S2. MALDI-TOF MS analysis of purified proteins was performed using a Bruker ultrafleXtreme MALDI-TOF. The observed mass was consistent with the expected protein mass.

S.9 UV/Vis of engineered proteins

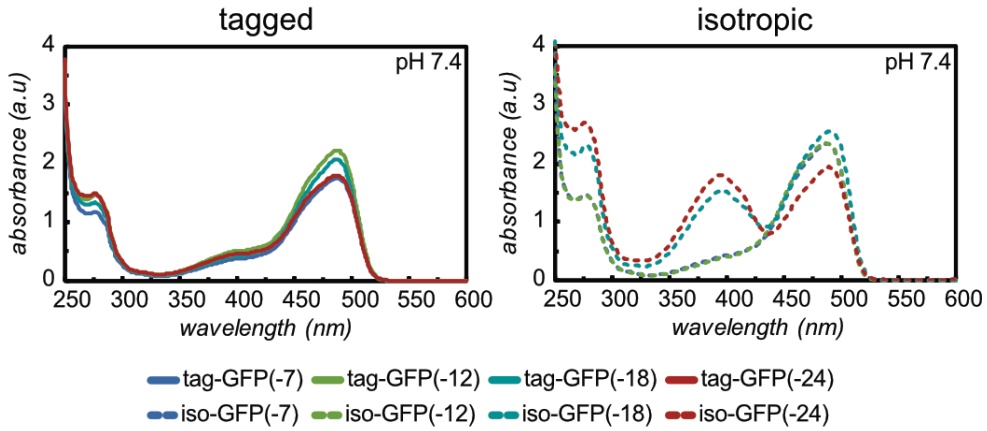

Figure S3. Absorbance scans of GFP at pH = 7.4. Absorbance was measured from 250 - 600 nm. Sample concentrations were prepared to 1 mg mL^{-1} in 10 mM tris calculated using the absorbance at 488 nm (ε = 83,300 L mol^{-1} cm^{-1}).

S.10 Phase separation of GFP with PEI and PLL

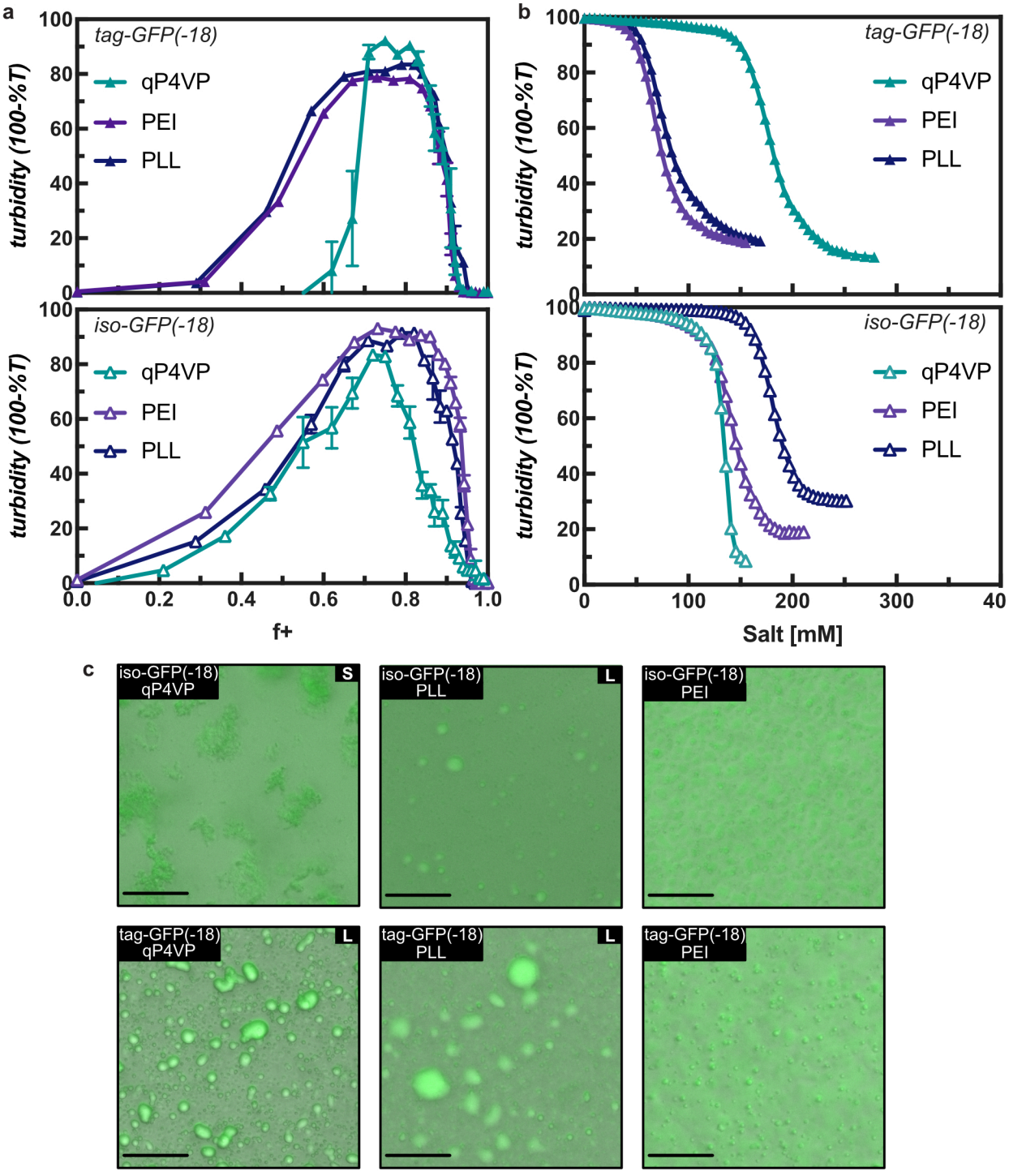

Figure S4. a) Turbidity studies of tag and iso-GFP(-18) with PLL and PEI. qP4VP data is replotted from Figure 3. **b)** Complex dissolution titration studies of tag and iso-GFP(-18) with PEI and PLL. qP4VP data is replotted from Figure 7. **c)** Optical microscopy studies of tag and iso-GFP(-18) with PLL and PEI at the midpoint of phase separation. Samples exhibited liquid-liquid (labeled L) or solid-liquid (labeled S) phase separation. The PEI samples are not labeled as they were not clearly solid or liquid. Scale bar represents 50 μm.

S.11 Comparison of turbidity of engineered proteins

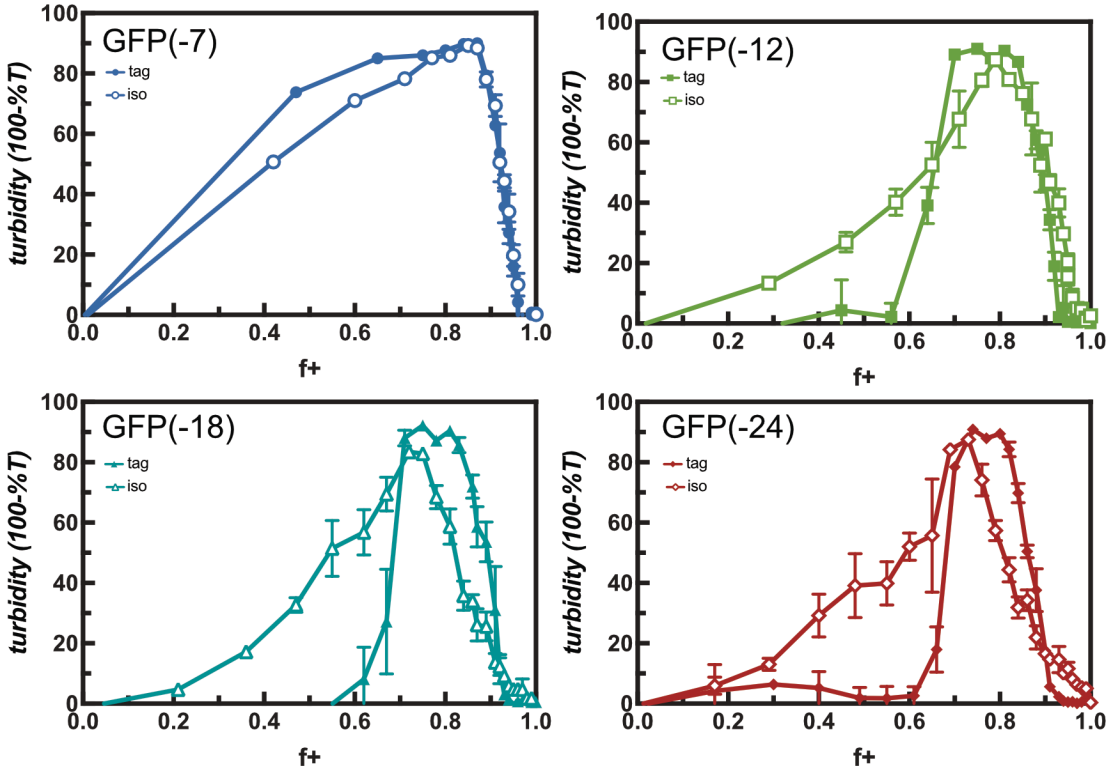

Figure S5. Turbidity studies of GFP and qP4VP solutions. Turbidity profiles as a function of positive charge fraction in 10 mM tris buffer (pH 7.4) for tagged mutants and isotropically charged mutants. Total macromolecule concentration is 1 mg mL^{-1}.

S.12 Fluorescence Recovery After Photobleaching (FRAP) of tag-GFP(-24)

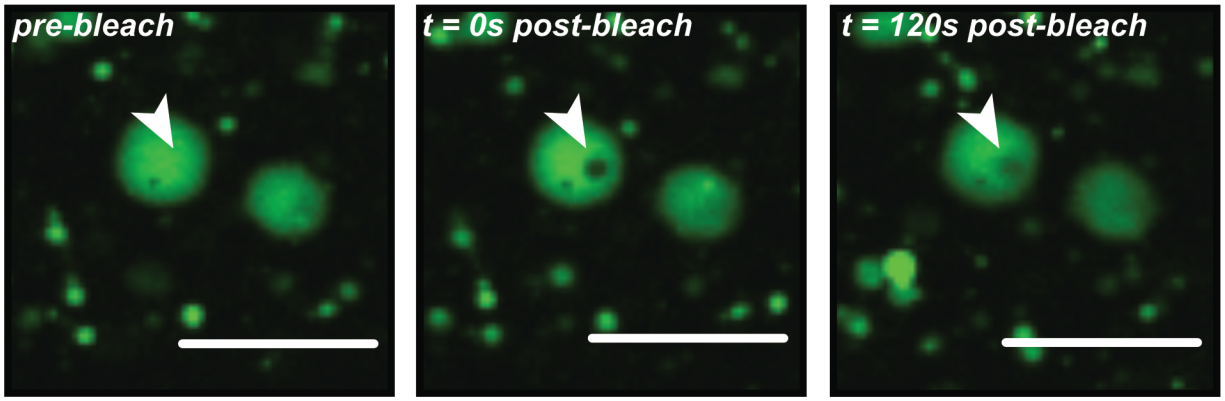

Figure S6. Fluorescence recovery after photobleaching (FRAP) of a liquid (tag-GFP(-24)). The sample was prepared with a total macromolecule concentration of 1 mg mL^{-1} in 10 mM tris (pH = 7.4) at the midpoint of phase separation on a CultureWell Multiwell Chambered Coverslip. Recovery was monitored on the GFP channel using the 20x air objective. Scale bar represents 5 μm.

S.13 Comparison of encapsulation of engineered proteins

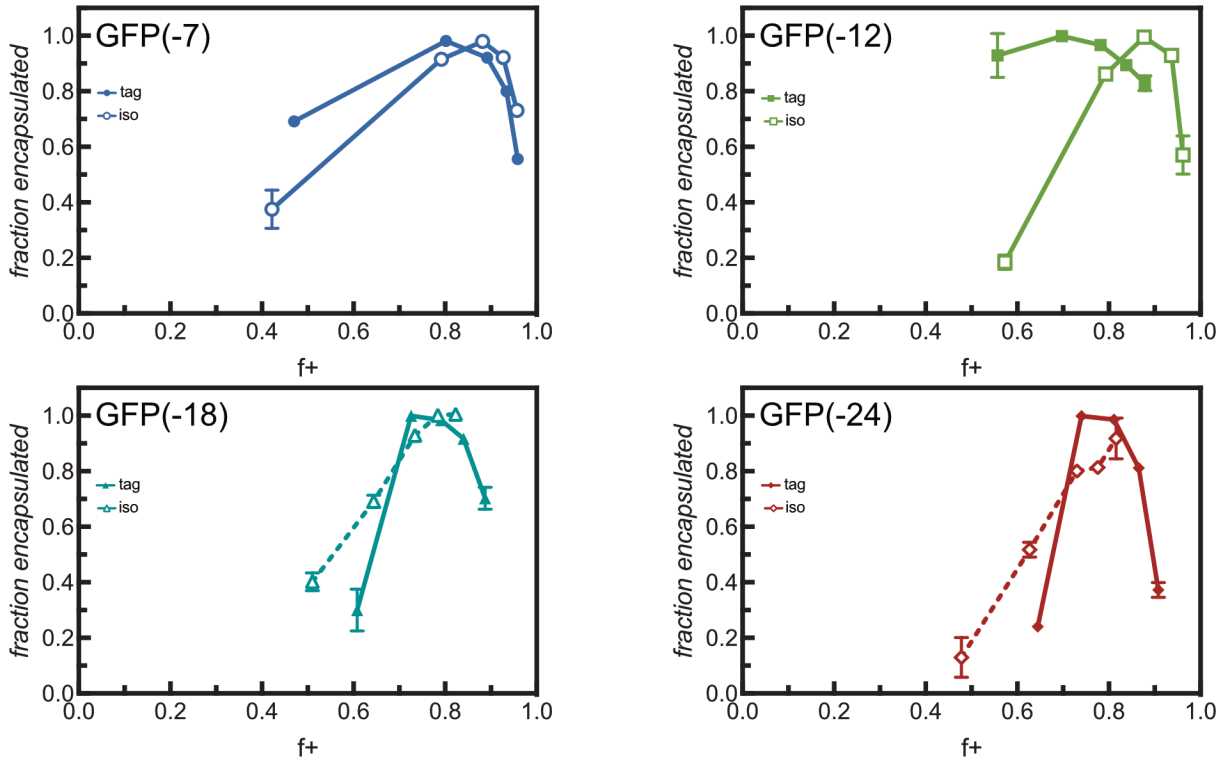

Figure S7. Encapsulation efficiency of tagged GFP and isotropic GFP with qP4VP in the coacervate (solid line) or precipitate (dashed line) phase at 5 macromolecule ratios determined by the initial turbidity screens.

S.14 Microscopy at alternate mixing ratios

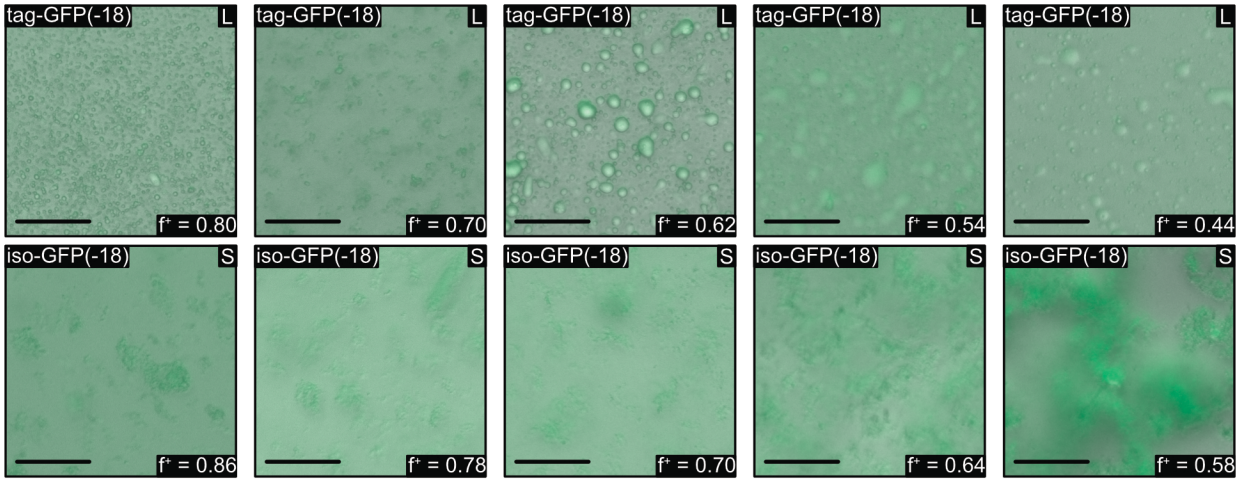

Figure S8. Optical microscopy images of tag-GFP(-18) (top row) and iso-GFP(-18) (bottom row) with qP4VP at different macromolecule mixing ratios (Tables S1 and S2). Samples were prepared with a total macromolecule concentration of 1 mg mL^{-1} in 10 mM tris (pH = 7.4). Samples were well mixed by pipetting. Samples exhibited liquid-liquid (labeled L) or solid-liquid (labeled S) phase separation. Scale bar represents 50 μm.

S.15 Non-normalized pH titration data

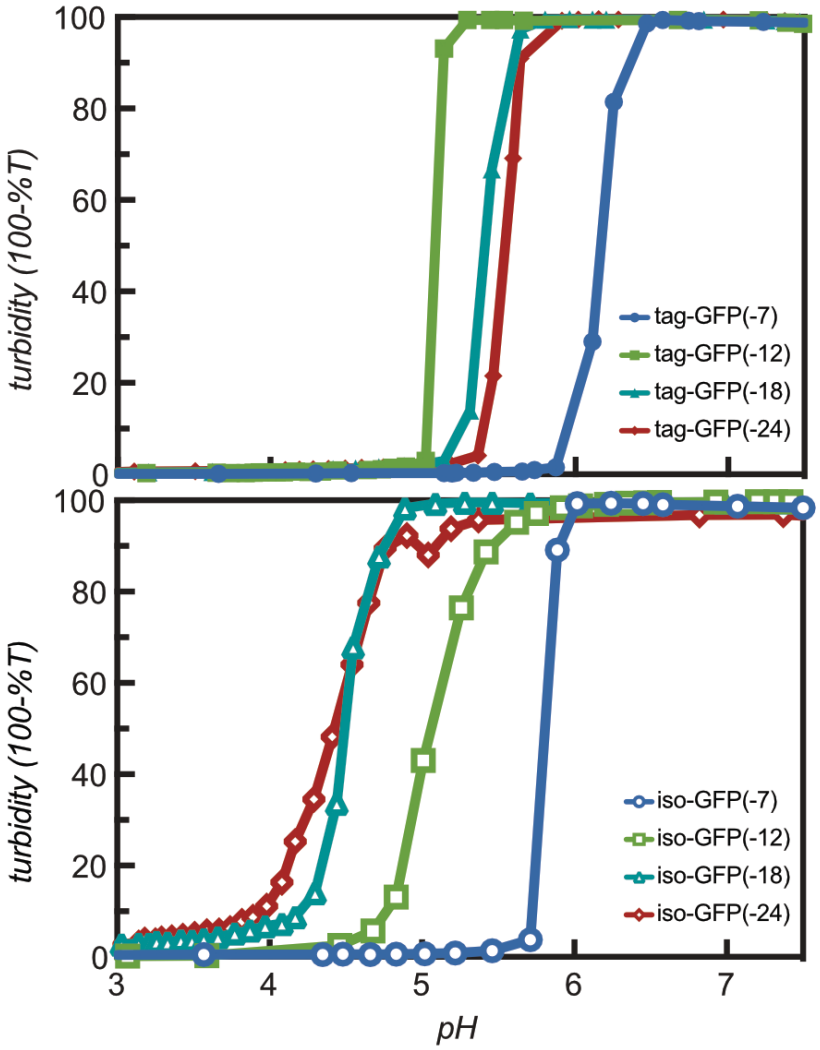

Figure S9. Non-normalized pH titration studies of GFP and qP4VP solutions for tagged mutants and isotropically charged mutants.

S.16 Critical Salt Concentration of GFP mutants

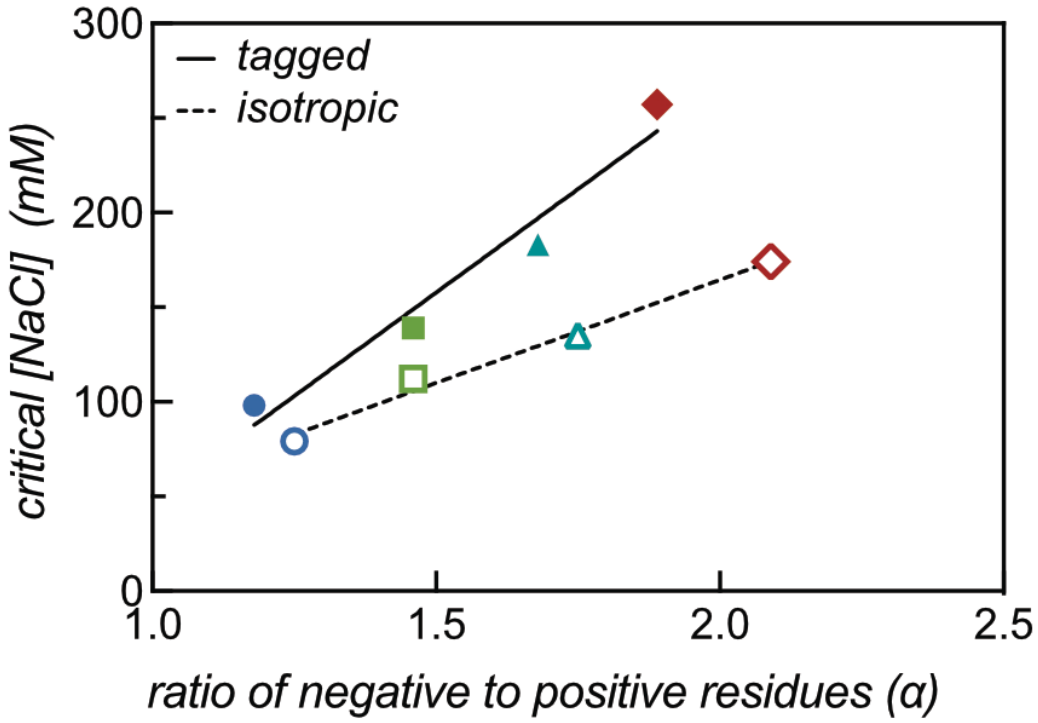

Figure S10. Critical salt concentration as a function of the ratio of negative to positive residues (α).

S.17 Optical microscopy of phase separation at high ionic strength

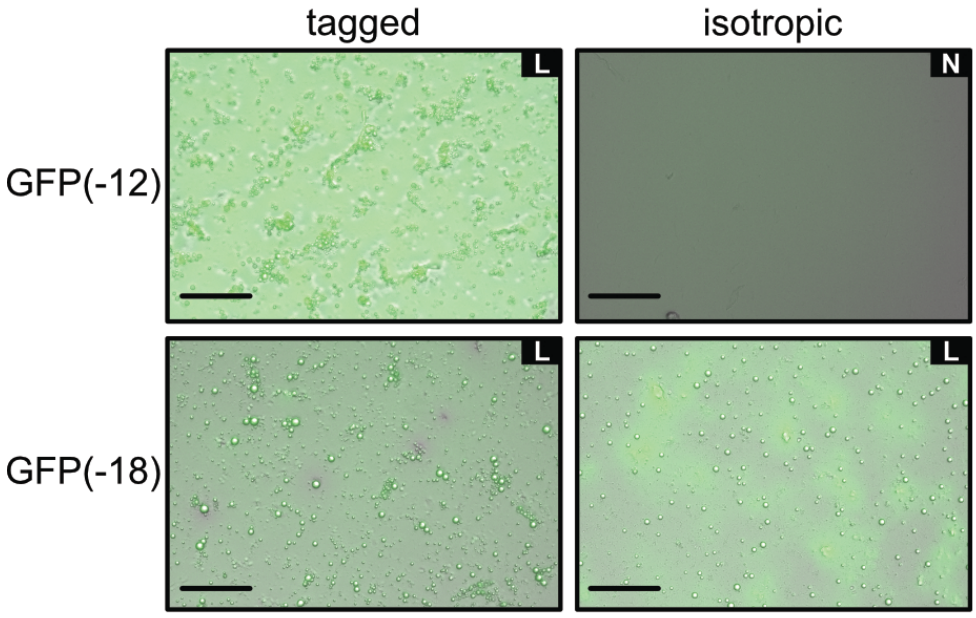

Figure S11. Effects of salt on complex formation. GFP and qP4VP were mixed at a total macromolecule concentration of 0.97 mg mL^{-1} in 10 mM tris buffer (pH 7.4) with 150 mM NaCl at the midpoint of phase separation as determined by the bulk turbidity studies. Microscopy images show no (labeled N) or liquid-liquid phase separation (labeled L). Scale bar represents 50 μm.