Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Rational Design of Elastin-Like Polypeptide Fusion Proteins to Tune Self-Assembly of Protein Vesicles

Yirui Li*, Dylan R. Dautel*, Mikaela A. Gray*, Michael E. McKenna, and Julie A. Champion

Supplemental Material

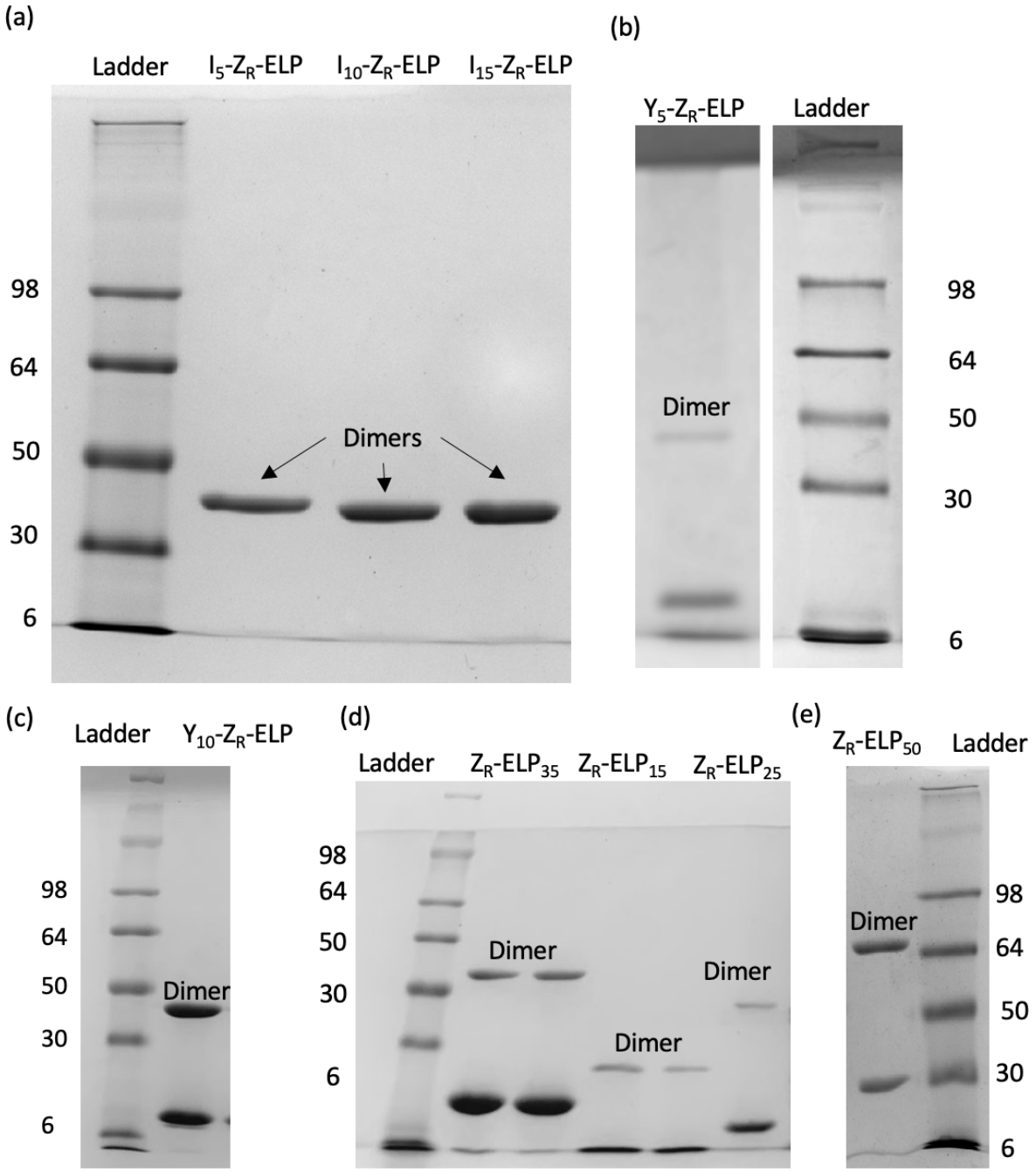

Figure S1. SDS-PAGE gel of each purified Z_R-ELP variant in this work, (a) I-Z_R-ELP variants, (b) Y_5-Z_R-ELP, (c) Y_{10}-Z_R-ELP, (d) Z_R-ELP$_{15}$, Z_R-ELP$_{25}$, and Z_R-ELP$_{35}$, and (e) Z_R-ELP$_{50}$. In each lane the lower band is protein monomer and the higher molecular weight band is dimer. Dimers tend to form due to disulfide bonds between the terminal cysteine in each protein.

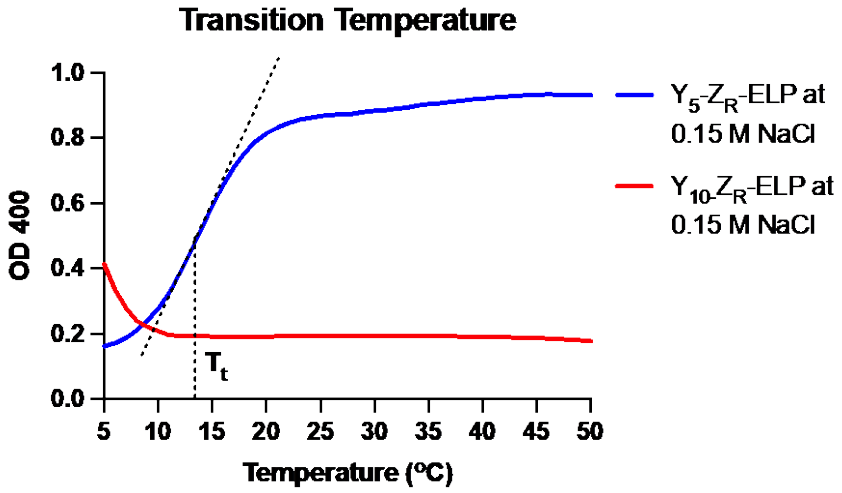

Figure S2. Turbidity curves (optical density (OD) at 400 nm) used for measuring the T_t of 1.5 µM mCherry-Z_E and 30 µM Y-Z_R-ELP mixtures at 0.15 M NaCl while increasing the temperature at a rate of 1 °C min^{-1}. Each T_t was calculated by the inflection point shown by dotted lines. The blue Y_5-Z_R-ELP curve is typical of all the other variants produced in this work except Y_{10}-Z_R-ELP (red).

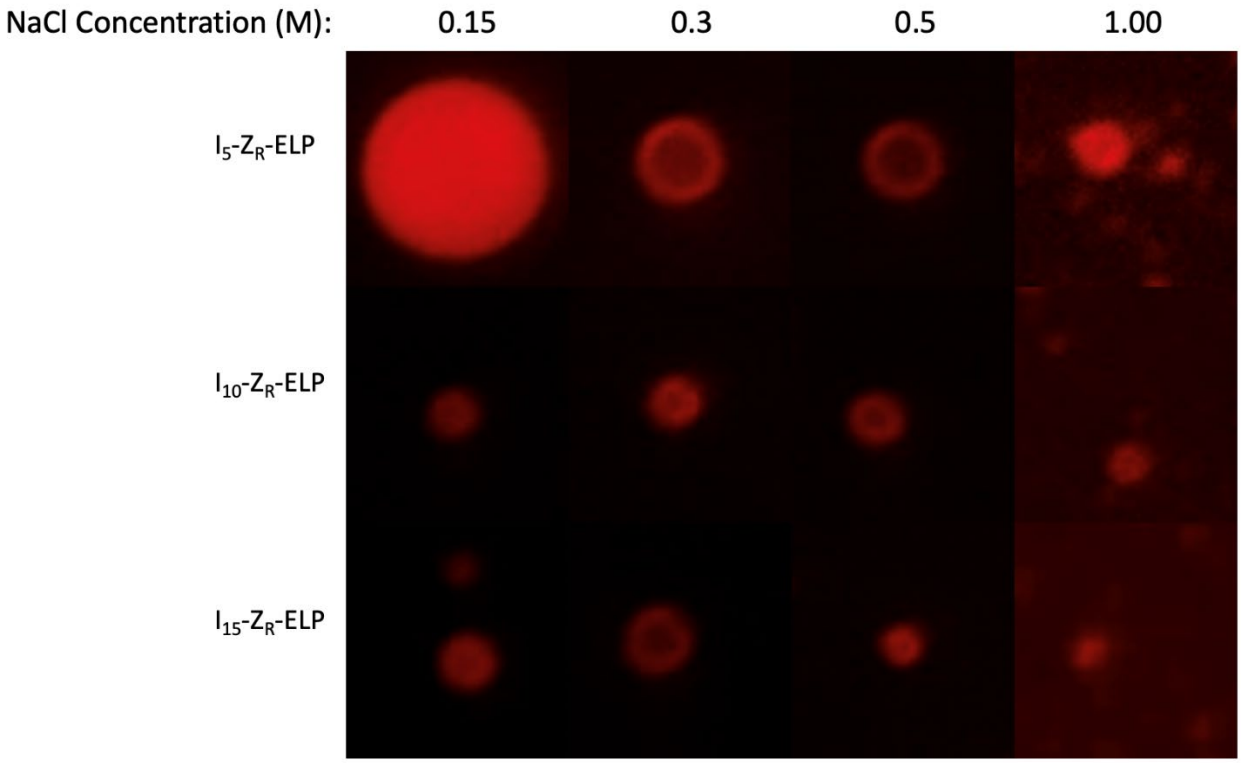

Figure S3. Enlarged insets from epifluorescence micrographs in Figure 3 of 1.5 µM mCherry-Z_E and 30 µM I_5-Z_R-ELP, I_{10}-Z_R-ELP, and I_{15}-Z_R-ELP solutions at 0.15, 0.3, 0.5, and 1.0 M NaCl after warming from 4 to 25°C for one hour.

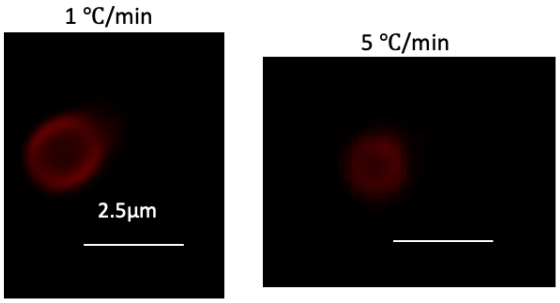

Figure S4. Characterization of vesicles made with slower (1 °C/min) and faster (5 °C/min) warming from 5 to 25 °C from 1.5 µM mCherry-Z_E and 30 µM I_{15}-Z_R-ELP with 0.5 M NaCl. Epifluorescence micrographs show smaller diameter with faster heating rate due to less time in the coacervation stage.

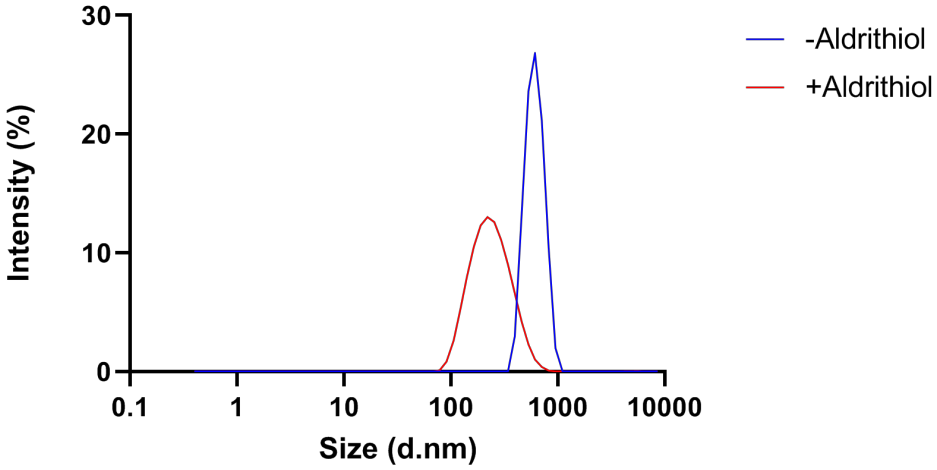

Figure S5. Dynamic light scattering analysis of 1.5 µM mCherry-Z_E and 30 µM I_{15}-Z_R-ELP at 0.5 M NaCl after warm from 4 to 25°C for one hour with and without aldrithiol, which prohibits disulfide bond formation between ELPs.

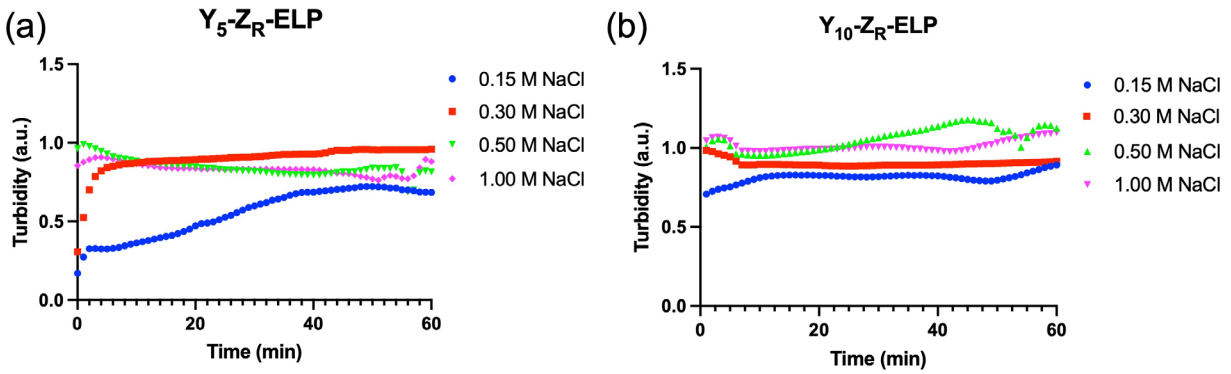

Figure S6. Turbidity profiles of mCherry-Z_E/Y-Z_R-ELP upon warming to 25 °C at 0.15, 0.30, 0.50, and 1 M NaCl. (a) Turbidity profiles of Y_5-Z_R-ELP. (b) Turbidity profiles of Y_{10}-Z_R-ELP.

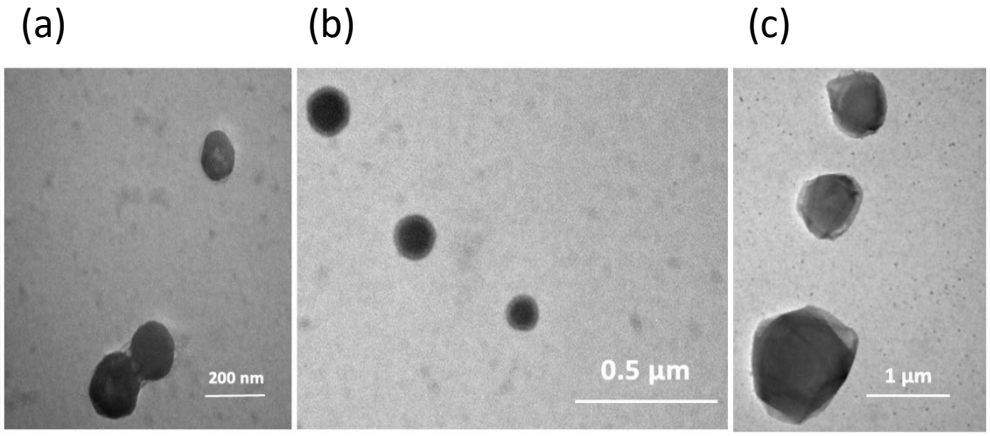

Figure S7. TEM images of structures made from mixing 1.5 µM mCherry-Z_E and 30 µM Y_5-Z_R-ELP at (a) 0.30 M, (b) 0.50M, and (c) 1.0 M NaCl after warmed from 4 to 25°C for one hour.

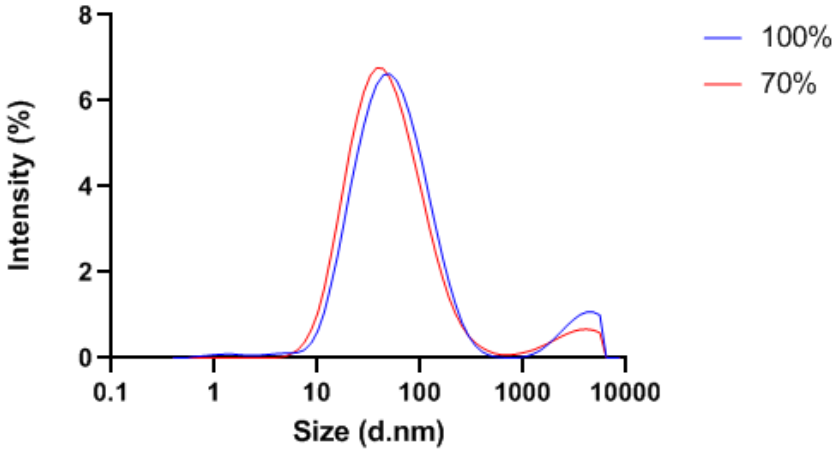

Figure S8. Dynamic light scattering (DLS) of 1.5 µM mCherry-Z_E and 30 µM Y_5-Z_R-ELP vesicles formed in 0.15 M NaCl after warmed from 4 to 25°C for one hour. 100% samples are not diluted. 70% samples are diluted to 70% of the original protein concentration with PBS. Further dilution resulted in insufficient concentration for DLS.

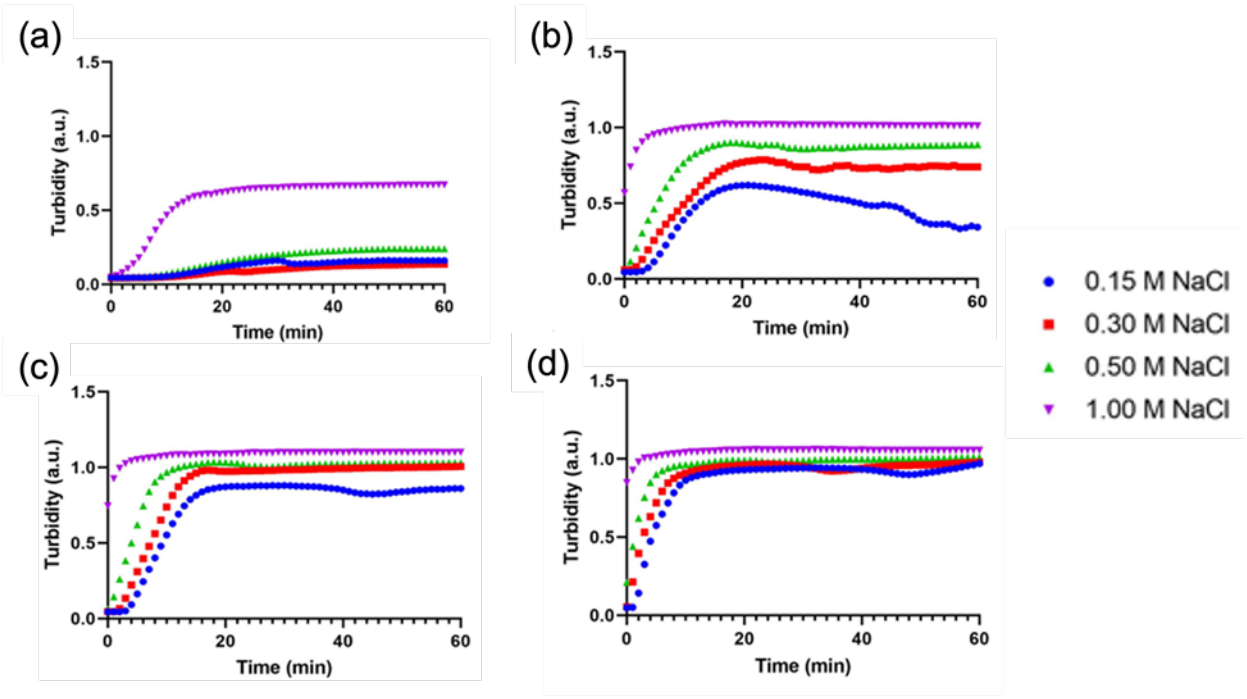

Figure S9. Turbidity measurements of Z_R-ELP$_n$ at NaCl concentrations of 0.15 M, 0.30 M, 0.50 M, and 1.00 M. (a) Turbidity profiles of Z_R-ELP$_{15}$. (b) Turbidity profiles of Z_R-ELP$_{25}$. (c) Turbidity profiles of Z_R-ELP$_{35}$. (d) Turbidity profiles of Z_R-ELP$_{50}$.

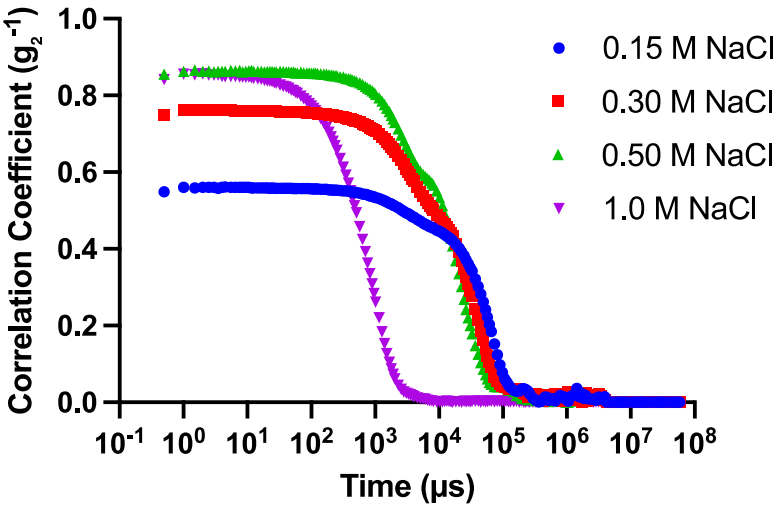

Figure S10. Correlogram from 1.5 μM mCherry-Z_E and 30 μM Z_R-ELP$_{15}$ mixtures measured by DLS.

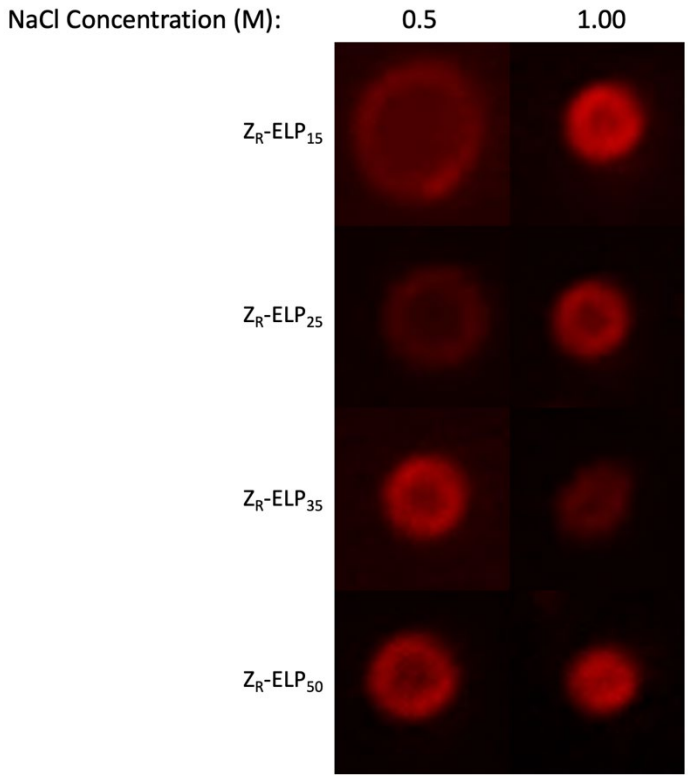

Figure S11. Enlarged insets from epifluorescence micrographs in Figure 3 of 1.5 μM mCherry-Z_E and 30 μM I_5-Z_R-ELP, I_{10}-Z_R-ELP, and I_{15}-Z_R-ELP solutions at 0.15, 0.3, 0.5, and 1.0 M NaCl after warming from 4 to 25°C for one hour.

Table S1. Sequences of Z_R-ELP proteins.

ELP Name	ELP Sequence
Z_R-ELP$_{(25)}$	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)$_2$VPGFG(VPGVG)$_2$]$_5$C
I_5-Z_R-ELP	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG) (VPGFG) (VPGIG) (VPGVG)$_2$]$_5$C
I_{10}-Z_R-ELP	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)(VPGIG)(VPGFG)(VPGIG)(VPGVG)]$_5$C
I_{15}-Z_R-ELP	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)(VPGIG) (VPGFG)(VPGIG)$_2$]$_5$C
Y_5-Z_R-ELP	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)$_2$(VPGFG)(VPGYG)(VPGVG)]$_5$C

Y_{10}-Z_R-ELP	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)(VPGYG)(VPGFG)(VPGYG)(VPGVG)]$_5$C
Y_{15}-Z_R-ELP	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)(VPGYG)(VPGFG)(VPGYG)$_2$]$_5$C
Z_R-ELP$_{15}$	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)$_2$VPGFG (VPGVG)$_2$]$_3$C
Z_R-ELP$_{35}$	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)$_2$VPGFG(VPGVG)$_2$]$_7$C
Z_R-ELP$_{50}$	MGGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPL GGGGSGGGGSG[(VPGVG)$_2$VPGFG(VPGVG)$_2$]$_{10}$C