

Supporting Information for

Protein Vesicles Self-Assembled from Functional Globular Proteins with Different Charge and Size

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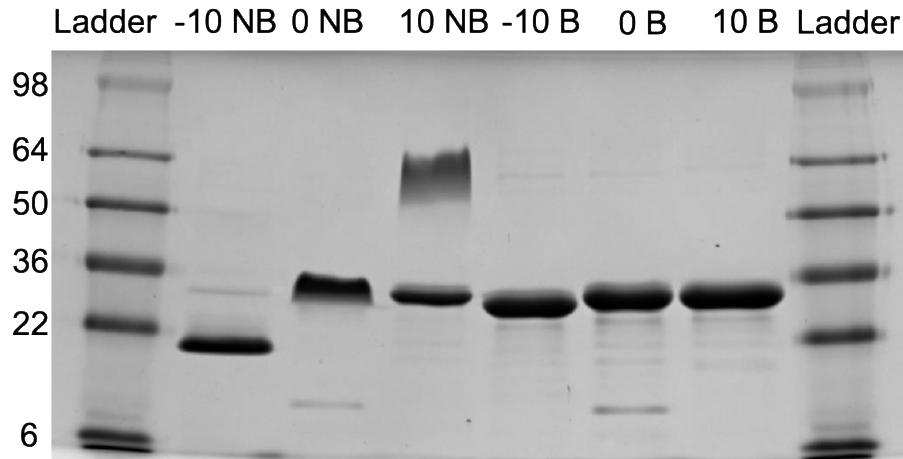


Figure S1. SDS-PAGE gel of sfGFP-Z_E. Each charged variant of sfGFP runs differently when not denatured by boiling. The negative variant runs further because there are more negatively charged amino acids on the surface of the protein. The neutral variant runs nearly the same MW as the denatured proteins and the positive variant has two bands one that arrests at a higher MW and another that gets denatured by SDS and runs at the expected MW. The molecular weights of each band on the ladder is shown in kDa. NB- not boiled, B- boiled.

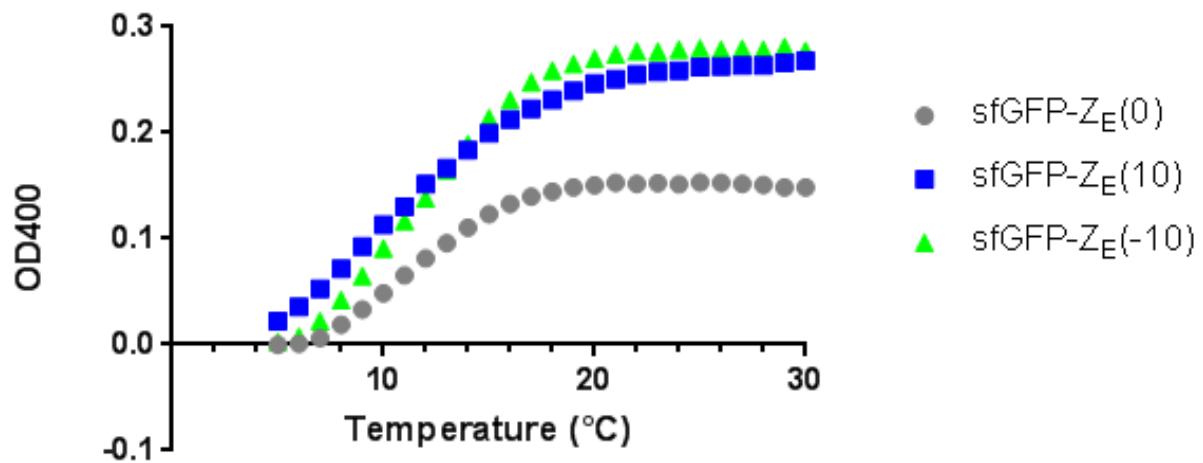


Figure S2. Transition temperature of sfGFP-Z_E charge variants. Solutions contain 30 μ M Z_R-ELP, 1.5 μ M sfGFP-Z_E, and 1.0 M PBS. The T_t (point of largest slope) of each variant is 12 °C.

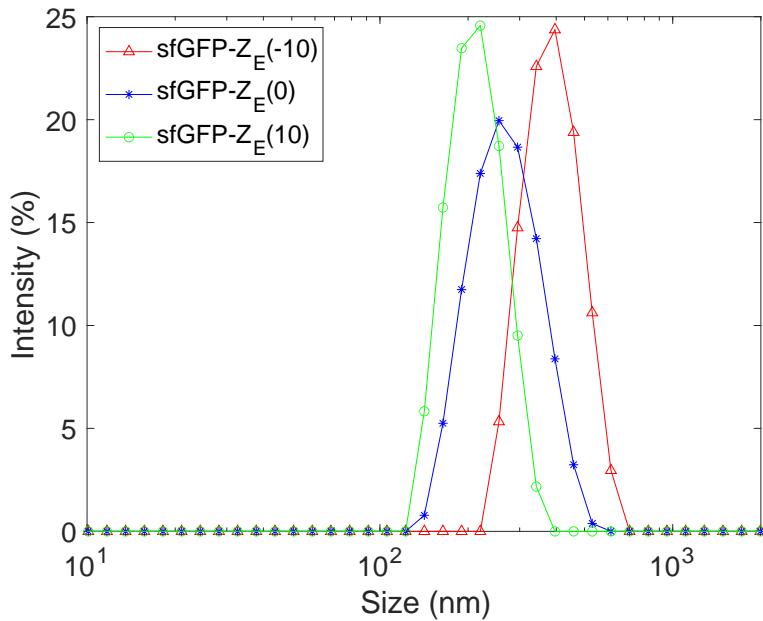


Figure S3. Size intensity plot of sfGFP-Z_E vesicles assembled 1.5 M PBS, 30 μ M Z_R-ELP, and Z_E/Z_R of 0.10. Increasing the salt concentration decreases the size of vesicles from greater than 1 μ m to ~400 nm.

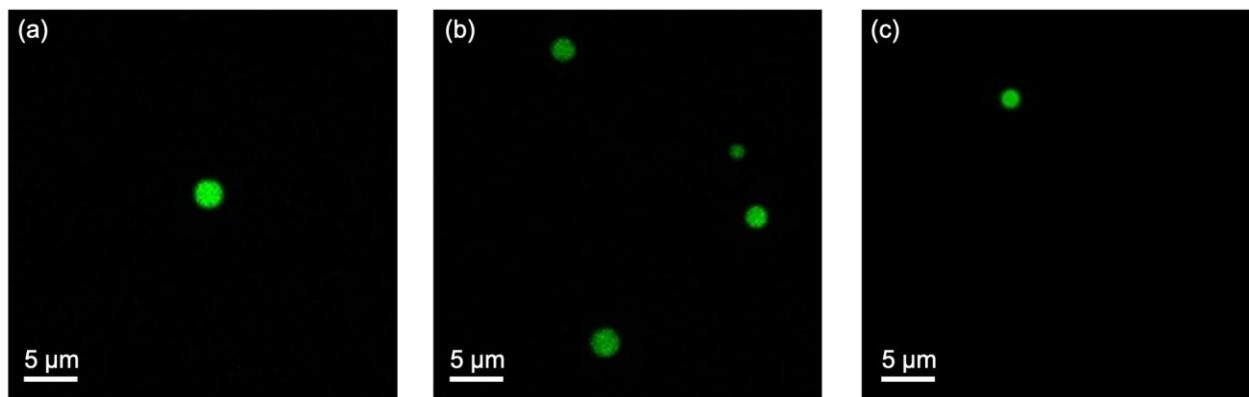


Figure S4. Fluorescent micrographs of HCA-Z_E coacervates. HCA-Z_E coacervates made at 25 °C in solutions containing 0.15 M PBS, 10 μ M Z_R-ELP, and (a) 0.01 (b) 0.05 (c) 0.10 Z_E/Z_R molar ratio.

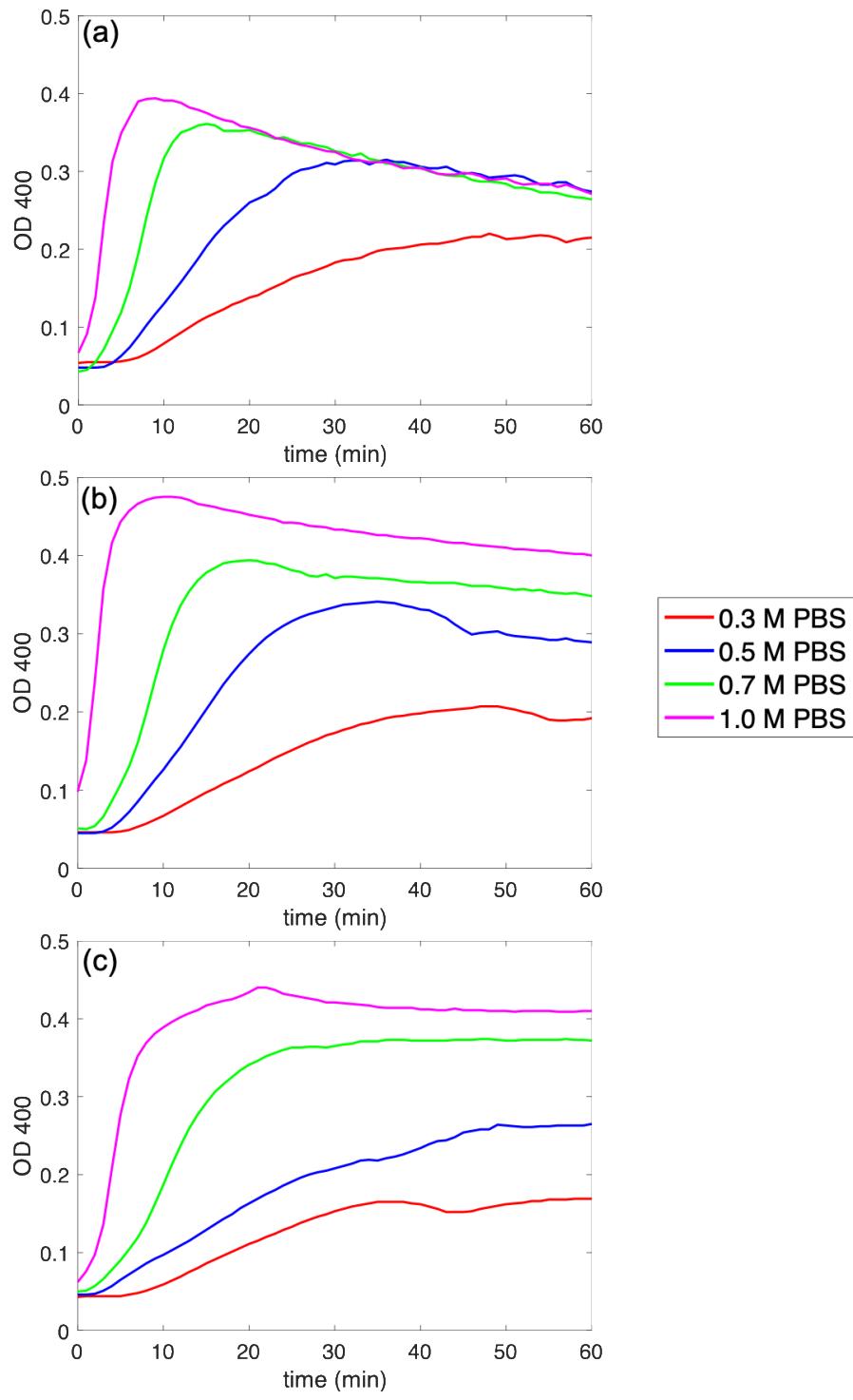


Figure S5. HCA-Z_E turbidity profiles of solutions containing 10 μ M Z_R-ELP with varying Z_E/Z_R. (a) The turbidity decreases over time for solutions containing 0.01 Z_E/Z_R, signaling the formation of unstable structures. (b) As salt concentration increases, the structures with 0.05 Z_E/Z_R become more stable. (c) Stable HCA-Z_E vesicles with 0.1 Z_E/Z_R form from solutions containing salt concentrations above 0.3 M PBS.

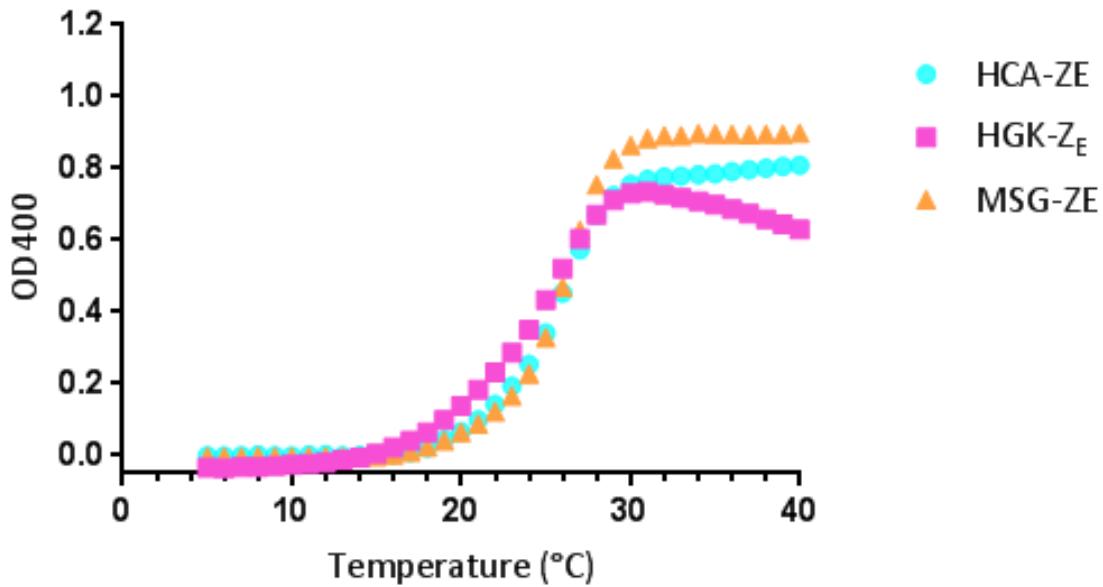


Figure S6. The transition temperature of solutions containing 30 μM Z_R -ELP, 1.5 μM Globule- Z_E , and 0.3 M PBS. The T_t of HCA- Z_E , HGK- Z_E , and MSG- Z_E is 27 °C, 25 °C and 27 °C, respectively.

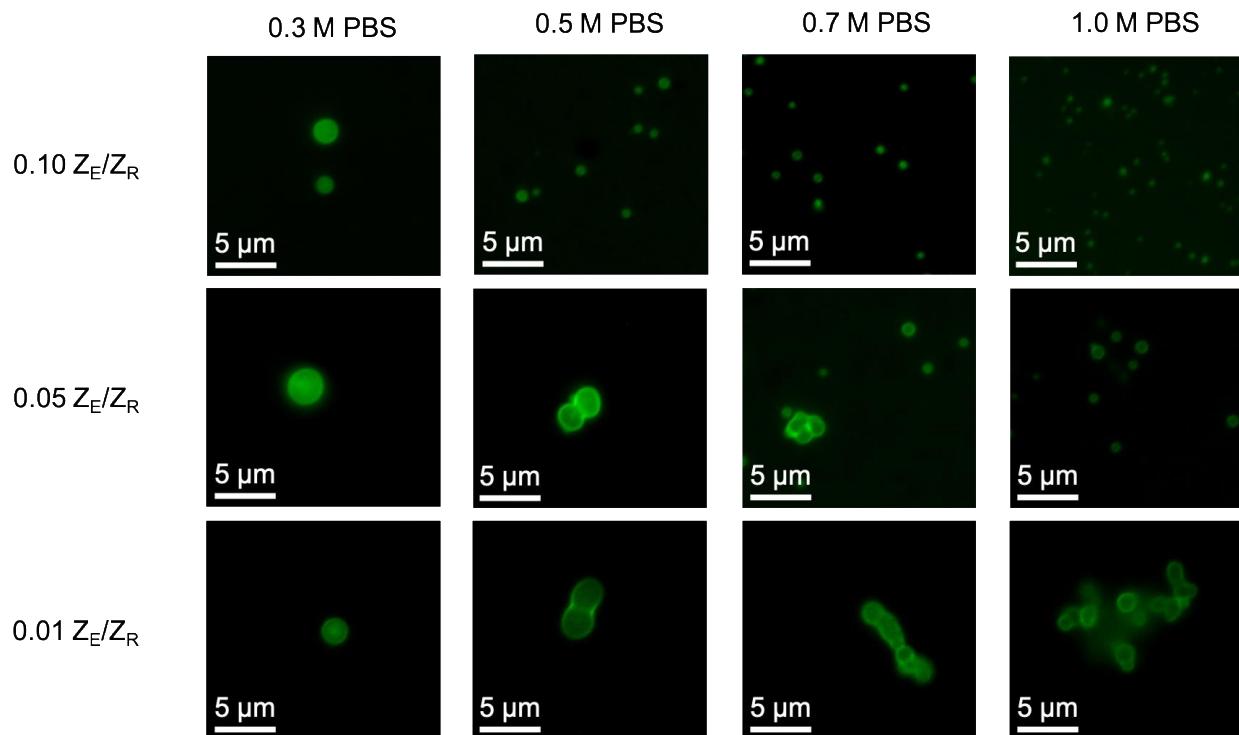


Figure S7. Epifluorescent micrographs of structures in the HCA- Z_E phase diagram containing 10 μM Z_R -ELP.

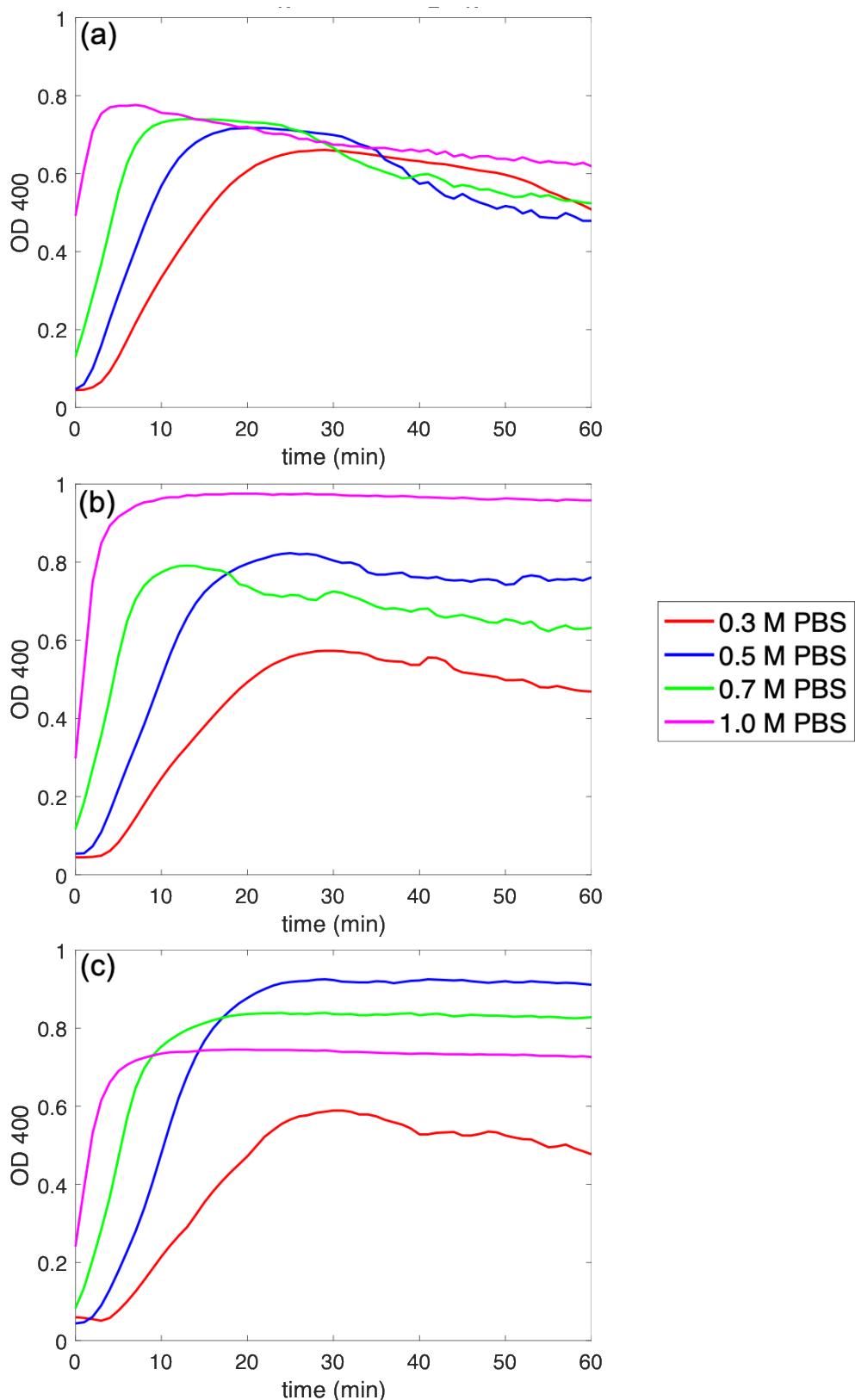


Figure S8. Turbidity profiles of structures assembled at 25 °C from HCA-Z_E with 30 μM Z_R-ELP at (a) 0.01, (b), 0.05, and (c) 0.10 Z_E/Z_R ratio.

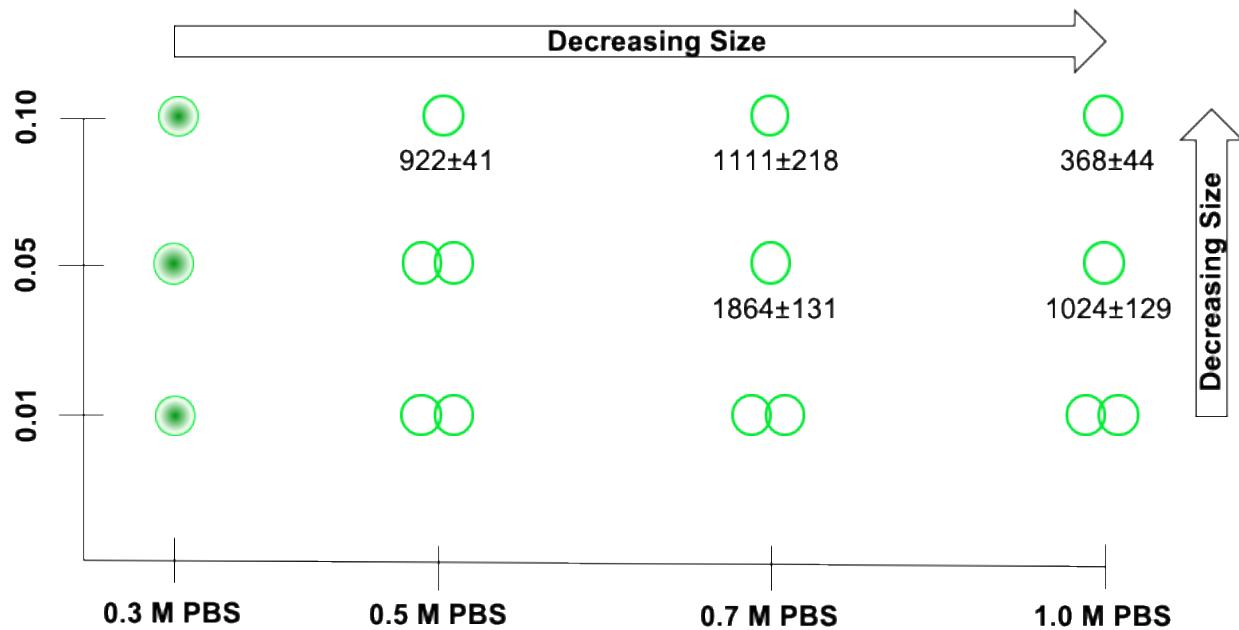


Figure S9. Phase diagram of structures assembled from HCA-Z_E at 25 °C with 30 μM Z_R-ELP. Partially filled circles represent the formation of coacervate-vesicle hybrid structures. Fused rings represent the formation of fused vesicles. Hollow circles represent the formation of vesicles. The average hydrodynamic diameters and standard deviation of stable structures are shown in nm.

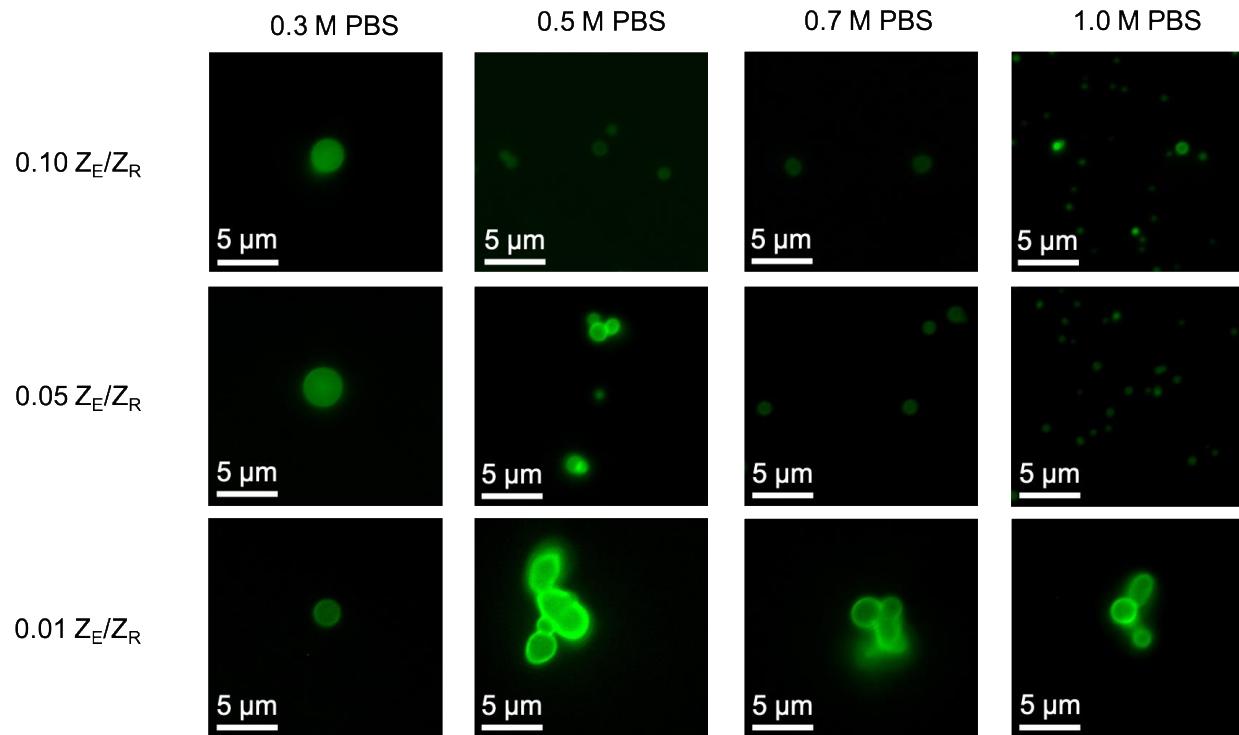


Figure S10. Epifluorescent micrographs of structures in phase diagram assembled from HCA-Z_E at 25 °C with 30 μM Z_R-ELP.

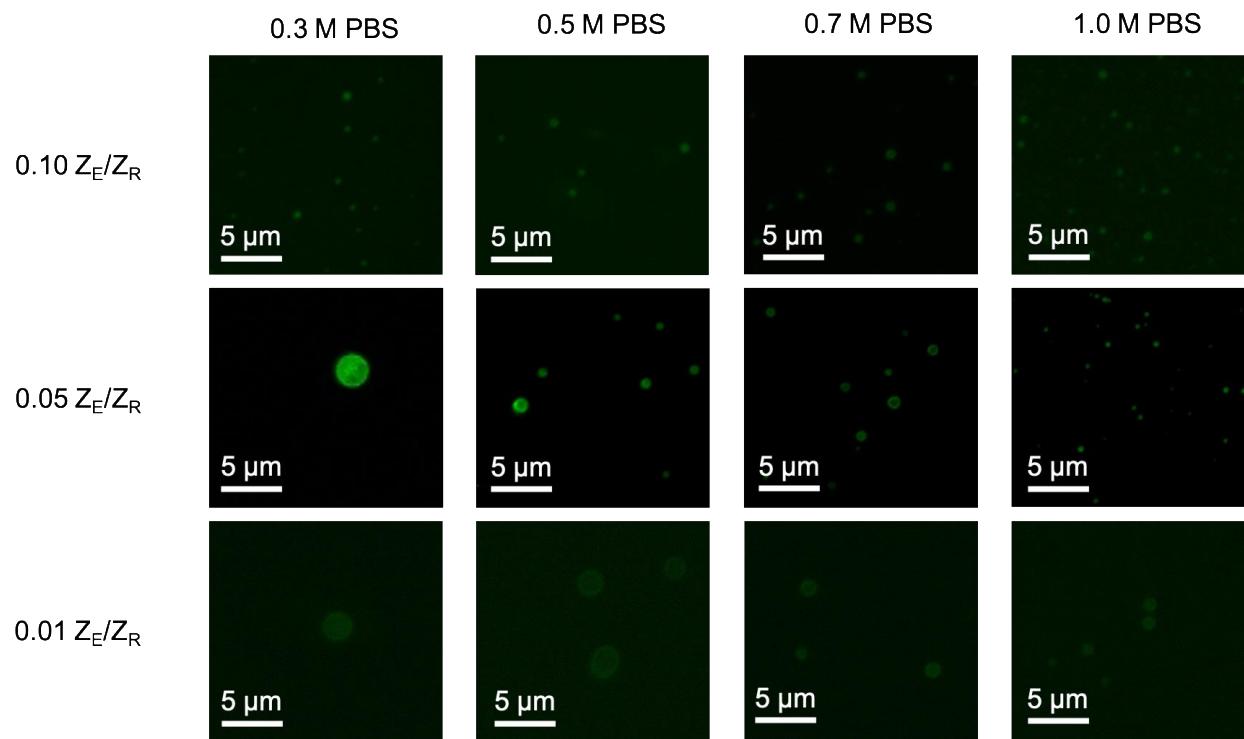


Figure S11. Epifluorescent micrographs of structures in the MSG- Z_E phase diagram at 25 °C with 10 μM Z_R -ELP.

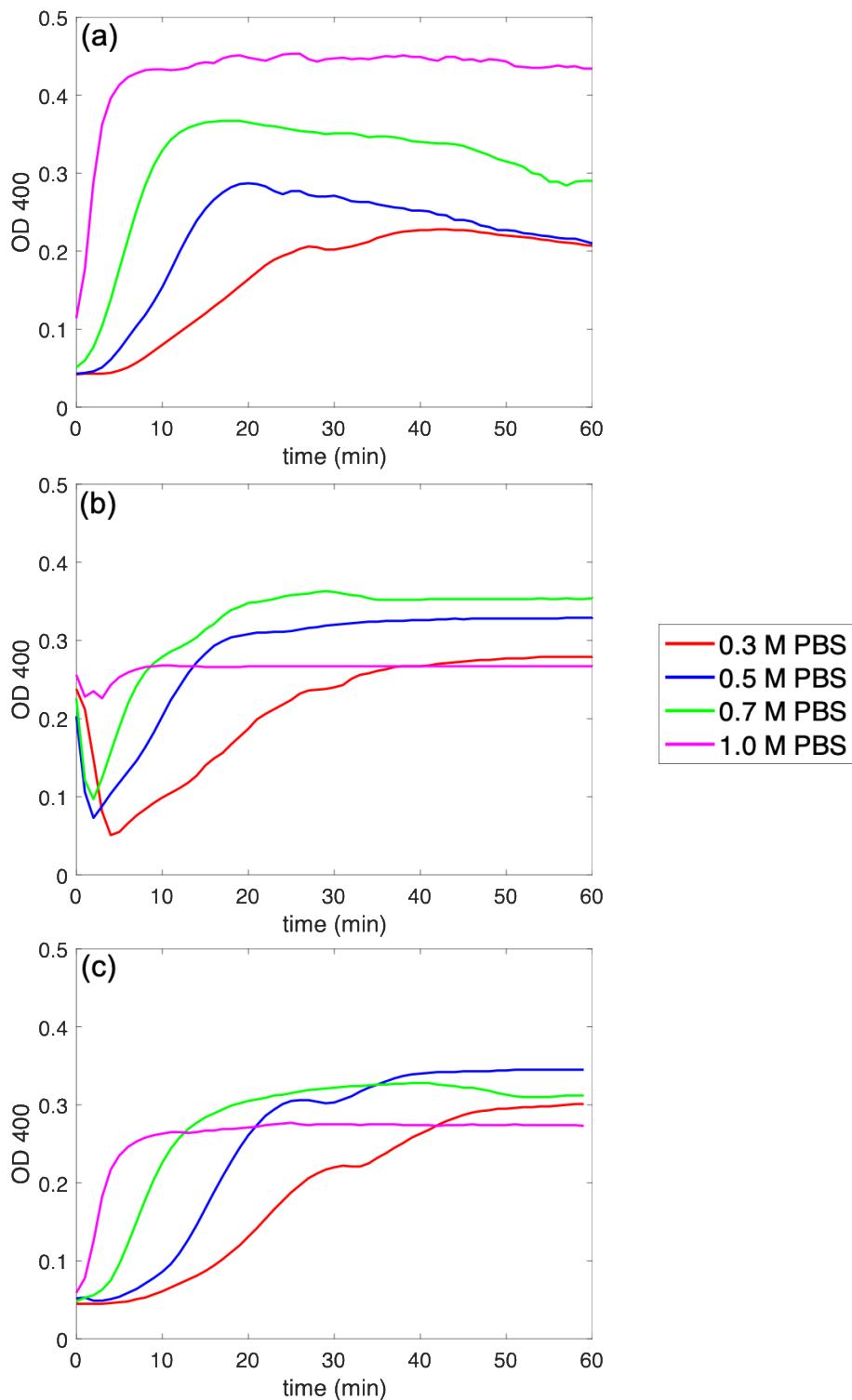


Figure S12. MSG-Z_E turbidity profiles of solutions containing 10 μ M Z_R-ELP. (a) The turbidity decreases over time for solutions containing 0.01 Z_E/Z_R , signaling the formation of unstable structures except at 1.0 M PBS. (b) As Z_E/Z_R increases to 0.05 and salt concentration increases, the structures become more stable. (c) Stable HCA-Z_E vesicles form from solutions of all salt concentrations above 0.10 Z_E/Z_R .

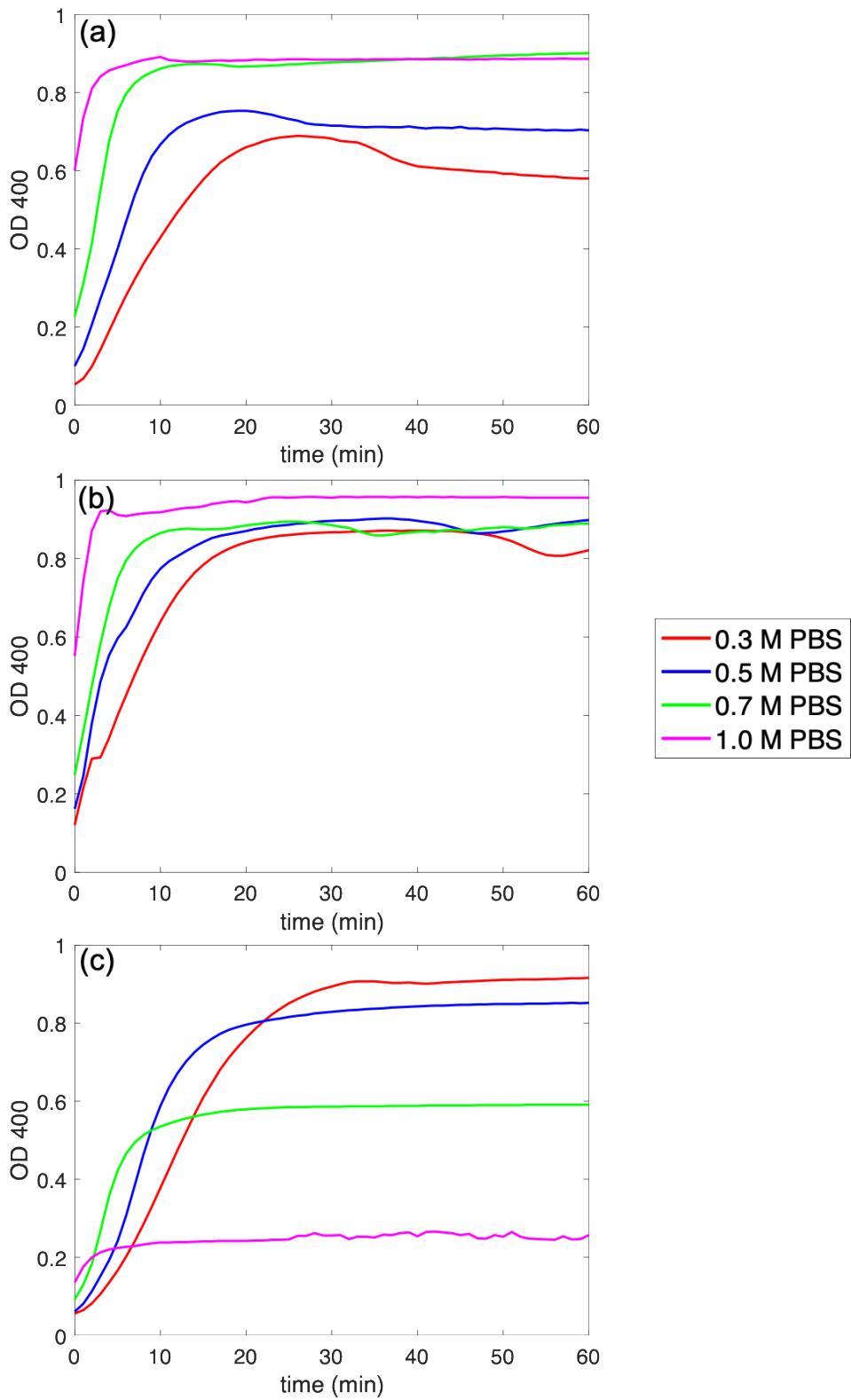


Figure S13. Turbidity profiles of structures assembled from MSG-Z_E at 25 °C with 30 μM Z_R-ELP at (a) 0.01, (b) 0.05, and (c) 0.10 Z_E/Z_R ratio.

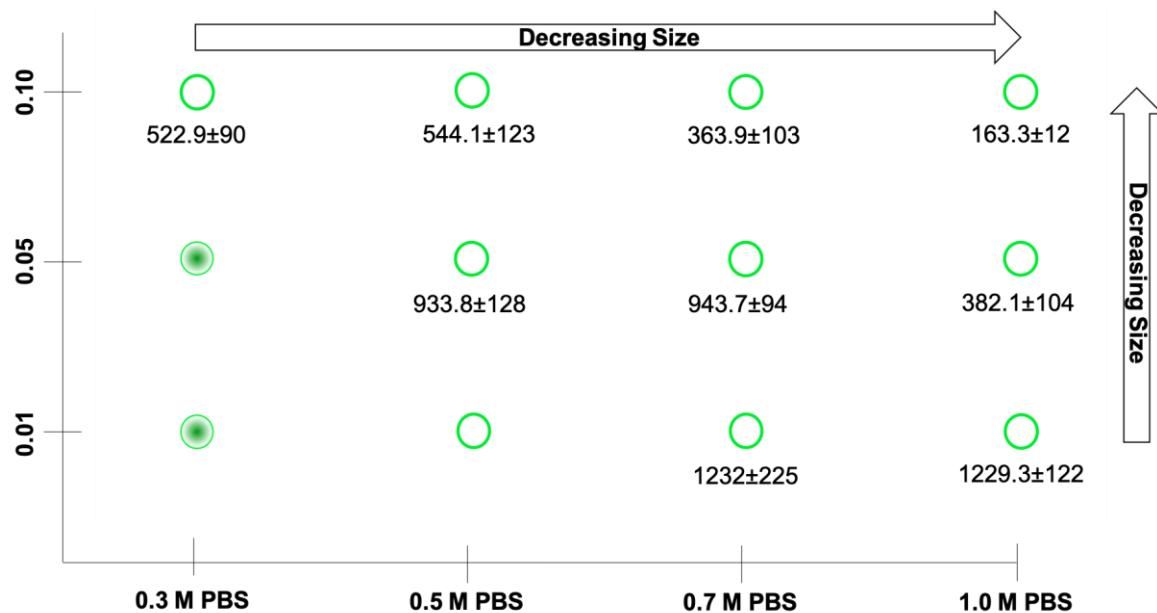


Figure S14. Phase diagram of structures assembled from MSG-Z_E at 25 °C with 30 μM Z_R-ELP. Partially filled circles represent coacervate-vesicle hybrids. Hollow circles represent the formation of vesicles. The average hydrodynamic diameters and standard deviation of stable structures are shown in nm.

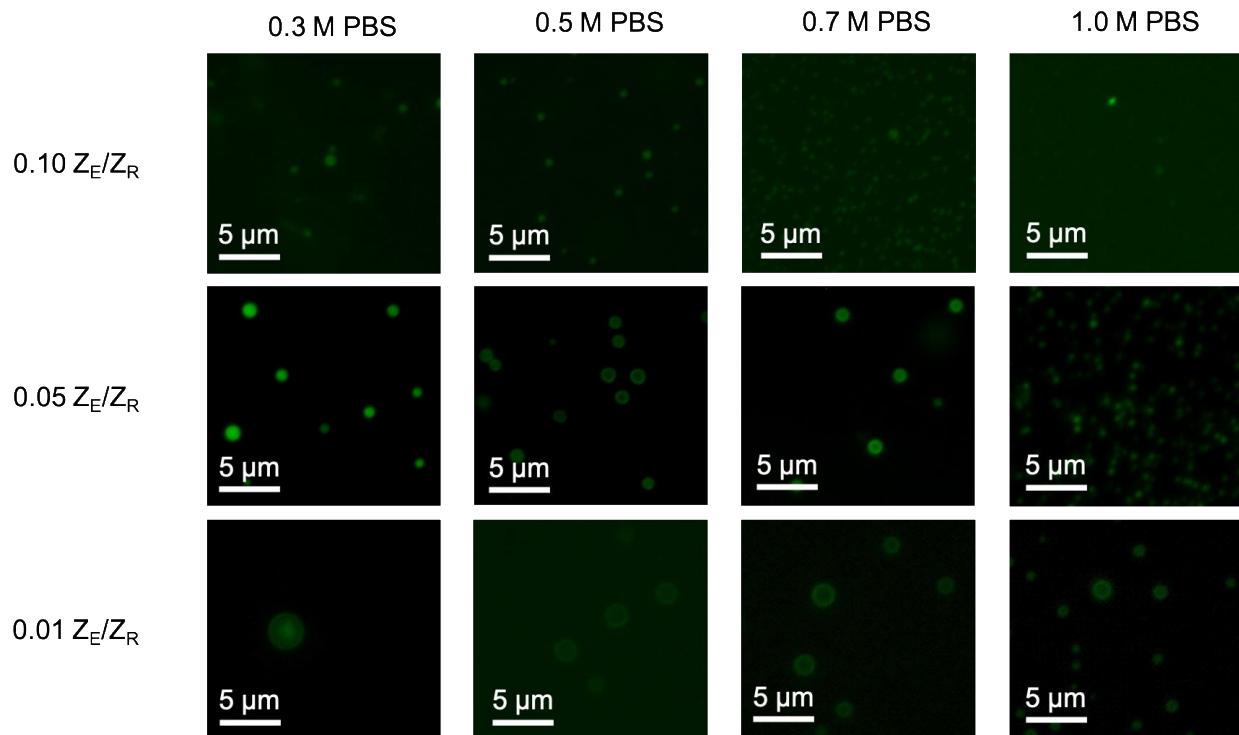


Figure S15. Epifluorescent micrographs of structures in phase diagram assembled from MSG-Z_E at 25 °C with 30 μM Z_R-ELP.

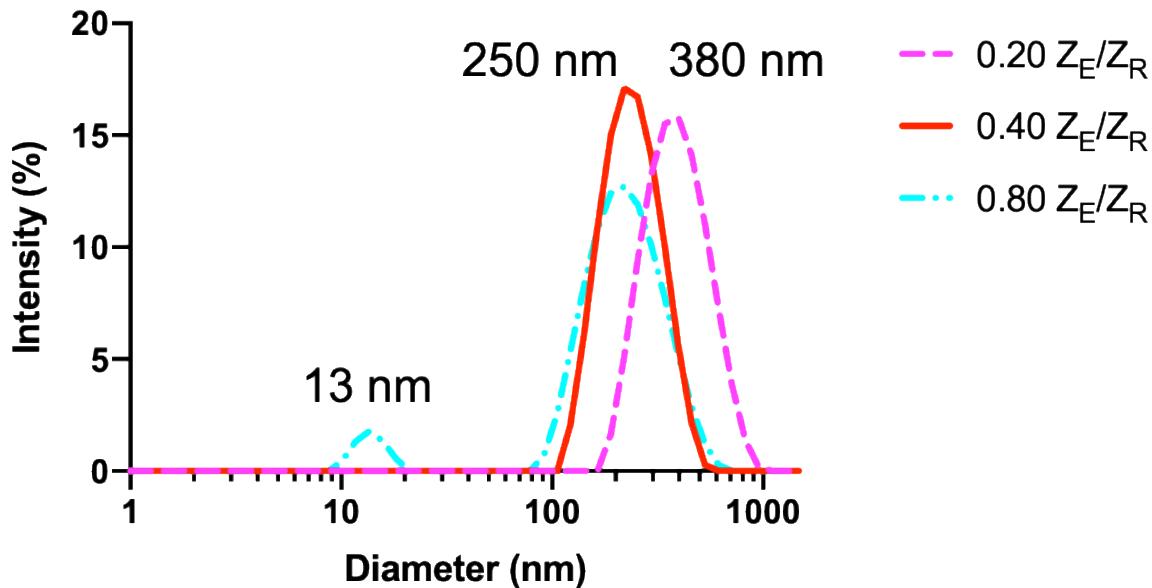


Figure S16. DLS histogram of MSG-Z_E vesicles with high Z_E/Z_R ratio formed with 10 μ M Z_R-ELP. Increasing the Z_E/Z_R ratio decreases the size of vesicles until at least 0.40 Z_E/Z_R. Above 0.80 Z_E/Z_R, smaller structures are not formed, instead there is a soluble peak and a vesicle peak.

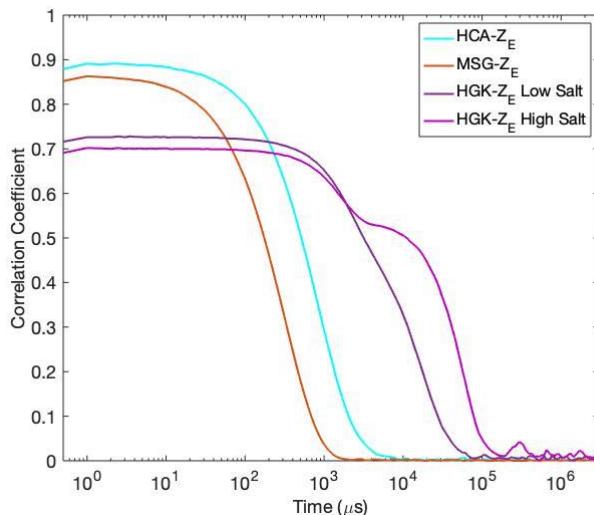


Figure S17- DLS correlograms for solutions containing 10 μ M Z_R-ELP and 0.10 Z_E/Z_R incubated at 25 °C for 1 hour. Good quality correlograms are shown for HCA-Z_E and MSG-Z_E in 1.0 M PBS. Poor quality correlograms are shown for HGK-Z_E at 0.5 and 1.0 M PBS. The good quality correlograms have a high correlation coefficient, a steep slope, and a correlation coefficient of zero at high correlation times, which are indicative of low polydispersity and little sedimentation of stable vesicles. The correlograms for poor quality DLS results from HGK-Z_E have a gentler slope that spikes up and down at high correlation times, indicative of high polydispersity and sedimentation.

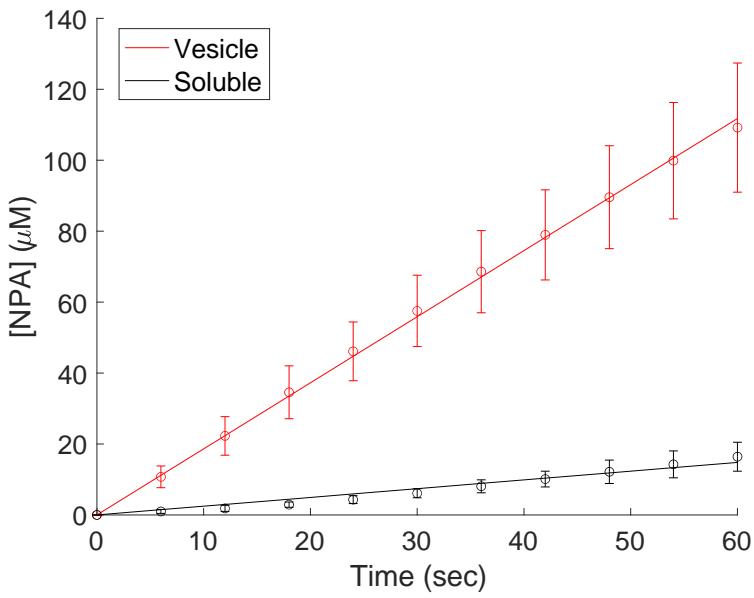


Figure S18. The activity of HCA-Z_E was assayed at 30 °C in the presence of 10 mM ZnSO₄, and 1 mM 4-nitrophenyl acetate. The specific activity of HCA-Z_E under these conditions was 0.34 ± 0.01 and 2.23 ± 0.38 μmol product/min/mg enzyme for the soluble enzyme and vesicles, respectively.

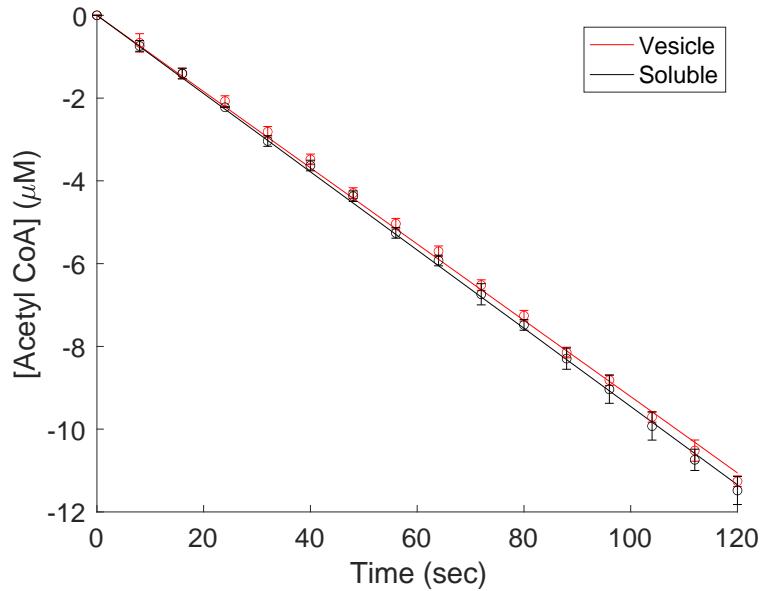


Figure S19. Activity of MSG-Z_E at 30 °C in the presence of 5 mM MgCl₂, 0.25 mM acetyl CoA, and 1 mM glyoxylate. The specific activity of MSG-Z_E under these conditions was 4.53 ± 0.13 and 4.42 ± 0.06 μmol product/min/mg enzyme for the soluble enzyme and vesicles, respectively.

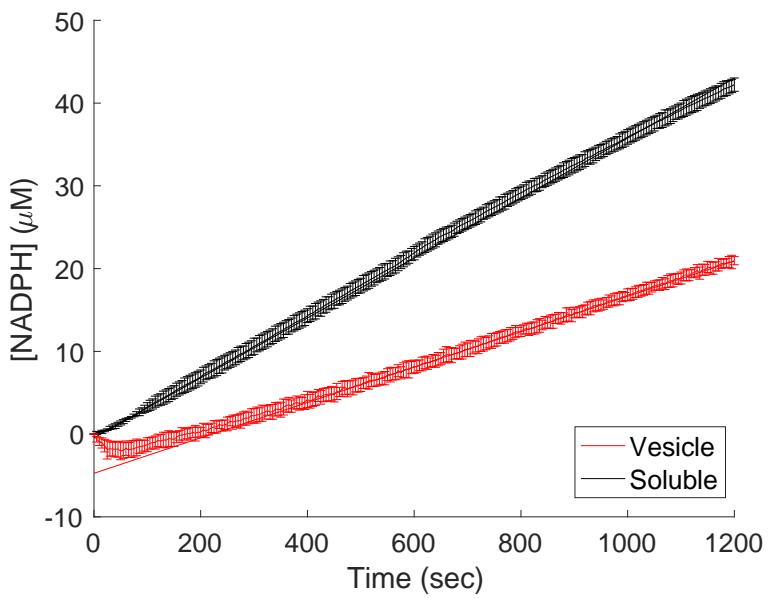


Figure S20. Activity of HGK-Z_E at 30 °C in the presence of 50 mM glucose, 1 mM ATP, and 5 mM MgCl₂. To detect HGK-Z_E activity, two units of glucose-6-phosphate dehydrogenase and 0.5 mM NADPH were added to the solution to obtain an absorbance reading for NADPH. The specific activity of HGK-Z_E under these conditions was 0.10 ± 0.002 and 0.06 ± 0.0006 μmol product/min/mg enzyme for the soluble enzyme and vesicles, respectively.

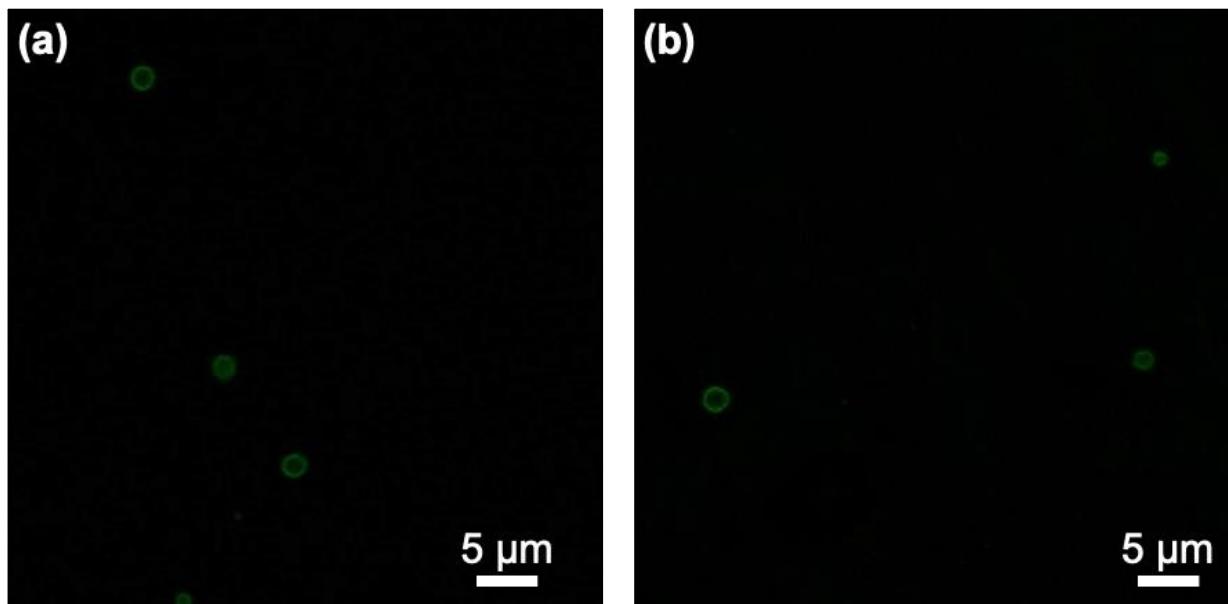


Figure S21. Effect of presence of hydrophobic substrate on HCA-Z_E vesicles. (a) HCA-Z_E vesicles incubated with 1 mM 4-nitrophenyl acetate and 10 mM ZnSO₄ after 1 hour of incubation under reaction conditions. (b) HCA-Z_E vesicles incubated under reaction conditions without 4-nitrophenyl acetate substrate after 1 hour.

sfGFP-Z _E Variant	-10	0	+10
Zeta Potential (mV)	-15.7 ± 1.1	-10.1 ± 0.8	9.32 ± 0.1

Table S1. Zeta potential values for soluble sfGFP-Z_E(-10), sfGFP-Z_E(0), and sfGFP-Z_E(10). The values for each variant are lower than the sfGFP alone because they are bound to a negatively charged glutamate rich leucine zipper.

DNA and amino acid sequences for all 7 proteins used in this work. The altered amino acids for sfGFP are highlighted in red for positive changes and blue for negative changes.

Protein	DNA Sequence	Amino Acid Sequence
Z _R -ELP	ATGAAAGGATCCCTGGAAATCCGTGC GGCGCGCT GCGTC GTCGTAACACCGCGCTCGTACCCGTGTTGCGGAACTCGT CAGCGTGTTCAGCGTCTCGCGTAACGAAGTTCTAGTACGA AACCCGTTACGGTCCGCTGGGTGGTGGCGGTTCTGGTGGC GGTGGATCCGGGTGCCGGGTGTGGCGTCCGGCGTGTG GGTGTACCGGGCTCGGTGTCCC CGGCGTAGGTGTTCCGG GTGTCGGGGTGC CGGGTGTGGCGTCCCGGGCGTGGGTG TACCGGGCTTCGGTGTC CC CGGCGTAGGTGTTCCGGGTG CGGGGTGC CGGGTGTGGCGTCCCGGGCGTGGGTGTACCGGG GGGCTCGGTGTC CC CGGCGTAGGTGTTCCCGGGTGTACCGGG GTGCCGGGTGTGGCGTCCCGGGCGTGGGTGTACCGGGCT TCGGTGTC CC CGGCGTAGGTGTTCCCGGGTGTGGGTG GGGTGTGGCGTCCCGGGCGTGGGTGTACCGGGCTCGGT GTCCCGGGCGTAGGTGTTCCCGGGTGTGGGTGCCCGGG GCTAAA	MKGSLIRAAALRR RNTALRTRVAELRQ RVQRLRNEVSQYET RYGPLGGGGGGGG GSGVPGVGVPVG VPGFGVPGVGVP VGVPVGVPVG PGFGVPGVGVP GVPGVGVPVG GFGVPGVGVP VPGVGVPVG FGVPGVGVP PGVGVPVG GVPGVGVP GC
sfGFP-Z _E (-10)	ATGGGCCACCACCACCA CACCACCGTAGCAGCAAAGGCG AGGA ACTGTTCACCGGTGTGGTCCGATCCTGGTGGAACTG GACGGCGATGTTAACGGTCACAAGTTAGCGTTCTGGT AGGGCGAAGGTGACCGACCAACGGCAAGCTGACCTGA AATTCA TTGACCA CCGTAAACTGCCGTGCCGTGGCCG ACCTGGTTACCACCC TGACCTACGGCGTTAGTGCTT CGTTATCCG GACCACATGAAGCGTCAGGATTTAAAG CGCGATGCCGGAAGGCTACGTGCAAGAGCGTACCATCAGC TTCAAGGACGATGGTACCTATAAAACCGTGCGGAAGTTAA ATTGAGGGCGACACCCTGGTGAACCGTATCGAACTGAAG GGTATTGACTTCAAAGAGGATGGCAACATCCTGGTCACA AGCTGGAATACAACCTAACAGCCACAACGTGTATATTACC GCGGATGAAACAGAAAAACGGCATCAAGGCGAACCTTAAAA TTCGTACAACGTGGAGGACGGTAGCGTTCAACTGGCGGA TCACTACCAGAAAACACCCGATTGGTATGGTCCGGTTC TGCTGCCGGATAACCACTATCTGAGCACCCAGAGCGTGCTG	MGHHHHHHRSSK GEELFTGVVPILVEL DGDVN GHKF SVRG EGEGDATNGKLTK FIC TTGKLP VPWPT LVTTL TYGVQCFSR YPDHMKRHDFFKS AMPEGYVQERTISF KDDGTYKTRAEVKF EGDTLVNRIELKGID FKEDGNILGHKEY NFNSHN VYITADEQ KNGIKANF KIRHNV EDGSVQLADHYQQ NTPIGDGPVLLPDN HYLSTQSVL SKDPD

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	TCACCTTAGCTCCGGTGCACGAGGACATCGATAAG GGTATTCTGCTGAACGGACAAAGGGCTTAAAGCGAGCG GCGCGGAGGGTAACAACGTGGTTGGTCTGCTGCGTGACGC GATTAAACGTCGTGGCGACTTCGAGATGGATGTGGTTGCG ATGGTGAACGACACCGTTGCGACCATGATCAGCTGCTACTA TGAGGATCACCAGTGCAGTGGGTATGATTGGTACCG GGCTGCAACCGTGCTACATGGAGGAAATGCAAACGTGG AACTGGTTGAGGGCGATGAAGGTCGTATGTGCGTGACAC CGAATGGGGCGCGTTGGTACAGCGGTGAACTGGATGAA TTCCTGCTGGAGTATGACCGTCTGGTTGATGAAAGCAGCGC GAACCCGGGTCA GCAACTGTACGAAAAGCTGATCGGTGGC AAATATATGGCGAGCTGGTGCCTGGTTCTGCTGCGTCT GGTGACGAGAACCTGCTGTTCACGGTAAGCGAGCGAA CAGCTCGTACCCGTGGCGCGTTGAAACCGTTCTCGT GAG CCAGGTTGAGAGCGACACCGGTGATCGTAAGCAAATCTAC AACATTCTGAGCACCCCTGGGCTGCGTCCGAGCACCCGA CTCGATATTGTGCGTCGTGCGTGC GAAAGCGTTAGCACCC GTGCGGCGCACATGTGAGCGCGGGTCTGGCGGGCGT GAT CAACCGTATGCGT GAGAGCGTAGCGAAGACGTTATGCGT ATTACCGTGGGTGTTGATGGCAGCGTGATAAGCTGACCC GAGCTTAAAGAGCGTTCCACCGGAGCGTTCTCGTCTGA CCCCGAGCTCGAGATCACCTCATTGAAAGCGAGGAAGG TAGCGGTCTGGTGC GGCGCTGGTGAGCGCGGGTCTCGT G AAGAAAGCGTGCATGCTGGGT CAGCATATGGG CAGCGGTA GCCTGGAGATTGAAGCGGGCGCTGGAGCAAGAAAACA CCCGCGCTGGAAACCGAGGTGGCGGAGCTGGAACAGGAAG TGCAACGTCGGAGAACATTGTGAGCCAATACCGTACCCGT TATGGCCCGCTGCATCACCATACCATCACTAA	FTFSFPV RHEDIDKG ILLNWTKGFKASGA EGNNVVG LRLDAIK RRGD FEMDVAM VN DTVATMIS CYYE DHQCEVGMIVGTG CNAC YMEEMQNV ELVEGDEGRMCVN TEWGAFGD SGE LD EFLLEYDRLVDESSA NP GQQQLYEKLIGGK YMGELVRLVLLRV DENILFHGEASEQL RTRGAFETRFVSQV ESDTGDRKQIYNILS TLGLRPSTTDCD I VR RACESVSTRAAHM CSAGLAGVINRMR ESRSEDVMRITVGV DGSVYKLHPSFKER FHASVRLTPSCEIT FIESEEGSGRGAALV SAVACKACMLGQ HMGSGS LEIAAAL EQENTALETEVAEL EQEVQRLENIVSQY RTTRYGPLHHHHHH
MSG-Z _E	ATGAGCCAGACCATCACCCAAAGCGCTGCGTATTGACGC GAACCTCAAGCGTTTGTGGATGAGGAAGTTCTGCCGGGTA CCGGCCTGGATGCGCGGGCGTTCTGGCGTAACCTTGACGA AATCGTTCACGATCTGGCGCCGGAGAACCGTCAGCTGCTG GCGGAACGTGACCGTATTCAAGCGGCGCTGGATGAGTGGC ACCGTAGCAACCCGGGCCGGTTAAGGACAAAGCGGGCGTA CAAAGCTTCCCTGCGTGAACTGGTTATCTGGTGCCG CAGC CGGAGCGTGTGACCGTTGAAACCACCGGCATCGATAGCGA GATTACCAGCCAAGCGGGTCCGCAACTGGTGGTCCGGCG ATGAACGCGCGTTATGCGCTGAACGCGCGAACGCGCGTT GGGGCAGCCTGTATGATGCGCTGTATGGTAGCGATATCATT CCGCAGGAAGGTGCGATGGTTAGCGGTTACGACCCG CAGC GTGGCGAACAGT GATCGCGTGGGTTCGTCGTTCTGGAT GAGAGCCTGCCGCTGGAAAACGGTAGCTATCAGGACGTGG TTGCGTTAAGGTGGTTGATAAACAGCTGCGTATCCA ACTG AAGAACGGCAAAGAAACCACCCCTGCGTACCCCGCGCAGT	MSQTITQSRLIDA NFKRFVDEEVLPGT GLDAAAFWRNFDE IVHDLAPENRQLLA ERDRIQAALDEWH RSNPGPVKDKAAYK SFLRELGYLVPQPER VTVETTGIDSEITSQ AGPQLVVPAMNAR YALNAANARW GSL YDALYGSIDIIPQEGA MVSGYDPQRGEQ VIAWVRRFLDESLP LENGSYQDVVAFKV VDKQLRIQLKNGKE TTLRTPAQFVGYRG

	TTGTTGGTTATCGTGGCGATCGGGCGGCCGACCTGCATC CTGCTGAAGAACAAACGGTCTGCACATCGAACTGCAAATTGA CGCGAACGGTCGTATTGGCAAAGACGATCCGGCGCACATC AACGATGTGATTGTTGAGGCGGCGATCAGCACCATTCTGG ACTGCGAGGATAGCGTGGCGCGTTGATCGGAAAGATA AGATCCTGCTGTACCGTAACCTGCTGGGTCTGATCGAGGGC ACCCTGCAAGAAAAGATGGAGAAAAACGGTCGTCAGATTG TTCGTAAACTGAACGACGATCGTCACTATACCGCGGCGGAT GGCAGCGAGATCAGCCTGCACGGTCGTAGCCTGCTGTTAT TCGTAACGTTGCCACCTGATGACCATCCCGGTATTGGG ATAGCGAGGGTAACGAGATCCCGAAGGTATTCTGGACGG CGTTATGACCGGTGCGATCGCCTGTACGATCTGAAGGTGC AGAAAAAACAGCCGTACCGGCAGCGTGTATATTGTTAACGCC GAAAATGCACGGTCCGCAAGAGGTGGCGTTCGCGAACAAAG CTGTTACCGTATCGAAACCATGCTGGCATGGCGCCGAA CACCCCTGAAAATGGGTATTATGGATGAGGAACGTCGTACC AGCCTGAACCTGCGTAGCTGCATCGCGCAGGCCTGTAACC GTGTTGCCTTCATTAACACCGGCTTCTGGACCGTACCGGT GATGAGATGCACAGCGTGATGGAAGCGGGCCGATGCTGC GTAAGAACCAAGATGAAAAGCACCCCGTGGATCAAGGCCTA CGAGCGTAACAACGTTCTGAGCGGTCTGTTCTGCGGTCTGC GTGGCAAGGCGLAAATTGGTAAAGGCATGTGGCGATGCC GGACCTGATGGCGGATATGTACAGCCAGAAAGGCCTGACCAA CTCGTGCCTGCGAACACCCGCTGGTGCCAGCCC CCCGCGCGACCTGCATGCGCTGCACTATCACCAGACCAAC GTGCAGAGCGTTCAAGCGAACATCGCGAACCCGAGTTCA ACCGGGAGTTGAACCGCTGCTGGACGATCTGCTGACCATT CCGGTGGCGAAAACCGCAACTGGAGCGCAGGAGATC CAGCAAGAACTGGATAACAACGTTCAAGGTATTCTGGCTA CGTGGTTCGTTGGGTTGGAGCAGGGTATCGGCTGAGCAAG GTGCCGGACATTACAACGTTGCGCTGATGGAAGATCGTG CGACCCCTGCGTATCAGCAGCCAACACATTGCGAACCTGGCTG CGTCACGGTATCCTGACCAAGGAGCAGGTTCAAGCGAGCC TGGAAAACATGGCGAAAGTGGTTGACCAGCAAAACCGCGG GCGATCCGGCGTACCGTCCGATGGCGGGTAACCTCGCGAA CAGCTGCGCGTTAAGGCGGCGAGCGACCTGATCTTCTGG GCGTGAACACGCCAACCGTTAACCGAACCCTGCTGCA TGCCTGGCGTCTGCGTGAGAAAGAAAGCCACCATATGGGT AGCGGCAGCCTGGAGATTGAAGCGGCGGCGCTGGAGCAG GAAAACACCGCGCTGGAAACCGAGGTGGCGGAGCTGGAA CAGGAAGTGAACGTCTGGAGAACATTGTGAGCCAATACC GTACCCGTTATGGTCCGCTGCACCAACCAACCAACTAA	DAAAPTCILLKNNG LHIELQIDANGRIGK DDPAHINDVIVEAA ISTILDCEDSVAAVD AEDKILLYRNLLGL MQGTLQEKMEN GRQIVRKLNDDRHY TAADGSEISLHGRSL LFIRNVGHLMTIPVI WDSEGNEIPEGILD GVMTGAIALYDLKV QKNSRTGSVYIVKP KMHGPQEVAFANK LFTRIETMLGMAPN TLKMGIMDEERRTS LNLRSCIAQARNRV AFINTGFLDRTGDE MHSVMEAGPMLR KNQMKSTPWIKAY ERNNVLSGLFCGLR GKAQIGKGMWAM PDLMADMYSQKG DQLRAGANTAWV PSPTAATLHALHYH QTNVQSVQANIAQ TEFNAEFEPLLDLL TIPVAENANWSAQ EIQQELDNNVQGIL GYVVRWVEQGIGC SKVPDIHNVALME DRATLRISSQHIAN WLRHGILTKEQVQ ASLENMAKVVDQQ NAGDPAYRPMAG NFANSCAFKAASDL IFLGVKQPNGYTEP LLHAWRLREKESHH MGSGSLEIEAAALE QENTALETEVAELE QEVRLENIVSQYR TRYGPLHHHHHH
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