Electronic Supplementary Information

Incorporation of Short, Charged Peptide Tags Affects the Temperature Responsiveness of Positively-Charged Elastin-Like Polypeptides

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Table S1. Amino acid analysis results of I-tag[YKV-48].

Amino acid	Expected mol%	Measured mol%	Error (%)
Asp + asn	1.15	1.58	0.48
Thr	0.38	0.65	0.27
Ser	0.38	0.50	0.12
Glu + gln	0.38	1.36	0.98
Pro	19.23	18.23	1.00
Gly	38.46	36.99	1.47
Ala	0.38	1.07	0.69
Val	25.38	23.73	1.65
Ile	0.00	0.26	0.26
Leu	0.77	1.37	0.60
Tyr	6.15	5.83	0.32
Phe	0.00	0.49	0.49
His	0.00	0.11	0.11
Lys	6.54	6.96	0.42
Arg	0.77	0.86	0.09

Table S2. Fitted T_t values at 1 μM protein and concentration dependence parameter (b_{pH}) of I-tag[YKV-48] and S-tag[YKV-48].[a]

pH	I-tag[YKV-48]		S-tag[YKV-48]	
	$T_t\|_{1\ \mu M,\ pH}$ (°C)	b_{pH} (°C/ln[μM])	$T_t\|_{1\ \mu M,\ pH}$ (°C)	b_{pH} (°C/ln[μM])
5.5	82.7 ± 1.0	6.4 ± 0.2	130.2 ± 6.8	14.2 ± 1.4
6.0	80.0 ± 0.6	5.9 ± 0.1	114.2 ± 3.4	12.0 ± 0.7
6.5	79.1 ± 1.0	5.7 ± 0.2	100.9 ± 4.9	10.5 ± 1.0
7.0	80.5 ± 0.7	5.9 ± 0.1	88.7 ± 3.7	9.0 ± 0.7
7.5	83.6 ± 0.6	6.4 ± 0.1	82.8 ± 2.5	8.4 ± 0.5
8.0	81.8 ± 0.6	6.1 ± 0.1	79.7 ± 2.8	8.2 ± 0.6

[a] Data in Figure 2 were fitted to equation 1. Parameters were reported as the fitted value with standard error. The coefficient covariance matrix was reported using the regstats function in MATLAB (R2018a, MathWorks, Natick, MA). The square root of the diagonals of the coefficient covariance matrix was reported as the standard error of each fitted parameter. We note that some of the fitted T_t values were ≥100 °C and would not be observable in aqueous solutions under normal pressure.

A

M-SKGPG-VDGTL-*(PGYGVPGKGVPGVGV)*$_{16}$-PVADRGMRLDKEFLE

I-tag charge estimation — *YKV charge estimation* — I-tag charge estimation

M-MASMTGGQQMG-HHHHHHH-DDDDK-LDGTL-*(PGYGVPGKGVPGVGV)*$_{16,\ 24,\ or\ 32}$-PVADRGMRLE

S-tag charge estimation — *YKV charge estimation* — S-tag charge estimation

B

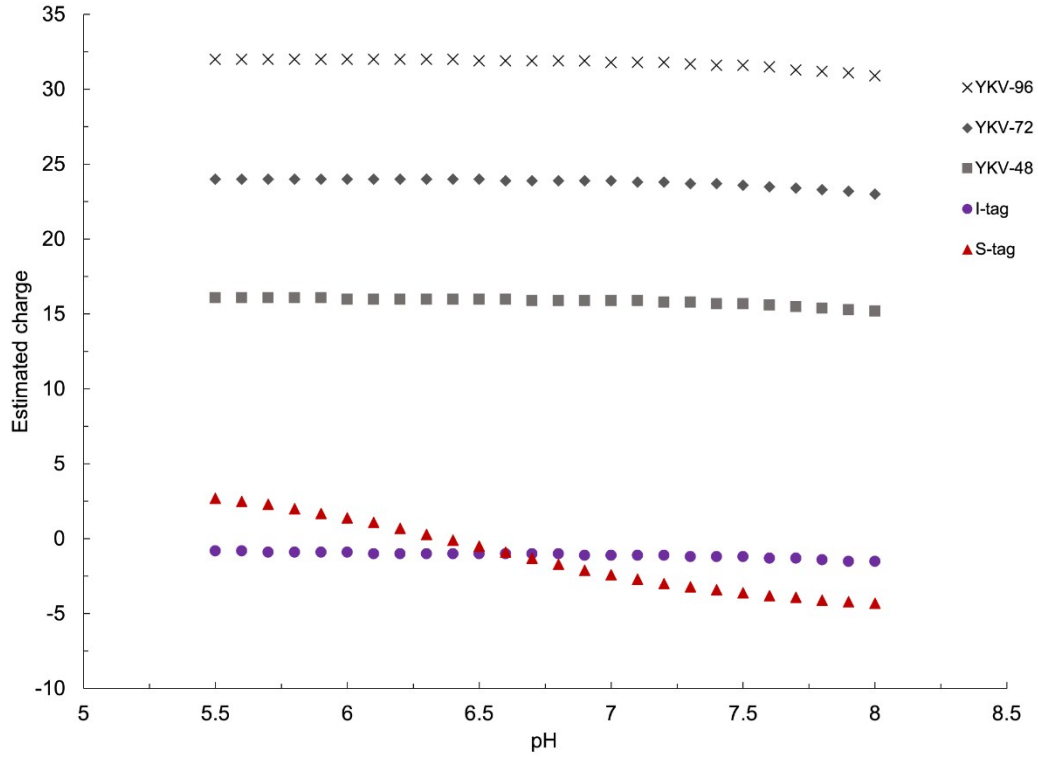

Figure S1. (A) Amino acid sequences of I-tag[YKV-48] and S-tag[YKV] proteins. Each repeat of (PGYGVPGKGVPGVGV) contains three ELP pentapeptide sequences with guest residues being Y, K, and V. The 16, 24, or 32 repeats contain a total of 48, 72, or 96 pentapeptide sequences, respectively. The C-terminal sequences (PVADRGMRLDKEFLE for I-tag[YKV-48] and PVADRGMRLE for S-tag[YKV-48/72/96]) resulted from the restriction sites used in the cloning steps. (B) Charge estimation of I-tag, S-tag, and YKV sequences as indicated in (A). The charge estimation of the amino acid sequence was calculated using the Henderson-Hasselbalch equation by assuming that the pKa value of each residue was the same as the isolated amino acid and was not affected by the protein structure.

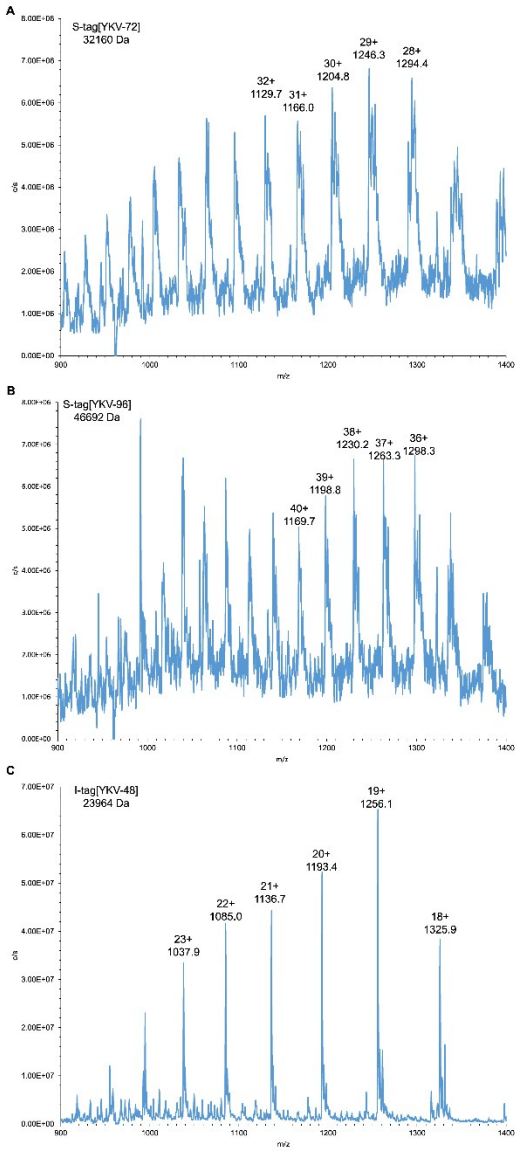

Figure S2. Electrospray ionization mass spectroscopy spectra of (A) S-tag[YKV-72] (expected MW: 32160 Da), (B) S-tag[YKV-96] (expected MW: 46692 Da), and (C) I-tag[YKV-48] (expected MW: 23964 Da). Peaks were labeled with the m/z ratios and the corresponding number of charges on the polymer chains.

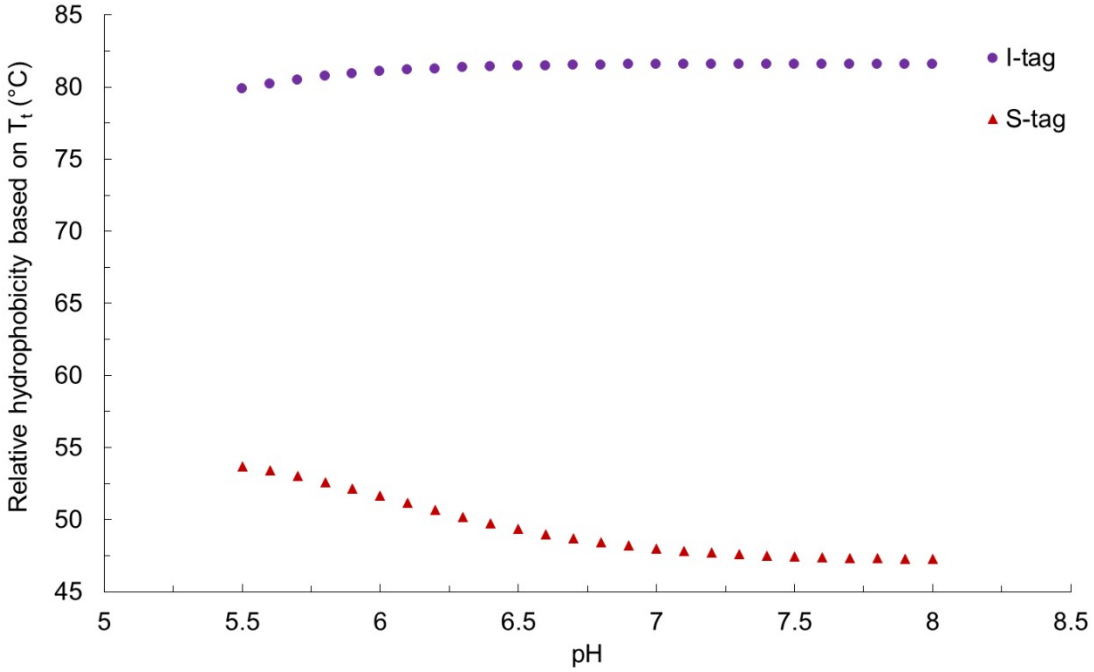

Figure S3. Relative hydrophobicity of the I-tag and the S-tag sequences. The sequences of each domain used for the estimation were indicated in Fig. S1A. The relative hydrophobicity was calculated using a method proposed by Trabbic-Carlson *et al.*[1] This estimation was based on the hydrophobicity scale established by Urry *et al.*[2] that was originally used to predict the T_t of an ELP with a known guest residue composition. More hydrophobic guest residue compositions resulted in lower T_t values. It should be noted that this estimation had no direct physical meaning and was only used to demonstrate the difference in the relative hydrophobicity between the tags in an ELP-fusion protein context.

References

1. K. Trabbic-Carlson, D. Meyer, L. Liu, R. Piervincenzi, N. Nath, T. LaBean and A. Chilkoti, *Protein Eng. Des. Sel.*, 2004, **17**, 57-66.
2. D. W. Urry, D. Gowda, T. M. Parker, C. H. Luan, M. C. Reid, C. M. Harris, A. Pattanaik and R. D. Harris, *Biopolymers*, 1992, **32**, 1243-1250.