

Phase behavior and self-assembly of perfectly sequence-defined and monodisperse multiblock copolypeptides

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Supporting Information

Table S1. Nomenclature and sequence of block copolymer ELPs.

Abbreviation	Sequence
ELP-S _{comp}	G-(SGVPG) ₁₂₀ -Y
ELP-V _{comp}	G-(VGVPD) ₁₂₀ -Y
ELP-SVB ₁	G-[(SGVPG)-(VGVPD)] ₆₀ -Y
ELP-SVB ₅	G-[(SGVPG) ₅ -(VGVPD) ₅] ₁₂ -Y
ELP-SVB ₁₀	G-[(SGVPG) ₁₀ -(VGVPD) ₁₀] ₆ -Y
ELP-SVB ₂₀	G-[(SGVPG) ₂₀ -(VGVPD) ₂₀] ₃ -Y
ELP-SVB ₃₀	G-[(SGVPG) ₃₀ -(VGVPD) ₃₀] ₂ -Y
ELP-SVT _{VSV}	G-(VGVPD) ₃₀ -(SGVPG) ₆₀ -(VGVPD) ₃₀ -Y
ELP-SVT _{SVS}	G-(SGVPG) ₃₀ -(VGVPD) ₆₀ -(SGVPG) ₃₀ -Y
ELP-SVD _{SV}	G-(SGVPG) ₆₀ -(VGVPD) ₆₀ -Y
ELP-SVG _L	G-(SGVPG) ₃₀ -(VGVPD) ₅ -(SGVPG) ₁₀ -(VGVPD) ₅ -(SGVPG) ₅ - (VGVPD) ₅ -(SGVPG) ₅ -(VGVPD) ₅ -(SGVPG) ₅ -(VGVPD) ₁₀ - (SGVPG) ₅ -(VGVPD) ₃₀ -Y
ELP-SVG _I	G-(SGVPG) ₃₅ -(VGVPD) ₅ -(SGVPG) ₁₀ -(VGVPD) ₁₀ - (SGVPG) ₁₀ -(VGVPD) ₁₀ -(SGVPG) ₅ -(VGVPD) ₃₅ -Y
ELP-SVG _H	G-(SGVPG) ₄₀ -(VGVPD) ₅ -(SGVPG) ₁₀ -(VGVPD) ₅ -(SGVPG) ₅ - (VGVPD) ₁₀ -(SGVPG) ₅ -(VGVPD) ₄₀ -Y

A) ELP-S_{comp}

[illegible]

B) ELP- V_{comp}

[illegible]

C) ELP-SVB₁

[illegible]

D) ELP-SVB₅

[illegible]

E) ELP-SVB₁₀

[illegible]

F) ELP-SVB₂₀

[illegible]

G) ELP-SVB₃₀

[illegible]

H) ELP-SVT_{vsv}

[illegible]

I) ELP-SVT_{SVS}

[illegible]

J) ELP-SVD_{SV}

[illegible]

K) ELP-SVG_L

[illegible]

L) ELP-SVG_I

[illegible]

M) ELP-SVG_H

M G S G V P G S G V P G S G V P G S G V P G S G V P G S G V
 atg ggc agc ggt gtt ccg ggc tct ggt gtc cca ggt tcc ggc gta ccg ggc agc ggt gtt cct ggt tct ggc gtg ccg ggc agc ggt gtt
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 P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
 cct ggt gtc ggc gtg ccg ggc gtg ggt gtt ccg ggc gta ggt gtc cca ggt gtg ggc gta ccg ggc gtt ggt gtt cct ggt gtc ggc gtg
 P G Y
 ccg ggc tac

Figure S1. DNA and polypeptide sequences. All ELPs were recombinantly synthesized from short oligomers using concatemerization and recursive directional ligation by plasmid

reconstruction (PRe-RDL).¹ Each ELP gene included a leader composed of a methionine and glycine residue that preceded the ELP sequence. Each ELP gene also included a trailer composed of a tyrosine residue that succeeded the ELP sequence, providing optical activity at 280 nm with an extinction coefficient of $1280 \text{ M}^{-1}\text{cm}^{-1}$. Hydrophilic monomers composed of the pentapeptide SGVPG are marked in blue, while hydrophobic monomers composed of the pentapeptide VGVPG are marked in red.

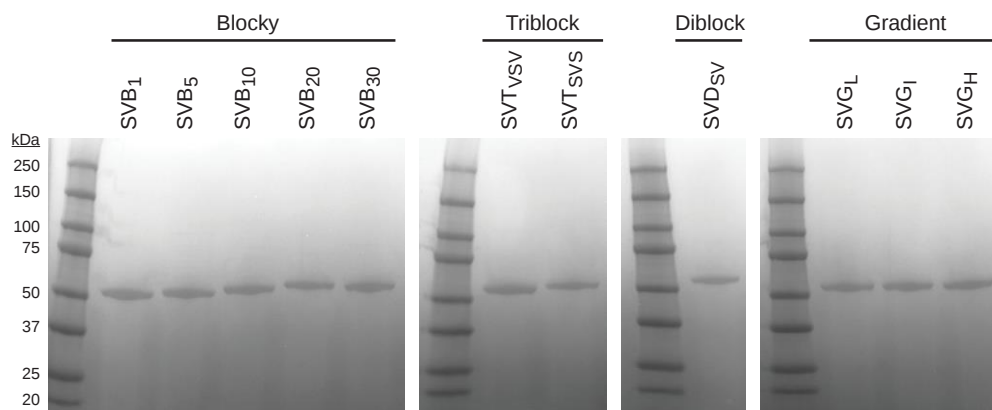


Figure S2. Evaluation of purity and size by gel electrophoresis. The purity and size of all ELPs was confirmed with SDS-PAGE and CuCl_2 staining. All ELPs ran slightly higher than their expected MW of 48.66 kDa, which has been reported previously for other ELPs.² There were slight variations in the migration of the bands, likely influenced by differences in polypeptide architecture, where trends in migration were apparent within classes of ELPs. For instance, the bands appeared to migrate more slowly in accordance with increasing block size.

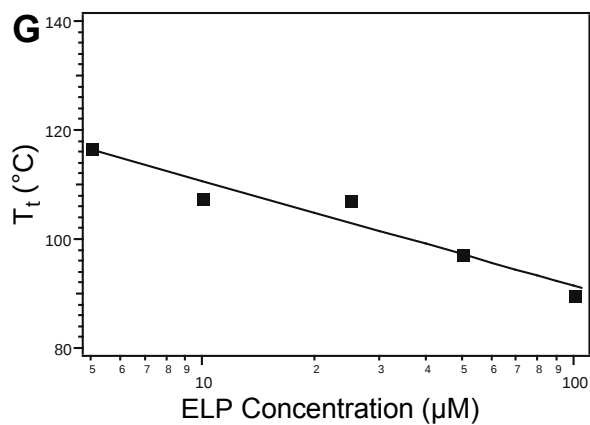
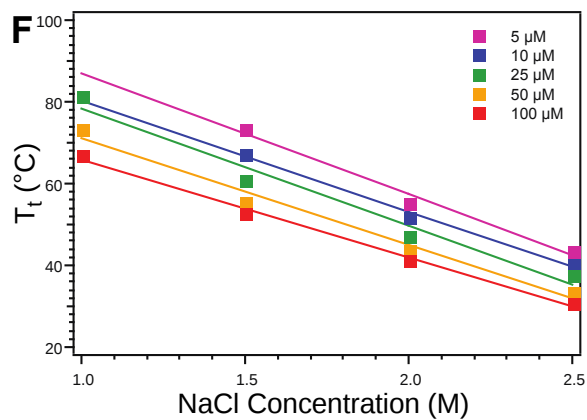
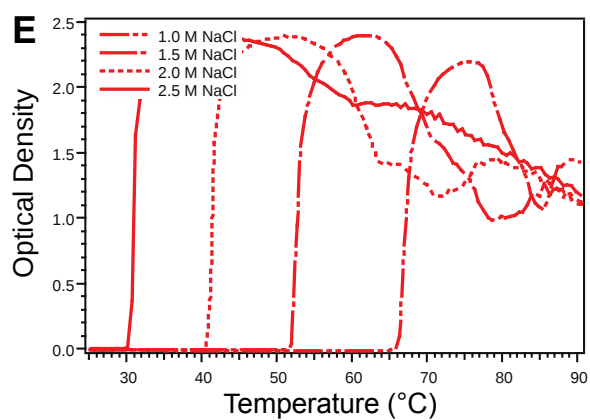
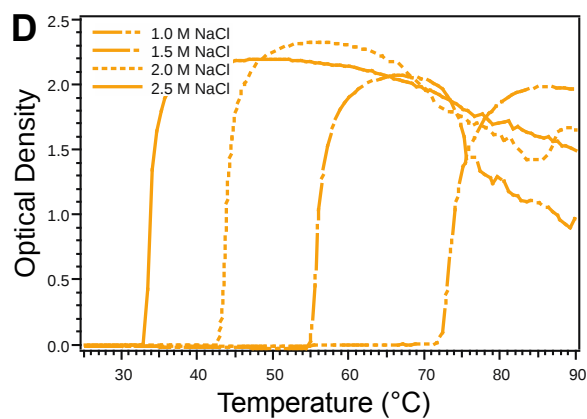
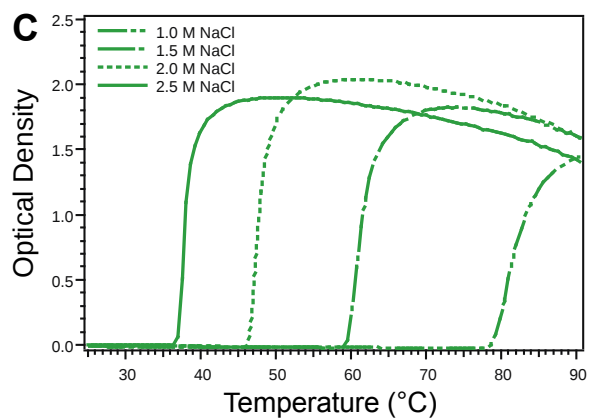
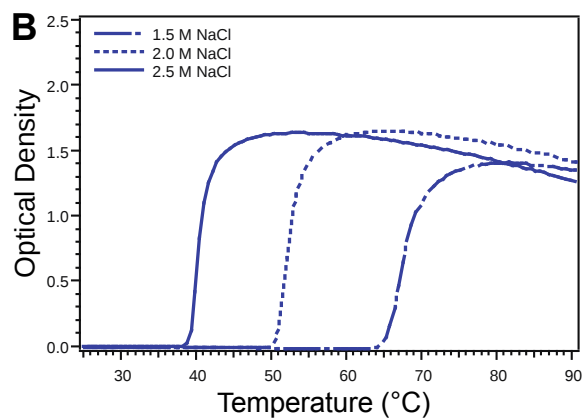
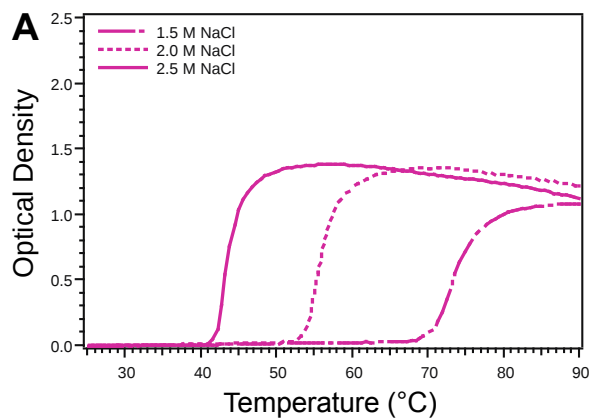


Figure S3. Thermal characterization of ELP-S_{comp} by temperature-regulated turbidimetry. The T_t of ELP-S_{comp} exceeded the upper thermal limit of the UV-VIS spectrophotometer (90°C). NaCl was added to lower the T_t such that it could be observed in the measurable temperature range. ELP-S_{comp} was characterized by temperature-regulated turbidimetry in 1.0-2.5 M NaCl in PBS at ELP concentrations of A) 5 μ M, B) 10 μ M, C) 25 μ M, D) 50 μ M, and E) 100 μ M. F) The experimentally measured T_t s at each ELP concentration (squares) were fit to linear approximations (lines) whose extrapolation defined intercepts that were interpreted as the T_t s in PBS without NaCl. G) The approximated T_t s in PBS (squares) at each concentration were fit to a logarithmic relationship (line).

Table S2. T_t s of ELP-S_{comp} in NaCl.¹

ELP concentration (μ M)	NaCl concentration (M)			
	1.0	1.5	2.0	2.5
5	NM ²	73.22	55.41	43.53
10	NM ²	67.35	52.00	40.19
25	81.42	60.79	47.35	37.89
50	73.32	55.65	43.83	33.73
100	66.94	52.64	41.27	30.89

¹ T_t s calculated as the temperature corresponding to the maximum of the Gaussian fit of the derivative of the OD, with respect to temperature, reported in °C.

²Not measurable (NM) in the temperature range 20-90°C. The T_t at these conditions exceeded 90°C.

The T_t s of ELP-S_{comp} in NaCl solutions were fit to a linear relationship of T_t , with respect to NaCl concentration,³ at a constant ELP concentration as according to Equation S1:

$$T = T_0 + c[M] \quad (\text{S1})$$

where T is the experimentally measured T_t , T_0 is the extrapolated T_t in the absence of salt, c is a constant of units °C/M and $[M]$ is the molar concentration of NaCl.

Table S3. Fit parameters of ELP-S_{comp} T_t s in NaCl.¹

ELP concentration (μ M)	c (°C/M)	T_0 (°C)	r^2
5	-29.69	116.77	0.987
10	-27.16	107.50	0.994
25	-28.80	107.27	0.970
50	-26.12	97.34	0.982
100	-23.91	89.77	0.994

¹Parameters according to Equation S1.

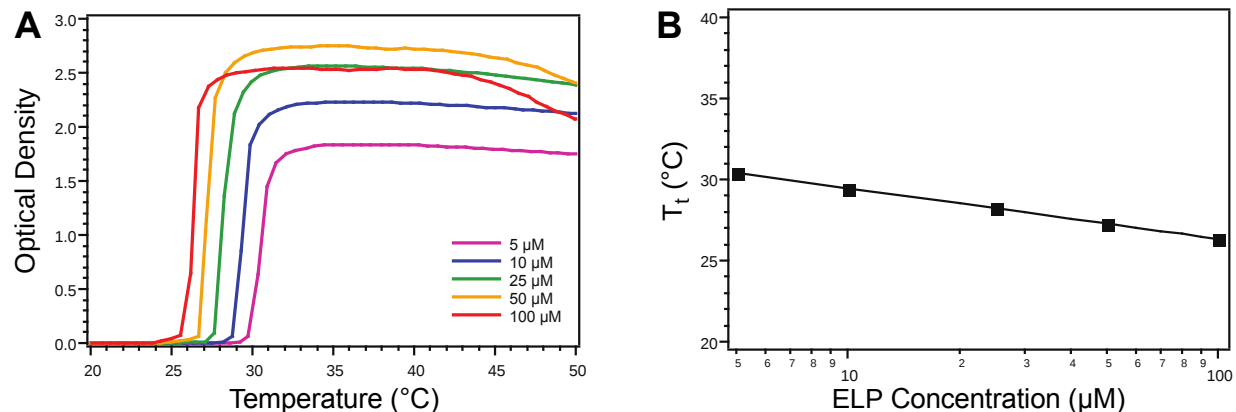


Figure S4. Thermal characterization of ELP-V_{comp} by temperature-regulated turbidimetry. A) ELP-V_{comp} was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM. B) The T_ts at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line).

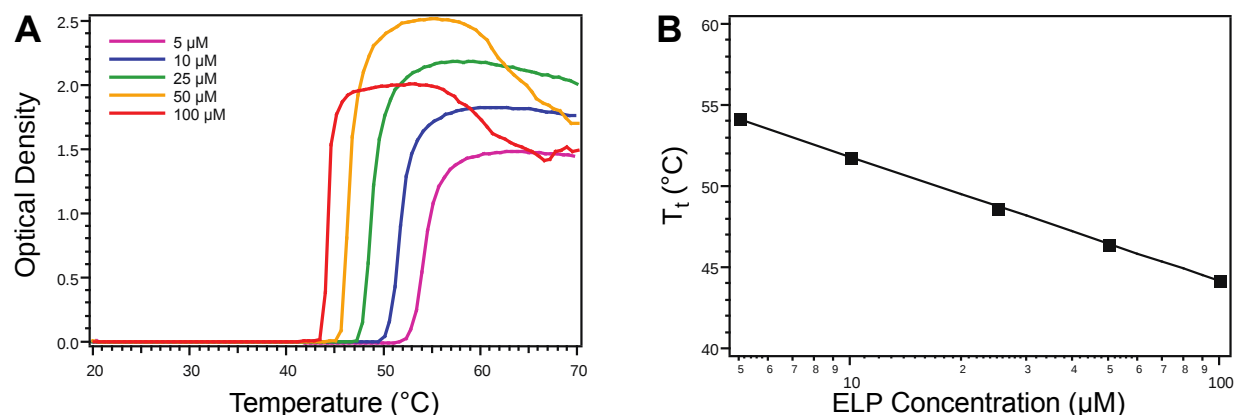


Figure S5. Thermal characterization of ELP-SVB₁ by temperature-regulated turbidimetry. A) ELP-SVB₁ was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM. B) The T_ts at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line).

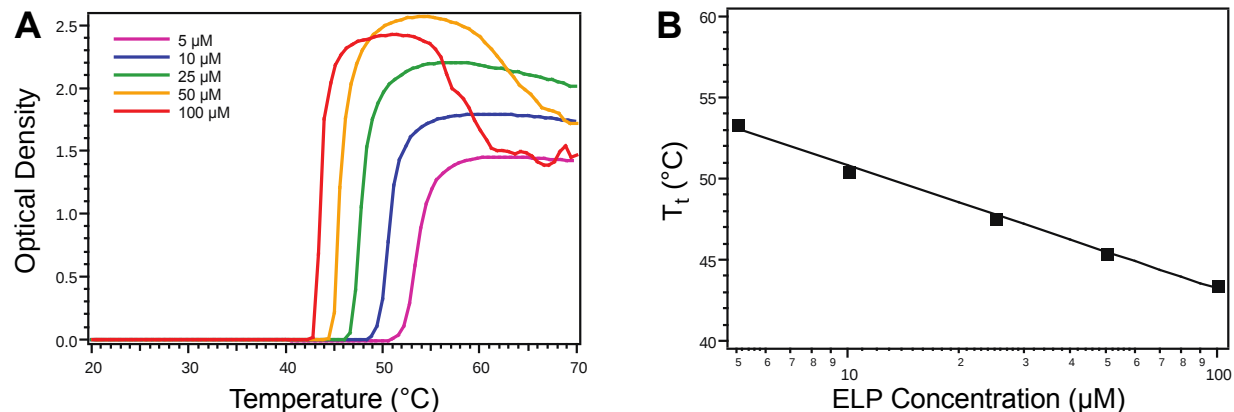


Figure S6. Thermal characterization of ELP-SVB₅ by temperature-regulated turbidimetry. A) ELP-SVB₅ was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM. B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line).

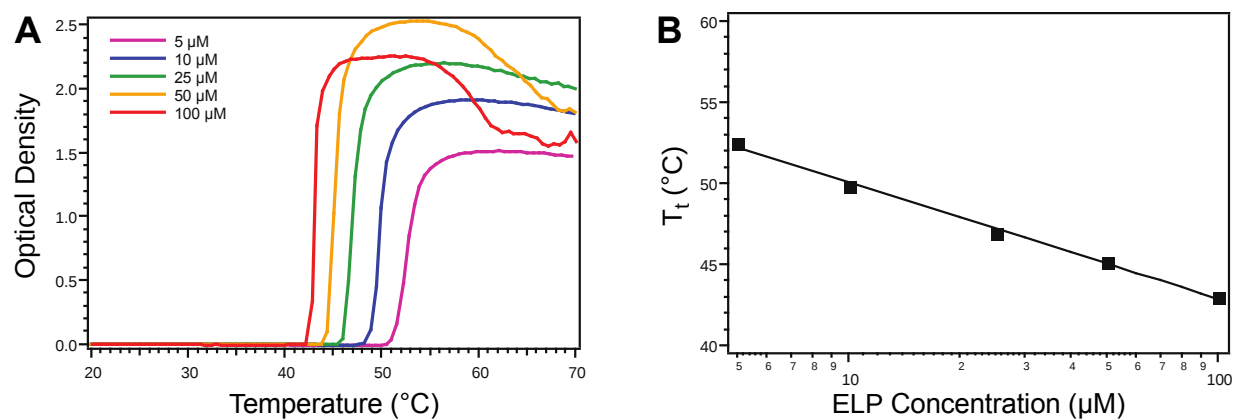


Figure S7. Thermal characterization of ELP-SVB₁₀ by temperature-regulated turbidimetry. A) ELP-SVB₁₀ was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM. B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line).

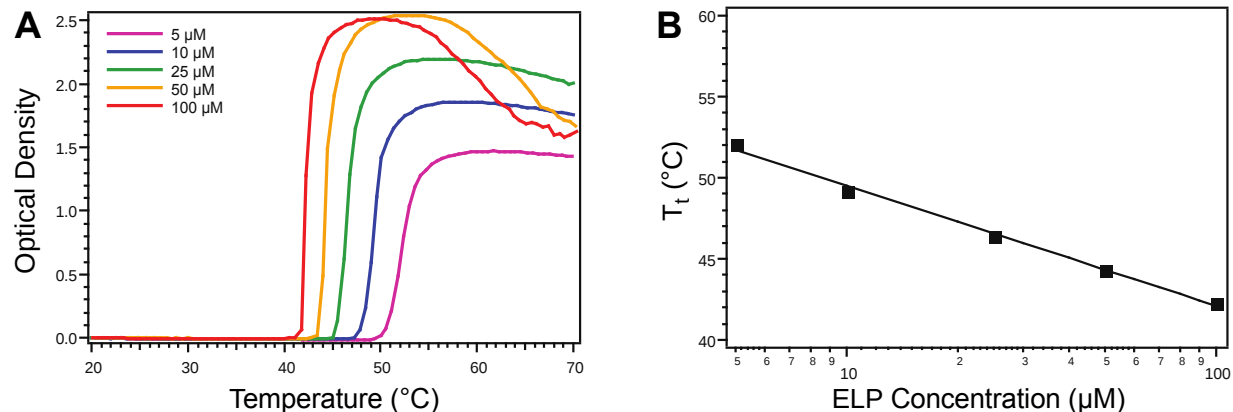


Figure S8. Thermal characterization of ELP-SVB₂₀ by temperature-regulated turbidimetry. A) ELP-SVB₂₀ was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM. B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line).

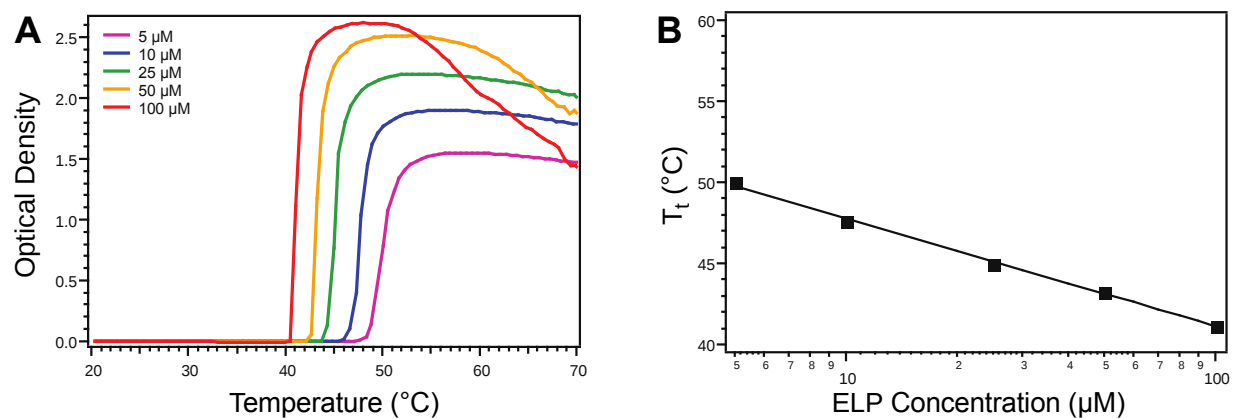


Figure S9. Thermal characterization of ELP-SVB₃₀ by temperature-regulated turbidimetry. A) ELP-SVB₃₀ was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM. B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line).

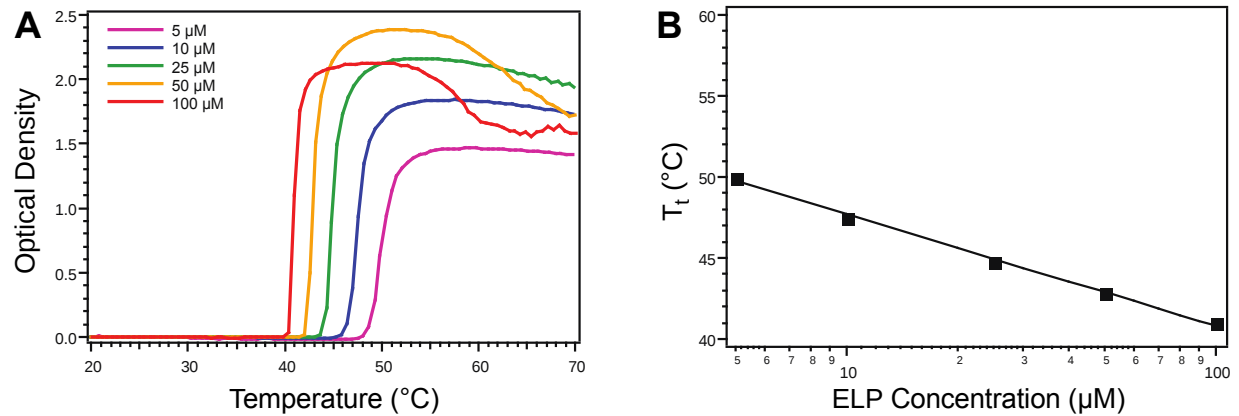


Figure S10. Thermal characterization of ELP-SVT_{VSV} by temperature-regulated turbidimetry. A) ELP-SVT_{VSV} was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM . B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line).

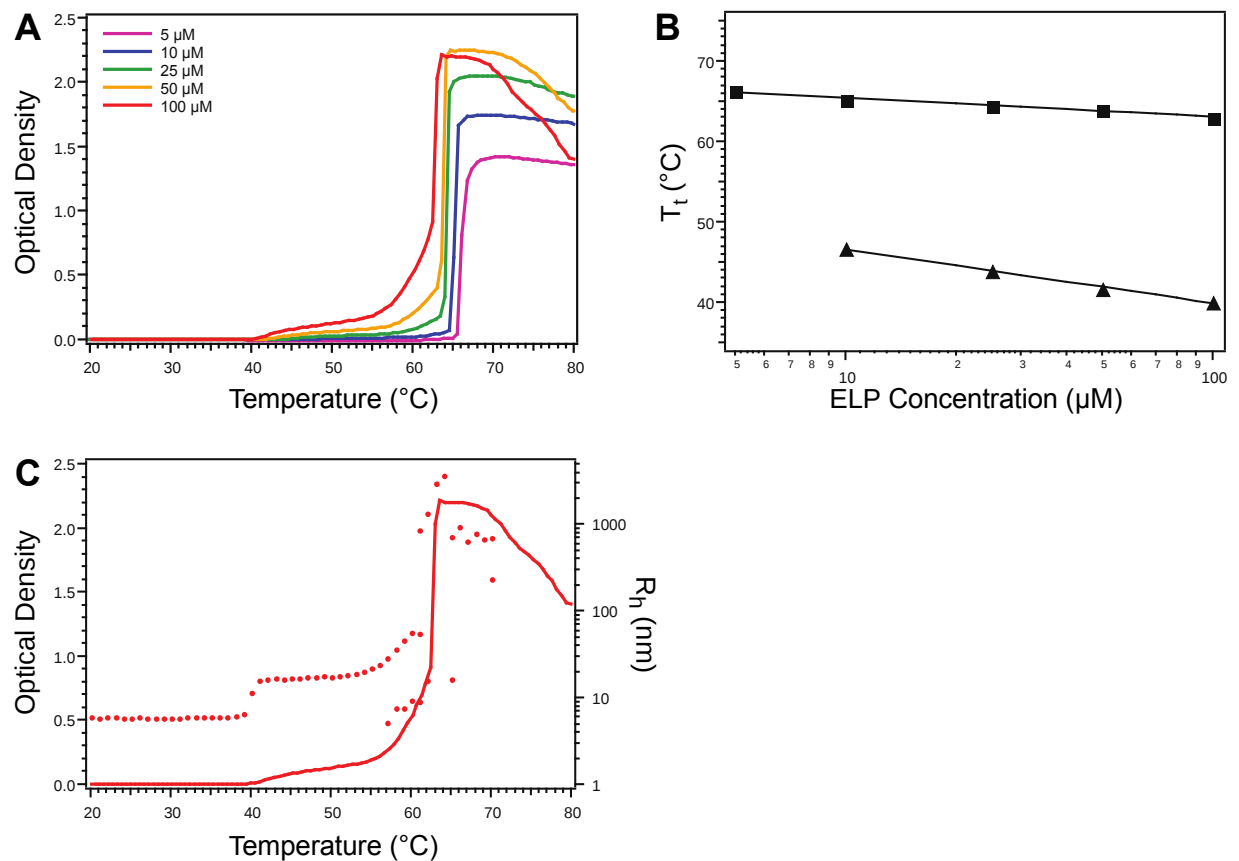


Figure S11. Thermal characterization of ELP-SVT_{SVS} by temperature-regulated turbidimetry and DLS. A) ELP-SVT_{SVS} was characterized by temperature-regulated turbidimetry in PBS at ELP

concentrations of 5, 10, 25, 50, and 100 μM . B) The T_{ts} at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line). The CMTs at each concentration were approximated as the temperatures corresponding to the positive divergences of OD from baseline (triangles), which were fit to a logarithmic relationship (line). Turbidimetry was not sensitive enough to determine the CMT below 10 μM . C) DLS measurement of R_{h} (dots, right ordinate) at 100 μM corroborated the self-assembly with respect to temperature as determined by turbidimetry (line, left ordinate).

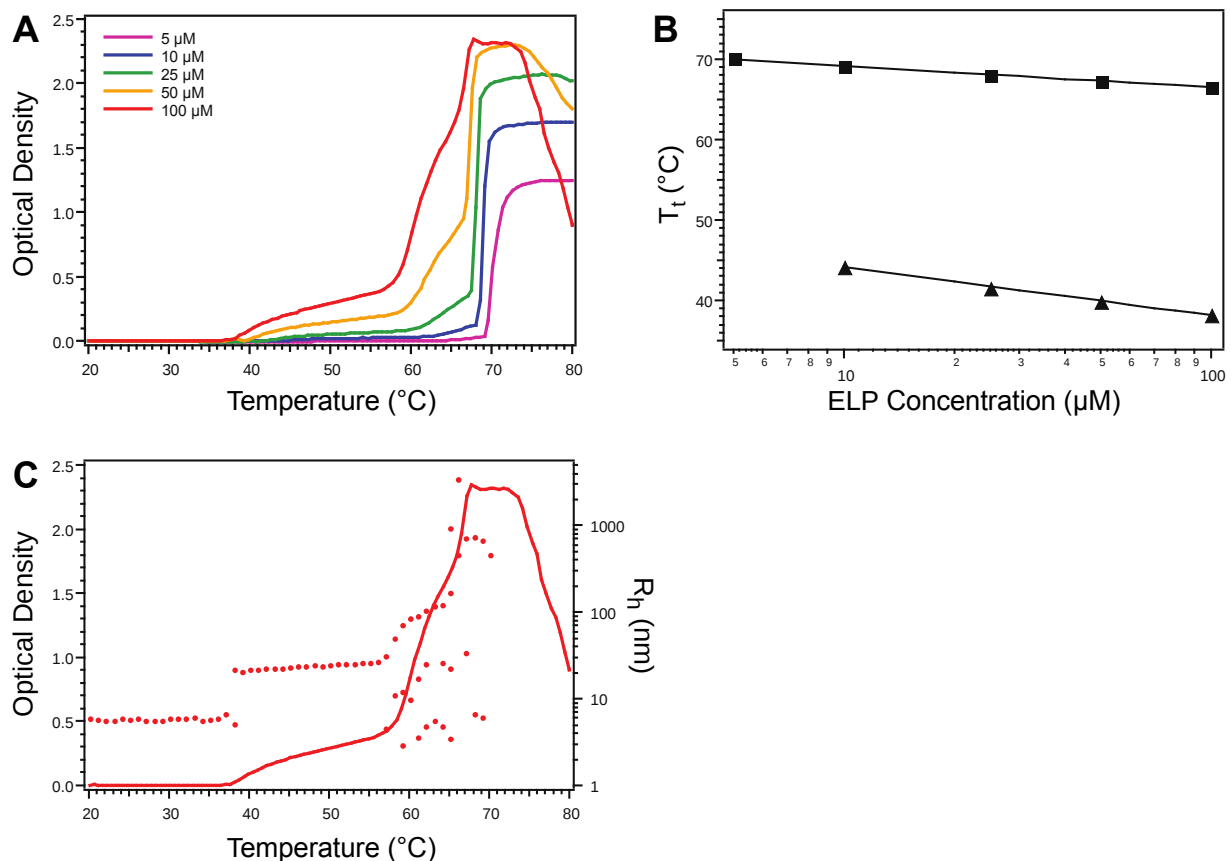


Figure S12. Thermal characterization of ELP-SVD_{SV} by temperature-regulated turbidimetry and DLS. A) ELP-SVD_{SV} was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM . B) The T_{ts} at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line). The CMTs at each concentration were approximated as the temperatures corresponding to the positive divergences of OD from baseline (triangles), which were fit to a logarithmic relationship (line). Turbidimetry was not sensitive enough to determine the CMT below 10 μM . C) DLS measurement of R_{h} (dots, right ordinate) at 100 μM corroborated the self-assembly with respect to temperature as determined by turbidimetry (line, left ordinate).

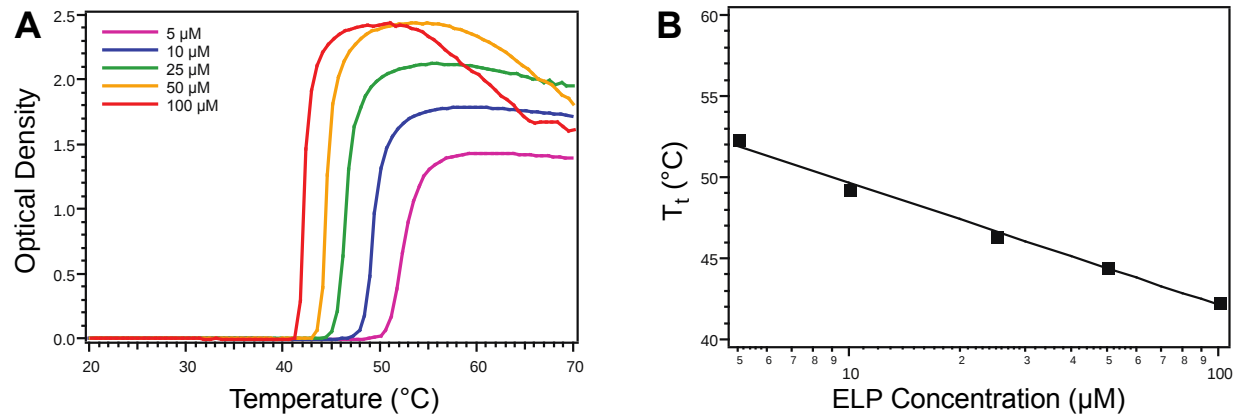


Figure S13. Thermal characterization of ELP-SVG_L by temperature-regulated turbidimetry. A) ELP-SVG_L was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM . B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line).

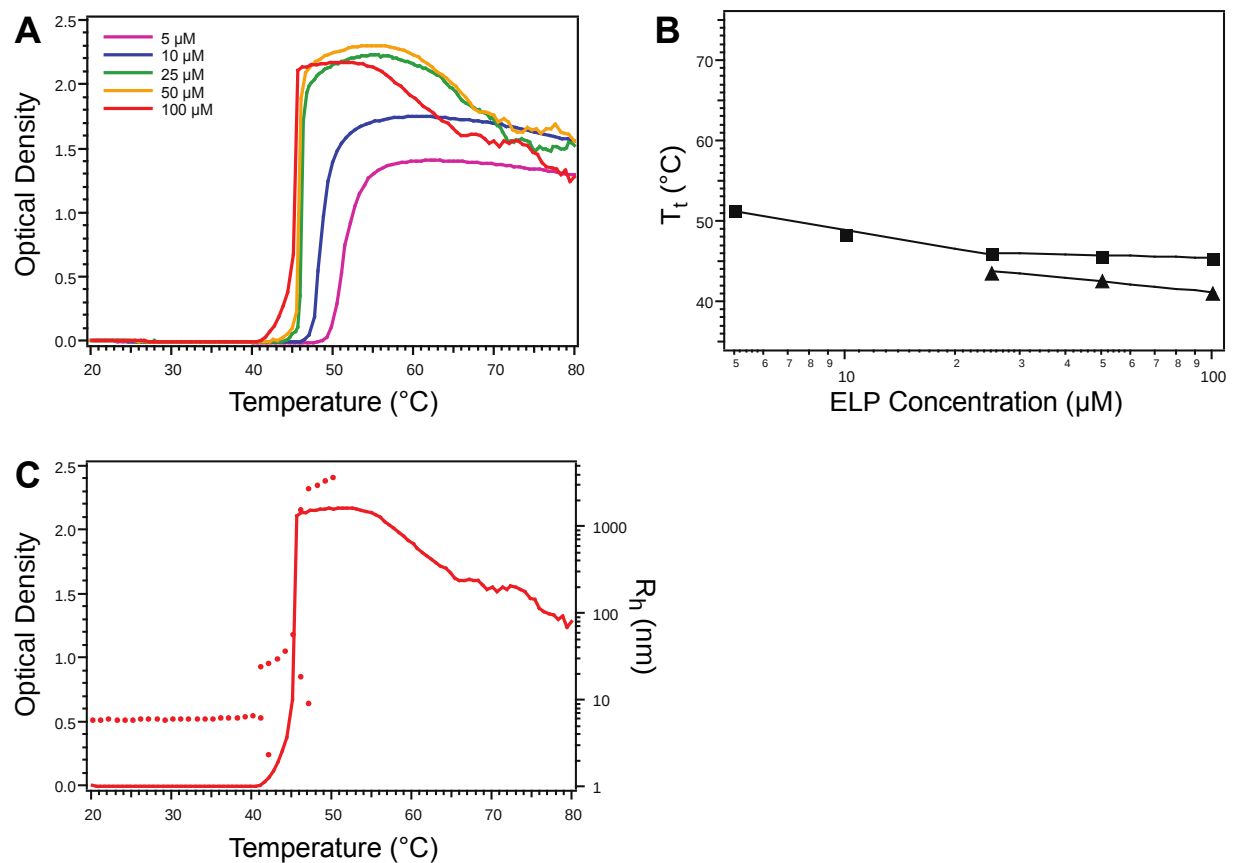


Figure S14. Thermal characterization of ELP-SVG_I by temperature-regulated turbidimetry and DLS. A) ELP-SVG_I was characterized by temperature-regulated turbidimetry in PBS at ELP

concentrations of 5, 10, 25, 50, and 100 μM . B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to two separate logarithmic relationships for 5–25 μM and 25–100 μM (line). The CMTs at each concentration were approximated as the temperatures corresponding to the positive divergences of OD from baseline (triangles), which were fit to a logarithmic relationship (line). No CMT was evident below 25 μM by turbidimetry, which was supported by the difference in slope of the logarithmic fit to the measured T_t s above and below 25 μM . C) DLS measurement of R_h (dots, right ordinate) at 100 μM corroborated the self-assembly with respect to temperature as determined by turbidimetry (line, left ordinate).

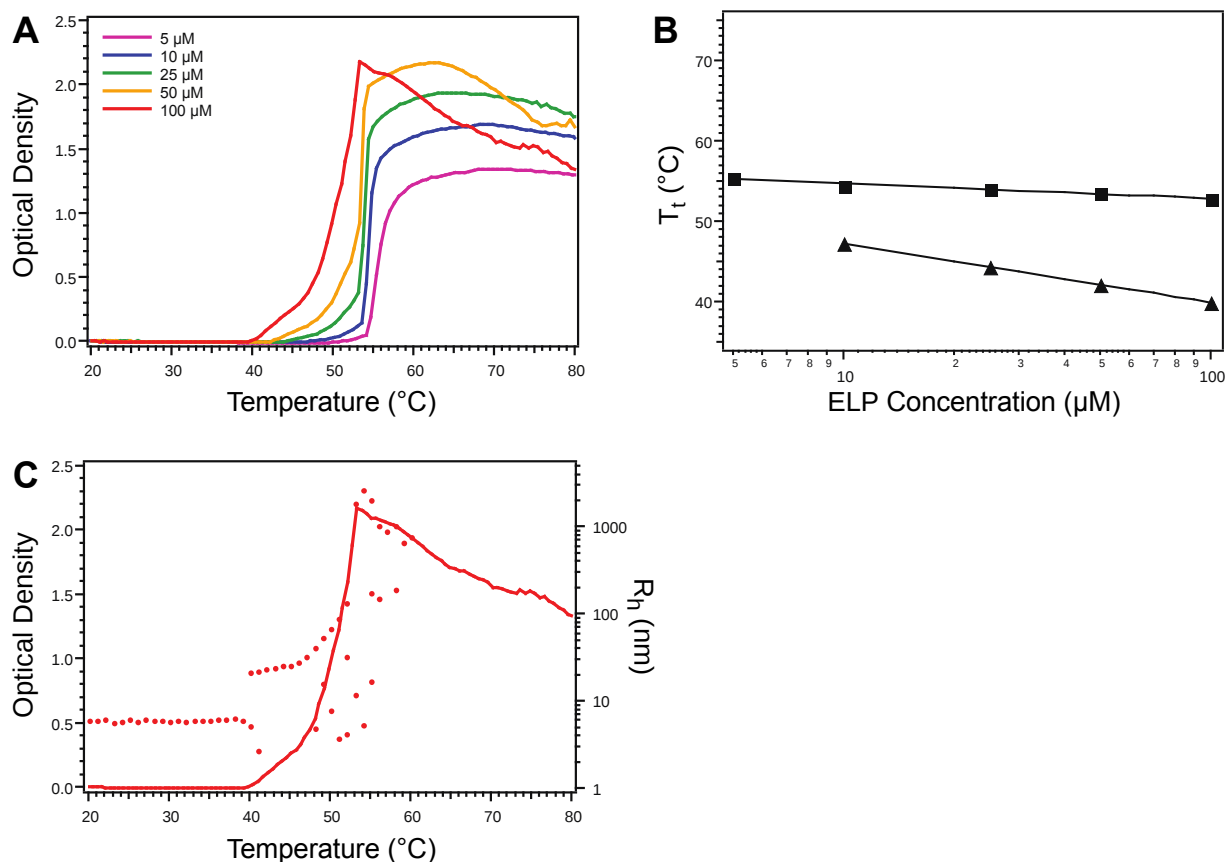


Figure S15. Thermal characterization of ELP-SVG_H by temperature-regulated turbidimetry and DLS. A) ELP-SVG_H was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM . B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line). The CMTs at each concentration were approximated as the temperatures corresponding to the positive divergences of OD from baseline (triangles), which were fit to a logarithmic relationship (line). Turbidimetry was not sensitive enough to determine the CMT below 10 μM . C) DLS measurement of R_h (dots, right ordinate) at 100 μM corroborated the self-assembly with respect to temperature as determined by turbidimetry (line, left ordinate).

Table S4. ELP T_t s.¹

ELP	ELP concentration (μM)				
	5	10	25	50	100
S_{comp} ²	116.77	107.50	107.27	97.34	89.77
V_{comp}	38.84	36.93	34.77	33.54	31.93
SVB_1	54.23	51.80	48.66	46.45	44.24
SVB_5	53.40	50.52	47.60	45.42	43.46
SVB_{10}	52.50	49.83	46.93	45.17	42.99
SVB_{20}	52.08	49.14	46.40	44.29	42.30
SVB_{30}	49.97	47.62	44.97	43.26	41.17
SVT_{VSV}	49.94	47.52	44.82	42.86	40.95
SVT_{SVS}	66.20	65.12	64.50	63.91	62.95
SVD_{SV}	70.07	69.10	68.03	67.39	66.50
SVG_L	52.33	49.34	46.42	44.46	42.30
SVG_I	51.45	48.39	46.04	45.71	45.41
SVG_H	55.37	54.39	54.09	53.44	52.80

¹ T_t s calculated as the temperature corresponding to the maximum of the Gaussian fit of the derivative of OD, with respect to temperature, reported in °C. This T_t corresponded to the phase transition of ELP into unstable micron-scale coacervates, from either a unimer or micelle state.

² T_t s of ELP- S_{comp} were determined from extrapolation of T_t s measured in NaCl dilutions (see Table S2 and S3).

Table S5. ELP CMTs.¹

ELP	ELP concentration (μM)			
	10	25	50	100
SVT_{SVS}	46.62	43.92	41.72	40.00
SVD_{SV}	44.22	41.57	39.92	38.22
SVG_I	NM ²	43.67	42.67	41.07
SVG_H	47.18	44.37	42.14	39.87

¹For those ELPs exhibiting temperature-triggered self-assembly, the CMT, corresponding to the temperature at which the unimer-to-micelle transition occurred, was approximated as the temperature at which the OD deviated positively from baseline, reported in °C. Due to the limited sensitivity of turbidimetry to self-assembled nanoparticles at low concentrations, approximations were not made at 5 μM .

²No CMT was evident for measurement of ELP- SVG_I at concentrations of 10 μM and lower.

The ELP T_t (or CMT) exhibited a logarithmic relationship to concentration⁴ as described by Equation S2:

$$T_t = b + m \ln C \quad (\text{S2})$$

which is a simplification of the relationship that more precisely describes the dependence of T_t on the ELP's sequence, size, and concentration,⁴ as described in Equation S3:

$$T_t = T_{t,c} + \frac{k}{L} \ln \frac{C_c}{C} \quad (\text{S3})$$

where L is the ELP length in pentapeptides, k is a constant of units $^{\circ}\text{C} \cdot \text{pentapeptides}$, and $T_{t,c}$ and C_c are a critical temperature and concentration, respectively, that are determined at a limit of high concentration and high ELP length, which is unique to each ELP sequence. The length was conserved over the family of ELPs investigated here, such that $T_{t,c}$, C_c , and k could not be experimentally determined. Therefore the simplified Equation S2 was used to fit the relationship between ELP concentration and T_t (or CMT).

Table S6. Fit parameters of ELP T_t s.¹

ELP	m ($^{\circ}\text{C}$)	b ($^{\circ}\text{C}$)	r^2
S_{comp}^2	-8.33	129.78	0.928
V_{comp}	-2.27	42.30	0.996
SVB_1	-3.34	59.51	0.999
SVB_5	-3.29	58.37	0.996
SVB_{10}	-3.12	57.25	0.996
SVB_{20}	-3.21	56.90	0.995
SVB_{30}	-2.89	54.45	0.998
SVT_{SVS}	-2.98	54.54	0.998
SVT_{SVS}	-1.01	67.70	0.980
SVD_{SV}	-1.17	71.87	0.997
SVG_L	-3.29	57.25	0.994
SVG_I	-3.32^3	56.53^3	0.976^3
	-0.45^4	47.48^4	0.999^4
SVG_H	-0.80	56.51	0.967

¹Parameters according to Equation S2, fit to T_t s measured at 5-100 μM .

²Fit based on T_t s determined from extrapolation of T_t s measured in NaCl (see Table S2 and S3).

³Fit to T_t s at concentrations 5-25 μM .

⁴Fit to T_t s at concentrations 25-100 μM .

Table S7. Fit parameters of ELP CMTs.¹

ELP	m ($^{\circ}\text{C}$)	b ($^{\circ}\text{C}$)	r^2
SVT_{SVS}	-2.91	53.27	0.998
SVD_{SV}	-2.59	50.09	0.998
SVG_I	-1.88^2	49.81^2	0.983^2
SVG_H	-3.18	54.53	0.999

¹Parameters according to Equation S2, fit to CMTs approximated at 10-100 μM .

²Fit to CMTs approximated at 25-100 μM .

Table S8. Refractive index increment of self-assembling ELPs.

ELP	dn/dc (mL/mg)	r^2
-----	-----------------------------------	-------

SVT_{SVS}	1.797764×10^{-4}	0.999
SVD_{SV}	1.653665×10^{-4}	0.999
SVG_{H}	1.729193×10^{-4}	0.999
SVG_{I}	1.749068×10^{-4}	0.985

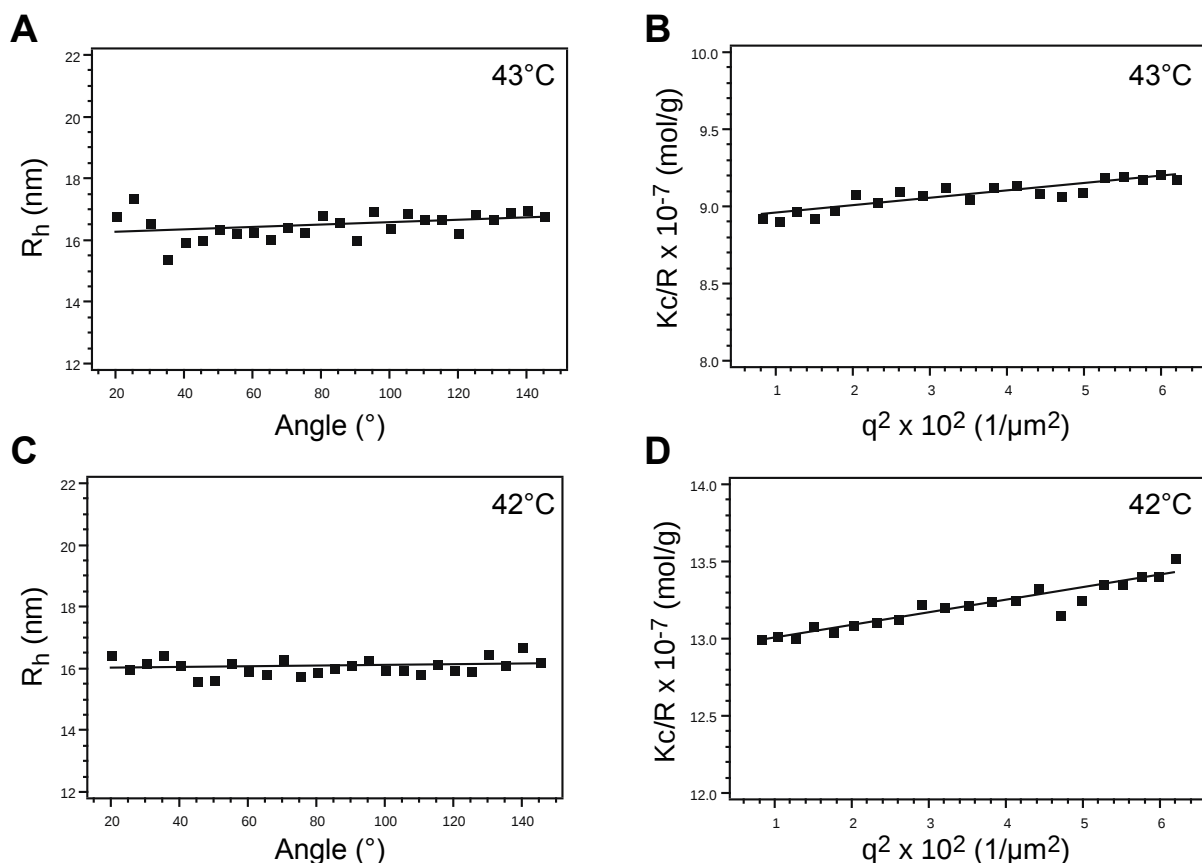


Figure S16. Characterization of ELP-SVT_{SVS} by SLS and DLS. A) R_h was determined from DLS measurements and B) R_g and MW was determined from SLS measurements of 100 μM solution at 43°C . C) R_h was determined from DLS measurements and D) R_g and MW was determined from SLS measurements of 100 μM solution at 42°C , 2°C above the CMT of ELP-SVT_{SVS}. Markers represent individual measurements while solid lines represent linear fits to the data.

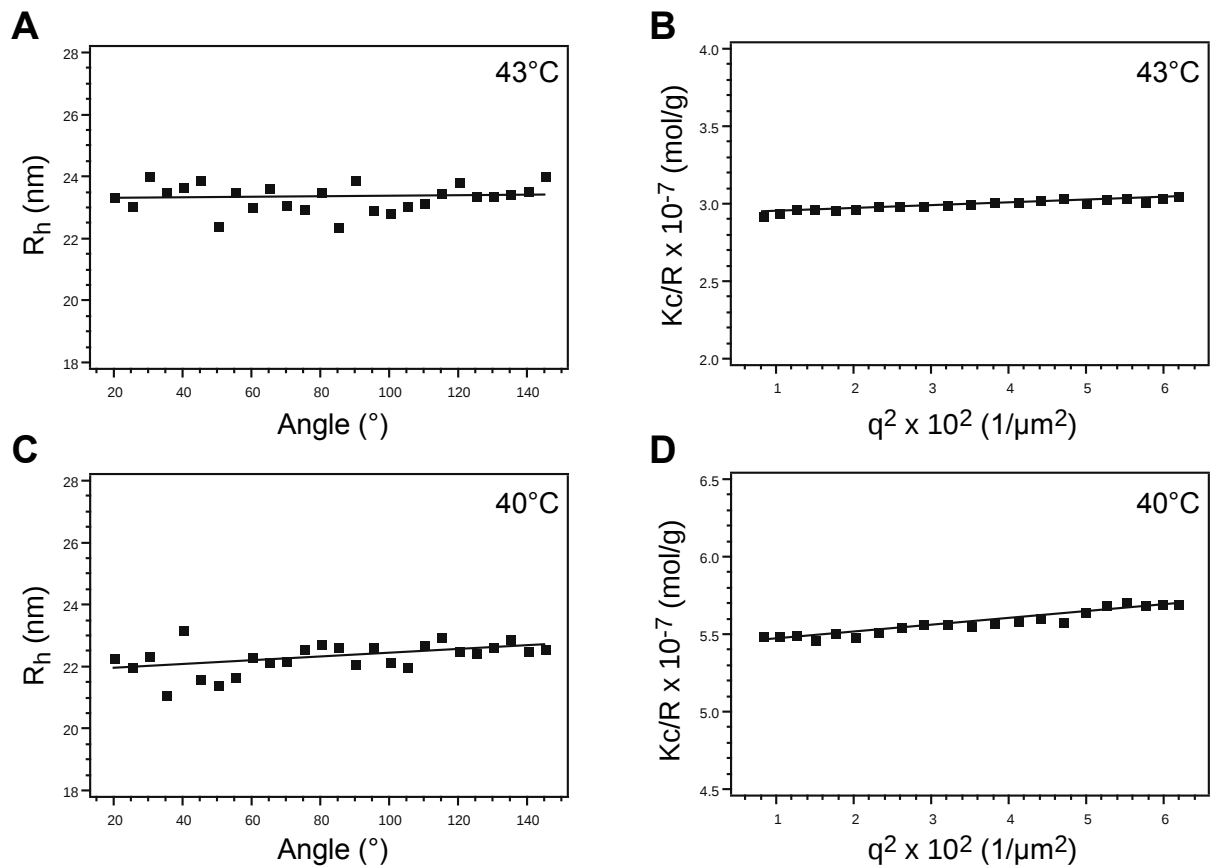


Figure S17. Characterization of ELP-SVD_{SV} by SLS and DLS. A) R_h was determined from DLS measurements and B) R_g and MW was determined from SLS measurements of 100 μM solution at 43°C. C) R_h was determined from DLS measurements and D) R_g and MW was determined from SLS measurements of 100 μM solution at 40°C, 2°C above the CMT of ELP-SVD_{SV}. Markers represent individual measurements while solid lines represent linear fits to the data.

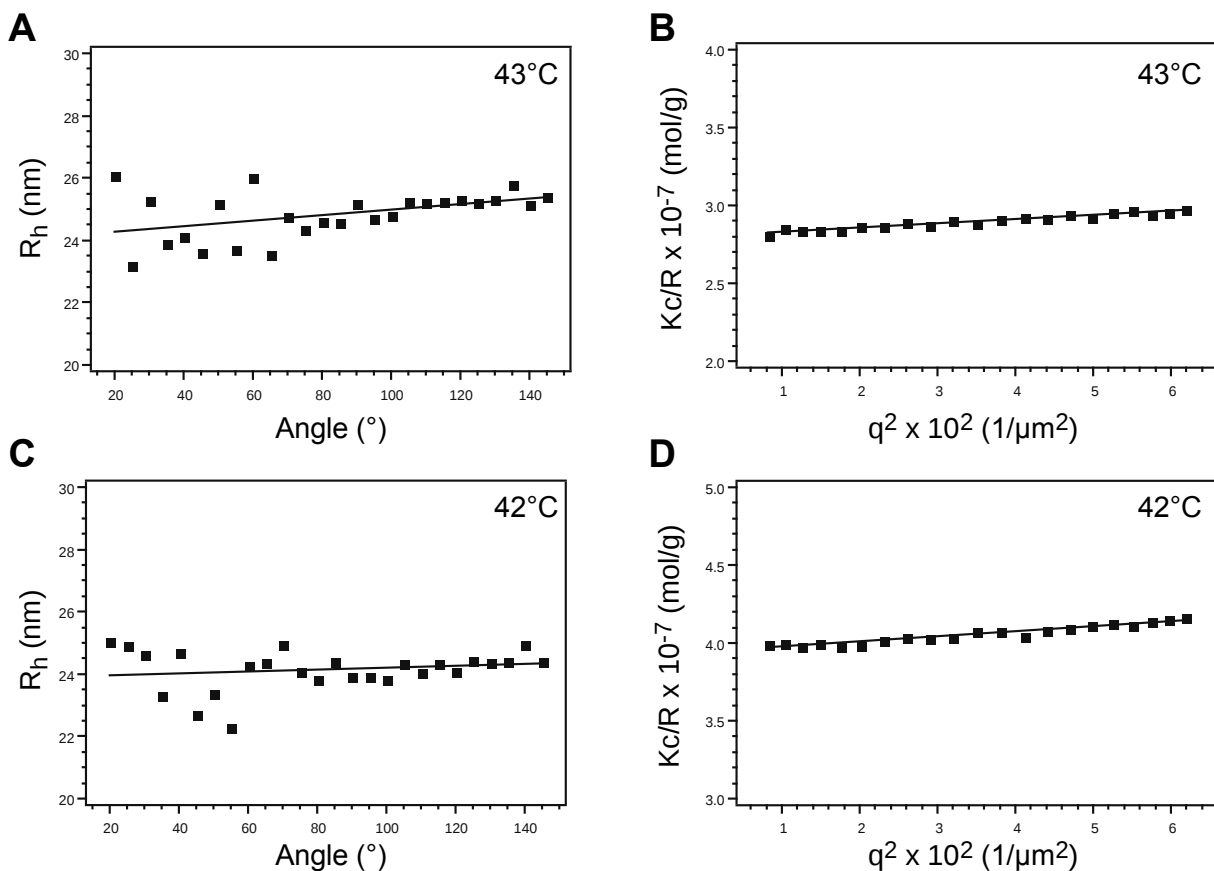


Figure S18. Characterization of ELP-SVG_H by SLS and DLS. A) R_h was determined from DLS measurements and B) R_g and MW was determined from SLS measurements of 100 μM solution at 43°C. C) R_h was determined from DLS measurements and D) R_g and MW was determined from SLS measurements of 100 μM solution at 42°C, 2°C above the CMT of ELP-SVG_H. Markers represent individual measurements while solid lines represent linear fits to the data.

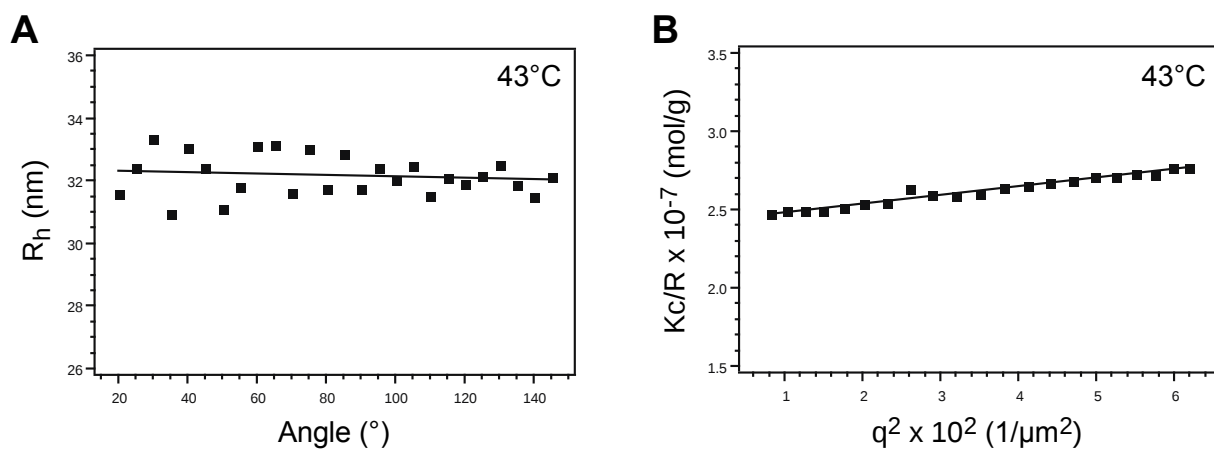


Figure S19. Characterization of ELP-SVG_I by SLS and DLS. A) R_h was determined from DLS measurements and B) R_g and MW was determined from SLS measurements of 100 μ M solution at 43°C, 2°C above the CMT of ELP-SVG_I. Markers represent individual measurements while solid lines represent linear fits to the data.

Table S9. Summary of SLS and DLS characterization of ELP self-assembly at 43°C.

ELP	R_g (nm)	R_h (nm)	ρ (R_g/R_h)	MW (g/mol)	N_{agg}
SVT _{SVS}	12.7 ($\pm 5.9\%$)	16.2	0.78	1.122×10^6 ($\pm 0.25\%$)	23
SVD _{SV}	13.8 ($\pm 4.2\%$)	23.3	0.59	3.408×10^6 ($\pm 0.21\%$)	70
SVG _H	17.0 ($\pm 2.8\%$)	24.1	0.70	3.564×10^6 ($\pm 0.21\%$)	73
SVG _I	26.1 ($\pm 2.0\%$)	32.4	0.81	4.118×10^6 ($\pm 0.35\%$)	85

Table S10. Summary of SLS and DLS characterization of ELP self-assembly at 2°C above the CMT.¹

ELP	R_g (nm)	R_h (nm)	ρ (R_g/R_h)	MW (g/mol)	N_{agg}
SVT _{SVS}	13.7 ($\pm 4.0\%$)	16.0	0.86	7.735×10^6 ($\pm 0.20\%$)	16
SVD _{SV}	15.7 ($\pm 3.4\%$)	21.8	0.72	1.842×10^6 ($\pm 0.22\%$)	38
SVG _H	15.9 ($\pm 3.1\%$)	23.9	0.67	2.537×10^6 ($\pm 0.20\%$)	52
SVG _I	26.1 ($\pm 2.0\%$)	32.4	0.81	4.118×10^6 ($\pm 0.35\%$)	85

¹Measurements were made at 2°C above the CMT: ELP-SVT_{SVS} at 42°C, ELP-SVD_{SV} at 40°C, ELP-SVG_H at 42°C, and ELP-SVG_I at 43°C.

A) ELP-V-SVB₁-S

[illegible]

B) ELP-V-SVB₅-S

[illegible]

C) ELP-V-SVB₁₀-S

M G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
 atg ggc gtg ggt gtt ccg ggc gta ggt gtc cca ggt gtg ggc gta ccg ggc gtt ggt gtt cct ggt gtc ggc gtg ccg ggc gtg ggt gtt

 P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
 ccg ggc gta ggt gtc cca ggt gtg ggc gta ccg ggc gtt ggt gtt cct ggt gtc ggc gtg ccg ggc gtg ggt gtt ccg ggc gta ggt gtc

 P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
 cca ggt gtg ggc gta ccg ggc gtt ggt gtt cct ggt gtc ggc gtg ccg ggc gtg ggt gtt ccg ggc gta ggt gtc cca ggt gtg ggc gta

 P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
 ccg ggc gtt ggt gtt cct ggt gtc ggc gtg ccg ggc gtg ggt gtt ccg ggc gta ggt gtc cca ggt gtg ggc gta ccg ggc gtt ggt gtt

 P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
 cct ggt gtc ggc gtg ccg ggc gtg ggt gtt ccg ggc gta ggt gtc cca ggt gtg ggc gta ccg ggc gtt ggt gtt cct ggt gtc ggc gtg

 P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
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 P G V G V P G V G V P G V G V P G V G V P G S G V P G S G V
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 P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V
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 P G S G V P G S G V P G S G V P G V G V P G V G V P G V G V P G V G V
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 P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
 cct ggt gtc ggc gtg ccg ggc gtg ggt gtt ccg ggc gta ggt gtc cca ggt gtg ggc gta ccg ggc gtt ggt gtt cct ggt gtc ggc gtg

 P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V
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 P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V P G V G V
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 P G V G V P G V G V P G S G V P G S G V P G S G V P G S G V P G S G V
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 P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V
 cct ggt tct ggc gtg ccg ggc agc ggt gtt ccg ggc tct ggt gtc cca ggt tcc ggc gta ccg ggc agc ggt gtt cct ggt tct ggc gtg

 P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V
 ccg ggc agc ggt gtt ccg ggc tct ggt gtc cca ggt tcc ggc gta ccg ggc agc ggt gtt ccg ggc tct ggt gtc cca ggt tcc ggc gta

 P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V
 ccg ggc agc ggt gtt cct ggt tct ggc gtg ccg ggc agc ggt gtt ccg ggc tct ggt gtc cca ggt tcc ggc gta ccg ggc agc ggt gtt

 P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V P G S G V
 cct ggt tct ggc gtg ccg ggc agc ggt gtt ccg ggc tct ggt gtc cca ggt tcc ggc gta ccg ggc agc ggt gtt cct ggt tct ggc gtg

 P G Y
 ccg ggc tac

Figure S20. DNA and polypeptide sequences of blocky interface ELPs. ELP composition and length was equivalent to all other ELPs with various block architectures.

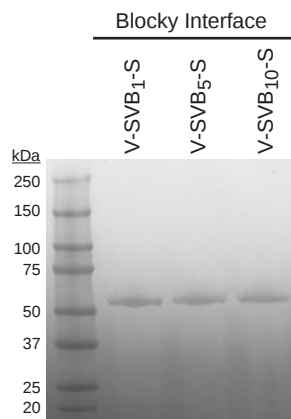


Figure S21. Evaluation of blocky interface ELP size and purity by SDS-PAGE and CuCl_2 staining. These ELPs ran slightly higher than their expected MW of 48.66 kDa, which has been previously reported for other ELPs.²

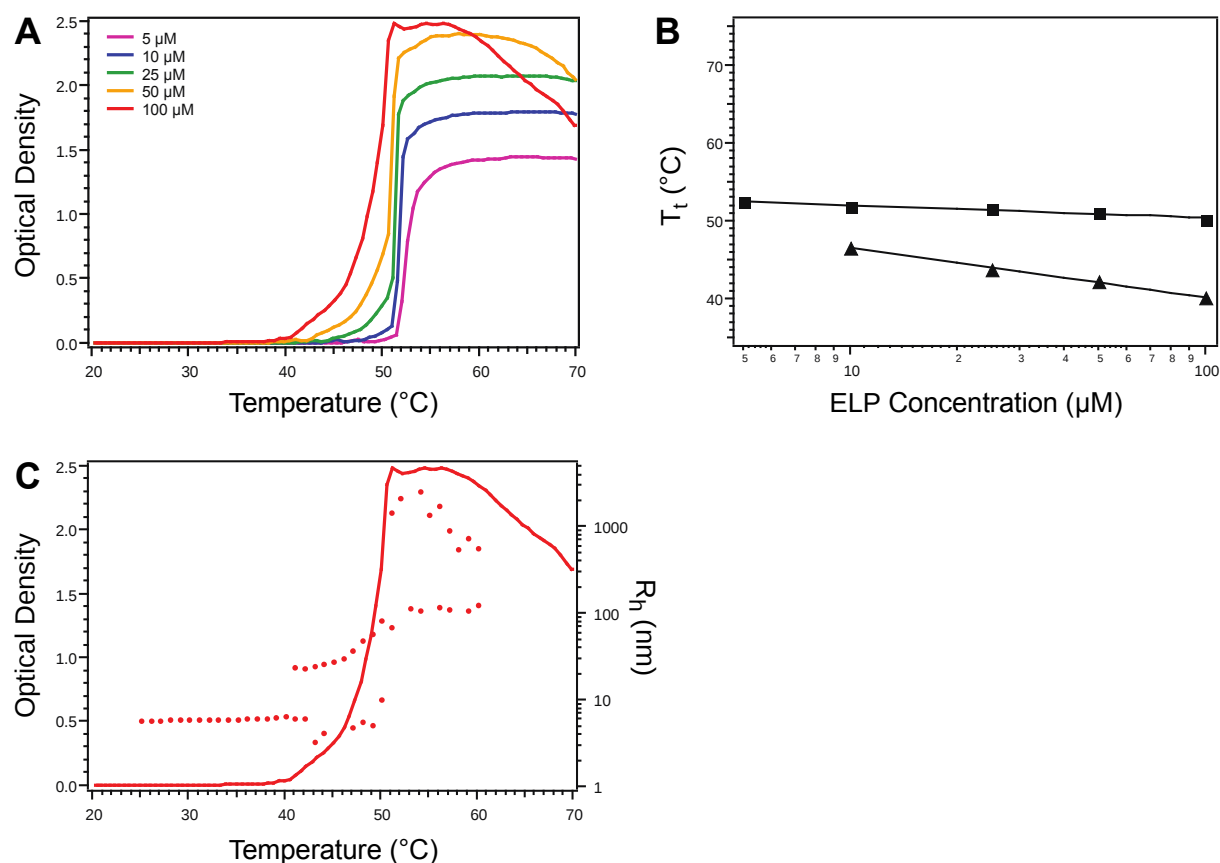


Figure S22. Thermal characterization of ELP-V-SVB₁-S by temperature-regulated turbidimetry and DLS. A) ELP-V-SVB₁-S was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM. B) The T_t s at each concentration were

determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line). The CMTs at each concentration were approximated as the temperatures corresponding to the positive divergences of OD from baseline (triangles), which were fit to a logarithmic relationship (line). Turbidimetry was not sensitive enough to determine the CMT below 10 μM . C) DLS measurement of R_h (dots, right ordinate) at 100 μM corroborated the self-assembly with respect to temperature as determined by turbidimetry (line, left ordinate).

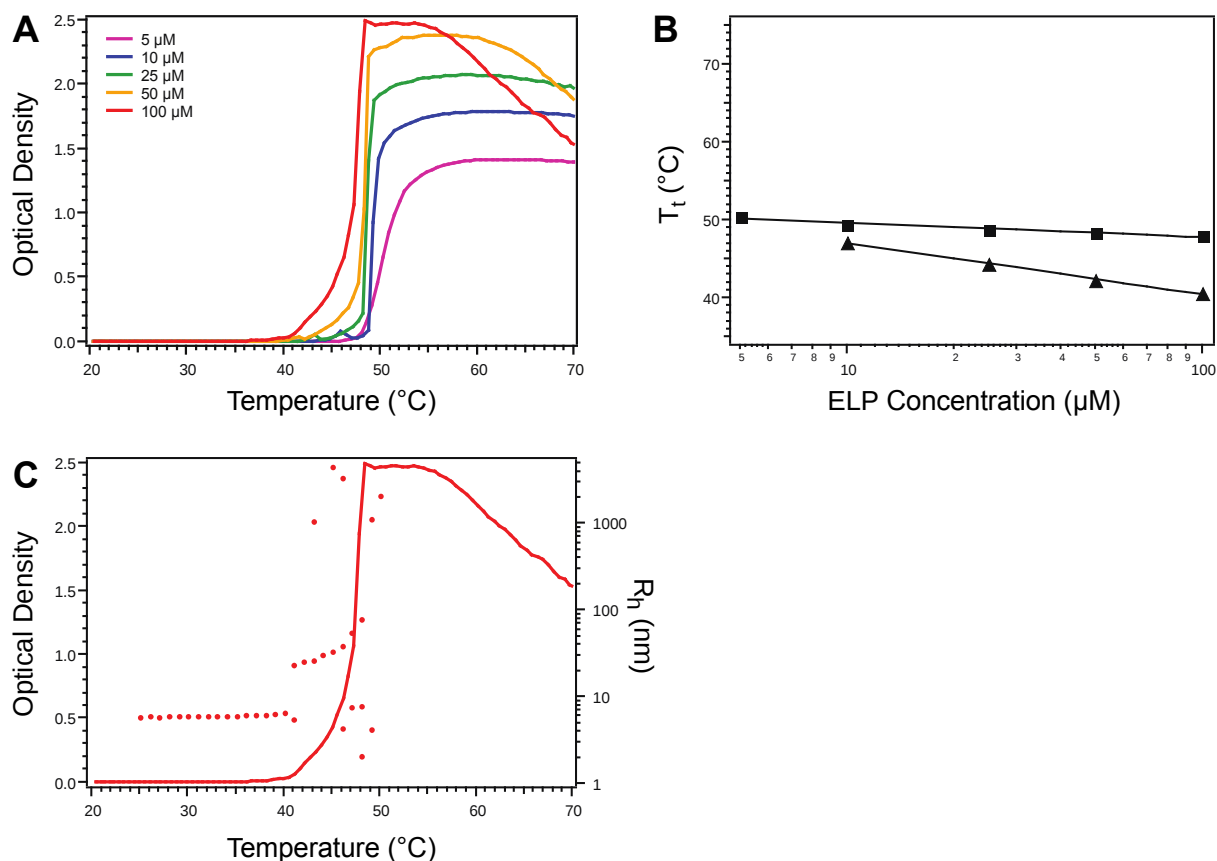


Figure S23. Thermal characterization of ELP-V-SVB₅-S by temperature-regulated turbidimetry and DLS. A) ELP-V-SVB₅-S was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM . B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to a logarithmic relationship (line). The CMTs at each concentration were approximated as the temperatures corresponding to the positive divergences of OD from baseline (triangles), which were fit to a logarithmic relationship (line). Turbidimetry was not sensitive enough to determine the CMT below 10 μM . C) DLS measurement of R_h (dots, right ordinate) at 100 μM corroborated the self-assembly with respect to temperature as determined by turbidimetry (line, left ordinate).

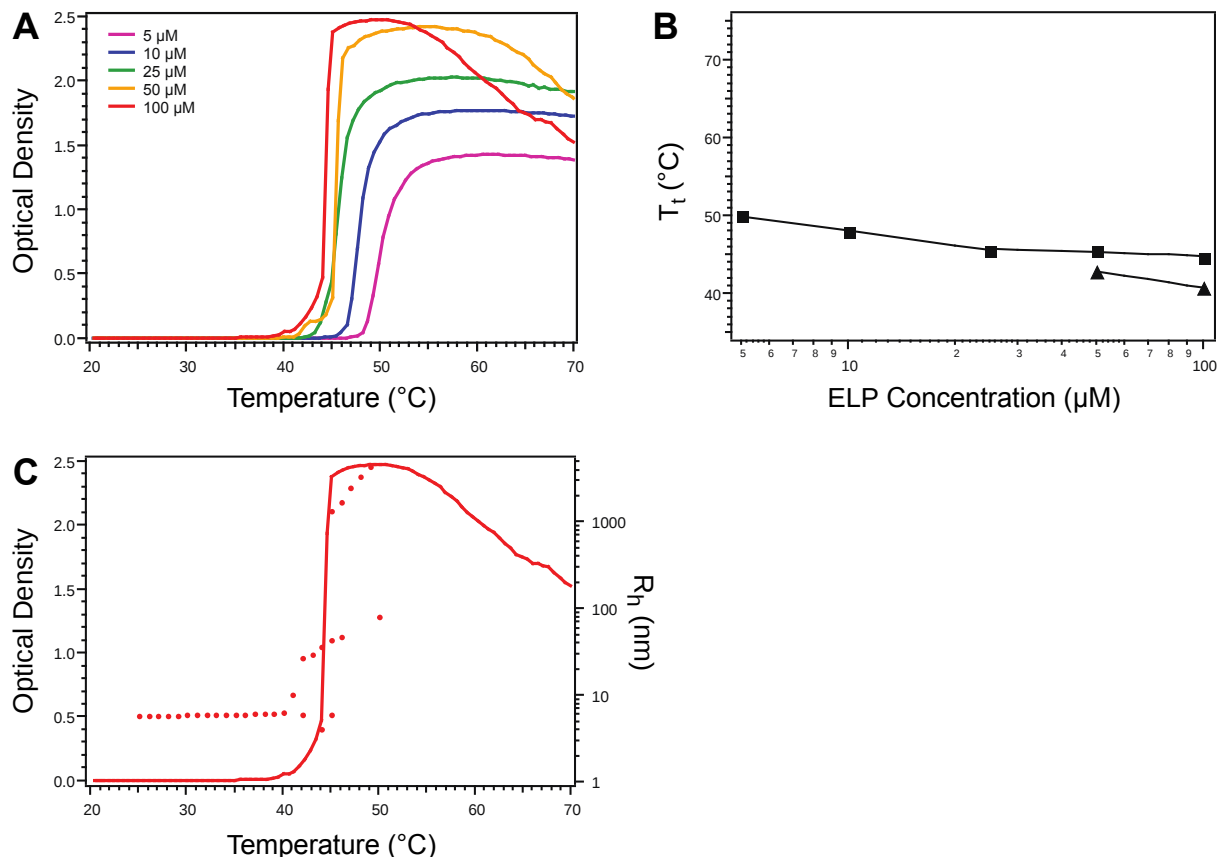


Figure S24. Thermal characterization of ELP-V-SVB₁₀-S by temperature-regulated turbidimetry and DLS. A) ELP-V-SVB₁₀-S was characterized by temperature-regulated turbidimetry in PBS at ELP concentrations of 5, 10, 25, 50, and 100 μM. B) The T_t s at each concentration were determined as the temperatures corresponding to the maximum of the Gaussian fit of the derivative of OD with respect to temperature (squares), which were fit to two separate logarithmic relationships for 5-25 μM and 25-100 μM (line). The CMTs at each concentration were approximated as the temperatures corresponding to the positive divergences of OD from baseline (triangles), which were fit to a logarithmic relationship (line). No CMT was evident below 50 μM by turbidimetry, which was supported by the difference in slope of the logarithmic fit to the measured T_t s above and below 50 μM. C) DLS measurement of R_h (dots, right ordinate) at 100 μM corroborated the self-assembly with respect to temperature as determined by turbidimetry (line, left ordinate).

Table S11. T_t s of blocky interface ELPs.¹

ELP	ELP concentration (μM)				
	5	10	25	50	100
V-SVB ₁ -S	52.48	51.83	51.52	51.16	50.12
V-SVB ₅ -S	50.35	49.32	48.71	48.35	47.85
V-SVB ₁₀ -S	49.97	47.92	45.56	45.60	44.62

¹ T_t s were calculated as the temperature corresponding to the maximum of the Gaussian fit of the derivative of OD, with respect to temperature, reported in °C. This T_t corresponded to the

aggregation of ELP into micron-scale coacervates, indicative of the nanoparticle-to-aggregate transition.

Table S12. CMTs of blocky interface ELPs.¹

ELP	ELP concentration (μM)			
	10	25	50	100
V-SVB ₁ -S	46.52	43.77	42.17	40.07
V-SVB ₅ -S	47.07	44.32	42.17	40.57
V-SVB ₁₀ -S	NM ²	NM ²	42.77	40.67

¹The CMT, corresponding to the temperature at which the unimer-to-nanoparticle transition occurred, was approximated as the temperature at which the OD deviated positively from baseline, reported in °C. Due to the limited sensitivity of turbidimetry to self-assembled nanoparticles at low concentrations, approximations were not made at 5 μM .

²No CMT was evident for measurement of ELP-V-SVB₁₀-S at concentrations of 25 μM and lower.

Table S13. Fit parameters of blocky interface ELP T_t s.¹

ELP	m (°C)	b (°C)	r^2
V-SVB ₁ -S	-0.70	53.61	0.930
V-SVB ₅ -S	-0.79	51.38	0.961
V-SVB ₁₀ -S	-2.74 ²	54.32 ²	0.999 ²
	-0.68 ³	47.90 ³	0.715 ³

¹Parameters according to Equation S2, fit to T_t s measured at 5-100 μM .

²Fit to T_t s at concentrations 5-25 μM .

³Fit to T_t s at concentrations 25-100 μM .

Table S14. Fit parameters of blocky interface ELP CMTs.¹

ELP	m (°C)	b (°C)	r^2
V-SVB ₁ -S	-2.76	52.82	0.998
V-SVB ₅ -S	-2.86	53.56	0.997
V-SVB ₁₀ -S	-3.03 ²	54.62 ²	1.000 ²

¹Parameters according to Equation S2, fit to CMTs approximated at 10-100 μM .

²Fit to CMTs approximated at 50-100 μM .

Table S15. Refractive index increment of blocky interface ELPs.

ELP	dn/dc (mL/mg)	r^2
V-SVB ₁ -S	1.703658×10^4	0.999
V-SVB ₅ -S	1.718044×10^4	0.999
V-SVB ₁₀ -S	1.692355×10^4	0.999

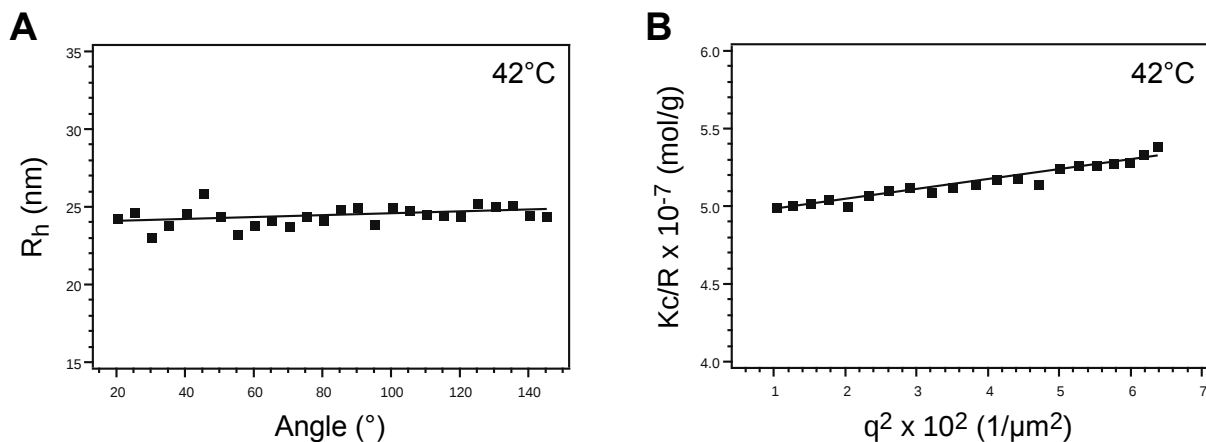


Figure S25. DLS and SLS characterization of ELP-V-SVB₁-S. A) R_h was determined from DLS measurements and B) R_g and MW was determined from SLS measurements of 100 μ M solution at 42°C, 2°C above the CMT of ELP-V-SVB₁-S. Markers represent individual measurements while solid lines represent linear fits to the data.

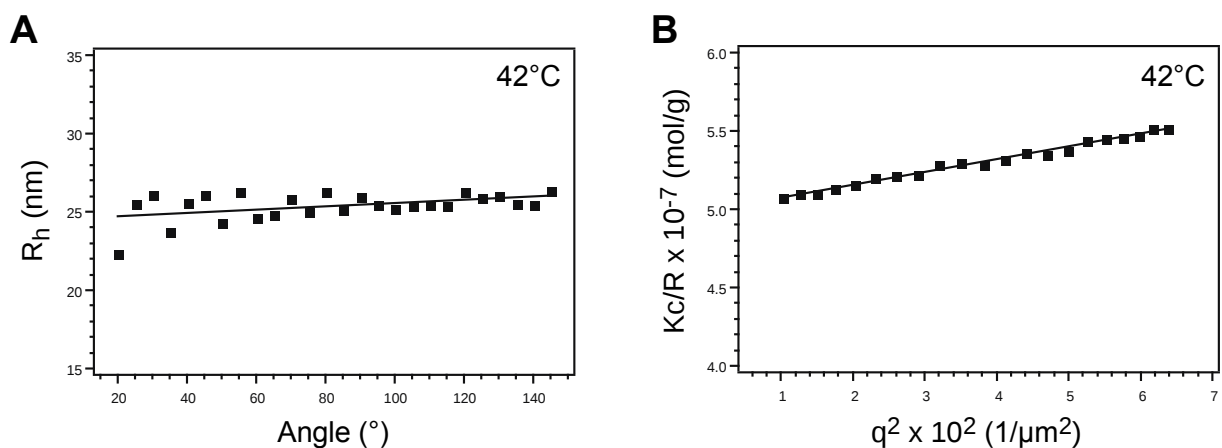


Figure S26. DLS and SLS characterization of ELP-V-SVB₅-S. A) R_h was determined from DLS measurements and B) R_g and MW was determined from SLS measurements of 100 μ M solution at 42°C, 2°C above the CMT of ELP-V-SVB₅-S. Markers represent individual measurements while solid lines represent linear fits to the data.

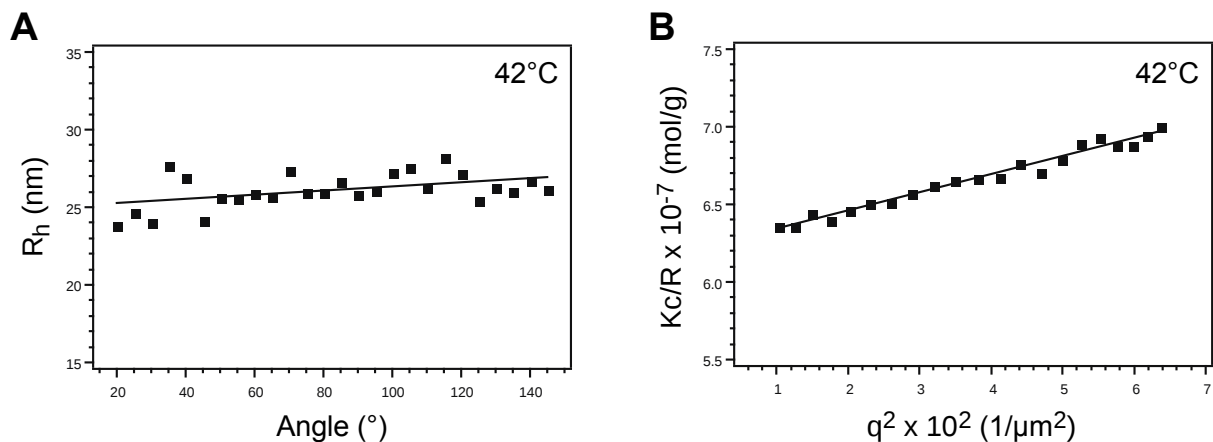


Figure S27. DLS and SLS characterization of ELP-V-SVB₁₀-S. A) R_h was determined from DLS measurements and B) R_g and MW was determined from SLS measurements of 100 μM solution at 42°C, 2°C above the CMT of ELP-V-SVB₁₀-S. Markers represent individual measurements while solid lines represent linear fits to the data.

Table S16. Summary of SLS and DLS characterization of blocky interface ELP self-assembly at 42°C.

ELP	R_g (nm)	R_h (nm)	ρ (R_g/R_h)	MW (g/mol)	N_{agg}
V-SVB ₁ -S	19.9 ($\pm 2.9\%$)	24.0	0.83	2.033×10^6 ($\pm 0.31\%$)	42
V-SVB ₅ -S	22.1 ($\pm 1.3\%$)	24.5	0.90	2.001×10^6 ($\pm 0.17\%$)	41
V-SVB ₁₀ -S	23.7 ($\pm 1.8\%$)	25.0	0.95	1.605×10^6 ($\pm 0.28\%$)	33

Table S17. Molecular volume, scattering length, and scattering length density values for block copolypeptides and H₂O.

ELP	Molecular volume (nm ³)	Scattering length (nm)	Scattering length density (nm ⁻²)
SVT _{SVS}	62.9	7.31×10^{-2}	1.16×10^{-3}
SVD _{SV}	62.9	7.31×10^{-2}	1.16×10^{-3}
H ₂ O	3.0×10^{-2}	2.82×10^{-5}	9.40×10^{-4}

The form factor for a core-shell spherical micelle was developed by Gerstenberg and Pedersen,⁵ and consists of four contributions: the self-correlation of the micelle core, the self-correlation of the chains that form the corona, cross-correlation between the core and chains, and cross-correlation between different chains.

$$P(q) = N_{agg}^2 \beta_s^2 P_s(q) + N_{agg} \beta_c^2 P_c(q) + 2N_{agg}^2 \beta_s \beta_c S_{sc}(q) + N_{agg}(N_{agg} - 1) \beta_c^2 S_{cc}(q) \quad (S4)$$

where N_{agg} is the aggregation number of the micelle, β_s and β_c are the scattering lengths of the spherical core and corona chains, respectively. P_s and P_c are the form factors of the spherical core

and corona chains, S_{sc} is the cross-correlation between the spherical core and chains, and S_{cc} is the cross-correlation between the chains.

The form factor of the spherical core is given by:

$$P_s(q) = \Phi^2(qR) \quad (S5)$$

where

$$\Phi(qR) = \frac{3[\sin(qR) - qR\cos(qR)]}{(qR)^3}. \quad (S6)$$

Corona chains with radius R_g have a self-correlation term as follows:

$$P_c(q) = \frac{2[\exp(-x) - 1 + x]}{x^2} \quad (S7)$$

which = $q^2 R_g^2$.

Gerstenberg and Pedersen mimicked non-penetration of the corona chains into the core by modeling them to start at distance R_g from the surface of the core. Thus:

$$S_{sc}(q) = \Phi(qR)\varphi(qR_g) \frac{\sin(q[R+dR_g])}{q[R+dR_g]}. \quad (S8)$$

The function $\varphi(x) = [1 - \exp(-x)] / x$ is the form factor of a Gaussian polymer chain. The interaction term between the chains is:

$$S_{cc}(q) = \varphi^2(qR_g) \left[\frac{\sin(q[R+dR_g])}{q[r+dR_g]} \right]^2. \quad (S9)$$

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