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Predicting Transition Temperatures of Elastin-Like Polypeptide Fusion Proteins

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Abstract

Elastin-like polypeptides (ELPs) are thermally sensitive peptide polymers that undergo thermally triggered phase separation and this behavior is imparted to soluble proteins when they are fused to an ELP. The transition temperature of the ELP fusion protein is observed to be different than that of a free ELP indicating that the surface properties of the fused protein modulate the thermal behavior of ELPs. Understanding this effect is important for the rational design of applications that exploit the phase transition behavior of ELP fusion proteins. We had previously developed a biophysical model that explained the effect of hydrophobic proteins on depressing the transition temperature of ELP fusion proteins relative to free ELP. Here, we extend the model to elucidate the effect of hydrophilic proteins on the thermal behavior of ELP fusion proteins. A linear correlation was found between overall accessible surface residue composition of a protein weighted by a characteristic transition temperature for that residue and the difference in transition temperatures between the ELP protein fusion and the corresponding free ELP. In breaking down the contribution of residues to polar, non-polar and charged, the model revealed that charged residues are the most important parameter in altering the transition temperature of an ELP fusion relative to the free ELP.

Keywords

Elastin-like polypeptides; ELP fusion proteins; fusion effect; transition temperature; accessible surface area

Introduction

Fusions of an elastin-like polypeptide (ELP) to a target protein at the gene level enable their simple purification by a simple, inexpensive, non-chromatographic batch technique developed in our laboratory, termed Inverse Transition Cycling (ITC) ^{1–4}. ITC relies on the phase behavior of ELP fusion proteins which stems from the thermally triggered demixing behavior of the ELP domain. ELPs are repetitive polypeptides composed of multiple repeats of the pentameric motif Val-Pro-Gly-Xaa-Gly where Xaa is the guest residue and can be any amino acid except proline^{5–7}. At a critical temperature, the ELP undergoes a reversible phase transition within a very narrow temperature range (2–3 °C) in aqueous solvents; below

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Supporting Information Available. Additional data on temperature-programmed turbidimetry and light scattering data and analysis is available. This information is available free of charge via the Internet at <http://pubs.acs.org>.

this transition temperature (T_t) ELPs are structurally disordered, highly solvated and soluble in aqueous solution. However, when the solution temperature is increased and the T_t is reached, the polymers collapse and coalesce resulting in the formation of large, micron sized aggregates, as visually seen by the change in turbidity of sufficiently concentrated solutions. The phase transition of ELPs and their fusion proteins can also be isothermally triggered by depressing the T_t below solution temperature by the addition of kosmotropes from the Hofmeister series^{8–11}.

In addition to external factors such as concentration and ionic strength, ELP transition temperatures are controlled by the composition and length of an ELP. As compared to synthetic polymers, recombinant synthesis of ELPs from a synthetic gene enables precise control over composition and length at the molecular level. The T_t can be tuned by the composition and mole fraction of the guest residue; hydrophobic amino acids lower the T_t while polar and charged residues raise the T_t . The molecular weight (MW) of the ELP is the second molecular parameter that also controls the T_t ; longer ELPs have lower transition temperatures compared to shorter ELPs with the same composition.

While optimizing the ITC purification process, we observed that different target proteins altered the phase transition temperature of the fused ELP compared to the free ELP and sought to understand this behavior in greater detail for two reasons^{1, 4, 12}. First, a quantitative understanding of this effect would allow *a priori* prediction of the phase transition temperature of new ELP fusion proteins, so that the ITC purification process could be rationally optimized for each ELP fusion protein. Second, a useful corollary to understanding this behavior is that it would enable rational development of stimulus responsive molecular switches in which the ELP phase transition can be triggered by changes in the surface properties of a fused protein via a molecular binding event.

The observed change in T_t was termed the fusion $\Delta T_{t,fusion}$ effect and is defined in Equation 1.

$$\Delta T_{t,fusion} = T_{t,protein-ELP} - T_{t,ELP} \quad (1)$$

where $T_{t,protein-ELP}$ and $T_{t,ELP}$ are the transition temperatures for the ELP fusion protein and the free, un-fused ELP, respectively. Previously, we have shown that the T_t of an ELP fusion protein is depressed in proportion to the fraction of exposed non-polar area of the surface of the folded target protein⁴. However, our earlier model could only explain the fusion $\Delta T_{t,fusion}$ effect for relatively hydrophobic target proteins that depressed the T_t of the fusion relative to the ELP (negative $\Delta T_{t,fusion}$ effect). In several instances, we have observed that fusion can *increase* the T_t of the ELP fusion relative to the ELP— which we term the positive $\Delta T_{t,fusion}$ effect. This study expands the model to include target proteins with a wider range of hydrophathy, extending from relatively hydrophobic to hydrophilic and encompasses values of $\Delta T_{t,fusion}$ that range from negative to positive. The results of this study have implications for the rational design of ELP fusion proteins that exploit the phase transition behavior of the ELP.

Materials and Methods

Materials

Expression vectors pET25b, pET24d, and pET32b, bacteria strain BLR(DE3), and thrombin were purchased from Novagen, Inc. (Milwaukee, WI); bacteria strain BL21(DE3) was from EdgeBio (Gaithersburg, MD); restriction nucleases were from New England Biolabs (Beverly, MA). DNA plasmids were purified using the QIAGEN, Inc. (Valencia, CA) spin

miniprep and gel purification systems. The barstar gene was purchased from Integrated DNA Technologies (Coralville, IA). Cultures were grown in Terrific Broth (TB) media from MoBio Laboratories (Carlsbad, CA). Precast SDS-PAGE Mini-PROTEAN 4–20 % Tris/HCl gels were from Bio-Rad (Hercules, CA).

Gene Synthesis and Protein Expression

The ELP used in all fusions is a 36 kDa peptide with 90 pentapeptide repeats where the guest residue composition is Val, Ala, Gly at a ratio of 5:2:3, respectively (ELP[V₅A₂G₃-90]). ELP gene synthesis has been described earlier². The synthesis of genes for CAT-ELP, BFP-ELP, IL1Ra-ELP, Trx-ELP, ELP-Trx, and Trx-ELP-tendamistat has been reported previously^{1, 3, 4, 13, 14}. The barstar-ELP gene was synthesized containing NdeI and SalI sites at the 5' and 3' ends, respectively. Both the vector containing the barstar gene and the pET25b vector (already containing the ELP gene) were digested with NdeI and SalI, and the barstar DNA insert was gel purified. After digestion, the pET25b vector containing the ELP gene was treated with calf intestinal alkaline phosphatase (CIP) before it was purified on a spin column. The DNA containing the barstar gene was ligated into the pET25b vector containing the ELP gene and transformed into BL21(DE3) cells. All ELP fusion protein vectors were sequenced.

Each fusion protein were expressed in *E. coli* BLR(DE3) (BFP-ELP, CAT-ELP, IL1Ra-ELP, Trx-ELP, ELP-Trx, and Trx-ELP-tendamistat) or BL21(DE3) (barstar-ELP). All ELP fusion proteins (except for barstar-ELP) were grown in 1 L TB media supplemented by either 100 µg/ml ampicillin or 45 µg/ml kanamycin. The 1 L cultures were each inoculated with 10 mL from a 50 mL overnight starter culture (overnight starter cultures were inoculated from frozen DMSO stocks stored at –80 °C). Barstar-ELP expressed in higher yields from 50 mL cultures. Barstar-ELP expressed in BL21 cells was induced after 6–7 hours of growth with 1 mM IPTG (final concentration). The rest of the cultures were not induced³. These cultures were grown for 24 h at 37 °C, after which the cells were harvested by centrifugation at 4 °C for 15 min. Each bacterial pellet was resuspended in ~35 mL PBS buffer and frozen at –80 °C. One exception was barstar-ELP; the pellets from 50 mL growth were resuspended in 3 mL PBS and purification of the fusion protein was initiated right after harvesting the cells. Barstar cold denatures and so was not stored in pellet form at –80 °C. In general, cell pellets were thawed and lysed by ultrasonic disruption (Sonicater 3000, Misonix, Farmingdale, NY) on ice. Two mL of a 10 % poly(ethyleneimine) solution was added to the lysed cell suspension per 1 L of growth. Insoluble cell debris was separated from the soluble fusion protein by centrifugation at 16120 *g* for 15 min.

All ELP fusion proteins were purified by ITC using NaCl or (NH₃)₂SO₄ to trigger the phase transition at room temperature. In the first round of ITC, NaCl was added to the soluble cell lysate to a final concentration of 3 M (5 M for ELP-Trx, 0.5 M (NH₃)₂SO₄ for barstar-ELP) before centrifugation at room temperature. The pellets were resuspended in cold PBS buffer (typically 4–6 mL) and transferred to eppendorf tubes before the cold spin. The supernatant from the cold spin was transferred to clean eppendorf tubes. These two steps constitute one round of ITC. In the following rounds of ITC, saturated NaCl in water (or (NH₃)₂SO₄ in the case of barstar-ELP) was added drop wise to the tubes until the phase transition was induced. Five to six rounds of ITC were typically carried out and the final resuspension volumes would be 500–1000 µL depending on the concentration of the fusion protein. After purification, barstar-ELP was stored at 4 °C, while the other fusion proteins were stored at –20 °C.

Purity of the proteins was verified on SDS-PAGE (data not shown). Tendamistat was expressed as a double fusion with Trx (Trx-ELP-tendamistat); expression of tendamistat as a single fusion is poor and, because of the very hydrophobic nature of the protein, the pellets

obtained during the ITC purification process are difficult to resuspend. Therefore, Trx was fused as the N-terminal protein to overcome these problems¹⁴. A Trx-ELP-tendamistat construct was engineered with a thrombin cleavage site between Trx and the ELP. The Trx-ELP-tendamistat ternary fusion was purified by ITC, and after ITC purification the double fusions were proteolytically cleaved by thrombin followed by one more round of ITC leaving the products Trx-ELP and tendamistat-ELP. Because tendamistat is a very hydrophobic protein, in the first rounds of ITC, the pellets containing the double fusion obtained from the hot spins were resuspended in ionized water instead of buffer. Free ELP was obtained by thrombin cleavage of BFP-ELP followed by two rounds of ITC to separate the ELP from BFP and thrombin.

Concentrations of purified fusion protein were measured on a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE) corresponding to absorbance at 280 nm using the following extinction coefficients: Free ELP, $5690 \text{ M}^{-1}\text{cm}^{-1}$; Trx-ELP and ELP-Trx, $1.975 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$; BFP-ELP, $2.418 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$; CAT-ELP, $4.95 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$; ELP-tendamistat, $1.361 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$; IL1Ra-ELP, $2.231 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$; and barstar-ELP $2.66 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$. The extinction coefficients for all fusion constructs were estimated from the method of Gill and von Hippel¹⁵. The number of disulfide bonds were found in the PDB files; 2trx (Trx), 1gfl (BFP), 1pd5 (CAT), 1ok0 (tendamistat), 1ilr (IL1Ra) and 1a19 (barstar).

The primary sequences of the target proteins fused to the ELP were compared to those of the crystal structures used to calculate the extinction coefficients and the SI. In most cases the sequences were identical or deviated by one or two amino acids. However the primary sequence corresponding to the crystal structure of BFP was somewhat different from our BFP sequence, our BFP sequence was more similar to that corresponding to the GFP crystal structure. Therefore the PDB file for GFP was used to calculate the extinction coefficient and the SI for BFP.

Turbidity Measurements

The transition temperatures were measured by measuring the turbidity at 350 nm in the temperature range between 15–90 °C using a scan rate of 1 °C per minute. The data were collected on a Cary 300 Bio UV-Visible spectrophotometer equipped with a multi-cell thermoelectric temperature controller from Varian (Palo Alto, CA). The fusion protein concentrations for the model studies were 25 μM in PBS buffer. Three repeats were performed and averaged. To distinguish between ELP phase transition and target protein unfolding and aggregation turbidity profiles were carried out at different fusion protein concentrations between 2–30 μM . The phase transition temperatures were determined as the temperature corresponding to the maximum of the first derivative of the turbidity versus temperature.

Computer Modeling to Calculate the Surface Indices

Explicit hydrogen atoms were added to protein data bank (PDB) molecular structure files, and the orientations of resulting OH, SH, NH_3^+ , Met methyl groups, Asn and Gln side chain amides, and His rings were optimized using the program REDUCE (<http://molprobability.biochem.duke.edu/>). The amino acid composition of the solvent accessible surface area was calculated using the program PROBE^{16, 17}. We assume that the ELP does not interfere with the ASA of the target proteins. Briefly, a probe sphere having a 1.4 Å radius, corresponding to the molecular size of a water molecule, was virtually rolled over the outside surface of the reduced PDB structure and a contact dot was placed on the atomic surface of the PDB structure where the probe contacted the van der Waals surface of the protein without simultaneously intersecting the van der Waals surface of another atom. The

fraction of solvent accessible surface area attributable to each of the 20 amino acids was calculated from the ratio of the number of dots placed on each type of amino acid relative to the total number of dots on the whole protein structure. PDB files used were 2trx (Trx), 1gfl (BFP), 1pd5 (CAT), 1ok0 (tendamistat), 1ilr (IL1Ra) and 1a19 (barstar).

Results and Discussion

ELP Fusion Protein Constructs

The ELP chosen for fusion to the target proteins consists of 90 repeats of a VPGXG repeat unit, and the guest residue at the fourth position (X) is a mixture of Val, Ala, and Gly at a ratio of 5:2:3, respectively, herein referred to as ELP[V₅A₂G₃-90]. Six proteins were included in this study: blue fluorescent protein (BFP), chloramphenicol acetyl transferase (CAT), barstar, interleukin 1 receptor antagonist (IL1Ra), thioredoxin (Trx), and tendamistat. The architecture of the ELP fusion proteins in this study including their fusion order and the sequence of the linkers between the target proteins and the ELP are summarized in Supplementary Figure S1. Because the linkers connecting the target proteins and the ELP vary in these constructs, three of the fusions contain the same target protein Trx and the same ELP but different linker sequences to test the role of the linker.

All ELP fusion proteins were purified by ITC. During the ITC purification process, the proteins were cycled through their soluble and insoluble phases by adding sodium chloride or ammonium sulfate, to isothermally trigger their phase transition^{1-4, 12, 13}. The micron-sized aggregates were separated from contaminants by centrifugation followed by dissolution of the ELP fusion protein-rich pellets in low salt buffer. The purity of each ELP fusion protein was verified by SDS-PAGE (data not shown).

Transition Temperatures of ELP Fusion Proteins

The increase in turbidity resulting from the phase transition was followed spectrophotometrically at 350 nm as a function of temperature for free ELP and for ELP fusion proteins. Figure 1A shows a turbidity profile as a function of solution temperature for CAT-ELP and free ELP; these turbidity profiles are typical of all the ELP fusion proteins studied herein. The T_s were determined as the maximum of the first derivative of turbidity with respect to temperature. The experimentally measured T_s and $\Delta T_{t,fusion}$ s, determined at a concentration of 25 μ M of ELP fusion protein, are listed in Table 1.

The observed increase in turbidity with increasing temperature can arise from two sources: unfolding of the target protein followed by irreversible aggregation or the ELP phase transition. To avoid the artifact of thermal denaturation and aggregation of the ELP fusion proteins, we only heated each protein to a maximum temperature below its melting temperature (Table S1 in Supplementary Material). In addition, the transition temperatures were measured for the all ELP fusion proteins at different protein concentrations and a significant change in T_t is observed (Fig. 1B). Whereas the ELP phase transition from monomer to large, micron-sized aggregates is concentration dependent, most proteins show no concentration dependence on temperature-induced unfolding and aggregation, indicating that the observed aggregation is due to the phase transition of the ELP rather than unfolding of the protein. Two fusion proteins IL1Ra-ELP and Trx2-ELP showed small, temperature independent increases in turbidity before the larger, temperature dependent formation of aggregates (Figure S2 in Supplementary Materials). However, because the second transition was concentration dependent and the melting temperatures for the two target proteins were significantly higher than the measured T_b , both ELP fusion constructs were included in the study.

The “Surface Index versus $\Delta T_{t, \text{fusion}}$ Effect” Model

Next, we developed an analytical model from which the T_t of an ELP fusion protein can be predicted for a protein with a known crystal structure. The model uses two parameters: 1) the summed solvent-exposed surface area of each amino acid from the crystal structure of the folded protein and 2) the characteristic transition temperature (T_{tc}) for that amino acid, as measured by Urry and co-workers. To determine the T_{tc} of the 20 naturally occurring amino acids, Urry and coworkers synthesized and measured the T_t of a set of ELPs with an increasing ratio of each residue to valine at the guest residue (X) position in the VPGXG motif. By extrapolation to 100 % substitution, a characteristic transition temperature for each amino acid was calculated, thus providing an ELP-based hydrophobicity scale for the 20 naturally occurring amino acids (Table S2 in Supplementary Material)^{6, 7, 18, 19}.

In this model, we further distinguish between polar and non-polar amino acids based on the guest residue composition of the ELP fused to the target proteins. The guest residue composition of the ELP used in this study was Val, Ala, and Gly at a ratio of 5:2:3, so the weighted T_{tc} average of the guest residues in this ELP was 37.7 °C. Amino acids that have a T_{tc} below 37.7 °C (the calculated hydrophobicity of the ELP) on the Urry hydrophobicity scale were classified as non-polar; these are: Cys, Val, Met, Ile, Leu, His, Pro, Phe, Tyr, and Trp. From the remaining residues with T_{tc} higher than 37.7 °C, Gln, Gly, Ser, Thr, Asn, and Ala were classified as uncharged polar, and are simply referred to as polar (p) in the remainder of the paper. For ionizable residues, the T_{tc} was selected for the dominant charge state at pH 7.4, the pH at which the T_t of the ELP fusion proteins was measured. Hence, in the case of the carboxylic acids Asp and Glu with pK_a values of 3.9 and 4.3, respectively, the charged deprotonated state was chosen as their pK_a s are far removed from the working pH of 7.4²⁰. Lys and Arg, which have pK_a values of 10.4²⁰ and ~12²¹, respectively, are protonated at pH 7.4. Histidine with a pK_a value of 6.0 is also partially protonated at pH 7.4 and T_{tc} for both states were reported by Urry and coworkers; therefore, the characteristic transition temperature for that amino acid was calculated as a weighted average between the protonated and deprotonated T_{tc} s using the Henderson-Hasselbalch equation²². Based on these considerations, Glu, Asp, Lys, Arg, were classified as charged.

Next, the accessible surface area (ASA) of solvent accessible residues for the eight target proteins were calculated using the program PROBE¹⁷ using crystal or NMR structures deposited in the Protein Data Bank. The PDB files used are reported in the Materials and Methods section below. A virtual ball corresponding to the size of a water molecule (1.4 Å radius) was rolled over the van der Waal's surface of the protein and a dot was placed each time the ball contacted the surface of only a single atom. Hence the number of dots placed on each amino acid was used as a measure of the accessible surface area for that particular residue. The total number of dots was summed over all the exposed residues to determine the total solvent accessible surface area of the protein. The number of dots on each amino acid and the total number of dots for each target protein are shown in Table S3 (Supplementary Material). A visual representation of the dots generated for each of the six proteins is shown in Figure S3 in the Supplementary material.

We defined a Surface Index (SI) for each protein (Equation 2), as follows:

$$SI = \sum_{Xaa} \left(\frac{ASA_{Xaa}}{ASA_p} T_{tc} \right) \quad (2)$$

where ASA_{Xaa} and ASA_p correspond to the accessible surface areas measured in dots for a particular amino acid Xaa and that of the protein (p), respectively. Using Equation 2, four different SI's were determined: (1) SI for the total surface of the protein (SI_{total}), (2) non-polar (SI_{np}), (3) polar (SI_p), and (4) charged (SI_c) wherein the last three SI's were

determined using the specific classification of amino acids as non-polar, polar and charged, relative to the hydrophobicity of the ELP, as discussed previously. The results are shown in Table 2.

The $\Delta T_{t,fusion}$ (Table 1) obtained from each fusion protein were plotted *versus* the SI_{total} values calculated for the respective target proteins (Table 2) and the graph is shown in Figure 2. We found a linear correlation with $R^2 = 0.95$ as given by the following equation: $\Delta T_{t,fusion} = -56.4 + 0.75 \times SI$.

Breaking Down the Contributions to the Surface Index

We next sought to determine the relative contribution of different classes of residues –non-polar, polar and charged– to the correlation between SI_{total} and $\Delta T_{t,fusion}$. Therefore, SI_{total} was divided into its non-polar, polar, and charged SI components, as shown in Table 2 and the $\Delta T_{t,fusion}$ was plotted as a function of SI_{np} , SI_p , and SI_c (Figure 3). Interestingly, no correlation is observed between the non-polar or the polar residues and the fusion $\Delta T_{t,fusion}$ effect. Instead, we found that the contribution to SI_{total} arises almost exclusively from the four charged amino acids: Glu, Asp, Lys, and Arg.

Our previous model explained the depression in the T_t of an ELP fusion protein as compared to free ELP for predominantly hydrophobic target proteins; however, it could not account for the positive fusion $\Delta T_{t,fusion}$ effect⁴. We speculated that analogous to elevating the T_t in free ELPs by introducing more hydrophilic amino acids at the guest residue position, there is likely a parallel mechanism by which hydrophilic or charged residues on the surface of a target protein in an ELP fusion elevate the T_t , resulting in the observed positive fusion $\Delta T_{t,fusion}$ effect. Therefore, we define herein a Surface Index (SI) where the calculated accessible surface area contribution of each amino acid was weighted by the residue specific characteristic transition temperatures – T_{tc} – measured by Urry and coworkers. Using this definition of SI, we find a clear correlation between the SI and the fusion $\Delta T_{t,fusion}$ effect that encompasses proteins that range from predominantly hydrophobic to hydrophilic in terms of their solvent accessible residues. However, more than polarity of solvent accessible residues, we find that the charged residues Glu, Asp, Lys, and Arg are by far the largest contributors to the total SI (Figure 3C) and are the single most important predictors of the elevation in T_t . This is because three of the charged residues Glu, Asp, and Lys have large T_{tc} s (Table S2, Supplementary Material), so that even though these residues do not have an unusually large solvent accessible surface area as compared to other residues, their large T_{tc} s result in a significant contribution to the SI.

In assessing the value of this model, it is important to understand its limitations. First, the model is based on fusions to ELP[Val₅Ala₂Gly₃-90], so that it does not allow quantitative prediction of the effect of fusion to other ELPs. Nevertheless, even when fused to different ELPs, this model will allow a semi-quantitative prediction of effect of fusion of a protein to an ELP, in that it will predict whether the T_t of the fusion is likely to be higher or lower than that of the parent ELP, which in itself is useful information. Second, the model was developed for a single concentration of ELP fusion protein. Both of these limitations can, and will be addressed in future work where we plan to include the effect of ELP composition and concentration on the $\Delta T_{t,fusion}$ similar to our efforts in quantifying the effect of these variables on the T_t of ELPs²³.

Conclusions

We have developed a simple biophysical model that explains the effect of protein fusion on the phase transition behavior of ELP fusion proteins. A linear relationship was found between the difference in T_t of the ELP fusion protein and free ELP, and a surface index

defined as the solvent accessible surface area fraction of each amino acid weighted by a characteristic T_f for that amino acid. The model predicts that the solvent accessible charged area on the protein is the most important parameter in altering the transition temperature of an ELP fusion protein, relative to that of the free ELP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Turbidity profiles of 25 μ M CAT-ELP ($\square$) and free ELP ($\times$) in PBS at pH 7.4. The turbidity traces show significant differences in the T_m of the ELP fusion protein and the free ELP. (B) The T_m as a function of ELP protein fusion concentration.

Figure 2.
 $\Delta T_{t, fusion}$ as a function of SI_{total} .

Figure 3.

$\Delta T_{t,fusion}$ versus SI_{np} , SI_p , and SI_c (A, B, and C, respectively). No correlation between the fusion effect and SI_{np} and SI_p are found whereas a strong correlation between $\Delta T_{t,fusion}$ versus SI_c is observed ($\Delta T_{t,fusion} = -47.4 + 0.77 \times SI$ with $R^2 = 0.93$). Glu, Asp, Lys, and Arg are the most important amino acids to predict the phase transition temperature of ELP protein fusions.

Table 1

The T_t and the $\Delta T_{t,fusion}$ of ELP fusion proteins. All measurements were carried out in PBS at a concentration of 25 μ M of ELP fusion protein or ELP (control).

Target Protein	T_t ($^{\circ}$ C)	$\Delta T_{t,fusion}$ ($^{\circ}$ C)
Free ELP	50.0 ± 0.4	N/A
BFP	51.1 ± 0.5	1.1 ± 0.1
CAT	38.3 ± 0.8	-11.7 ± 0.3
Barstar	59.4 ± 0.4	9.4 ± 0.1
IL1Ra	48.0 ± 0.3	-2.0 ± 0.1
Trx1	54.0 ± 0.5	4.0 ± 0.1
Trx2	58.1 ± 0.8	8.1 ± 0.1
Trx	56.4 ± 0.4	6.4 ± 0.1
Tendamistat	35.6 ± 0.7	-14.4 ± 0.3

Table 2

The Surface Index has been divided into non-polar (SI_{np} : Cys, Val, Met, Ile, Leu, His, Pro, Phe, Tyr, and Trp), polar (SI_p : Gln, Gly, Ser, Thr, Asn, and Ala), and charged (SI_c : Glu, Asp, Lys, Arg) contributions. The PDB files used to calculate the Surface Indices are listed in Materials and Methods.

Target Protein	SI_{total} (°C)	SI_{np} (°C)	SI_p (°C)	SI_c (°C)
BFP	80.4	−2.2	15.6	67.0
CAT	60.9	−8.8	20.4	49.2
Barstar	87.1	−6.9	17.8	76.2
IL1Ra	76.7	1.1	19.7	55.9
Trx1	82.4	−1.9	17.0	67.3
Trx2	82.4	−1.9	17.0	67.3
Trx	82.4	−1.9	17.0	67.3
Tendamistat	54.5	−6.3	18.0	42.9