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Elastin-like Polypeptide Diblock Copolymers Self-Assemble into Weak Micelles

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Abstract

The self-assembly of synthetic diblock copolymers has been extensively studied experimentally and theoretically. In contrast, self-assembly of polypeptide diblock copolymers has so far been mostly studied experimentally. We discovered that the theory developed for synthetic diblock copolymer does not fully explain the self-assembly of elastin-like polypeptide diblock copolymers, leading us to generalize the theory to make it applicable for these polypeptides. We demonstrated that elastin-like polypeptide diblocks self-assemble into weak micelles with dense cores and almost unstretched coronas, a state not previously observed for synthetic diblock copolymers. Weak micelles form if the surface tension at the core–corona interface is low compared to that expected of a micelle with a dense core. The predictions of the theory of weak micelles for the critical micelle temperature, hydrodynamic radius, and aggregation number of elastin-like polypeptide diblocks are in reasonable agreement with the experimentally measured values. The unique and unprecedented control of amphiphilicity in these recombinant peptide polymers reveals a new micellar state that has not been previously observed in synthetic diblock copolymer systems.

Graphical abstract

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Supporting Information

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Notes

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1. INTRODUCTION

Peptide polymers are an important class of biomaterials for a multitude of biomedical applications, such as drug delivery, protein separation, biosensors, and tissue engineering.^{1–6} Many examples of hydrogels, vesicles, and micelles have been engineered from this class of materials for various applications.^{2,7–13} A quantitative understanding of the self-assembly behavior of peptide polymers is an important asset in the intelligent design of these materials. Herein, we focus on diblock copolymers of elastin-like polypeptides (ELPs) that display temperature-triggered self-assembly into spherical micelles^{1,7,14,15} (Figure 1). ELPs are composed of the pentapeptide repeat [valine-proline-glycine-X-glycine]_n, where X is a guest residue that can be any amino acid except proline and *n* is the number of pentapeptide repeats.^{16,17} These polypeptides exhibit lower critical solution temperature (LCST) behavior in aqueous solutions, such that they phase separate upon heating above their cloud point (otherwise referred to as the transition temperature, *T*_l). The phase separation is sharp and reversible, as the sediment formed above the cloud point dissolves upon cooling the solution below the cloud point. At a given solution composition, the cloud point for a given ELP sequence depends on the number of repeats, *n* (i.e., the length of the chain), and the guest residue composition, *X*.^{16–20} ELPs with longer chains and more hydrophobic guest residues have lower cloud points.

The unique feature of these peptide polymers is the ability to synthesize them by expression in *Escherichia coli* from a synthetic gene,^{21,22} so that these recombinant polymers have a precise molecular weight and composition that can be specified *a priori*, and as such are perfectly monodisperse. Genetically encoded synthesis also enables the construction of architectures such as diblocks that self-assemble into micelles by imparting amphiphilicity through block composition—i.e., choice of the guest residue—and block lengths. Two features differentiate ELP diblock copolymers from their synthetic counterparts that provide a strong motivation to develop a theoretical understanding of their self-assembly behavior: first, the change in composition between the hydrophilic and hydrophobic block is fine-tuned with a precision that is simply not possible with synthetic polymers, as only the guest residue within the pentapeptide needs to be varied to impart amphiphilicity. The

hydrophobic block shares with the hydrophilic block the same four invariant repeating amino acids (V, P, and G in the VPGXG repeat) within the pentapeptide repeat unit. In addition, for the set of ELP diblocks considered here, the hydrophilic and hydrophobic block guest residue composition both share a guest residue, valine, which is present in different percentages in the two blocks, thereby imparting amphiphilicity to the polymer. Second, ELPs are polypeptides and hence have the propensity to form secondary structures as they undergo their phase transition, which could add another energetic component to the free energy as well as result in a change in the rigidity of the chain for both blocks with increasing temperature.^{23,24}

To develop a theoretical framework of their temperature triggered self-assembly that would enable the rational, *a priori*, design of ELP micelles with desired properties, we first tested the applicability of a self-assembly theory developed for synthetic diblocks on these peptide polymers.^{25–27} This theoretical model had been previously developed and applied to describe the micellization of polystyrene–polyisoprene (PS–PI) diblocks in heptane. The PS–PI diblock micelles were found to have stretched corona and core blocks as expected for strong micelles, and the theoretical predictions were found to be in good agreement with experimentally determined values of micelle radii and aggregation numbers.²³ This theoretical framework was used at the outset of this study to predict the properties of micelles formed from ELP diblocks and to compare them to the experimentally determined values. In particular, for a chosen set of ELP diblock samples we measured (i) the critical micelle temperature (CMT) above which ELP diblocks self-assemble into micelles, (ii) the hydrodynamic radius (R_h), and (iii) the aggregation number Q (i.e., number of unimers per micelle). We separately measured the parameters used in the theoretical model: thermodynamic quality of the solvent for the corona segment of the ELP diblock and the surface tension of the core segment related to the tension of the core–corona interface of the micelle. The solvent quality for the corona block was derived from experimental measurements of the second virial coefficient (A_2) of the hydrophilic ELP monoblock in a dilute solution, which was determined by static light scattering. The surface tension of the hydrophobic block was obtained from the temperature–concentration phase diagrams of solutions of hydrophobic ELP monoblocks with different molecular weights that were determined by measurements of turbidity as a function of temperature.

We found, unexpectedly, that the theory developed for synthetic diblock copolymers failed to describe the physical properties of ELP micelles. This prompted us to re-examine the underlying assumptions of the model and to generalize it to incorporate novel states of spherical micelles with unstretched corona and core blocks that we call weak micelles. Our theory predicts that weak micelles emerge in ELP diblock copolymers because of their composition and sequence peculiarities that considerably decrease the core–corona surface tension γ as compared to the values expected from their relatively high core density.

This paper is organized as follows: we start with a description of the theoretical model and delineate the diagram of self-assembly states of diblock copolymers. We demonstrate that the regime of weak micelles appears in the diagram of states only if the surface free energy per area of a diblock copolymer is smaller than kT . We then specify the model parameters,

predict the critical micelle temperature (CMT), hydrodynamic radius (R_h) of the micelle, and the aggregation number (Q), and compare these predictions to the experimental data.

2. THEORETICAL MODEL OF DIBLOCK SELF-ASSEMBLY: STRONG AND WEAK MICELLES

Below, we present a generalization of the theory of diblock copolymer self-assembly based on the previously described scaling models.²⁵ A conventional model envisions a micelle as a self-assembled aggregate with a denser core composed of hydrophobic blocks B with N_B monomers, and a solvated corona composed of hydrophilic blocks A with N_A monomers. The solvent is theta or good for the hydrophilic block A and poor for hydrophobic block B. The corona of the micelle is envisioned as a solvated brush of hydrophilic blocks A. The monomers of the insoluble hydrophobic block prefer to interact with each other more than with the solvent molecules. Therefore, these blocks prefer to associate with each other forming the core of the micelle. In classical models of diblock copolymer micelles, the chains in the core and the corona are noticeably extended with respect to their Gaussian sizes, and these assemblies are called strong micelles.

An approximation that the core–corona interface is narrow compared to both the radius of the core and thickness of the corona allows the total free energy (F) of the micelle to be expressed as the sum of three contributions: the corona, the interface, and the core free energies.

$$F = F_{\text{corona}} + F_{\text{interface}} + F_{\text{core}} \quad (1)$$

The balance of the three contributions to the free energy determines the properties of the micelles. Typically, the variations in the corona free energy and the interface free energy are the two dominant contributions that determine the aggregation number and the radius of micelles formed by synthetic polymers. In this strong micelles scenario the equilibrium micelles have extended corona and core blocks. The elastic free energy of the core determines the transitions between the various micelle morphologies: spherical to cylindrical to lamellar.²³

We define effective symmetric monomers for the hydrophilic block A, a_A , and hydrophobic block B, a_B , which are repeated N_A and N_B times in each block, respectively. We approximate a section of the chain with molecular weight M by a parallelepiped with contour length L and square cross-sectional area a^2 . By using the relationship between mass density, ρ , molecular mass and volume, the width a of cross-sectional area of the chain can be calculated as

$$a = \sqrt{\frac{M}{\rho N_{\text{av}} L}} \quad (2)$$

where N_{av} is Avogadro's number.

The dense core comprises the hydrophobic blocks B with almost constant volume fraction ϕ_B of monomers B. The value of ϕ_B is governed by the balance of attractive and repulsive

interactions between monomers in the interior of the core. For cores with small volume fraction ($\phi_B \ll 1$), the core constitutes a melt of thermal blobs with size $\xi_t \sim a_B/\phi_B$ and attraction energy density is kT per thermal blob, $kT/\xi_t^3 \sim \phi_B^3/a_B^3$. The surface tension at the core–corona interface is approximated as kT per thermal blob area, kT/ξ_t^2 due to the small concentration of monomers A at the core–corona interface (i.e., we assume pure block B at solvent boundary). As a result, the surface tension γ at the core–corona interface and volume fraction ϕ_B in the core of a micelle are coupled as $\gamma a_B^2/kT \simeq \phi_B^2$. This coupling is valid for small volume fractions $\phi_B \ll 1$, while for a dense core with $\phi_B \sim 1$ this scaling may not be valid. Furthermore, for block copolymers composed of complex amphiphilic monomers (as is the case with the pentapeptides comprising ELPs), the bulk monomer–monomer interactions—that govern the volume fraction ϕ_B inside the core—could differ from those at the core–corona interface where these monomers could exhibit surfactant-like properties.

The amphiphilicity of complex monomers could lead to a significant reduction in surface tension γ , resulting in novel states of micelles. If the core volume fraction ϕ_B and core–corona surface tension γ are decoupled ($\gamma a_B^2/kT \ll \phi_B^2$), then weak micelles with relatively dense cores and almost unstretched corona blocks emerge in the solution. The corona of such micelles comprises of weakly overlapping blocks A with free energy per corona block $F_{\text{corona}} \simeq kT$. We distinguish them from strong micelles with extended corona blocks ($F_{\text{corona}} \gg kT$). Below, we discuss the various states of self-assembly of diblock copolymers (see diagram in Figure 2).

2.1. Strong Micelles

The theory of strong micelles has been previously discussed in detail,²⁵ and here we only briefly review the basic results of the model. The aggregation number Q of strong micelles is determined by the balance between surface free energy at the core–corona interface which favors larger values of Q and the repulsive monomer–monomer interactions in the corona that become stronger with increasing Q and therefore favor lower values of Q . As a result, the equilibrium parameters of a strong micelle are governed by the balance $dF_{\text{interface}}/dQ = -dF_{\text{corona}}/dQ$. Equivalently, the interface and corona free energies per chain are both much larger than thermal energy and balance each other $F_{\text{interface}} \sim F_{\text{corona}} \gg kT$. The core free energy F_{core} comprises the contribution of attractive interactions between monomers (which scales with N_B similarly in unimer and micellar states and does not change upon micellization and therefore is not taken into consideration) and the elastic contribution $F_{\text{core,elastic}}$ due to stretching of blocks B in the core of the micelle. Typically, $F_{\text{core,elastic}}$ is noticeably smaller than F_{corona} . These strong micelles are thermodynamically stable in region III of the diagram of states presented in Figure 2. Depending on the ratio of the corona thickness, H , to the core radius, R , spherical micelles are distinguished as star-like ($H/R \gg 1$) or crew-cut ($H/R \ll 1$). The subregimes of strong star-like and crew-cut micelles are marked as III_s and III_c, respectively, in Figure 2. The asymptotic power law expressions for the boundaries between different spherical micelle regimes and for the binodals separating spherical micelles from different morphologies (cylindrical and lamellar aggregates) in these two limits are summarized in Table 3. Although in a strong micelle the core contribution ($F_{\text{core,elastic}} > kT$) is smaller than corona and interface contributions ($F_{\text{interface}} \simeq F_{\text{corona}} \gg kT$), this elastic stretching of core blocks governs the micelle

morphology and the precipitation of aggregates into sediment (regime IV). The decrease in length of the blocks (N_A , N_B) leads to the decrease in elastic stretching of chains in the core and corona of a strong micelle. The hydrophobic block B becomes unstretched at the boundary of subregime III_c along the thick solid line with slope 1. Here, the spherical micelles are expected to precipitate into sediment passing through sequence of morphological transformations (sphere–cylinder–lamella) upon the decrease in length of hydrophilic block A.^{25,28}

In addition to the previously described strong micelle regime (III), we expand the diagram of states to the unimer regime and the new regime of weak micelles, marked as I and II in the diagram in Figure 2.

2.2. Unimers

In regime I, the hydrophobic block is sufficiently short ($N_B < \phi_B(\gamma a_B^2/kT)^{-3/2}$) so that no self-assembly is observed. The surface free energy of the collapsed hydrophobic block is not sufficient to overcome the loss in translational entropy upon self-assembly, and the diblocks remain in their unimer state. The surface free energy of the unimer ($\simeq \gamma R_{\text{unimer}}^2 \simeq \gamma a_B^2(N_B/\phi_B)^{2/3}$) is less than kT and is hence not sufficient to drive self-assembly. In this regime, if $N_B < \phi_B^{-2}$, block B retains the conformation of a Gaussian coil, while if $N_B < \phi^{-2}$, block B collapses into a spherical globule. These coil and tadpole subregimes are separated by the vertical dash-dotted line at $N_B < \phi^{-2}$ in regime I of the diagram of states in Figure 2.

2.3. Weak Micelles

In regime II, where N_B is greater than $\phi_B(\gamma a_B^2/kT)^{-3/2}$, the surface free energy of the collapsed hydrophobic block in the unimer is larger than kT , driving the self-assembly of the unimers into micelles. However, upon aggregation, the surface free energy per chain in the micelle decreases below kT (shaded regime II in Figure 2). The low surface tension at the core–corona interface leads to important characteristics that distinguish these micelles, termed weak micelles, from the typical strong micelles. The corona blocks in a weak micelle are not strongly stretched as in strong micelles but instead remain close to their Gaussian size ($H \simeq a_A p_A^{1/2} N_A^{1/2}$ where $p_A = b_A/a_A$ is defined as the ratio of the Kuhn segment length b_A to the effective symmetric monomer size of the hydrophilic block a_A). This state corresponds to weak overlap of the hydrophilic blocks A in the corona of the micelle. The aggregation number in such micelles is governed by the balance of the repulsive interactions between monomers in the corona and the elasticity of the hydrophilic blocks (with both free energy contributions on the order of kT), while the surface free energy remains below kT per chain. The hydrophobic block B is confined within the core of the weak micelle with a radius R smaller than the Gaussian size of block B, and its free energy contribution is negligible. A more detailed formulation of the free energy F of a weak micelle is presented in the Appendix. Minimization of this free energy F with respect to two parameters (R and H) specifies the equilibrium values of the core radius R and corona thickness H of a weak micelle. Note that since the corona and interface contributions to the free energy per chain in a weak micelle are on the order of kT (see Figure 10), the distribution of aggregation numbers of weak micelles is noticeably broader than the distribution of strong micelles.

Nevertheless, one can still define the optimal aggregation number Q and the corresponding core and corona dimensions R and H of weak micelles. The calculated values of R and H can be used to obtain the aggregation number, $Q = 4\pi\phi_B R^3/(3N_B a_B^3)$, and the total micelle size, $R_{\text{total}} = R + H$, which can in turn be compared to the experimentally measured values.

Within regime II, similar to the case of strong micelles, there are two subregimes: star-like and crew-cut weak micelles. The power law asymptotes for the parameters of weak micelles in the limits of star-like ($H/R \gg 1$, subregime II_s) and crew-cut ($H/R \ll 1$, subregime II_c) micelles are summarized in Table 4 for the general case of $p_A > 1$. An additional subregime of weak micelles not present for strong micelles is the Gaussian weak micelle regime (II_G) where $N_A < \phi_B^{-1} N_B^{1/2}$. In this subregime, the thickness of the corona and radius of the core of weak crew-cut micelles coincide with their corresponding Gaussian sizes, $H \simeq a_A p_A^{1/2} N_A^{1/2}$ and $R \simeq a_B p_B^{1/2} N_B^{1/2}$, respectively. However, in the vicinity of the boundary between subregimes II_G and II_c, weak micelles lose their thermodynamic stability due to the van der Waals attraction and precipitate into a sediment (regime IV).

The weak micelles occupy a noticeable part of the diagram of states only for $\gamma a_B^2/kT \ll \phi_B^2$ and/or if the chains exhibit considerable thermodynamic rigidity; that is, the ratio p_A of the Kuhn segment length b_A to the effective symmetric monomer size a_A is considerably larger than unity ($p_A \gg 1$). For flexible chains with $p_A \simeq 1$, the area per chain $s \simeq a_A^2 N_A$, whereas for semiflexible chains with $p_A > 1$, the area per chain is smaller, $s \approx a_A^2 N_A p_A^{-1/2}$, leading to stronger overlap of the corona blocks without additional stretching, resulting in the formation of weak micelles. Therefore, for flexible chains with $p_A \simeq 1$, the diagram of states does not contain star-like weak micelles. Increasing N_B will result in a transition from unimers (regime I) to strong star-like micelles (III_s) or weak crew-cut micelles (II_c) depending on N_A , as the subregime II_s of star-like weak micelles in the diagram of states disappears. This regime II_s expands with a decrease in surface tension and an increase in chain stiffness, $p_A > 1$. We emphasize that for flexible diblocks ($p_A \simeq 1$) with coupled surface and/or bulk interactions ($\gamma a^2/kT \approx \phi_B^2$) the regimes of weak micelles (II_c, II_s, and II_G) shrink into the crossover regions between the regimes of strong micelles (III), unimers (I), and regime (IV).

3. RESULTS AND DISCUSSION

To examine the applicability of the theoretical model described above to the self-assembly of ELP diblocks, we compared the theoretical predictions of critical micelle temperature, hydrodynamic radius, and aggregation number to the experimental data of ELP diblocks with varying block lengths. A set of six ELP diblocks known to self-assemble into spherical micelles was chosen. The hydrophilic block contained the guest residues valine, glycine, and alanine in a ratio of 1:7:8, while the hydrophobic block solely contained valine as the guest residue. To obtain the theoretical predictions for this set, we determined the model parameters. We first defined an effective symmetric monomer for the heterogeneous ELP molecule. We then confirmed that the corona of the micelle is at theta solvent conditions in the chosen range of temperatures by measuring the value of the second virial coefficient, A_2 , of dilute solutions of the hydrophilic ELP monoblocks A. From the second virial coefficient A_2 we calculated the excluded volume v of monomer–monomer interactions in the corona of

the micelle. Next, the surface tension γ of the hydrophobic ELP monoblock B was determined from its phase boundary. The core volume fraction ϕ_B was delineated from literature data.

3.1. Effective Monomer of Elastin-like Polypeptides

ELP chains are composed of multiple amino acids and each amino acid is composed of three types of chemical bonds. We calculate the effective symmetric monomer sizes (eq 2) for the hydrophilic block A, a_A , and hydrophobic block B, a_B , which are repeated N_A and N_B times, respectively. A pentapeptide with the guest residue valine has a molecular mass $M = 427$ g/mol and its contour length $L = 1.82$ nm (each amino acid is assumed to have an average length of 0.365 nm as estimated from known peptide bond lengths and angles²⁹). By substituting ρ of approximately 1 g/cm³ into eq 2, the size a_B of the effective monomer was found to be 0.63 nm. However, while the hydrophobic block is composed of only the guest residue valine, the hydrophilic block is composed of guest residues valine, glycine, and alanine at a ratio of 1:7:8. The variation in pentapeptide guest residue composition from valine to glycine or alanine reduces the molecular mass of the pentapeptide from 427 to 385 or 400 g/mol, respectively, yielding a smaller monomer size a_A but still close to that of monomer B. Therefore, the effective monomer sizes of block A and block B are approximately equal, and the value of $a_A = a_B = a = 0.63$ nm was used in all the theoretical calculations. Each pentapeptide comprises of $L/a \simeq 3$ effective cubic monomers, and the number of these repeat units in each block (N_A , N_B) is equal to the number of pentapeptides in each block multiplied by L/a . Herein, N_A and N_B used in the model are the number of effective cubic monomers. However, in experimental plots (Figures 4–7) we use the number of pentapeptide repeat units denoting them by n_A and n_B . Throughout the whole paper the samples are identified by the numbers of pentapeptide repeat units (n_A and n_B) in the hydrophilic and hydrophobic blocks.

3.2. Solvent Quality of the Hydrophilic Block

To calculate the interaction energy of the corona, we determined whether two- or three-body interactions dominate in the corona by measuring the excluded volume of the hydrophilic block. The excluded volume determines at which length scale the magnitude of two-body interactions becomes larger than the thermal energy kT and causes swelling of the chain on length scales larger than the thermal blob.³⁰

Experimentally, the second virial coefficient, A_2 , was measured using static light scattering and was used to calculate the excluded volume parameter, v , of monomer–monomer interactions. The hydrophilic ELP monoblock (ELP[V₁:A₈:G₇]-96) was expressed, purified, and used for static light scattering experiments. The second virial coefficient was determined over a range of temperatures (23–45 °C) by measuring the average intensity over a range of scattering angles and solution concentrations to construct Zimm plots (Figure S1). Figure 3 shows the variation of A_2 with temperature. As expected for an LCST polymer, A_2 decreases with temperature. The change in the interaction from repulsion to attraction, as apparent from the change in sign of the second virial coefficient from positive to negative, indicates that the theta temperature for this polymer is between 40 and 45 °C.

The dimensionless excluded volume parameter v was calculated from A_2 using the relationship³⁰

$$v = \frac{2A_2 M_0^2}{a_A^3 N_{av}} \quad (3)$$

where $M_0 = 136$ g/mol is the average mass of the effective cubic monomer A. The excluded volume parameter was used to determine the thermal blob size

$$\xi_t \simeq a_A p_A^2 v^{-1} \quad (4)$$

where p_A is the ratio of the Kuhn length b_A to the effective symmetric monomer size a_A and is calculated to be 3.5 based on $b_A = 2.2$ nm as measured by Schmidt and co-workers.²⁹

Table 1 shows the values of A_2 , v , and ξ_t at different temperatures. The size of the thermal blob at all temperatures is much larger than the experimentally measured hydrodynamic radius of the micelles (30–40 nm). As the thermal blob is larger than the size of the corona and the chains are weakly overlapping, the excluded volume interactions are not strong enough to change the conformation of the corona block. As such, we conclude that the corona is in theta solvent at all studied temperatures, and three-body interactions dominate the interaction free energy of the corona.

3.3. Surface Tension

To derive the expression for the surface tension, γ , of the hydrophobic block, we considered the chemical potential, μ , of a two-phase solution of the hydrophobic ELP monoblock (ELP[V]) in which a sediment phase and a globule supernatant phase are in equilibrium. In a two-phase solution, the globules of collapsed ELP are in a dilute phase with a solution volume fraction ϕ' while the sediment is a dense phase of ELPs with volume fraction ϕ_B . The chemical potential of globules is composed of the sum of their translational entropy ($kT \ln(\phi')$), surface energy ($4\pi R_{unimer}^2 \gamma$), and interaction energy due to monomer–monomer attraction. The chemical potential of hydrophobic block in the sediment is composed of the sum of its translational entropy ($kT \ln(\phi_B)$) and the interaction energy (because the sediment surface energy per chain is negligible). This interaction energy per chain depends only on the monomer volume fraction, ϕ_B , and is assumed to be the same in the sediment and inside the globule. Equating the chemical potential of chains in the globule supernatant and sediment phases ($\mu_{globule} = \mu_{sediment}$), we obtain an expression for the surface tension:

$$\gamma = \frac{kT \ln\left(\frac{\phi_B}{\phi'}\right)}{4\pi R_{unimer}^2} \quad (5)$$

The radius of a globule R_{unimer} can be expressed in terms of the number of hydrophobic block monomers N_B and the volume fraction ϕ_B inside the globule as follows:

$$R_{unimer} = \left(\frac{3a_B^3 N_B}{4\pi \phi_B} \right)^{1/3} \quad (6)$$

Using eqs 5 and 6, we obtain the expression for the surface tension γ in terms of the volume fraction ϕ' of globules in the supernatant and the number of monomers in hydrophobic block N_B as follows:

$$\frac{\gamma}{\phi_B^{2/3}} = \frac{kT}{(36\pi)^{1/3} a_B^2} \frac{1}{N_B^{2/3}} \ln\left(\frac{\phi_B}{\phi'}\right) \approx \frac{kT}{(36\pi)^{1/3} a_B^2} \frac{1}{N_B^{2/3}} \ln\left(\frac{1}{\phi'}\right) \quad (7)$$

The contribution of the sediment entropy was ignored as the sediment volume fraction ϕ_B is assumed to be much larger than that of the dilute globules in the supernatant ($\phi_B \gg \phi'$) and thus its entropy is negligible. As a result, $\ln(\phi_B/\phi')$ is replaced by $\ln(1/\phi')$ in eq 7.

The value of ϕ' as a function of temperature T , and thereby the right-hand side of eq 7, can be determined from the turbidity measurements of the lower concentration branch of the phase boundary, $\phi'(T)$. Therefore, the ratio $\gamma/\phi_B^{2/3}$ on the left-hand side of eq 7 is expected to have the same temperature dependence as $\ln(1/\phi')/N_B^{2/3}$.³¹ In our study, the relationship between ϕ' and the cloud point temperature was determined by measuring the change in absorbance of a hydrophobic ELP[V] monoblock solution with increasing temperature for a range of concentrations. For a given concentration, the cloud point was defined as the temperature at which the tangent through the steepest part of the absorbance curve intercepts the temperature axis.

As shown in Figure 4 (left), the cloud point was determined for all three hydrophobic monoblock lengths (60, 90, and 120 pentapeptides) at concentrations between 0.002 and 2 g/L. To verify that right-hand side of eq 7 is independent of N_B , we plotted the temperature dependence of $\ln(1/\phi')/N_B^{2/3}$. The collapse of all three curves for the three ELP lengths onto a single line (Figure 4 right) validates the approach. A linear fit of these data yields the temperature dependence of the ratio $\gamma/\phi_B^{2/3}$ in eq 7 as

$$\frac{\gamma}{\phi_B^{2/3}} = \frac{kT}{\text{nm}^2} \frac{T - 291\text{K}}{128\text{K}} \quad (8)$$

3.4. Critical Micelle Temperature

The self-assembly of diblocks into micelles with increasing temperature is driven by the increase in surface tension of the hydrophobic block. As we have determined the temperature dependence of the surface tension (eq 8), we can therefore calculate the expression for the CMT. The CMT depends on the volume fraction ϕ of unimers in the solution and the number of effective monomers in copolymer blocks (N_A and N_B).

At the CMT, the unimer chemical potential is equal to the chemical potential of a diblock in the micelle. The unimer chemical potential, μ_1 , is composed of its translational entropy ($kT \ln(\phi)$), the surface free energy of the core block ($F_{1,\text{surf}} = (36\pi)^{1/3} (N_B/\phi_B)^{2/3} \gamma a_B^2$, where ϕ_B is the volume fraction inside a collapsed hydrophobic block B), and the free energy of the corona block, $F_{1,\text{corona}}$. The latter is envisioned as a coil with ternary interactions between monomers controlled by the third virial coefficient, w , of monomer–monomer interactions. The expression for $F_{1,\text{corona}}$ is derived in the Appendix.

The chemical potential μ_m of the diblock in the micelle with core radius R and aggregation number Q is estimated as the sum of the surface free energy per chain ($F_{m,surf} = \gamma s = 4\pi\gamma R^2/Q$) and the corona free energy per chain ($F_{m,corona}$) (see Appendix for details). The translational entropy of the diblock within the micelle is negligible. Equating the chemical potentials of the diblock in the unimer state and in the micelle ($\mu_1 = \mu_m$), we obtain the following theoretical expression to specify the critical micelle temperature as a function of diblock volume fraction ϕ in solution, surface tension γ , and the parameters of diblocks (N_A , N_B , w , ϕ_B)

$$kT \ln(\phi) + F_{1,surf} + F_{1,corona} = F_{m,surf} + F_{m,corona} \quad (9)$$

Equation 9 was solved numerically, and the predicted values of the CMT are presented in Table 5 for all the experimentally examined samples.

An approximate analytical expression for CMT could be obtained by ignoring the corona contribution to the chemical potential μ_1 of unimer (because $F_{1,surf} \gg F_{1,corona}$) and by approximating the chemical potential μ_m of a block copolymer in the micelle as $\mu_m = 3kT$ for all the experimentally considered ELP diblocks. This approximation is obtained from the calculated free energy of the micelles of the ELP diblocks (Figure 10) where the free energy is close to $3kT$ for the range of n_A and n_B values of interest (between 64 and 96 pentapeptides). Using these approximations, we can find a simple estimate for the CMT by solving the equation

$$\ln(\phi) + (36\pi)^{1/3} \left(\frac{N_B}{\phi_B} \right)^{2/3} \frac{\gamma a_B^2}{kT} = 3 \quad (10)$$

By inserting the temperature dependence for surface tension γ from eq 8 into eq 10, we find an approximate analytical expression for the CMT (valid in the considered limited range of molecular weights of ELP diblocks)

$$\text{CMT} = \frac{3 - \ln(\phi)}{N_B^{2/3}} \times 67 \text{ K} + 291 \text{ K} \quad (11)$$

In Figure 5, we compared the theoretical predictions of the CMT (eq 11 and first column in Table 5) to the experimentally measured CMTs (determined by observing changes in hydrodynamic radius as measured by dynamic light scattering). The predicted values of the CMT were found to be in good agreement with the experimental values for all six ELP diblocks. Note that approximate analytical expression in eq 11 predicts CMT with the accuracy of 1 K (Table 5).

3.5. Physical Properties of ELP Diblock Micelles

The hydrodynamic radii and the aggregation numbers of micelles formed by ELP diblocks were determined experimentally from dynamic and static light scattering (DLS/SLS) measurements respectively (Table 2) at a concentration of $25 \mu\text{M}$ and at 43°C . At this temperature, the largest subset of ELP diblocks (four out of six) was found to assemble into a spherical morphology. These spherical micelles are thermodynamically stable as discussed

in the Supporting Information (see Figure S2). The remaining two ELP diblocks (ELP 64, 90 and ELP 64, 120) are composed of two populations at 43 °C: spherical nanoparticles (~30–40 nm) and larger aggregates (~100–200 nm).

To fit the experimental results using the theoretical model of weak micelles, we fixed three parameters: core volume fraction, ϕ_B , numerical coefficient in the expression for elastic free energy of the corona block (see Appendix), and the three-body interaction parameter, w . The core of ELP micelles is assumed to be dense, with a volume fraction $\phi_B \approx 0.6$ estimated at the temperature of interest, 43 °C, from the previously determined phase diagram of temperature versus composition of the hydrophobic ELP monoblock by Urry and co-workers.^{17,19,20} The numerical coefficient 3/2 of the deformation free energy of an ideal chain was used in the corona elastic free energy. The dimensionless three-body interaction parameter was fit empirically to the experimental data and was estimated to be $w = 1.5$. Given this set of parameters of the theoretical model, the equations $\partial F / \partial R = 0$ and $\partial F / \partial H = 0$ were solved numerically and the predications of micelle properties at 43 °C were compared to the experimental values as described below.

3.6. Hydrodynamic Radii of Micelles

The hydrodynamic radius (solid lines in Figure 6, left) was calculated from the predicted total radius (R_{total} : dashed lines in Figure 6, left) by subtracting the size of the last correlation blob as solvent drains through the edge of the corona at this length scale. As the micelles are within the weak regime, the size of the correlation blob ξ was estimated by considering the hydrophilic chains in the corona as a semidilute theta solution. The calculated correlation blob size for each ELP diblock is summarized in Table 6. Figure 6 (left) shows the experimentally measured hydrodynamic radii (points) and the predicted hydrodynamic radii (solid lines). The predicted hydrodynamic radii were found to be in good agreement with the experimentally determined hydrodynamic radii.

Figure 6 (right) compares the predicted corona thickness H and core size R to their Gaussian sizes H_G and R_G (equal to $a(pN)^{1/2}$) with increasing n_A (for a hydrophobic block of 60 pentapeptides). This comparison gives rise to several observations regarding the state of ELP micelles. The core was found to be slightly compressed in comparison with the Gaussian end-to-end size of the core block. The core size decreases with increasing n_A as the larger size of the corona block reduces the number of chains that pack within the micelle (aggregation number) resulting in a smaller micelle core. Furthermore, the core size is smaller than the corona thickness indicating that the ELP micelles are star-like for $n_A > 40$ pentapeptides and, as expected, become more so as n_A increases. Most importantly, however, the model predicted that the corona thickness is close to its Gaussian size. This observation did not depend on the fit parameters used in predicting the core and corona thicknesses (data not shown).

3.7. Aggregation Number of Micelles

The experimentally measured aggregation numbers of the four ELP diblocks are compared to the theoretical predictions in Figure 7. The solid lines depict the theoretical dependence of the aggregation number on the number of pentapeptides in the hydrophilic block, while the

points show the experimentally measured aggregation number. The theoretical variations of the aggregation number with diblock parameters matched those observed experimentally. As expected, the aggregation number decreased with increasing hydrophilic block length. This dependence stems from the larger volume occupied by a larger hydrophilic chain within the corona, and thus a smaller number of chains can pack in each micelle. However, the aggregation number was observed to increase with the increase in the length of the hydrophobic block. Larger hydrophobic blocks increase the volume of the core, and thus more chains are able to pack into the micelle to reduce the surface area per chain at the core–corona interface. The qualitative trends confirmed that the properties of the ELP micelles change with block lengths similarly to properties of synthetic diblocks. The quantitative comparison of the physical properties indicated that all energetic contributions are correctly accounted for within the weak micelle model, as we found the predicted aggregation numbers to be in good agreement with experimental values.

3.8. Temperature Dependence of Micelle Properties

The results presented in the previous section compared the model prediction of micelle properties with the experimental data at only one temperature, 43 °C. However, ELP diblocks form stable spherical micelles over a range of temperatures between their CMT and aggregation temperature (see red points in the diagram of states in Figure 5). The CMT of each ELP diblock and the temperature at which the spherical micelles aggregate depend on the length of each block. Below we describe the temperature dependence of the hydrodynamic radius (Figure 8) and aggregation number (Figure 9) for two ELP diblocks (ELP 96, 60 and ELP 96, 90) over the range of temperatures at which spherical micelles are observed for these two diblocks. The results for the four remaining ELP diblocks are shown in Figures S3 and S4 in the Supporting Information. As predicted by the theoretical model, the hydrodynamic radius does not change significantly with increasing temperature for all six ELP diblocks (increases by ~1–2 nm over the entire temperature range). The trends in temperature dependence of R_h predicted by the model are in good agreement with experimental data for all ELP diblocks. However, the aggregation numbers were observed to increase significantly for all six ELP diblocks (an increase of 20–30 diblocks/micelle per 1 °C increase). This experimentally observed variation is 2–5 times stronger than the theoretical prediction.

These results indicate that additional factors lead to the strong increase in the aggregation numbers. A single fixed set of model parameters does not predict the observed strong temperature dependence of the aggregation number. It is possible that the strong increase in aggregation number is caused by the increase in chain stiffness in the corona due to the increase in secondary structure (leading to a change in p_A). Additional experimental measurements to quantify the formation of the secondary structure leading to the increase in Kuhn length with increasing temperature must be conducted and integrated into the model to determine their effect on the temperature dependence of the aggregation number.

4. CONCLUSIONS

We have developed a theoretical model for the micellization of diblock copolymers in which the interactions in the core of the micelle are significantly stronger than interactions at the

core–corona interface. The development of this model was driven by the inability of a previously developed model for synthetic diblock copolymers to fully describe the properties of spherical micelles of peptide diblock copolymers that are chemically more complex with repeat units consisting of Val-Pro-Gly-X-Gly pentapeptides. For low surface tension at the core–corona interface, this model predicts the appearance of weak micelles in which the hydrophilic block A stays at the threshold of stretching in the corona while the hydrophobic block B is confined in the relatively dense core with a radius smaller than the Gaussian size of block B. This is in contrast to the case of strong micelles with stretched corona and core blocks observed for most synthetic diblock copolymers.

The weak micellization model successfully predicts the micelle properties, such as their critical micelle temperature, hydrodynamic radius, and aggregation number that are in good agreement with experimental data for a set of ELP diblock copolymers. This finding is the first systematic study of such weak micelles. The weak micellization model complements the scaling theory of strong micelles developed previously²⁵ and provides a basis to describe the self-assembly of ELPs and potentially other peptide polymers in dilute solutions. The model also suggests that the thermodynamic origin of this behavior stems from the amphiphilic nature of the chemically complex repeat units in these peptide polymers that are composed of both polar and nonpolar amino acids.^{32–35} The preferential surfactant-like orientation of these groups at the core–corona interface results in the low surface tension that leads to weak stretching of the corona blocks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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APPENDIX

A1. Free Energy of Weak Micelles

The free energy of micelles is composed of the corona free energy, the core free energy, and the interface free energy. The derivation of the interaction free energy of the corona of weak micelles is described below. In theta solvent, three-body interactions dominate, and the interaction free energy per block can be expressed as follows:

$$F_{\text{corona, interaction}} = kT w N_A \phi_A^2 \quad (\text{A1})$$

where w is the three-body interaction parameter, ϕ_A is the volume fraction of the hydrophilic block in the corona, and N_A is the number of effective monomers in the hydrophilic block. The volume fraction ϕ_A in the corona can be written within the box-like approximation as

$$\phi_A = \frac{N_A a_A^3 Q}{\frac{4}{3}\pi[(R+H)^3 - R^3]} \quad (\text{A2})$$

where a_A is the effective symmetric monomer size of the hydrophilic A block, Q is the aggregation number of the micelle, R is the radius of the core, and H is the thickness of the corona. The volume fraction ϕ_B in the core can be similarly written as

$$\phi_B = \frac{N_B a_B^3 Q}{\frac{4}{3}\pi R^3} \quad (\text{A3})$$

where N_B is the number of effective monomers in the hydrophobic block, and a_B is the effective symmetric monomer size of the hydrophobic block. By combining the two above equations, we can relate the volume fraction ϕ_A in the corona to the volume fraction ϕ_B of the core as follows:

$$\phi_A = \frac{\phi_B N_A}{N_B \left[\left(1 + \frac{H}{R}\right)^3 - 1 \right]} \quad (\text{A4})$$

Substituting this expression of ϕ_A into the expression for the corona interaction free energy (eq A1), we obtained the equation

$$F_{\text{corona, interaction}} = kT w N_A^3 \frac{\phi_B^2}{N_B^2 \left[\left(1 + \frac{H}{R}\right)^3 - 1 \right]^2} \quad (\text{A5})$$

The elastic free energy of corona blocks can be written as the balance of stretching and confinement energies as follows:

$$F_{\text{corona, elastic}} = \frac{3}{2} kT \left[\left(\frac{H}{H_G} \right)^2 + \left(\frac{H_G}{H} \right)^2 \right] \quad (\text{A6})$$

where H_G is the Gaussian size of the hydrophilic block. While for strong micelles, the confinement penalty of the corona elastic free energy was ignored, as the corona is assumed to be highly stretched, the corona elastic energy for weak micelles is on the order of thermal energy kT , and thus it is important to balance both of its components to yield an equilibrium state with a Gaussian end-to-end distance in the absence of the interaction free energy ($w = 0$). We note that this expression is limited in its validity, as thermal fluctuations are on the same order as the elastic free energy of the corona.

The interface free energy per chain is related to the surface area per chain, s , and the surface tension, γ , as follows:

$$F_{\text{interaction}} = \gamma s = \frac{3\gamma a_B^3 N_B}{\phi_B R} \quad (\text{A7})$$

The core free energy comprises of the monomer–monomer interactions and the elastic energy of the hydrophobic block B. As the monomer–monomer interaction within a single collapsed unimer is the same as within the core of a micelle, this energetic contribution was omitted from further consideration. The elastic energy of the core is related to the confinement of the hydrophobic block. However, this energy is much smaller than kT and can be safely neglected.

Using the equations above, we obtain the expression of the total free energy F per chain in a weak spherical micelle under theta solvent conditions for hydrophilic blocks as follows:

$$\frac{F}{kT} = \frac{3\gamma a_B^3 N_B}{kT \phi_B R} + \frac{3}{2} \left(\frac{H^2}{p_A N_A a_A^2} + \frac{p_A N_A a_A^2}{H^2} \right) + w N_A^3 \frac{\phi_B^2}{N_B^2 \left[\left(1 + \frac{H}{R}\right)^3 - 1 \right]^2} \quad (\text{A8})$$

where p_A is the ratio of the Kuhn monomer to the effective monomer of the hydrophilic block. The free energy minimum is determined by taking the partial derivative of free energy F with respect to the core radius R and the corona thickness H . The result is two nonlinear equations that determine R and H ($\partial F / \partial R = 0$ and $\partial F / \partial H = 0$) and can be solved numerically.

$$\frac{1}{kT} \frac{\partial F}{\partial H} = 3 \left(\frac{H}{p_A N_A a_A^2} - \frac{p_A N_A a_A^2}{H^3} \right) - 6w \frac{N_A^3}{R} \times \frac{\phi_B^2 \left(1 + \frac{H}{R}\right)^2}{N_B^2 \left[\left(1 + \frac{H}{R}\right)^3 - 1 \right]^3} = 0 \quad (\text{A9})$$

$$\frac{1}{kT} \frac{\partial F}{\partial R} = \frac{-3\gamma a_B^3 N_B}{kT \phi_B R^2} + 6w N_A^3 \frac{\phi_B^2 \frac{H}{R^2} \left(1 + \frac{H}{R}\right)^2}{N_B^2 \left[\left(1 + \frac{H}{R}\right)^3 - 1 \right]^3} = 0 \quad (\text{A10})$$

Upon determining the radius of the core and the corona thickness, the total radius, R_{total} and the aggregation number, Q , can be calculated as follows:

$$R_{\text{total}} = H + R \quad (\text{A11})$$

$$Q = \frac{4\pi \phi_B R^3}{3 N_B a_B^3} \quad (\text{A12})$$

A2. Calculation of Critical Micelle Temperature

The chemical potential of a unimer μ_1 in a solution of diblocks with volume fraction ϕ is

$$\mu_1 = kT \ln(\phi) + F_{1,\text{surf}} + F_{1,\text{corona}} \quad (\text{A13})$$

The surface free energy $F_{1,\text{surf}}$ associated with the condensed hydrophobic block B of the unimer is given by

$$F_{1,\text{surf}} = (36\pi)^{1/3} \left(\frac{N_B}{\phi_B} \right)^{2/3} \gamma a_B^2 \quad (\text{A14})$$

where a_B is the size of effective ELP monomer (assumed to be the same for both ELP blocks, A and B), N_B is the number of effective monomers in the hydrophobic block, γ is the surface free energy per unit area at the surface of condensed block B, and ϕ_B is volume fraction of B in the condensed state.

The free energy of corona block comprises of the elastic free energy $F_{1,\text{elastic}}$ of hydrophilic block A and the free energy of interactions between the monomers $F_{1,\text{interaction}}$.

$$F_{1,\text{corona}} = F_{1,\text{elastic}} + F_{1,\text{interaction}} \quad (\text{A15})$$

The corona block in the unimer state is envisioned as a polymer coil with radius H_1 and average volume fraction of monomers $\phi_A = 3N_A a_A^3 / (4\pi H^3)$. Under theta solvent conditions, the interaction free energy $F_{1,\text{interaction}}$ is dominated by ternary contacts between monomers

$$F_{1,\text{interaction}} = kT w \phi_A^2 N_A \quad (\text{A16})$$

The volume fraction ϕ_A of monomers in a single corona block A is assumed to be much less than unity.

The elastic free energy $F_{1,\text{elastic}}$ of the hydrophilic block A is approximated as

$$F_{1,\text{elastic}} = \frac{3}{2} \left(\frac{H^2}{p_A N_A a_A^2} + \frac{p_A N_A a_A^2}{H^2} \right) \quad (\text{A17})$$

The total free energy per chain is

$$F_{1,\text{corona}} = \frac{3}{2} \left(\frac{H^2}{p_A N_A a_A^2} + \frac{p_A N_A a_A^2}{H^2} \right) + kT \frac{9w a_A^6 N_A^3}{16\pi^2 H^6} \quad (\text{A18})$$

By minimizing $F_{1,\text{corona}}$ in eq A18 with respect to H , that is, by solving the equation $F_{1,\text{corona}} / H = 0$ or equivalently

$$\frac{H^8}{a_A^2 p_A N_A} - a_A^2 p_A N_A H^4 - \frac{18w a_A^6 N_A^3}{16\pi^2} = 0 \quad (\text{A19})$$

We find the equilibrium value H_{eq} of corona thickness

$$H_{\text{eq}} = a_A \sqrt{p_A N_A} \left(\frac{1 + \sqrt{1 + 9w / (2\pi^2 p_A^3)}}{2} \right)^{1/4} \quad (\text{A20})$$

By substituting H_{eq} in the expression of $F_{1,\text{corona}}$, we find the equilibrium value of $F_{1,\text{corona}} + (H_{\text{eq}})$. The equilibrium chemical potential of unimer is given by

$$\mu_1 = kT \ln(\phi) + (36\pi)^{1/3} \left(\frac{N_B}{\phi_B} \right)^{2/3} \gamma a_B^2 + F_{1,\text{corona}}(H_{\text{eq}}) \quad (\text{A21})$$

The chemical potential of a diblock in the micelle μ_m is specified as the sum of the surface and the corona free energy contributions, while the translational entropy of the diblock in the micelle is negligible. The derivation of micelle chemical potential is described in section A1. Equations A9 and A10 were solved numerically for all experimentally investigated ELP samples for the following set of the system parameters: $p_A = p_B = 3.5$; $a_A = a_B = 0.63$ nm, $w = 1.5$, $\varphi = 25$ μM , and for the experimentally evaluated temperature dependence of the surface tension (eq 8). The chemical potentials μ_m and μ_1 were calculated for each of the samples at equilibrium. The critical micelle temperature was found from the equation $\mu_1 = \mu_m$ which is equivalent to eq 11. The theoretical results are presented in Table 5.

A3. Free Energy of Weak Micelles

The different micellar regimes differ in their total free energy. The free energy within the weak micelle regime is expected to be smaller than in the strong micelle regime. The total free energy of ELP micelles increases from 2 to 3.5 kT with increasing N_A (for hydrophobic block of $n_B = 60$ pentapeptides) and from 2 to 4 kT with increasing N_B (for hydrophilic block of $n_A = 96$ pentapeptides) within the relevant range of block lengths (20–140 pentapeptides) as seen in Figure 10. The predicted total free energy of the ELP micelles is consistent with the expected free energy of weak micelles, as all three energy contributions are expected to be $\sim kT$.

A4. Hydrodynamic Radius of Weak Micelles

The hydrodynamic radius was calculated by subtracting the size of the outer correlation blob ξ from the total radius R_{total} . The correlation blob was estimated from the corresponding value of the correlation blob in a semidilute theta solution. The validity of this assumption stems from the weak overlap between the Gaussian chains in the corona. The size of a correlation blob in a semidilute solution is given by the expression

$$\xi \approx H_G \frac{\phi^*}{\phi_A} \quad (\text{A22})$$

where ϕ^* is the overlap volume fraction and H_G is the Gaussian end-to-end distance of the corona block. Each of the three variables was determined as follows:

$$H_G = a_A \sqrt{p_A N_A} \quad (\text{A23})$$

$$\phi^* = \frac{3N_A a_A^3}{4\pi(H_G/2)^3} \quad (\text{A24})$$

$$\phi_A = \frac{N_A a_A^3 Q}{\frac{4}{3}\pi((R+H)^3 - R^3)} \quad (\text{A25})$$

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Figure 1.

Schematic of self-assembly of ELP diblock copolymers. Below the CMT, the ELP diblocks are soluble as unimers. Above the CMT, the hydrophobic blocks form the core of the micelles while the hydrophilic blocks remain hydrated and form the corona of the micelles. Upon further heating, the hydrophilic blocks also become insoluble, and the ELP diblocks phase separate.

Figure 2.

Diagram of states of ELP diblocks with degrees of polymerization of hydrophilic block N_A and hydrophobic block N_B for surface tension $\gamma \ll \phi_B^2 kT/a_B^2$, where ϕ_B is core volume fraction. ELP diblocks are unimers in regime I, weak micelles in regime II, and strong micelles in regime III. Regime IV represents regions where ELP diblocks self-assemble into nonspherical morphologies or separate into two-phase solutions.

Figure 3.

Dependence of the second virial coefficient A_2 of hydrophilic ELP[V₁:A₈:G₇]-96 monoblock on temperature as determined from Zimm plots obtained from static light scattering. A_2 decreased with increasing temperature as expected for LCST polymers. A_2 changes signs from positive to negative between 40 and 45 °C (shaded area). This indicates that the monomer–monomer interaction changes from repulsion to attraction and that the theta temperature is between 40 and 45 °C.

Figure 4.

(left) Transition temperatures of hydrophobic ELP[V] monoblock as a function of its concentration for chains with $n_B = 60, 90$, and 120 pentapeptides (blue triangle, black circle, and red square symbols, respectively). The absorbance was measured at 350 nm while the temperature was increased at a rate of 1 °C/min. The transition temperature was defined as the temperature at which the tangent through the steepest part of the absorbance curve intercepts the temperature axis. (right) The temperature dependence of the surface tension γ normalized by $kT\phi_B^{2/3}$, where ϕ_B is the volume fraction of the hydrophobic block. The data for three hydrophobic ELP monoblocks with $60, 90$, and 120 pentapeptides (same symbols as in the left figure) collapse onto a single temperature dependence fitted by the linear purple line, eq 8.

Figure 5.

Diagram of states of ELP diblocks. The dots represent experimentally observed states for all six ELP diblocks. Green dots represent unimers, red dots represent spherical micelles, and blue dots represent any particles larger than the spherical micelles. The surface represents the theoretically predicted critical micelle temperature (eq 11). n_A and n_B are the numbers of pentapeptide repeats in the hydrophilic and hydrophobic block, respectively. The critical micelle temperatures determined experimentally (boundary between green and red points) and predicted theoretically are in good agreement.

Figure 6.

(left) The variation of hydrodynamic radii of micelles with increasing hydrophilic block length. The solid lines represent the theoretical prediction of hydrodynamic radii of weak micelles R_h . The dashed lines represent the theoretical prediction of total radii of weak micelles R_{Total} . The points (squares and triangles) represent the experimentally measured hydrodynamic radii at 43 °C. The blue lines and triangles represent ELP diblocks with a hydrophobic block length of 60 pentapeptides. The red lines and square represent ELP diblocks with a hydrophobic block length of 90 pentapeptides. (right) Predicted corona thickness H (solid red) and core radius R (dashed double-dotted green) compared to their Gaussian end-to-end distance (dashed blue and dashed-dotted black lines respectively) with increasing n_A . The number of pentapeptides in the hydrophobic block is fixed at $n_B = 60$ for all four lines.

Figure 7.

Dependence of the aggregation number Q on the number of pentapeptides in hydrophilic blocks, n_A . The solid lines represent the theoretical predication of the aggregation number of weak micelles at 43 °C (exact numerical solution). The points indicate the experimentally measured aggregation numbers at 43 °C. The blue line and triangles represent ELP diblocks with a hydrophobic block containing $n_B = 60$ pentapeptides. The red line and square represent ELP diblocks with a hydrophobic block with $n_B = 90$ pentapeptides.

Figure 8. Temperature dependence of the hydrodynamic radius: (left) ELP 96, 60, (right) ELP 96, 90. The experimental hydrodynamic radii (black) are compared to the predictions from the weak micelle model (blue).

Figure 9.
Temperature dependence of the aggregation number: (left) ELP 96, 60, (right) ELP 96, 90.
The measured aggregation numbers (black) are compared to predictions of the weak micelle model in theta solvent (blue).

Figure 10.

Total free energy of ELP micelles with increasing n_A (red) with hydrophobic block length of $n_B = 60$ pentapeptides and with increasing n_B (blue) with a hydrophilic block length of $n_A = 96$ pentapeptides. The low total free energy indicates that the micelles are within the weak micelle regime.

Table 1

Solvent Quality of Corona Determined from Monomer–Monomer Interaction

temp (°C)	$\frac{dn}{dc}$ (mL/g)	M_w^a (kDa)	A_2 (mol L/g ²)	ν	ξ (nm)
23	0.160	39	1.4×10^{-7}	0.035	219
30	0.160	37	6.4×10^{-8}	0.016	486
35	0.159	39	3.9×10^{-8}	0.010	798
40	0.158	39	3.7×10^{-8}	0.010	850
45	0.156	40	-9.1×10^{-8}	-0.020	391

^aThe theoretical molecular weight of the polymer is 38.8 kDa. Variations in the measured molecular weight are within statistical error.

Table 2

Morphology, Aggregation Number Q , and Hydrodynamic Radius R_h As Determined Experimentally at 43 °C at Concentration 25 μ M

ELP diblock	n_A	n_B	morphology at 43 °C	Q	R_h (nm)
ELP 64, 60	64	60	spherical	125	24
ELP 96, 60	96	60	spherical	96	27.4
ELP 128, 60	128	60	spherical	83	28.9
ELP 96, 60	96	60	spherical	183	31.4

Table 3

Boundaries between Different Regimes in the Diagram of States in Figure 2 for the Parameter Values of $\gamma \ll \varphi_B^2 kT/a_B^2$, $w = 1$, and $p_A > 1$

regions	boundaries
I-II _s , I-II _c , I-II _G , I-IV	$N_B \sim \varphi_B(\gamma a_B^2/kT)^{-3/2}$
II _s -III _s	$N_B \sim \varphi_B(\gamma a_B^2/kT)^{-3/2} p_A^{3/4}$
II _s -II _c	$N_A \sim N_B^{2/3} \varphi_B^{-2/3}$
II _c -II _G -IV	$N_A \sim N_B^{1/2} \varphi_B^{-1}$
II _c -III _c	$N_A \sim \varphi_B^{-1} p_A^{1/2}$
III _c -III _s	$N_A \sim N_B^{4/5} \varphi_B^{-4/5} (\gamma a_B^2/kT)^{1/5} p_A^{-1/10}$
III _c -IV ($N_B > N_B^* \sim \varphi_B^{10/3} (\gamma a_B^2/kT)^{-8/3} p_A^{4/3}$)	$N_A \sim N_B^{8/11} \varphi_B^{-12/11} (\gamma a_B^2/kT)^{3/11} p_A^{-3/22}$
III _c -IV ($N_B < N_B^*$)	$N_A \sim N_B \varphi_B^{-2} (\gamma a_B^2/kT) p_A^{-1/2}$

Table 4

Asymptotic Scaling Dependences of the Physical Properties of Spherical Micelles in Different Regions of the Diagram of States in Figure 2 for the Parameter Values of $\gamma \ll \varphi_B^2 kT/a_B^2$, $w = 1$, and $p_A > 1$

region	Q	R/a	H/a	F/kT
I	1	$N_B^{1/3}/\varphi_B^{1/3}$	$\rho_A^{1/2}N_A^{1/2}$	$(\gamma a_B^2/kT)N_B^{2/3}/\varphi_B^{2/3}$
II _S	$\rho_A^{3/2}$	$N_B^{1/3}\rho_A^{1/3}/\varphi_B^{1/3}$	$\rho_A^{1/2}N_A^{1/2}$	~ 1
II _C	$N_B^2\rho_A^{3/2}/\varphi_B^2N_A^3$	$N_B\rho_A^{1/2}/\varphi_B N_A$	$\rho_A^{1/2}N_A^{1/2}$	~ 1
II _G	$\varphi_B\rho_A^{3/2}N_B^{1/2}$	$\rho_A^{1/2}N_A^{1/2}$	$\rho_A^{1/2}N_A^{1/2}$	~ 1
III _S	$N_B^{4/5}\rho_A^{9/10}(\gamma a_B^2/kT)^{6/5}/\varphi_B^{4/5}$	$N_B^{3/5}\rho_A^{3/10}(\gamma a_B^2/kT)^{2/5}/\varphi_B^{3/5}$	$N_A^{1/2}N_B^{1/5}\rho_A^{7/20}(\gamma a_B^2/kT)^{3/5}/\varphi_B^{1/5}$	$N_B^{2/5}(\gamma a_B^2/kT)^{6/5}/\varphi_B^{3/5}\rho_A^{3/10}$
III _C	$N_B^2\rho_A^{3/4}(\gamma a_B^2/kT)^{3/2}/\varphi_B^2N_A^{3/2}$	$N_B\rho_A^{1/4}(\gamma a_B^2/kT)^{1/2}/\varphi_B N_A^{1/2}$	$\rho_A^{3/8}N_A^{3/4}(\gamma a_B^2/kT)^{1/4}$	$N_A^{1/2}(\gamma a_B^2/kT)^{1/2}/\rho_A^{1/4}$

Table 5

Theoretical Predictions for CMT (in K); CMTs Calculated According to Full Equations for the Chemical Potentials μ_l and μ_m ; CMTs Calculated with Approximate Eq 11; and Experimentally Determined CMT As Measured by DLS

ELP diblock	CMT (K) theoretical (numerical solution)	CMT (K) theoretical (eq 11)	CMT (K) experimental
ELP 64, 60	313	313	312
ELP 64, 90	306	307	306
ELP 64, 120	303	304	304
ELP 96, 60	313	313	313
ELP 96, 90	307	307	307
ELP 128, 60	314	313	313

Table 6Size of the Correlation Blob ξ in Coronas of ELP

n_A	$n_B = 60$	$n_B = 90$	$n_B = 120$
64	7.5 nm	7.9 nm ^a	7.4 nm ^a
96	9.2 nm	8.4 nm	
128	10.6 nm		

^aThe correlation blob size for these ELP diblocks was calculated at 35 °C while the rest of the blob sizes were calculated at 43 °C.