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Peptide Contour Length Determines Equilibrium Secondary Structure in Protein-Analogous Micelles

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Abstract

This work advances bottom-up design of bioinspired materials built from peptide-amphiphiles, which are a class of bioconjugates in which a biofunctional peptide is covalently attached to a hydrophobic moiety that drives self-assembly in aqueous solution. Specifically, this work highlights the importance of peptide contour length in determining the equilibrium secondary structure of the peptide as well as the self-assembled (i.e. micelle) geometry. Peptides used here repeat a seven-amino acid sequence between one and four times to vary peptide contour length while maintaining similar peptide-peptide interactions. Without a hydrophobic tail, these peptides all exhibit a combination of random coil and α -helical structure. Upon self-assembly in the crowded environment of a micellar corona, however, short peptides are prone to β -sheet structure and cylindrical micelle geometry while longer peptides remain helical in spheroidal micelles. The transition to β -sheets in short peptides is rapid, whereby amphiphiles first self-assemble with α -helical peptide structure, then transition to their equilibrium β -sheet structure at a rate that depends on both temperature and ionic strength. These results identify peptide contour length as an important control over equilibrium peptide secondary structure and micelle geometry. Furthermore, the time-dependent nature of the helix-to-sheet transition opens the door for shape-changing bioinspired materials with tunable conversion rates.

INTRODUCTION

In the emerging fields of biomaterials and nanomedicine, engineered, self-assembled constructs display biological cues, such as peptides or proteins, on the surface of nanostructured objects in order to bind other proteins in solution or on the surface of cells and produce a desirable biological response. In vivo, these constructs often take the form of either insoluble network-forming nanofibers to influence cell behavior^{1,2} or small

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nanoparticles designed to circulate through the body as new types of ‘designer drugs’.^{3–7} In these applications, secondary (and often tertiary) peptide structure is integral to function, and the ability to program hierarchical structure is a powerful tool that may even allow for improvement upon nature’s protein designs.⁸ This theme is also found in a number of biomaterials applications such as molecular switches that rely on a secondary structure transition⁹ or inorganic nanoparticle assembly that is directed by the formation of a coiled-coil peptide structure.¹⁰ Despite successful demonstrations of secondary and tertiary structure to control material properties, the design of bioinspired materials is still evolving, with increasing focus on the use of soft interactions to drive self-assembly through non-covalent attractions among constituent monomers.

One type of building block for self-assembled biomaterials is the peptide amphiphile (PA) in which a hydrophilic, biofunctional, peptide head group is covalently attached to a hydrophobic tail. The tail is generally a lipid or fatty acid, but polymers, PEGylated lipids, and hydrophobic peptides have also been used to drive self-assembly in aqueous solution.^{11–13} PAs typically self-assemble into micelles or vesicles and micelle structure can vary from small spheroids to long cylindrical fibers and even planar sheets. Such objects are collectively termed protein-analogous micelles, or PAMs. Practically, PAMs are advantageous because the balance of dynamic intermolecular forces, rather than a fabrication process, defines the resulting material geometry and functional properties. The absence of covalent macromolecular structure can be exploited to create PAMs with stimuli-responsive properties such as pH-responsive shape changes^{14–16} and spontaneous degradation for improved clearance of nanoparticles from the body.^{17,18} Furthermore, mixing solutions that initially contain different components offers an easy path to multivalent and multifunctional PAMs.

Non-covalent structure also present challenges for bottom up PAM design because the desired PAM can only be achieved by controlling complicated intermolecular forces between PAs. In particular, intermolecular interactions between peptide head groups in the peptide corona of a PAM can play a large role in determining peptide secondary structure. Secondary structure can subsequently alter both the PAM geometry^{14–16} and the biological activity of the peptide;¹⁹ both properties are critical for PAM function. Several examples in the literature indicate that intermolecular β -sheet structure leads to long, cylindrical fibers that precipitate from solution^{2,20–22} while a range of geometries are compatible with helical secondary structure.^{12,19,23} What remains unclear, however, are the conditions under which α -helices or β -sheets are the stable, equilibrium secondary structure. For example, several helix-forming peptides have been observed in β -sheet conformations after incorporation into PAMs, indicating that there is not a direct correspondence between native peptide structure and secondary structure in a PAM.^{20,23}

There are some properties of a PAM, beyond the native structure of the amino-acid sequence, that have been found to influence secondary structure. For example, steric interactions between neighboring peptides in the PAM corona can drive random coil peptides towards a well-defined secondary structure.^{24,25} Consequently, aggregation of PAs into micelles has been shown to induce secondary structure formation in the peptide head groups that was absent in the unassembled free peptide.^{23,26} Recent work has also shown

that disruption of hydrogen bonding at the core-corona interface can inhibit β -sheet formation^{21,27} as can the incorporation of flexible spacers between peptide and tail.²³ Still, further understanding of general physical principles like these is necessary for bottom up PAM design.

In a crude sense, the PAM corona can be viewed as a polymer brush in which the peptides are analogous to (heterogeneous) polymers. Within this paradigm, the above examples address the effect of tethering density and the method of tethering to the core-corona interface. Contour length is another variable that can tune the properties of a polymer brush, yet studies of the effect of peptide length are largely absent from the PAM literature. This may be due to the fact that contour length is perceived as fixed for a given biofunctional peptide and the potential for secondary structure formation complicates experiments designed to assess the effect of contour length.

This work directly addresses peptide contour length for its effect on both secondary structure and the resulting PAM geometry. Peptide length is increased by repeating a heptad peptide sequence (Ala-Glu-Ile-Glu-Ala-Leu-Lys) of the PA in integer multiples such that a single heptad repeat unit is hereafter called “1hep”, two heptad repeats are termed “2hep” and so on. Dodecanoic acid (C₁₂) was conjugated to the N-terminus of each peptide to create a hydrophobic tail to drive self-assembly. The repeating heptad sequence forms an amphipathic α -helix with a hydrophobic face that further promotes the formation of coiled-coils through inter-helix electrostatic attraction.²⁸ Thus, this peptide was designed to form helices in the presence of aligned inter-peptide interactions, a similar situation to the PAM corona. Despite this strong helical preference, equilibrium peptide secondary structure and PAM geometry was found to depend on the number of heptad repetitions, providing physical insight into the importance of peptide contour length that will aid future design of self-assembled biomaterials.

RESULTS

Enhanced Secondary Structure Formation Due to Micellization

Circular dichroism (CD) was used to determine the secondary structure in phosphate buffer (PB) of both the free peptides and PAs at concentrations of 20 and 200 μ M, respectively. The resulting CD spectra are shown in Figure 1 for solutions less than 10 minutes after hydration. In order to quantify secondary structure content, polylysine basis spectra²⁹ were linearly combined to fit the experimental data, and these results are given in Table 1. Free peptides all exhibited a mixture of α -helical and random coil structure, with helical content increasing monotonically with peptide length. This result is similar to previous work by Su et al.²⁸ in which α -helical content increased significantly between a 19-residue peptide and a 23-residue peptide that are comparable to the 2hep and 3hep used in this work, respectively. Such a trend can be attributed to both the cooperative nature of helix formation as well as the amphipathic character of the α -helical faces that lead to stabilizing coiled-coil interactions. At concentrations above their CMCs, all PAs exhibited increased secondary structure relative to their peptide counterparts, as random coil content was $\sim$ 5% for all PA solutions. This observation is in good agreement with previous studies on micellized PAs^{13,19,26} and indicates that conformational restriction in a peptide brush of the PAM

corona makes it more difficult for the random coil to compete with hydrogen-bonded secondary structures like helices and sheets.²⁵

Beyond increased secondary structure upon micellization, an interesting observation is that the type of secondary structure depends on peptide length. While the secondary structure of peptides in C₁₂-3hep and C₁₂-4hep micelles was 100% α -helical, significant β -sheet content was observed in the peptide head group of C₁₂-1hep (100%) and C₁₂-2hep (35%) micelles. Thus, shorter peptides were more prone to β -sheet structure in the PAM corona even though no such structure was present in the free peptide. Comparing previously measured magnitudes of the mean residue ellipticity (θ , MRE) for polylysine β -sheets to what is shown in Figure 1b would indicate that the MRE for C₁₂-1hep is anomalously low. This discrepancy is due to noticeable precipitation of aggregates over the time necessary to record CD spectra. Precipitation lowered the effective PA concentration below what was used to calculate MRE. This hypothesis is verified by the observation that the shape of the C₁₂-1hep CD spectrum is more consistent with 100% β -sheet structure for a lower bulk concentration of PA than with any other combination of basis spectra.

In addition, we attempted to measure the critical micelle concentration (CMC) for each PA by detecting the partitioning of 1,6-diphenyl-1,3,5-hexatriene (DPH) into the micelle core. In this method, the fluorescence quantum yield of DPH is essentially independent of increasing PA below the CMC. Above the CMC, fluorescence should increase sharply with increasing PA concentration as DPH partitions from aqueous solution into hydrophobic micelle cores.³⁰ At a constant DPH concentration of 1 μ M in this work, DPH fluorescence obeyed a linear relationship with PA concentration for C₁₂-2hep, C₁₂-3hep, and C₁₂-4hep and no concentration-independent range was observed. This indicates that the CMC is in the sub-micromolar range for these PAs but that the exact value cannot be determined from these data. The lower limit of detection for this method is determined by the DPH concentration, as a CMC well below the DPH concentration will not induce a substantial shift in the fraction of DPH that partitions into the micelle core. In the case of C₁₂-1hep, noticeable precipitation occurred shortly after hydration of even the 0.17 μ M sample, indicating the presence of aggregates. It therefore seems likely that all PAs have sub-micromolar CMCs and that the free peptide amphiphile does not contribute significantly to the CD spectra for any PA.

Evidence for Coiled-Coil Tertiary Structure

Peptides used in this work are predisposed to coiled-coil interactions due to both amphipathic hydrophobic/hydrophilic character around the face of each α -helix and complementary placement of oppositely charged amino acids at neutral pH. Thus, peptides are designed to remain helical even in the presence of strong inter-peptide interactions. This effect is particularly apparent for the 3hep and 4hep peptides and we demonstrate this in the free peptide by showing a change in secondary and tertiary structure with increasing concentration in Figure 2. At higher concentrations, peptide-peptide collisions are more frequent, resulting in more peptides existing as dimeric species. Coiled-coil tertiary structure is apparent in the CD spectra when the depths of the minima at 222 and 208 nm are approximately equal while isolated α -helices dominate when the minimum at 208 nm is

significantly lower.^{31,32} This signature of coiled-coil structure is also apparent in the CD spectra of C₁₂-2hep provided in Figure 1b but little secondary structure was observed in the free peptide (Figure 1a), likely due to a coiled-coil dimerization constant significantly above 20 μ M.

At 20 μ M, 3hep exists primarily as isolated α -helices while 4hep exhibits some coiled-coil structure, a result that is likely due to a lower dimerization constant in 4hep. Increasing concentration to 200 μ M allows 3hep to also develop the coiled-coil spectral signature and overall helical content increases in both 3hep and 4hep. This indicates that coiled-coil tertiary structure helps to stabilize α -helical secondary structure even in the free peptides.

In addition to the increased collisions between peptides at higher concentration, PAM formation due to the addition of the C₁₂ tail increases the amount of coiled-coil structure in 3hep. This effect is not present in 4hep, indicating a lower dimerization constant for 4hep such that nearly all the free 4hep peptides exist as dimers at 200 μ M prior to the addition of the hydrophobic tail. Finally, to further confirm the presence of coiled-coil structure, the solvent, trifluoroethanol (TFE), was added to PB in order to inhibit the hydrophobic driving force to form coiled-coils while maintaining hydrogen bonds that promote α -helical structure.^{31,33} In both C₁₂-3hep and C₁₂-4hep, the CD spectra exhibited a decrease in the ratio of θ_{222} to θ_{208} and an overall decrease in helical content. In total, these data indicate that the coiled-coil interaction, in combination with micelle formation induced by addition of the C₁₂ tail, is responsible for the observation of high α -helical content in these PAMs.

Factors Affecting the Helix-to-Sheet Transition

The above observations of secondary structure were made within 10 minutes of hydrating the PAs. Samples were then tracked over time to determine whether the above results represented equilibrium behavior. Over a period of 3 hours at 20°C, CD showed that C₁₂-2hep transitioned to 100% β -sheet structure whereas C₁₂-1hep, C₁₂-3hep, and C₁₂-4hep preserved their initial secondary structures through a period of one week. After transitioning to the β -sheet conformation, C₁₂-2hep remained in this conformation at the end of one week. The stability of α -helical structure for C₁₂-3hep and C₁₂-4hep was confirmed by annealing each solution up to 80°C to attempt to induce β -sheet transitions in each PAM. As shown in Figure 3, elevated temperature caused helical structure to decrease in C₁₂-3hep and C₁₂-4hep, but the post-annealing spectrum matched the pre-annealing spectrum indicating that equilibrium was reached both before and after annealing. For comparison, annealing to 50°C immediately induced a β -sheet transition in C₁₂-2hep that was uninterrupted in subsequent heating cycles. This helix-to-sheet transition was the same phenomenon that occurred gradually over three hours at room temperature, and the fact that heating accelerated the helix-to-sheet transition implies an activated process. CD spectra of the free peptides at different temperatures are also shown in Figure 3, demonstrating that no β -sheet transition was observed in 2hep. Thus, incorporation into the PAM corona is the driving force for β -sheet formation rather than a spontaneous tendency of the peptide itself.

We also explored the role of electrostatics in the helix-to-sheet transition of the C₁₂-2hep PAM by varying ionic strength in the buffer solution and observing the secondary structure profile. Initially after hydration, PAMs in ultrapure water exhibited 64% α -helical content

and 36% β -sheet, which is comparable to what was observed in PB. High ionic strength (170 mM), provided by PBS, caused a significant shift in the secondary structure profile; these PAMs exhibited 29% α -helix and 71% β -sheet. Although a detailed temporal profile of the helix-to-sheet transition was not acquired, a reasonable conclusion is that the reduced role of electrostatics at high ionic strength leads to greater β -sheet content at similar post-hydration times. However, we do not believe that electrostatics play a detectable role in the equilibrium secondary structure of C₁₂-2hep as evidenced by the fact that C₁₂-2hep PAMs eventually transitioned to 100% β -sheet structure at both high and low ionic strengths. We also tested whether high ionic strength PBS could induce β -sheet formation in C₁₂-3hep PAMs and found α -helical secondary structure to persist after one week under these conditions. Thus, electrostatics are primarily kinetic, rather than thermodynamic considerations for the PAMs studied here.

Secondary Structure Correlates with PAM Geometry

PAM geometry was visualized using transmission electron microscopy (TEM) with negative staining, as shown in Figure 4. C₁₂-3hep and C₁₂-4hep, with stable α -helical secondary peptide structure, formed low aspect ratio, spheroidal micelles. In contrast, the β -sheet-forming C₁₂-1hep assembled into elongated rods. This correlation between secondary structure and PAM geometry was also seen in C₁₂-2hep PAMs as a function of time. Immediately after hydration, the helical peptides resulted in spheroidal micelles (Figure 4d). Over time, these micelles elongated (Figure 4e) and eventually coalesced into long fibers (Figure 4f). This shape transition corresponded to the helix-to-sheet transition observed using CD.

DISCUSSION

Bottom-up design of biomimetic assemblies of peptide amphiphiles is a challenging task due to the fact that peptide-peptide interactions in a 'peptide brush' are critically important in determining secondary and tertiary structure motifs that contribute to material or biofunctional properties. To this emerging field, this work adds the observation that there is a 'brush effect' that strongly favors the β -sheet conformation even if the preferred conformation of the untethered peptide is α -helical. Importantly, the ability of this brush effect to change the equilibrium secondary structure from α -helix to β -sheet depends on peptide contour length.

Brush Effects Favor β -sheet Secondary Structure

The behavior of the C₁₂-2hep PAM in this work is reminiscent of results obtained by Shimada et al.²⁰ for a 16-amino acid alanine- and lysine-containing PA with a palmitic acid (C₁₆) tail. These PAs initially formed spheroidal micelles with α -helical and random coil structure in the corona before transitioning to a β -sheet structure with a corresponding elongation of micelle geometry. Several other studies have also identified peptide amphiphiles that are dominated by β -sheet peptide conformations.^{2,21,22} In the present work, the heptad repeat sequence was designed specifically to form helices in the presence of inter-peptide interactions through a coiled-coil mechanism that was stabilized in part by inter-chain electrostatic interactions between lysine and glutamic acid residues.²⁸ It is

therefore surprising that inter-peptide interactions in coronae of C₁₂-1hep and C₁₂-2hep PAMs led to equilibrium β -sheet structure. In total, this evidence suggests that the crowding and alignment of peptides, which results from end-tethering them to the core-corona interface, provide a strong driving force towards the β -sheet conformation. Physical understanding of this driving force has been addressed in simulations by Tsonchev et al.³⁴ who found that the hydrogen bonding pattern of parallel β -sheets prefers to curve around a central axis, making cylindrical PAM geometry particularly good at satisfying all peptide-peptide hydrogen bonds. In the present work this argument is strengthened by the correlation between β -sheet structure and cylindrical micelle geometry and the contrast with α -helical peptides that adopted spheroidal geometry.

It is important to note here that while cylindrical geometry seems to be a consequence of β -sheet formation, there is evidence in the literature that helical PAs are compatible with spherical, cylindrical, and planar geometries.^{12,19,20,26} In these cases, the preferred geometry for helical PAs is determined by the packing parameter,³⁵ a quantity that differs for each individual PA. The packing parameter is defined as $P=v/(aL)$ where v is the amphiphile tail volume, L is the tail length normal to the hydrophobic-hydrophilic interface, and a is the equilibrium head group area. Each self-assembled geometry corresponds to a well-defined range of P such that subtle differences between helical PAs can cause variation in v , a , and L to achieve different geometries.

The observation of initial, non-equilibrium, α -helical peptide structure in C₁₂-2hep PAMs suggests that PAs incorporate into micelles with their preferred untethered secondary structure (coiled-coil helices in this case) rather than adopting the β -sheet as a prerequisite for micellization. The initial micelle formation process is therefore followed by a kinetic transition to equilibrium β -sheet structure. Importantly, this insight implies that one can control the micelle shape transition rate through both environmental conditions and the design of the peptide itself. In this work, we found qualitatively that the transition rate for the C₁₂-2hep PAM increased with temperature, which is a possible consequence of the activation energy required to break the α -helical peptide-peptide hydrogen bonding pattern in the brush in order to reform hydrogen bonds in the β -sheet conformation. In contrast, the C₁₂-1hep PAM, whose shorter contour length both decreased the number of potential hydrogen bonds and resulted in a greater fraction of non-hydrogen bonded random coil structure, transitioned to cylindrical β -sheet precipitates so rapidly that we could not detect any α -helical or random coil content. This faster conversion to β -sheet structure may be a consequence of having to break fewer intrapeptide hydrogen bonds before forming inter-peptide β -sheets in the C₁₂-1hep PAM.

In addition to breaking hydrogen bonds, the previously-mentioned electrostatic stabilization of coiled-coils must also be disrupted before β -sheet formation can proceed. This is evidenced by the fact that high ionic strength allows the helix-to-sheet transition to proceed more rapidly. It is interesting to note that, although electrostatics were designed to promote the formation of coiled-coil helical structure, it is also reasonable to expect favorable lysine-glutamic acid interactions in the β -sheet structure. We therefore conclude that electrostatic stabilization of helices over sheets is a useful kinetic, rather than thermodynamic strategy.

Preferred Secondary Structure Depends on Peptide Contour Length

We have focused the discussion thus far on C₁₂-1hep and C₁₂-2hep, which exhibited β -sheet structure and elongated, cylindrical PAM geometry at equilibrium. However, PAs with longer peptides, namely C₁₂-3hep and C₁₂-4hep, were stable as spheroidal PAMs with α -helical peptide conformations. We now turn our attention to understanding why the equilibrium secondary structure depends on peptide contour length.

One possible explanation for this observation is that longer peptides have greater cumulative electrostatic attractions between neighboring helices. However, we observed α -helices as the equilibrium secondary structure in longer peptides at high ionic strength and found that helices persist even when electrostatic contributions to stability are drastically reduced. This fact indicates that electrostatic interactions are not responsible for the length dependence of the equilibrium secondary structure and thus we seek other explanations for this phenomenon.

In micelles with finite positive curvature in at least one direction (e.g. spherical or cylindrical micelles), the area available to each peptide increases with distance from the micelle core. This curvature effect causes the amino acid density profile to decrease much faster with distance from the grafting surface than the well-known parabolic monomer density profile of a flat brush.^{36,37} This fast density drop-off causes the local environment of the peptide to increasingly resemble the bulk solution when farther from the grafting surface, thus mitigating any crowding-driven brush effects that favor β -sheet formation. It is therefore possible to have a peptide brush in which β -sheet structure is favored near the micelle core but disfavored (relative to α -helices or another conformation) at some finite distance away from the core-corona interface. The likelihood of crossing this threshold increases with contour length as longer peptides can extend farther from the micelle core to experience regions where β -sheet structure is less favorable.

Another geometrical argument for why β -sheet structure was not observed in long peptides also relates to the fact that the distance between neighboring chains increases with distance from the micelle core in curved geometries. In cylindrical micelles, this is true of the inter-chain distance when rotating around the cylindrical axis but not when moving parallel to the axis. However, given that the preferred hydrogen bonding pattern is in the direction rotating about the cylindrical axis,³⁴ the distal ends of the longer 3hep and 4hep peptides may be too far apart to participate in the optimal hydrogen bonding pattern. Much like the argument of the previous paragraph, this scenario could also result in a finite distance from the micelle core at which inter-chain hydrogen bonding in the β -sheet conformation is less energetically favorable than another peptide conformation.

Since either of the above arguments leads to the conclusion that β -sheet structure formation may become unfavorable at a given distance from the micelle core, the question becomes why do we not observe β -sheets near the core and either α -helices or random coils farther away from the core of C₁₂-3hep and C₁₂-4hep PAMs? We believe the cooperative nature of secondary structure formation can explain this observation. In cooperative secondary structure formation, boundaries between different secondary structure elements are unfavorable. In the case of a boundary between β -sheet and random coil amino acids, those

on the random coil side sacrifice significant conformational freedom without the full energetic benefit of β -sheet formation (e.g. they do not hydrogen bond with other amino acids and must remain fully solvated by nearby water molecules). This effect is typically accounted for in models of protein folding through the Zimm-Bragg cooperativity parameter.³⁸ For a boundary between β -sheet and α -helical structures, it is likely that the preferred bond angles for these different secondary structure elements are mismatched, preventing a perfectly sharp transition along the length of the chain. In this case, some amino acids will inevitably be excluded from either secondary structure element and incur penalties similar to those associated with β -sheet / random coil boundaries discussed above.

In contrast to the β -sheet conformation, α -helices and coiled-coils do not require inter-chain hydrogen bonds and hydrogen bonding patterns in these structures are relatively independent of micelle geometry or distance from a curved micelle core. In general, the effects of crowding may help to induce helix formation in peptides whose untethered preference is for the random coil state.²⁵ However, the 3hep and 4hep peptides in this work exhibited high helical content when free in solution and it is reasonable to expect them to maintain this conformation near the exterior of the micelle corona, when crowding is low.

In summary, although there seems to be a 'brush effect' that encourages β -sheet formation near the micelle core, sufficiently long peptides are expected to encounter regions farther from the micelle core in which β -sheet formation is no longer the preferred conformation. The energetic penalty that would be required to form an interface between two distinct secondary structure elements may be sufficiently high that the entire peptide adopts a non- β -sheet secondary structure. The 3hep and 4hep peptides in this work preferred α -helical structure, but other structural motifs may also be possible.

CONCLUSIONS

This work presents a systematic study of the effects of peptide contour length on secondary structure in peptide-amphiphile micelles. By using a heptad repeat sequence of amino acids, we sought to maintain similar peptide-peptide interactions throughout the PAM corona as peptide length varied. The repeating heptad peptide sequences exhibited coiled-coil interactions that helped to stabilize α -helical structure in the presence of strong inter-peptide interactions. Despite this strong preference for α -helical secondary structure in the free peptide, the assembly of a peptide brush in the PAM was able to drive formation of β -sheet structure in PAs with 9 and 16 amino acids while helical structure was preserved in PAs with 23 and 30 amino acids. This result suggests a strong 'brush effect' that favors the β -sheet conformation near the micelle core, but that this effect decreases with distance from the core. Thus, in the design of biomimetic PAMs where helical structure is required of a short peptide, a similar strategy of using repeat units of the relevant peptide may help to avoid β -sheet formation. Alternatively, a non-peptide spacer such as poly (ethylene glycol) may be used to increase the overall contour length of the hydrophilic portion of the PA while maintaining the same peptide sequence. Furthermore, this work, when combined with other examples in the literature of short peptides that form β -sheet coronae in cylindrical or fibrous micelles suggests that this phenomenon is a general feature of self-assembly in

protein-analogous micelles. If this combination of β -sheet structure and PAM geometry is undesirable, then special efforts^{21,23,27} must be undertaken to disrupt β -sheet formation.

The helix-to-sheet transition observed in PAs with short peptides (particularly C₁₂-2hep) was found to be a time-dependent process in which the PAM formed initially with peptide secondary structure that resembled its conformation when free in solution. The peptide then transitioned to a lower-energy β -sheet conformation likely by overcoming an activation barrier that involved the disruption of its α -helical secondary and coiled-coil tertiary structures. Inter-peptide electrostatic attractions were also found to play a role in the size of this barrier. This result suggests that shape-changing biomolecular assemblies can be built with tunable shape transition rates by precisely controlling the inter-peptide interactions between coiled-coil helices.

MATERIALS AND METHODS

Peptide and PA synthesis

Peptides were purchased (China Tech Peptide) fully protected and on the resin. The sequence of each repeat is listed below:

1hep: WKAEIEALK

2hep: WKAEIEALKAEIEALK

3hep: WKAEIEALKAEIEALKAEIEALK

4hep: WKAEIEALKAEIEALKAEIEALKAEIEALK

Note that, in addition to the repeating heptad sequence (AEIEALK), WK was prepended to the N-terminus of each peptide for spectroscopic quantification of concentration using the molar extinction coefficient of tryptophan absorbance. PAs were synthesized by adding a dodecanoic acid to the N-terminus of the peptide following Fmoc deprotection, as described previously.¹¹ Peptides and PAs were purified to >90% by reverse-phase high performance liquid chromatography on a C₈ column, and product identity was confirmed with MALDI-TOF mass spectrometry.

To form PAMs in aqueous solution, lyophilized PA powder was first dissolved in methanol, which was subsequently evaporated slowly under nitrogen to form a thin film on the interior wall of a glass vial. Films were dried overnight under vacuum, and then rehydrated in aqueous solution. Aqueous solutions used in this work included ultrapure water (purified to a resistivity of 18.2 M Ω -cm), phosphate buffer (PB, composition: 8.1 mM Na₂HPO₄ + 1.8 mM KH₂PO₄), and phosphate buffered saline (PBS, composition: PB + 138 mM NaCl + 2.7 mM KCl). The pH of all peptide and PA solutions was 7.5 $\pm$ 0.2.

Critical micelle concentration (CMC)

The DPH (1,6-diphenyl-1,3,5-hexatriene) fluorescence method was used to determine the CMC of each peptide amphiphile.³⁰ This method relies on an increase in DPH fluorescence as it partitions from aqueous solution into the non-polar micelle core. Solutions of each PA in PB were formed at PA concentrations ranging from 320 to 0.17 μ M. DPH was then

dissolved in tetrahydrofuran (THF) and a small amount of this solution was added to each PA solution to achieve 1 μ M DPH with a residual THF volume percentage of ~0.1. Solutions containing both PA and DPH were allowed to equilibrate for 1 hour prior to measurement using a Tecan Infinite 200 plate reader. DPH was excited at 350 nm and fluorescence emission was measured at 428 nm.

Circular dichroism (CD)

CD was performed in a 0.1 cm path length cuvette (Jasco J-815). Scans were run from 260 nm to 190 nm at a speed of 20 nm/min, and reported curves are an average of three scans. Temperature was set at 20°C and the buffer used for CD measurements was PB, unless otherwise noted. When a percent helix is reported, the CD spectra were fit by a linear combination of the polylysine basis spectra.²⁹

Transmission electron microscopy (TEM)

Peptide amphiphile solutions were prepared at 1 mg/mL in PB buffer. A volume of 1 μ L of each solution was loaded onto an ultrathin carbon type-A 400 mesh copper TEM grid and allowed to dry. Samples were negatively stained using 1 μ L of 1% phosphotungstic acid in water placed onto the dry grids for 1 minute, after which excess stain was removed. Electron microscopy imaging was performed using an FEI Tecnai 12 TEM at an accelerating voltage of 120 kV.

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Figure 1. CD determines secondary structure of free peptides and peptide amphiphiles
Mean residue ellipticity is shown as a function of wavelength for free peptides at 20 μM (a) and peptide amphiphiles at 200 μM (b).

Figure 2. Coiled-coil interactions in 3hep and 4hep peptides and PAs

CD spectra are shown for 3hep and C₁₂-3hep (a) and 4 hep and C₁₂-4hep (b) at various concentrations and solvent conditions. In both plots, spectra of the free peptide are shown at concentrations of 20 μM and 200 μM in PB while the PA is shown at a concentration of 200 μM in both pure PB and a 1:1 mixture of PB and TFE.

Figure 3. Annealing data for peptides and PAs in PB

(a) 2hep peptide (b) C₁₂-2hep (c) 3hep peptide (d) C₁₂-3hep PA (e) 4 hep peptide, and (f) C₁₂-4hep PA. The peptide concentration for each spectrum was 20 μM while that of the PA was 200 μM . The asterisk indicates the post-annealing spectrum and the solid black curve in each panel corresponds to the data shown in Figure 1 for that peptide or PA. Spectra of the 1hep peptide and PA are not shown due to precipitation of the C₁₂-1hep structures during the experiment.

Figure 4. Negatively stained TEM images of PAMs

(a) C₁₂-1hep (b) C₁₂-3hep (c) C₁₂-4hep (d) C₁₂-2hep, immediately after hydration (e) C₁₂-2hep, approximately 30 minutes after hydration (f) C₁₂-2hep, one week after hydration. The scale is constant in all images.

Table 1

Best fit secondary structure profile for CD spectra of peptides and PAs.

Species	% α -helix	% β -sheet	% random coil
1hep	14	0	86
2hep	21	0	79
3hep	56	0	44
4hep	88	0	12
C ₁₂ -1hep	0	100	0
C ₁₂ -2hep	60	35	5
C ₁₂ -3hep	100	0	0
C ₁₂ -4hep	100	0	0