Self-Assembly of Highly Asymmetric Polypeptide Amphiphiles

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Statement of Purpose: Elastin-like polypeptides are polymers based on the pentapeptide monomeric repeat VPGαG where the guest residue α can be any amino acid except proline. ELPs display a lower critical solution temperature (LCST) phase transition with a characteristic transition temperature that can be tuned by controlling the chain length and/or the hydrophobicity of the guest residue.[1] Due to their recombinant design, non-toxicity, biodegradability, and tunable stimulus-responsive behavior, ELPs have been used in many self-assembling drug delivery systems.[2-4] Here, the unusual self-assembly of highly asymmetric amphiphiles composed of a high molecular weight elastin-like polypeptide and a short hydrophobic assembly domain is characterized. The structure of these highly asymmetric amphiphiles follows the motif: $(M)SKGPG - (VPGAG)_n - (XG_z)_8$, where n is the number of pentameric repeats, X is the identity of the amino acid hydrophobe responsible for driving self-assembly, and y is the number of glycine (G) spacers. This work samples a subset of this population by independently modulating these variables to better understand how these sequence parameters affect the block copolymers' phase transition and self-assembly.[5] The increased understanding of biopolymer self-assembly developed here can be used to tune self-assembly and morphology for a specific drug delivery application.

Methods: All polypeptide amphiphiles were synthesized recombinantly and purified using inverse phase transition cycling, a technique that exploits the LCST phase transition of the ELP. Amphiphiles were characterized by dynamic and static light scattering (DLS and SLS) in order to assess their size and aggregation number, or number of polypeptide chains per nanostructure. Thermal turbidimetry was used to characterize the amphiphiles' LCST phase transition, and pyrene fluorescence was used to determine their critical aggregation concentration (CMC). Cryogenic transmission electron microscopy (cryo-TEM) was used to directly visualize the nanostructures in their near-native, hydrated state, and small-angle neutron scattering (SANS) was used to provide a detailed mesoscopic picture of their self-assembly.

Results: We report that despite their high degree of asymmetry (hydrophilic weight fraction >95%), many amphiphiles unexpectedly self-assembled into cylindrical micelles rather than the expected star-like morphology. Light scattering shape factor ($\rho = R_g/R_h$) values ranging from 1.3 to 1.4 suggested a non-spherical morphology, and a cylindrical morphology was confirmed by cryo-TEM and SANS (Figure 1). The cylindrical morphology and aspect ratio were conserved across a number of amphiphiles with different sequences. Additionally, we report that the self-assembly and morphology of these highly asymmetric amphiphiles is governed by both the hydrophobicity of the amino acid X and the number of glycine spacers z. Self-assembly and morphology could be highly tuned by varying the composition of the hydrophobic assembly domain. When coupled with an assembly domain of $(FGG)_8$, ELPs composed of 160, 80, and 40 repeats of the monomer (VPGAG) self-assembled into to nanostructures with a conserved morphology, but with aggregation numbers (N_{agg}) of 575, 290, and 111, respectively. Similarly, the same high molecular weight $(VPGAG)_{160}$ did not self-assemble with an assembly domain of $(YGG)_8$, but self-assembled into two distinct morphologies cylindrical morphologies with assembly domains of $(YG)_8$ and Y_8.

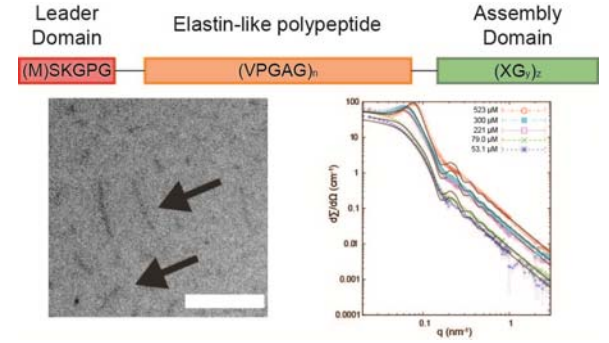

Figure 1. Asymmetric amphiphile sequence motif and cryo-TEM image and SANS spectra of cylindrical micelles. Scale bar is 200 nm.

Conclusions: We have shown that asymmetric amphiphiles unexpectedly self-assemble into a cylindrical morphology, despite their very high hydrophilic weight fraction. Additionally, we have used their recombinant design to show that their self-assembly can be precisely tuned by varying the length of the ELP block, the hydrophobicity of the amino acid X, and the number of glycine spacers z, enabling asymmetric amphiphile self-assembly to be specifically tuned for a desired drug delivery application. The morphological diversity within this subset of amphiphiles suggests that there may be vast and as-of-yet unexplored regions of sequence space with morphological diversity. Future studies will explore these regions, as well as address more fundamental questions regarding the mechanism of self-assembly for asymmetric amphiphiles.

References:

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