Temperature Triggered Self-Assembly of Elastin Like Polypeptides

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Statement of Purpose: Amphiphilic macromolecules that self-assemble into supramolecular structures such as micelles and gels have numerous applications in drug delivery and tissue engineering. Stimuli-responsive amphiphilic macromolecules are an especially interesting subset whose self-assembly can be triggered by an external trigger. We are interested in using one such class of stimulus responsive polymers –Elastin-like polypeptides (ELPs)– for the design of "smart" self-assembling systems. ELPs are temperature sensitive biopolymers composed of a Val-Pro-Gly-Xaa-Gly pentapeptide repeat (where the "guest residue" Xaa is any amino acid except Pro) derived from a structural motif found in mammalian elastin. These biopolymers undergo an inverse temperature phase transition; i.e. they are soluble at temperatures below their transition temperature (T_t) but become insoluble and aggregate at temperatures above their T_t.

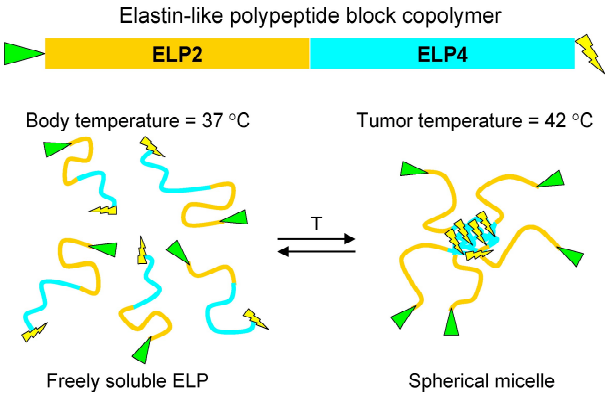

Figure 1. Schematic of an ELP_{BC} and its thermally triggered self-assembly. An ELP2 (hydrophilic, high T_t) and ELP4 gene (hydrophobic, low T_t) are seamlessly fused together to create an ELP_{BC}. When the size and ratio of the blocks are correctly selected, the ELP_{BC} self-assembles into a spherical micelle at 40 °C. Multiple copies of the ligand (green triangles) are presented at the exterior of the micelle in the corona while the radioactive payload (yellow) is sequestered within the core of the micelle.

We report the design of diblock ELPs that can be triggered to self-assemble into spherical micelles in a narrow, physiologically relevant temperature range of 37-42 ºC (**Figure 1**). The choice of this temperature range as the trigger for thermally driven self-assembly is dictated by the fact that regional hyperthermia is clinically approved in this temperature. We further show that version of the same polypeptides that bear terminal RGD or NGR targeting ligands also exhibit similar self-assembly behavior, leading to the formation of

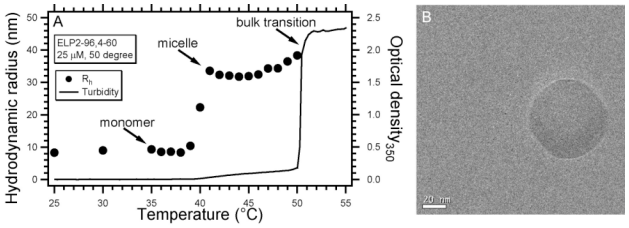

Figure 2. Thermally triggered self-assembly and cryo-TEM of an ELP-BC. A) DLS and UV-vis spectrophotometry show that ELP2-96,4-60 forms a micelle at temperatures between the T_t of both ELP blocks. B) Spherical micelles are confirmed by cryo-TEM of ELP2-96,4-60 at a temperature that induces micelle formation. Scale bar in (B) is 20 nm.

multivalent polymer micelles. The demonstration of reversible self-assembly in a narrow, clinically relevant range is significant because it opens up the possibility of designing macromolecular carriers of drugs and imaging agents that can be selectively targeted *in vivo* based on a clinically relevant physical trigger.

Results: We have synthesized 10 different ELP_{BC} with various MWs and hydrophilic to hydrophobic ratios, of which 6 form spherical micelles when heated to intermediate temperatures between the T_t of both ELP blocks. It was empirically determined that the ratio of hydrophilic to hydrophobic blocks must be between 1:2 and 2:1 in order for the ELP_{BC} to self assemble into spherical micelles triggered by an increase in temperature. The temperature dependent self-assembly of ELP_{BC} determined by UV-vis spectrophotometry and dynamic light scattering (DLS) is shown in **Figure 2A**. The ELP_{BC} is highly soluble as a monomer at low temperatures but forms a micelle at 40 °C due to the hydrophobic transition of the low temperature block (ELP4). This micelle persists up to 50 °C where the corona block (ELP2) undergoes its transition and a bulk aggregate is formed. The formation of spherical micelles was confirmed by vitrifying an ELP_{BC} at a temperature that induces micelle formation and imaging the samples with cryo-TEM as shown in **Figure 2B**. The micelle size of 60 nm determined by DLS and cryo-TEM are nearly identical. Angular dependent DLS studies confirmed the cryo-TEM results that the ELP_{BC} self-assemble into perfectly monodisperse spherical micelles as no angular dependence could be detected (data not shown).

Micelle formation temperature and size can rationally controlled. The micelle formation temperature (also called critical micelle temperature (CMT)) is determined by the MW of the low temperature block. It has been shown that the T_t of an ELP is inversely proportional to its MW; therefore, increasing the MW of the low temperature block reduces the CMT. The size of the micelle is influenced by the MW and the ratio of

hydrophilic to hydrophobic blocks. For example, as the hydrophobic block fraction becomes smaller, the R_h decreases for a consistent MW (see MW ≅ 74 kDa). Furthermore, as the total MW is raised and the ratio of blocks is held constant (e.g., 1:1) the average size of the micelle also increases. The R_g/R_h values are close to the predicted value of 0.775 for a solid sphere, consistent with the formation of a spherical micelle. The coordination numbers range between 60-80, and indicate that these ELP_{BC} self-assemble into spherical nanoparticles above 37 ºC that are capable of presenting polyvalent ligands.

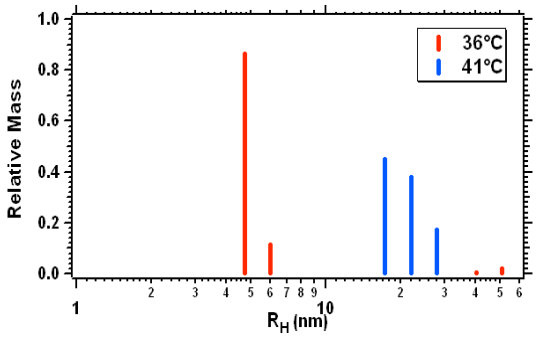

Figure 3. The hydrodynamic radius distribution of 25 µM RGD-ELP2-64,4-60 at 36°C and 41°C. At 36 ºC, greater than 98% of the mass in the sample are unimers (*i.e.,* soluble ELP_{BC} polymer chains). Upon raising the temperature to 41 ºC, these unimers self-assemble into spherical micelles.

RGD-presenting ELP_{BC} also undergo thermally triggered monomer-micelle transition. Having observed that the ELP_{BC} form spherical micelles in the 37-41 ºC, we turned our attention to the synthesis of the thermally triggered targeting vehicle – ELP_{BC} that present linear targeting peptides at their N-terminus. We modified the synthetic gene for the ELP_{BC} to encode the codons for RGD and NGR, and expressed the ELP_{BC} in *E. coli*. The thermally triggered self-assembly of the RGD-functionalized ELP_{BC} was studied by DLS as a function of temperature. **Figure 3** shows the distribution of hydrodynamic radius (R_h) for RGD-ELP2-64,4-60 at 36°C and 41°C (25 µM solution). The unimer (free ELP) comprises greater than 98% of the total mass at 36 ºC, while the micelle comprises greater than 95% of the mass at 41 ºC.

Conclusions. A series of ELP_{BC}s with a range of MWs and hydrophilic to hydrophobic ratios were successfully synthesized. These ELP_{BC}s form monodisperse spherical micelles when the hydrophilic to hydrophobic block ratio is between 1:2 and 2:1. These polypeptide micelles are fairly stable as they display a CMC in the range of 1-10 µM. In results not shown here, we have also observed that the rigidity of the ELP increases as it undergoes self-assembly into micelles, and the micelles' core is both more rigid than the ELP_{BC} unimer that is it is derived from as well as the aggregated ELP. The CMT is controlled by the length of the low T_t block and scales with the parent ELP block's T_t. The size of the micelle can be rationally controlled by both the total ELP_{BC} length and hydrophilic to hydrophobic block ratio.

In summary, these results provide the first set of empirical heuristics for the design of ELPs for temperature-triggered self-assembly. Although the results of this study are immediately useful in our quest to design thermally triggered multivalent nanoparticles with relevance for thermal medicine, critical questions that remain to be answered are: (1) What absolute chain lengths and ratios of the two blocks will yield other geometries such as wormlike micelles and vesicles; (2) How is micelle formation controlled by the difference in T_t between the two blocks? Answering these questions forms the basis for ongoing studies of these genetically encoded self-assembling polymers.