Genetically Encoded Inverse Bolaamphiphiles

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Introduction

- Post-translational modification of proteins is widely used in biological systems and plays an important role in modifying the function of polypeptides. The eukaryotic N-myristoyl transferase (NMT) genetically incorporates myristic acid at the N-terminus of proteins. The C-terminal domain of a hedgehog protein (HhC) can attach a cholesterol moiety to the C-terminus of the polypeptide by cleaving itself.
- Elastin-like-polypeptides (ELP) are a class of proteins with rapidly increasing importance due to their unique properties. By having a lower critical solution temperature (LCST), they exhibit temperature triggered hierarchical self-assembly.

Figure 1: Combining orthogonal PTM pathways to produce genetically encoded i-Bolas. (a) Schematic of our bicistronic vector that encodes the elements required for N-myristoylation and C-cholesterylation. (b) Comparison between the architecture of single tail amphiphiles and i-Bolas.

Overview

- We first demonstrated the feasibility of our approach to produce inverse bolaamphiphiles (i-bola), a hydrophilic domain flanked by two hydrophobic domains, by incorporating myristoylation and cholesterylation at the N- and C-termini of ELP, respectively.
- To understand the effect of these tandem PTMs, we then investigated the self assembly of the i-Bolas and compared them to single-tail amphiphile controls bearing either the N- or C-terminal lipid using turbidimetry, light scattering, and transmission electron microscopy (TEM).
- Finally, we established that these two PTMs can be combined in a prokaryotic expression system to genetically synthesize sequence-defined i-Bolas with high yield and facile purification.
- These genetic i-Bola structures exhibit hierarchical self-assembly that is significantly different from that of their single-tail counterparts. These results underscore the promise of our approach to repurpose biological machinery to develop unexplored lipoproteins with tunable nanoscale structures and morphologies.

In vitro Synthesis of i-Bola

- NMT and ELP-HhC were co-expressed from a bicistronic vector inside *E. coli*. The co-expressed NMT is active and modifies the N-terminal of its substrate (ELP-HhC) during expression.
- Myristoylated ELP-HhC reacts with cholesterol to form two new products, indicating that the N-myristoylation does not interfere with the function of the hedgehog domain.
- Constructs were purified by taking advantage of their temperature triggered phase transition using inverse transition cycling (ITC), followed by preparative HPLC.

Figure 2: In vitro synthesis of i-Bola by combining N-myristoylation with C-cholesterylation. (a) SDS-PAGE analysis of proteins demonstrates that NMT and ELP-HhC can be co-expressed from a bicistronic vector. Lane 1: ELP-HhC; lane 2: co-expression of NMT and ELP-HhC. (b) The peak corresponding to ELP-HhC shifts to longer retention times when it is expressed with NMT, consistent with the increased hydrophobicity of M-ELP-HhC. (c) Myristoylated ELP-HhC reacts with cholesterol to form two new products, (d) RP-HPLC chromatogram of the in vitro reaction between M-ELP-HhC and Cholesterol is consistent with the formation of M-ELP-Chol (marked with p3).

Characterization

- The LCST phase transition was quantified by measuring the turbidity at 350 nm at different temperatures (Figure 4).
- Turbidimetry confirms that lipidation reduces the transition temperature of ELPs. However, the identity and the topology of the lipidation influence how turbidity changes with temperature and concentration, suggesting that these molecular parameters influence the density or coarsening of the ELP coacervates.

(b) DLS is used to monitor the reversibility of LCST phase behavior of ELP constructs after heating and cooling the samples above LCST. Rh_i is the initial hydrodynamic radius below the LCST (15 °C); Rh_f is the hydrodynamic radius after heating the samples above LCST (40 °C) and cooling to the initial temperature. Unlike M-ELP and ELP-Chol, the i-Bola construct exhibited a hysteric increase in its hydrodynamic radius after the thermal cycle.

Morphological Characterization

- Morphology of ELP constructs was analyzed by TEM.
- M-ELP-Chol features larger, noticeably different spherical structures.
- The result demonstrate that PTM is a powerful approach to control the nanomorphology of protein-based materials.
- It also confirms our hypothesis that the architecture of lipidated protein (single-tail vs i-Bola) influences the self-assembly at nanoscale.

Figure 6: TEM reveals the effect of PTM on the nanomorphology of proteins in this study. (a) M-ELP and (b) ELP-Chol self-assemble into similar ~15 nm spherical nanoparticles. (c) M-ELP-Chol self-assembles into larger, ~55 nm, spherical nanoparticles.

Application of our Method with Different Protein

- We applied our method to another construct - (GSGVP)₈₀ in which the guest residues were changed from a mixture of hydrophobic Val and Ala to hydrophilic Ser residues (sELP).
- The construct is self-assembled into a mixture of spherical and ellipsoidal particles with an average diameter of 60.3 ± 11.4 nm (Figure 7c). This is possibly due to the lack of repulsive charged-charged interactions along the polypeptide backbone (as M-sELP-Chol does not contain positively charged lysine residues that are found in M-ELP-Chol).

Figure 7: Formation of M-sELP-Chol and its self-assembly in lysate. (a) M-sELP-HhC reacts efficiently with cholesterol even in the complex cellular environment of the cell lysate. (b) Modified products can be easily purified from endogenous proteins by leveraging the phase behavior of the ELP. HPLC chromatogram after two rounds of purification: p1: sELP, p2: M-sELP, p3: sELP-Chol, p4: M-sELP-Chol. (c) TEM reveals the morphology of the purified M-sELP-Chol aggregates, where a mixture of spherical and ellipsoidal particles is observed.

Conclusion

- We have demonstrated for the first time that it is possible to leverage the inherent regioselectivity of the PTM machinery to obtain post-translationally modified polypeptides with i-Bola structure.
- Our recombinant method provides a facile approach to synthesize protein-based nanoparticles and expands the phase-space of available nanostructures beyond single-tail amphiphiles.
- Our results also indicate that the architecture of lipidated protein and the sequence of the polypeptide can be used to control the hierarchical self-assembly of these protein-based materials across different length scales.
- The deeper understanding of molecular parameters controlling the assembly of this new class of biomaterials, achieved by the combination of computational and experimental tools, is the critical next step toward advancing their use in applications ranging from delivery vectors for the transport of hydrophobic therapeutic cargo to nanoreactors templating the formation of biominerals.

References

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