Biologically Inspired Engineering of Self-assembling Underwater Adhesives with Synthetic Biology

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Statement of Purpose: Several marine organisms achieve strong interfacial underwater adhesion in turbulent environments using hierarchically assembled amyloid nanostructures. Biomimetic adhesives that recapitulate the strong wet bonding strength, the robustness, and the structural and functional complexity of their natural counterparts would have broad applications in both medicine and biotechnology. Here, we demonstrate a modular genetic strategy for engineering underwater adhesives based on the fusion of mussel foot proteins (Mfps) from *Mytilus galloprovincialis* with CsgA, the major subunit of adhesive curli fibers from *Escherichia coli*. We expect that these self-assembling fibrous ultra-strong underwater adhesives will serve as advanced adhesive materials for medical and industrial applications in the future.

Methods: Recombinant genes coupling CsgA, Mfp 5(3), and a C-terminal six His-tag were constructed using Gibson assembly and inserted into plasmid pET-11d. The genes were expressed in the NEB C3016 strain and the recombinant proteins were purified under denaturing conditions using Tylon resin (cobalt). The morphology of adhesive proteins was assessed by TEM imaging via negative staining or gold staining. AFM force measurements were made at room temperature in pure water or PBS using an Asylum MFP-3D AFM (Asylum Research, Santa Barbara, CA, USA) mounted on top of an Olympus IX51 inverted optical microscope for visualizing and manually positioning regions to be probed. Force measurements were made at a rate of 0.3 to 0.5Hz, using $Si_3 N_4$ cantilevers with calibrated spring constants between 0.06~11 N/m. All AFM measurements were made with at least 200 force-extension curves taken for different regions.

Results: Incubation of freshly made protein solutions overnight at room temperature led to the formation of hierarchically assembled nanofibers for CsgA-Mfp3, as confirmed by TEM (Fig. 1B). The CsgA-Mfp3 nanofibers had an average fiber diameter about 30 nm, around three times the size of the unmodified CsgA nanofibers prepared under the same conditions (Fig 1A). Ni/NTA-gold-nanoparticle binding assays for the detection of His-tags directly fused to the C-terminal of Mfp-3 revealed that the His-tags and thus the Mfp-3 adhesive domains are mainly localized on fiber surfaces. In addition, more gold nanoparticles bound to the CsgA-Mfp3 nanofibers compared with control CsgA nanofibers, suggesting a potentially stronger adhesive interaction between gold nanoparticles and the modified nanofibers (Fig. 1C). Similar observations were found for Mfp5-CsgA nanofibers (data not shown). AFM force measurements (adhesive force) further revealed that Mfp5-CsgA and CsgA-Mfp3, respectively, exhibited >10-fold and >3-fold increases in adhesion forces compared with CsgA control nanofibers (data not shown).

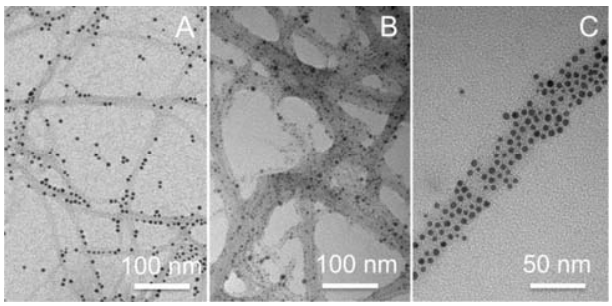

Figure 1. Morphology Characterization of CsgA and CsgA-Mfp3 proteins incubated in aqueous solution after one week: (A) TEM of CsgA, (B and C) TEM of CsgA-Mfp3. Gold nanoparticles were used to detect His-tags displayed on these proteins. The average fiber diameter for CsgA and CsgA-Mfp3 is about 10.2 ± 1.5 nm and 28.4 ± 3.3 nm, respectively.

Conclusions: This work introduces a new platform that for designing self-assembling amyloidogenic adhesive materials inspired by nature. We envision that adhesive biomaterials based on rational and modular fusion of natural functional domains will find broad applications and provide insights into the interfacial underwater adhesion phenomena in nature.

Acknowledgements: This research is supported by funding from the Office of Naval Research (ONR).