Designing Self-Assembling Protein Materials for Imaging and Energy Applications

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Project Goals: The project goals are to develop and test methods for designing novel self-assembling protein materials for applications that advance Department of Energy interests in high resolution imaging methods, green chemistry and metabolic engineering. Specific current goals include the design of a modular scaffold that will enable cryo-EM methods for smaller proteins, and the design of protein cages for the attachment of multiple sequentially-acting enzymes involved in cellulose degradation.

Abstract – Recent advances in designing proteins to self-assemble into specific architectures are opening up numerous exciting technology applications (1). One of these is in cryo-electron microscopy imaging. Recent advances in EM hardware and software have made it possible to image large protein and nucleic acid assemblies at near-atomic resolution detail. Notably, large and symmetric structures are the most easily studied, whereas smaller structures (e.g. below about 50 kD) are generally impossible owing to low contrast and low signal making particle identification and orientation unreliable. A long-standing goal has been to create scaffolding systems that would enable the cryo-EM imaging of smaller proteins, like those found throughout the cell, possible. An ideal scaffold would be large and symmetric and would hold many copies of a protein target to be imaged in rigid orientations in order to facilitate high resolution imaging. In this project we have demonstrated the first designed scaffold that meets those design requirements. A designed protein cage comprises the core. The core is then fused by a semi-rigid continuous alpha helical connection to a DARPin protein, which serves as a modular adaptor for selectable binding to other diverse target proteins. So far, we have imaged the scaffold itself (without target proteins attached) and have shown that the DARPin is attached rigidly enough to resolve this small 18 kD protein at resolutions from 3.5 to

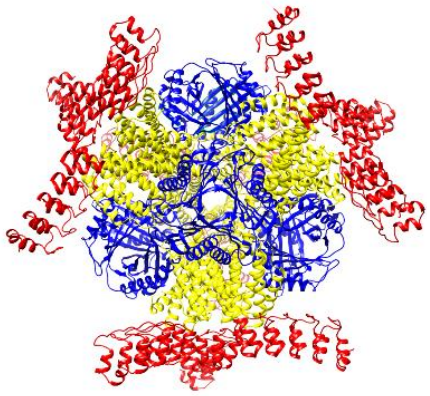

Figure 1. EM structure of a designed modular protein scaffold for cryo-EM imaging. The designed cage subunits are in blue and yellow. The DARPin adaptor domain (red) is fused to one of the two protein cage subunits by a rigid alpha helical linker. Modifying the loop sequences of the DARPin adaptor allow the rigid binding and presentation of diverse protein targets for imaging.

5 Å (2) (Fig. 1). Imaging of attached target proteins is underway. In a second set of distinct applications we are adapting designed protein cages for the display of multiple sequential enzymes. We are applying this idea to the problem of cellulose degradation. New methods based on sortase recognition sequences are being developed for the facile attachment of enzymes to designed cages (3). We have shown that a protein cage in which one component contains a C-terminal tag for recognition by the sortase enzyme SrtA can be covalently linked to the cellulase enzymes Cel8A and Cel48S. Preliminary data suggests that the cages remain intact after the modification and that both of these cellulase enzymes can be displayed on the cage surface simultaneously (Fig. 2). It is hypothesized that modifying designed protein cages in this way can increase pathway flux for the degradation of cellulose, a notoriously slow process, by forcing the enzymes that act sequentially in this pathway into close proximity. Tests of enzymatic efficiency in cellulose degradation are underway.

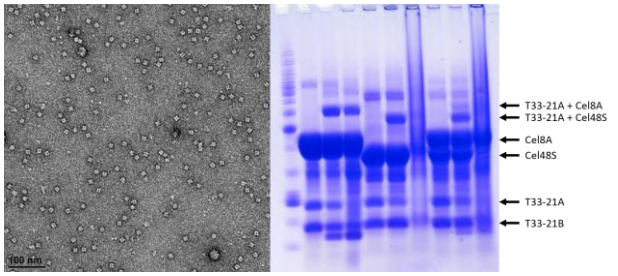

Figure 2. Left: Representative EM micrograph of T33-21, a 2-component tetrahedral cage, after attaching Cel8A via SrtA. Right: SDS-PAGE showing attachment of Cel8A and Cel48S, both separately and simultaneously, to the A component of T33-21.

References

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