

## **Design, Characterization, and Emerging Applications of Highly Symmetric Protein Nanostructures**

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**Project Goals: A primary goal of our work is to develop robust strategies for building novel materials and nanoscale structures using protein molecules as the building blocks. Several recent successes have been demonstrated in creating novel protein cages and ordered 2-D arrays. Our ongoing efforts are focused on improving design strategies to increase the currently low success rates, and on exploring applications of the designed protein materials in energy-related applications, including organizing sequentially acting enzymes. Current efforts toward achieving these goals will be described.**

Motivated by the rich diversity of protein molecules that have evolved by nature to form complex and highly symmetric supramolecular structures, recent engineering efforts in the field of protein design have exploited symmetry to create novel self-assembling protein structures of types unseen in biology. Such materials range from finite cages or shells to essentially unbounded two-dimensional and three-dimensional arrays (i.e. crystals) to linear or tubular filaments. Recent work in the Yeates laboratory and in collaborating groups has led to the successful design and characterization of several cage structures exhibiting tetrahedral, octahedral, and even icosahedral symmetries which show close agreement with design specifications. For certain design approaches, a complete list of allowable symmetry combinations that can be used for construction has been articulated, opening a path for creating a rich diversity of geometrically defined protein materials.

Multiple new lines of work are addressing current design challenges. Some of those efforts aim to improve upon the helix fusion strategy of cage design, wherein two naturally oligomeric proteins are fused together by an alpha helical linker at a specified geometry. New design variations are focused on reducing the polymorphism and flexibility of designed structures by optimizing design parameters to create more compact and rigid structures, especially to allow higher order symmetries such as icosahedral to be reliably achieved. Projects toward this goal include utilizing chemical cross-linkers to staple the alpha-helical fusion into a strictly defined geometry, as well as replacing the single helix linker with a coiled coil to make the structures more robust. Promising candidates from these approaches are currently undergoing characterization.

Further work in the lab has been inspired by the large microcompartment shells that exist in many bacterial cells. Our recent experiments have led to the formation of a novel dodecahedral nanocage made from one redesigned microcompartment shell protein. This result has inspired efforts to utilize these shell proteins for further cage designs as well as for gaining further insight into the evolution of symmetric proteins in general.

Following the exciting successes of protein nanoarchitectural designs in the last few years, the lab has also begun to turn its attention to the ways in which these protein nanomaterials can be endowed with properties useful for applications in materials science, energy and medicine. Ongoing efforts include decorating the outside of previously characterized cages with new amino acid sequences that correspond to bioactive peptides or enzymatic recognition tags. Our recent experiments have shown success in using sortase to covalently attach other proteins to the outside of a tetrahedral cage by incorporating a short amino acid sequence that is recognized by sortase at the C-terminal end of one of the cage's component proteins. The success of this method could make it possible to attach a wide range of other proteins to the outside of cages, and the availability of up to 4 unique termini exposed on the surface could lead to applications where multiple enzymes that act in sequence are attached to the same cage in order to increase the pathway flux.

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