Methods for the Synthesis of Defined Protein Nanotubes

Julie M. Silverman[1], James W. Wollack[2], Edward R. Ballister[3], Angela H. Lai[4], Yifan Cheng[4], Ronald N. Zuckermann[3], Tamir Gonen[5], Mark D. Distefano[2], and Joseph D. Mougous[1]

Departments of [1]Microbiology and [5]Biochemistry, University of Washington, Seattle, WA 98195; [2]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455; [3]Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; [4]W. M. Keck Advanced Microscopy Laboratory, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158

Proposal Title:

Flexible Protein Building Blocks for Nanotechnology

Research Achievement:

Biological molecules fulfill a unique and important niche in nanoscale materials. Unlike synthetic inorganic and organic materials, biological molecules have evolved over eons to possess properties such as exquisite molecule recognition and highly efficient, selective catalysis. Proteins, arguably the most versatile natural polymers, are a particularly flexible nanoscale material – their templated synthesis makes them easily modified and many possess the ability to self-assemble into high-order arrays. We have developed a novel, flexible *in vitro* self-assembled protein nanotube system using the *Pseudomonas aeruginosa* secreted ring protein Hcp1. Hcp1 nanotubes differ from other protein nanotubes in that they are non-helical and are stabilized by covalent bonds (Figure 1). In addition, the nanotubes have an outer/inner diameter ratio of < 2.0, which is exceedingly small for protein nanotubes. We exploited these properties of the Hcp1 system in order to control nanotube length, specify terminating subunits, and to generate Hcp1 nanocapsules (Ballister 2008). Most recently, we have begun to develop methods that will allow for the synthesis of Hcp1 nanotubes of discrete length and composition. We have generated Hcp1 ring subunits site-specifically modified at their C-termini with assorted DNA oligonucleotides (Wollack 2009). These subunits will be brought together in a specified number and order using an appropriate

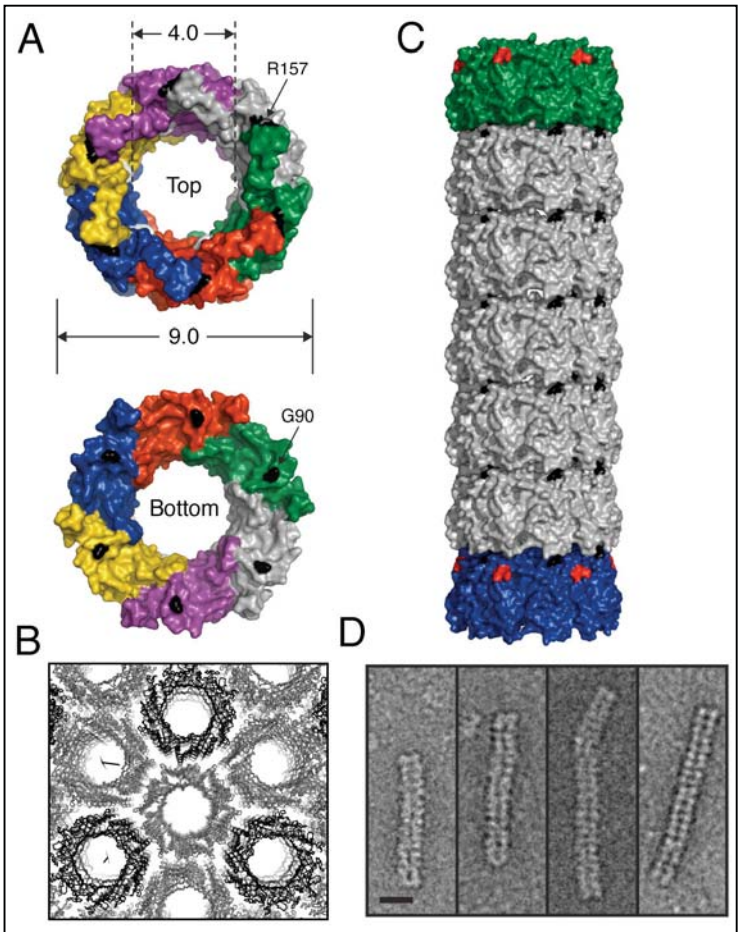

Figure 1. Overview of the Hcp1 nanotube system. (A) Molecular surface representation of the "top" and "bottom" faces of the Hcp1 hexamer. Monomers are colored for clarity. Cysteine mutation sites (black) and key dimensions (in nm) are provided. X-Y crossection of the honeycomb Hcp1 P6 crystal lattice. The two nanotube orientations are indicated with arrows (left) and gray shading. (C) Molecular model of a 7-mer Hcp1 nanotube (side and top view). Top and bottom "capping" subunits are green and blue, respectively. The position of the outer surface-exposed C-terminus of these subunits is colored red. (D) Transmission electron micrograph of Hcp1 nanotubes prepared from Hcp1 subunits containing double cysteine substitutions at R157 and G90. In the absence of these mutations, only Hcp1 rings are observed (Ballister and Mougous *PNAS* 2008).

complementary templating DNA strand (Figure 2). The ability to generate monodisperse populations of tailored Hcp1 nanotubes in high yield will represent an important step forward for realizing the potential of proteins in various nanotechnogical devices.

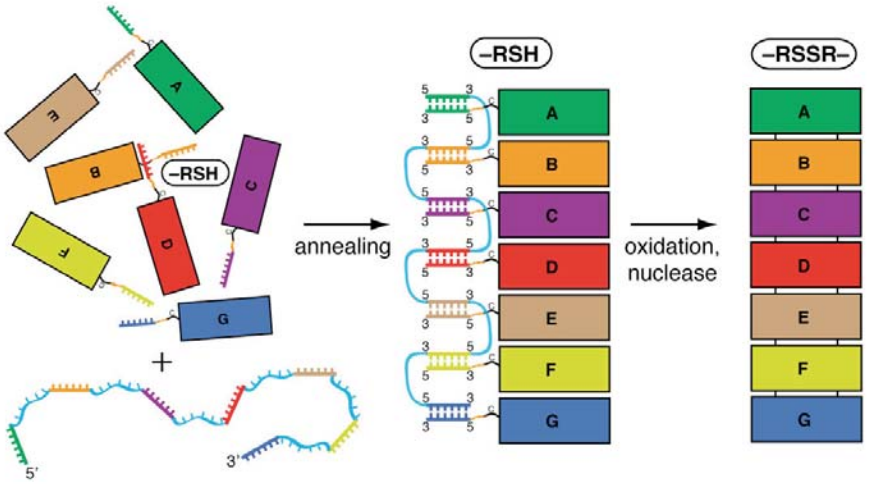

Figure 2. Schematic depiction of method for generating ordered, defined Hcp1 nanotubes by DNA templating. Hcp1 hexameric rings (rectangles) are labled A-G and are colored to indicate the uniqueness of each subunit and conjugated DNA oligo sequence (DNA covalently attached using "Click" chemistry following protein farnesyl transferase treatment). For clarity, we show only one conjugated DNA oligo and templating DNA strand. In actual experiments, each subunit of the hexamer will have the same DNA oligo attached and six templating DNA strands would be present on a single nanotube. This method will be the first to permanently trap the information from DNA-based assembly in a protein structure.

Future Work:

Following successful synthesis of second generation (DNA templated) Hcp1 nanotubes, we will adapt the scaffold for a diverse array of applications including targeted drug and DNA delivery. For cellular delivery applications, the nanotube exterior will be modified with molecules that target it to a specified cell type. For instance, we will use a poly-histidine-binding peptoid conjugated to folate in order to target nanotubes to folate receptor-expressing cancer cells. The interior of Hcp1 nanotubes will also be modified, such that terminal subunits of the nanotubes can be selectively "plugged" and middle subunits are loaded with covalently attached cargo.

Publications:

In vitro self-assembly of tailorable nanotubes from a simple protein building block.
Ballister E.R., Lai A.H., Zuckermann R.N., Cheng Y., and Mougous J.D.
Proc. Natl. Acad. Sci. U.S.A. 2008 Mar 11;105(10):3733-8.

A minimalist substrate for enzymatic peptide and protein conjugation.
Wollack J.W., Silverman J.M., Petzold C.J., Mougous J.D., and Distefano M.D.
ChemBioChem 2009 *in press*