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Rational Design of Helical Nanotubes from Self-assembly of Coiled-coil Lock Washers

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Structurally defined materials on the nanometer length-scale have been historically the most challenging to rationally construct and the most difficult to structurally analyze. Sequence-defined polypeptides represent attractive candidates as design elements for construction of these nano-scale materials, in that correlations can be drawn between sequence and higher order structure. However, the diversity of sequence space and the current limitations of theoretical approaches to reliably define the relationship between sequence and supramolecular structure present a significant challenge to the *de novo* design of novel materials architectures. Herein, we define a principle for the design of protein-based assemblies that we designate the re-coding hypothesis, in which we suggest that the sequences of natively folded protein structures can be re-designed to accommodate the formation of structurally defined supramolecular assemblies. In support of this hypothesis, we introduce modifications into the sequence of a structurally characterized seven-helix bundle that promote the self-assembly of the helical protomers into fibrillar nano-tubes that are capable of binding shape-appropriate small-molecules in the central channel.

The crystal structure of **GCN4-pAA** (PDB ID: 2HY6), a *de novo* designed peptide derived from the leucine zipper region of the *S. cerevisiae* transcription factor **GCN4**, displays a discrete seven-helix bundle structure that constitutes the largest, freely standing coiled-coil oligomer that has been structurally characterized thus far (Figure 1A). The heptameric assembly defines a continuous central channel with an internal diameter of approximately 7 Å. Computational analysis using the program CASTp indicated the presence of an internal void volume of 1,880 $Å^3$ associated with the central channel. The presence of several hexane-1,6-diol molecules in the central channel of the heptamer indicates that it is capable of binding appropriately shaped small-molecules within the cavity. The **GCN4-pAA** structure provides the opportunity to design tubular peptide fibrils of defined internal dimension through self-assembly. In addition, crystallographic analysis of the **GCN4-pAA** structure indicated a single residue shift in registry between adjacent helices, which resulted in an overall displacement of seven residues (i.e., one coiled-coil heptad repeat) upon closure of the bundle structure (Figure 1A). In contrast, most coiled-coil structures have no corresponding shift in helix registry and result in blunt-ended helical bundles. The structure of the seven-helix bundle of **GCN4-pAA** resembles a screw (or lock washer), in which the displaced edges at the seam provide an additional interface for complementary interactions between the coiled-coil protomers.

We hypothesized that if the sequence of the peptide was appropriately modified to promote end-to-end association between the complementary surfaces perpendicular to the super-helix axis of the lock washer structure, then the helical bundle would self-associate into a high aspect-ratio fibril with a continuous

channel throughout the assembly that would correspond in lateral dimensions to that of the seven-helix bundle observed in the crystal structure of the original peptide. To test our hypothesis, peptide **7HSAP1** was synthesized and structurally characterized.

Circular dichroism spectropolarimetry showed strong α-helicity with MRE values that exceed those of the control peptide **GCN4-pAA** under identical conditions. The solution of **7HSAP1** displayed a flow linear dichroism spectrum with a strong positive signal at 207 nm, which indicates the formation of an extended assembly in which the α-helices align parallel to the flow direction of the Couette cell. The LD results are consistent with the hypothesis that **7HSAP1** assembles in solution to form extended arrays that stack along the direction of the super-helical axis (Figure 1B). STEM and Cryo-TEM of solutions of **7HSAP1** demonstrated the presence of fibrils of circa 3 nm in diameter. The observed diameter of the majority of **7HSAP1** fibrils compared well with the diameter of 3.1 nm that was determined from the crystal structure of **GCN4-pAA**. Solid-state NMR measurements on the **7HSAP1** fibrils were consistent with an assembly based on seven-helix bundles with displaced edges. In x-ray fiber diffraction, the meridianal reflections observed at 5.1 and 10.1 Å are consistent with the regular repeat that arises from α-helices and the position on the meridian supports the view that the α-helices are aligned parallel to the fiber axes. Solid-state NMR measurements on the isotopically labeled **7HSAP1*** fibrils were consistent with an assembly based on seven-helix bundles with displaced edges. SAXS/WAXS data support the presence of nanotube assemblies in solutions of **7HSAP1** with lateral dimensions that approximate those observed in the crystal structure of the seven-helix bundle of **GCN4-pAA**.

References:

[1] Liu, J.; Zheng, Q.; *et al*, *P Natl Acad Sci USA* 2006, **103** (42), 15457-15462.

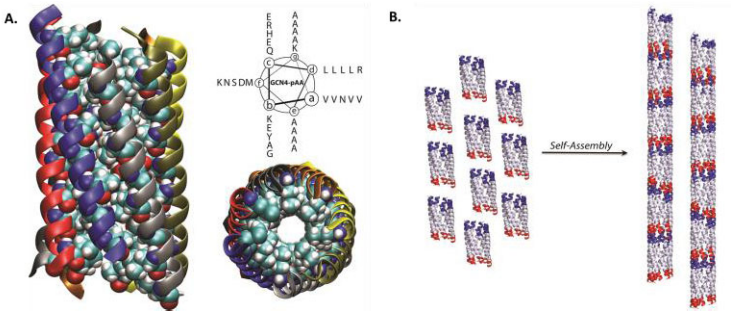

Figure 1. A. Crystal structure (PDB ID: 2HY6) of **GCN4-pAA** and helical wheel projection of the amino acid sequence of **GCN4-pAA** (upper right). B. Schematic representation of the proposed mode of self-assembly of lock washer structures derived from the 7-helix bundle of peptide **7HSAP1** into helical nanotubes.

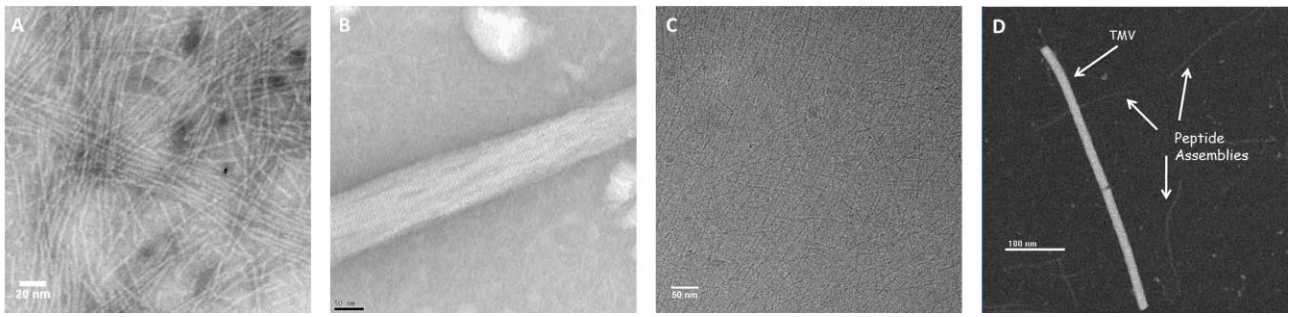

Figure 2. Electron microscopy of **7HSAP1** assemblies. A. Negative staining STEM. B. Conventional TEM. C. Cryo-EM. D. Cryo-STEM.

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