Engineered self-assembling coiled-coil protein fibers

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1 Abstract

Research towards biomaterials has become increasingly popular, as scientists look for energy efficient alternatives to electronic components commonly used in medical and biological applications. Using protein-derived materials as electronic components, biological engineers are given design options that are easier and less expensive to achieve than using synthetic methods. In this project, our goal is to design and construct self-assembling protein fibers capable of metal nanoparticle templation. We have engineered two variants of the α-helical coiled-coil of cartilage oligomeric matrix protein (COMPcc) to self-assemble longitudinally. Characterizations of these protein fibers are conducted using circular dichroism (CD), transmission electron microscopy (TEM) and fluorescence microscopy (FM). Additionally, confocal microscopy provides evidence that our protein fibers demonstrate small molecules binding capabilities, specifically to the small fluorescence molecule curcumin. The aim of our research is to functionalize protein nanowire materials as components of electronic devices with the ability to deliver the same level of functionality and effectiveness as their synthetic equivalents.

2 Cartilage Oligomeric Matrix Protein coiled-coil (COMPcc)

- Crystal structure of a COMPcc parallel pentameric coiled coil, defining the oligomerization domain of COMPcc
- The pentamer is stabilized by electrostatic interactions between heptad units, generating a hydrophobic core
- Core is 73 Å long and 2-6 Å in diameter between subunit chains

Malashkevich VN et al. Science. 1996 Nov 1;274(5288):761-5

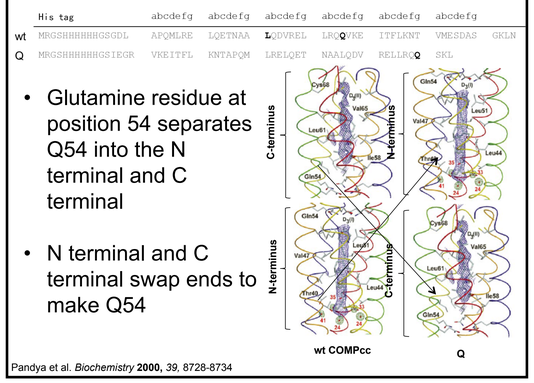
3 α-helical proteins form coiled-coil structures

Blue arrows show hydrophobic and polar interactions between residues in *a/d* positions and *red arrows* show electrostatic interactions between residues in *e/g* positions

- Antiparallel α- helical proteins interact to assemble into coiled-coils
- Each α-helix contains heptad residue repeats that can assemble to form coiled-coils

Y. Zimenkov et al. Tetrahedron 60 (2004) 7237–7246

4 Design of Q54

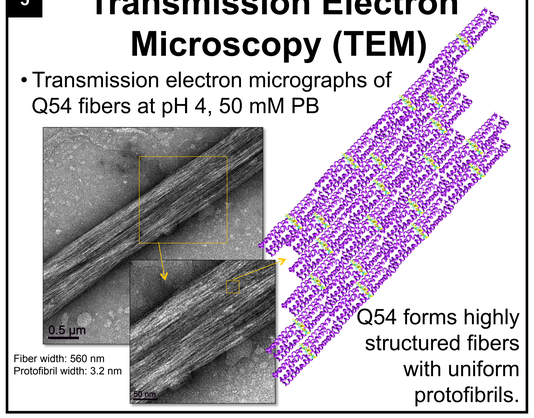


- Glutamine residue at position 54 separates Q54 into the N terminal and C terminal
- N terminal and C terminal swap ends to make Q54

Pandya et al. *Biochemistry* **2000,** *39,* 8728-8734

5 Transmission Electron Microscopy (TEM)

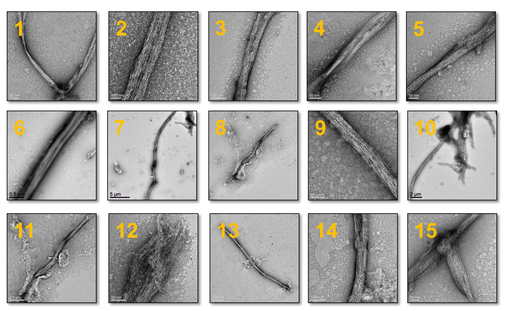
- Transmission electron micrographs of Q54 fibers at pH 4, 50 mM PB



Fiber width: 560 nm
Protofibril width: 3.2 nm

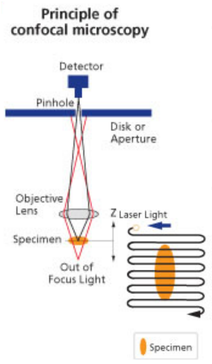
Q54 forms highly structured fibers with uniform protofibrils.

6 TEM images of Q54 fibers



Average fibers width: 20 – 560 nm (*n* = 14)
Average protofibril width: 3.5 ± 0.5 nm (*n* = 210)

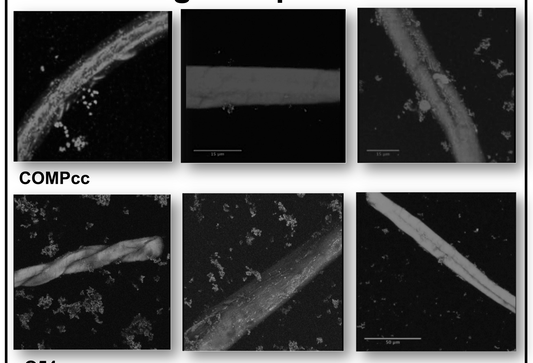
7 Confocal Microscopy



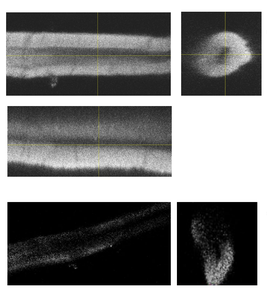
- Scanning of one or more focused beams of light across a specimen
- Automated collection of 3D data in Z-stacks
- Results in a focused, 3D representation of the material
- COMPcc and Q54 fibers were studied in the presence of a 5:1 curcumin to protein ratio

Confocal graphical illustration: http://www.biovis.com/carv-ii.htm

8 3D images of protein fibers



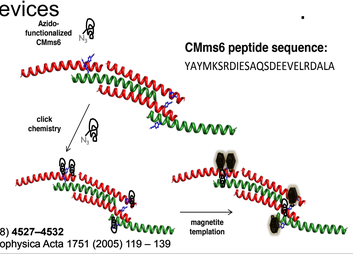
9 Confocal provides insight on fiber assembly



- Orthogonal views show a hollow cavity within the COMPcc fiber bound to curcumin
- Q54 fibers bound to curcumin also exhibit a cavity

10 Protein nanowires for electronic applications

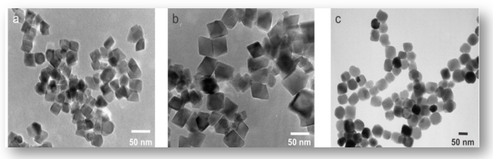
- Nanoparticles and nanowires are seen as an effective components useful for emerging nanometer scale biosensors and small scale electronic devices .



Scheibel et al. PNAS 2003 100 (8) **4527–4532**
S.M. Kelly et al. Biochimica et Biophysica Acta 1751 (2005) 119 – 139

11 Magnetite templation

- Our goal is to template magnetic nanoparticles with defined spatial orientation allowing for effective and dense energy storage
- Our ability to specifically tailor the structure of the magnetic materials by altering chemical and physical properties of proteins will give us control of the function of such materials



Y. Amemiya et al. Biomaterials 28 (2007) 5381–5389

12 Conclusions and Future Work

- Q54 regularly assembles into highly structured fibers, in 50 mM phosphate buffer at pH 4
  - Average fibers width measure 20 – 560 nm (*n* = 14)
- In the presence of curcumin, Q54 fiber assemblies were more abundant (width range: 35 ± 8 μM) than fibers of COMPcc (width range: 15 ± 2 μM)
- Our future work will focus on functionalizing protein fibers with magnetite templating peptides (CMMs6)

ACKNOWLEDGEMENTS

THIS WORK WAS SUPPORTED BY THE ARMY RESEARCH OFFICE W911NF-11-1-0449, THE NATIONAL SCIENCE FOUNDATION GK-12 FELLOWS GRANT DGE-0741714 AND IN PART BY THE NSF MRSEC PROGRAM DMR-0820341

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