

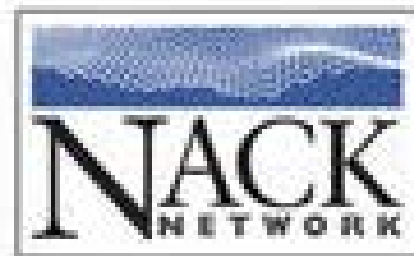
Protein Engineered Nanomaterials

The Webinar Will Begin at 1 PM Eastern Time

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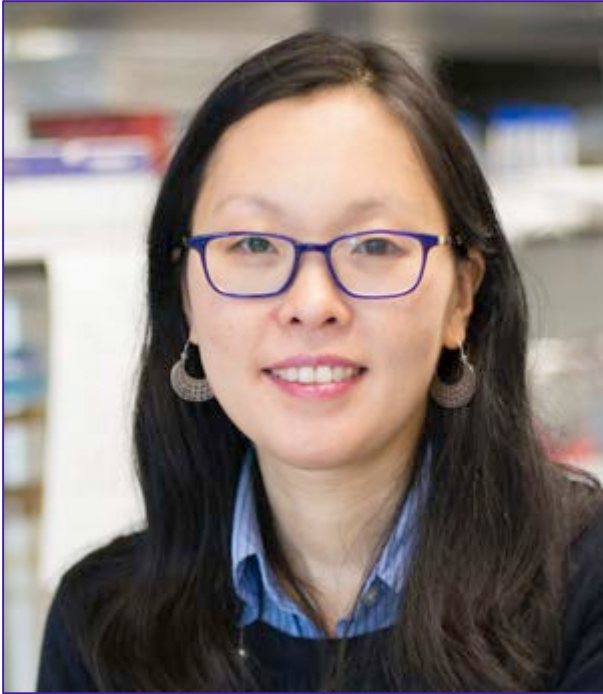
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Presenters



Dr. Jin Kim Montclare
Associate Professor
Director, Convergence for Innovation
& Entrepreneurship (CIE) Institute
New York University



Dr. Priya Katyal
Postdoctoral Associate
New York University



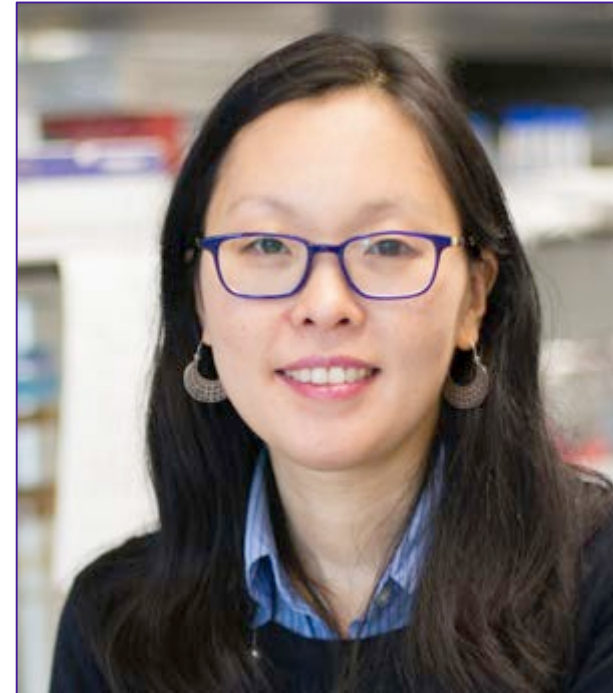
Lindsay K. Hill
MD/PhD candidate
New York University
SUNY Downstate Medical Center

Protein Engineered Nanomaterials

- Proteins as building blocks
- Engineered protein fibers
- Engineered protein nanoparticles

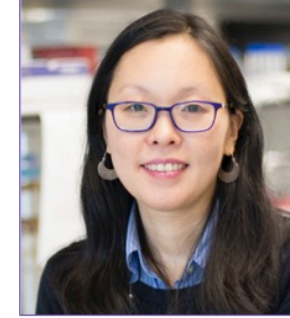
Protein Engineered Nanomaterials

- **Proteins as building blocks**
- Engineered protein fibers
- Engineered protein nanoparticles

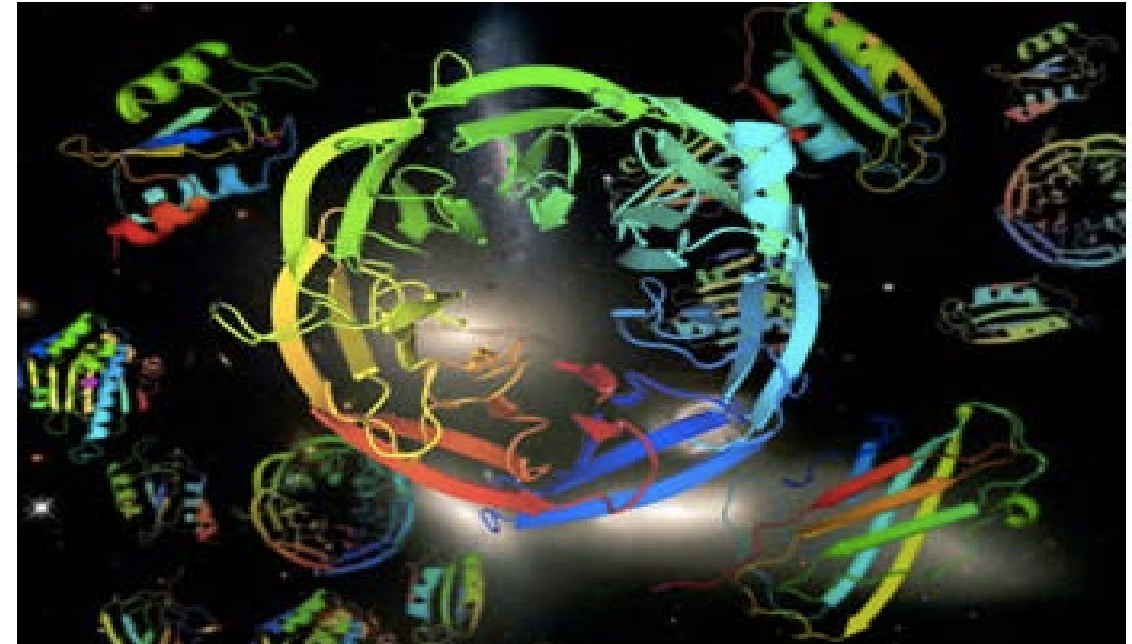
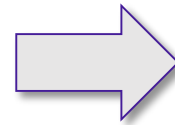


Dr. Jin Kim Montclare

Building Blocks of Nature: Proteins



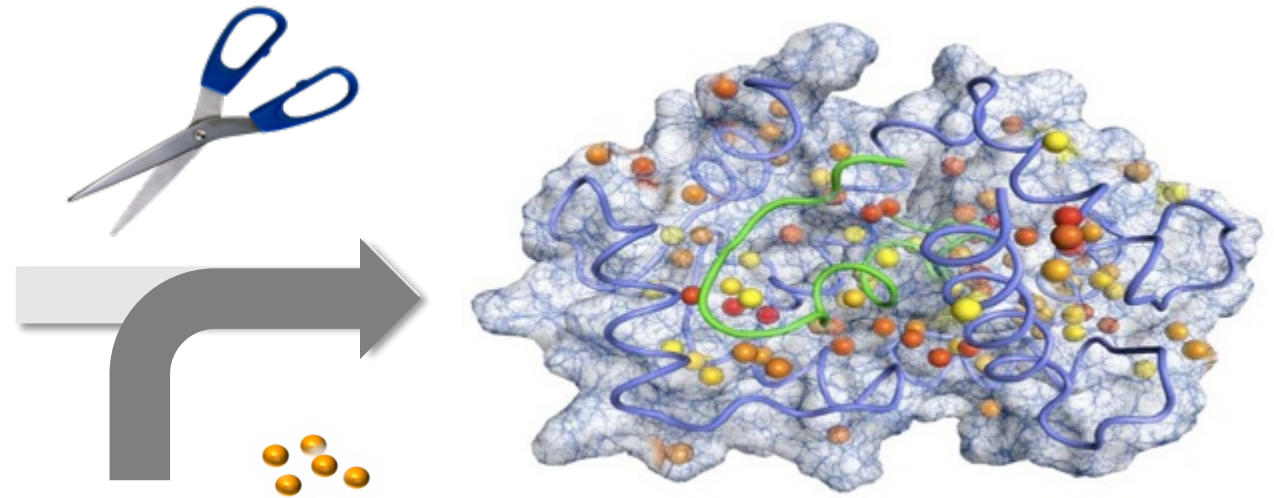
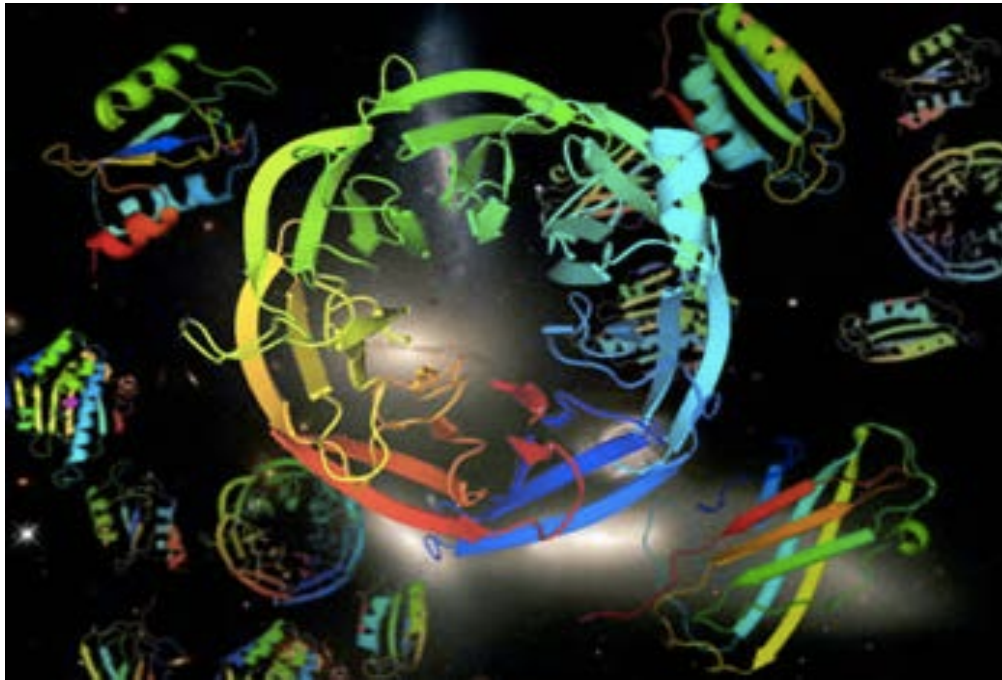
Dr. Jin Kim Montclare



Complex functions in organisms are achieved through proteins

Building Blocks of Nature: Proteins

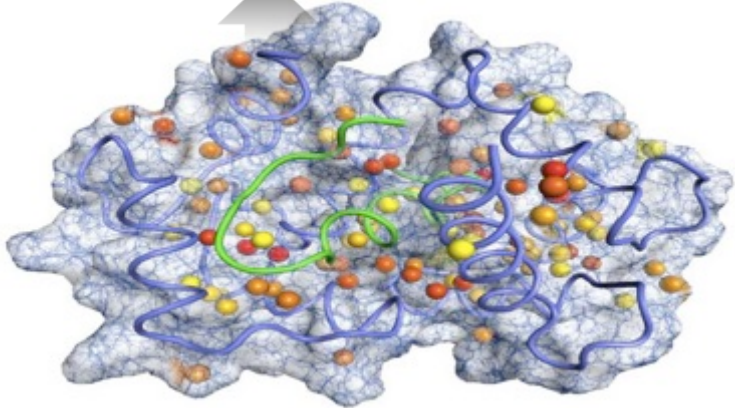
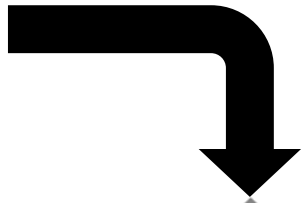
20 amino acid alphabet with defined order and number of secondary structural elements



Non-canonical amino acids

Novel functions achieved through “artificial” proteins

Program Bacteria: Living Factories for Artificial Proteins



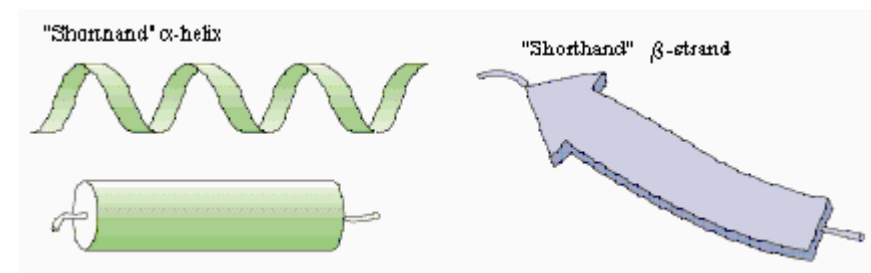
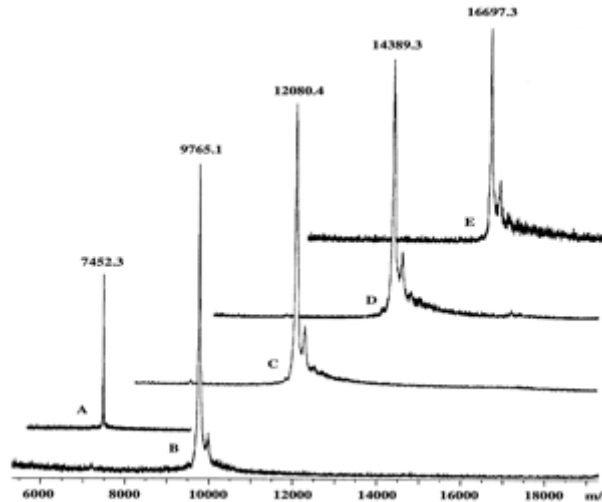
Advantages of Biopolymer Synthesis

Chain length, sequence and stereochemistry

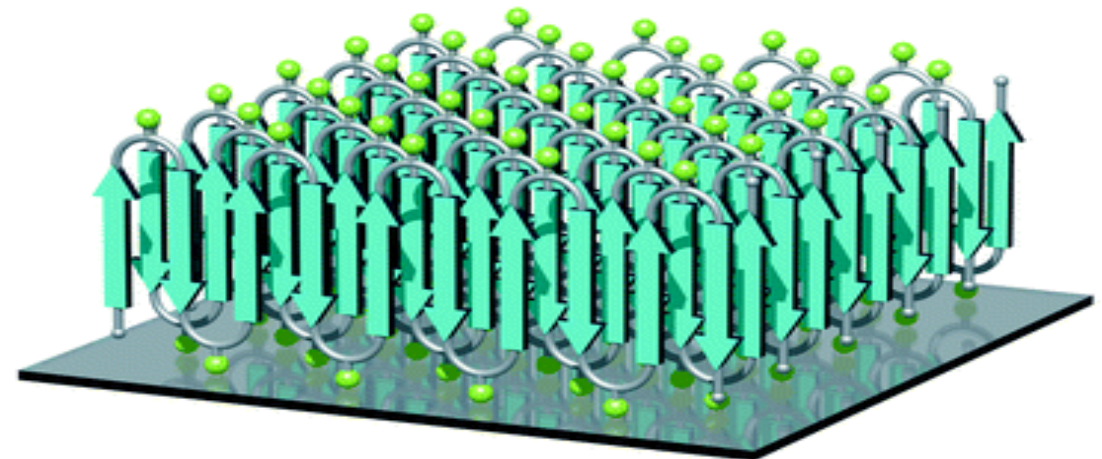
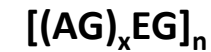
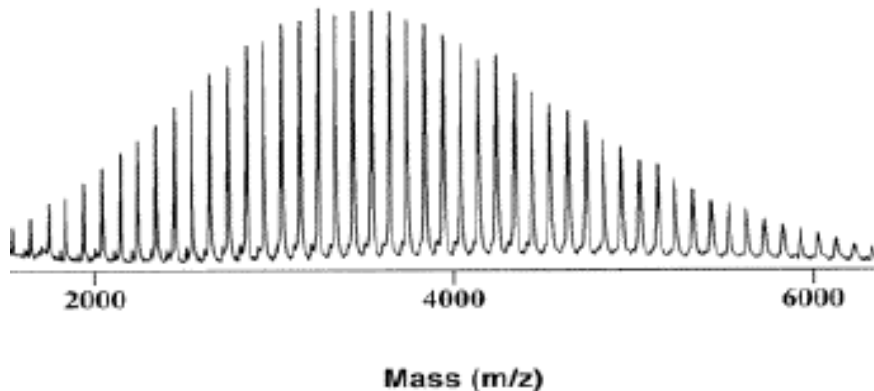
- Mono-dispersity

- Well defined secondary structures

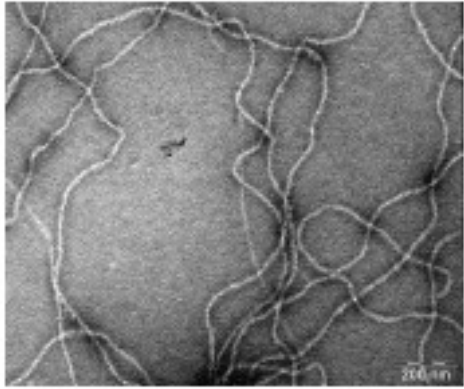
Proteins
(PLGAs)



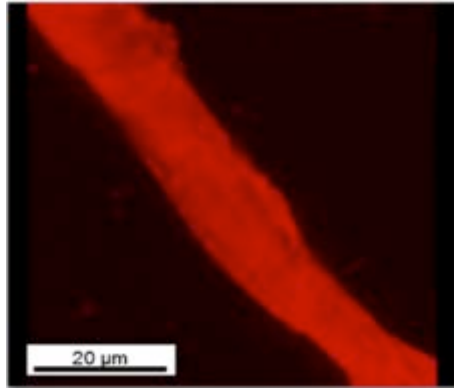
Synthetic Polymer
(PMMA)



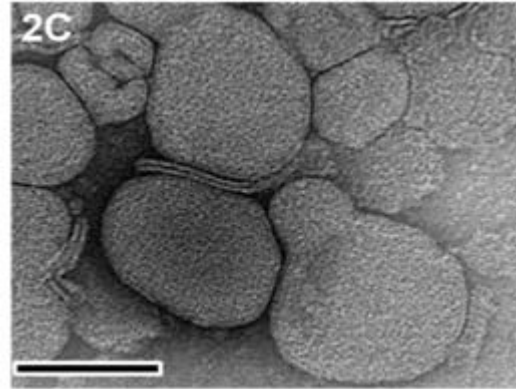
Nanofibers



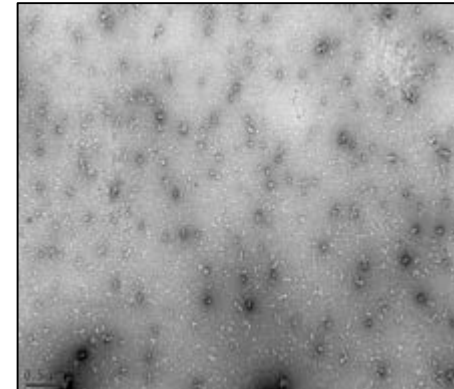
Mesofibers



Lipoproteoplexes



Nanoparticles



Hydrogels



...for treating various disorders:

- Osteoarthritis
- Breast cancer
- Diabetic wound healing
- Diagnostic and imaging

Rabbani, P., et al. *Biomaterials* 2017; 132: 1-15.

Hume, J, et al. *Biomacromolecules* 2014; 15: 3503-3510.

Gunasekar S.K. et al., *Biochemistry*, 2009, 48, 8559-8567

Liu, C.F., Chen, R. et al. *Biomacromolecules* 2017; 18: 2688-2698.

More, H.T., et al. *Biomaterials* 2014; 35: 7188-7193.

Haghpanah, J. *et al.*, *Mol. BioSyst.*, 2010; 6:1662-1667

Dai, M, et al. *Biomacromolecules* 2011; 12: 4240-4246.

Haghpanah, J. *et al.*, *ChemBioChem*, 2009; 10:2733-2735

Dai, M., Frezzo, J.A. *et al.* *J. Nanomed Nanotechnol.* 2016; 7:356.

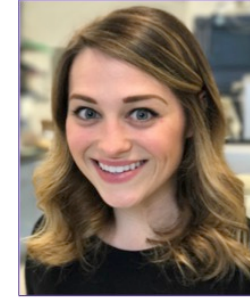
Objectives

- Proteins as building blocks
- **Engineered protein fibers**
- Engineered protein nanoparticles

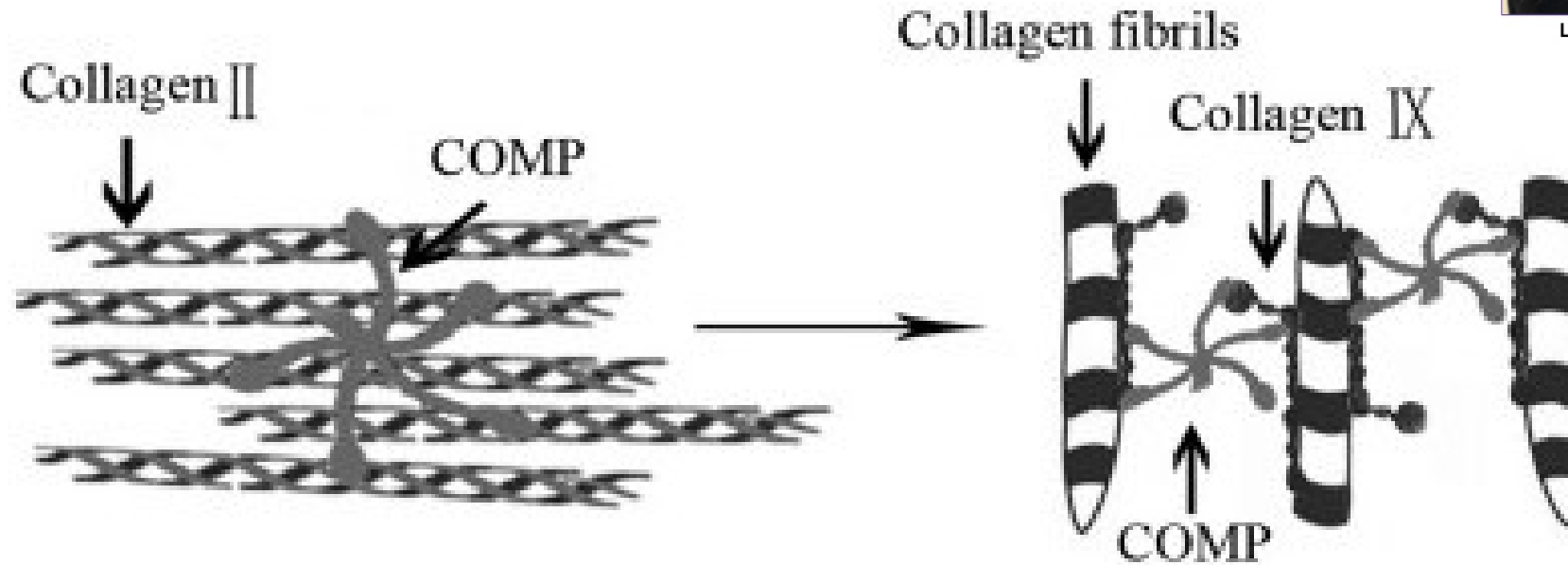


Lindsay K. Hill

Cartilage Oligomeric Matrix Protein

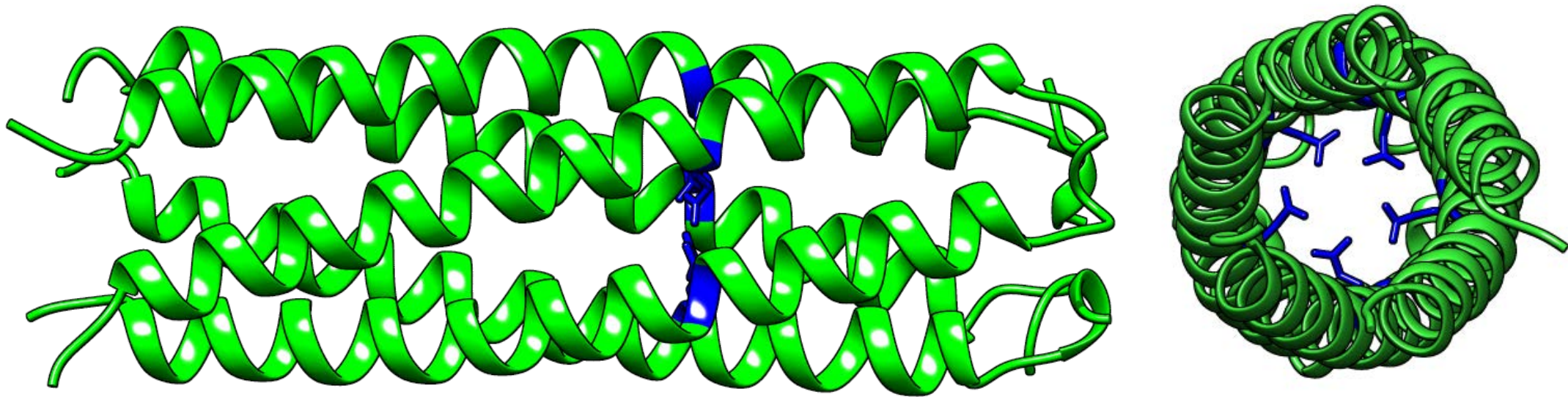


Lindsay K. Hill



- Four-domain non-collagenous extracellular matrix protein
- Found in cartilage, ligaments, and tendons
- N-terminal maintains a coiled-coil structure

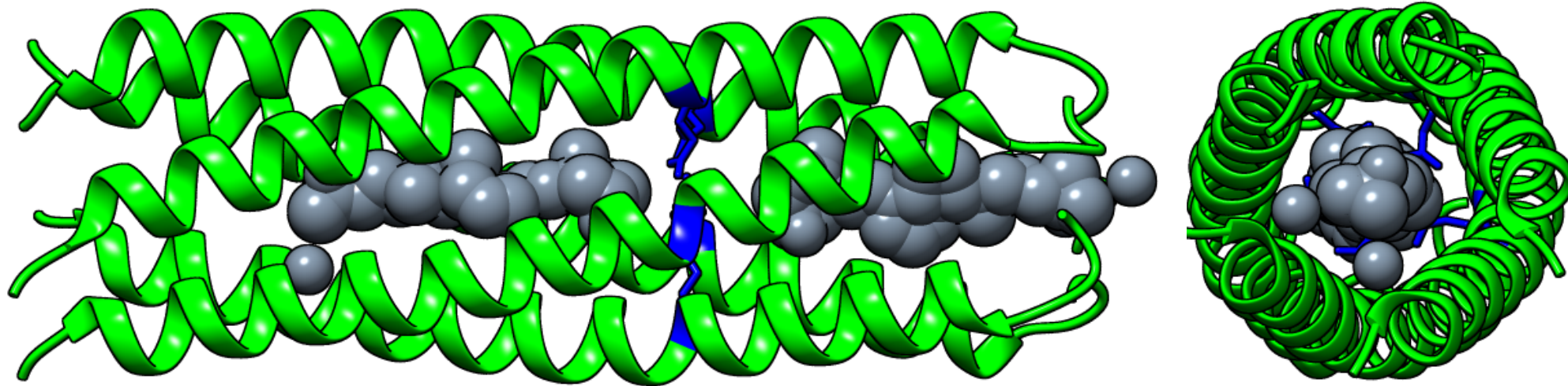
Cartilage Oligomeric Matrix Protein, Coiled-coil Domain (COMP_{cc})



COMP_{cc}: MRGSHHHHHHGSGDL APQMLRE LQETNAA LQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

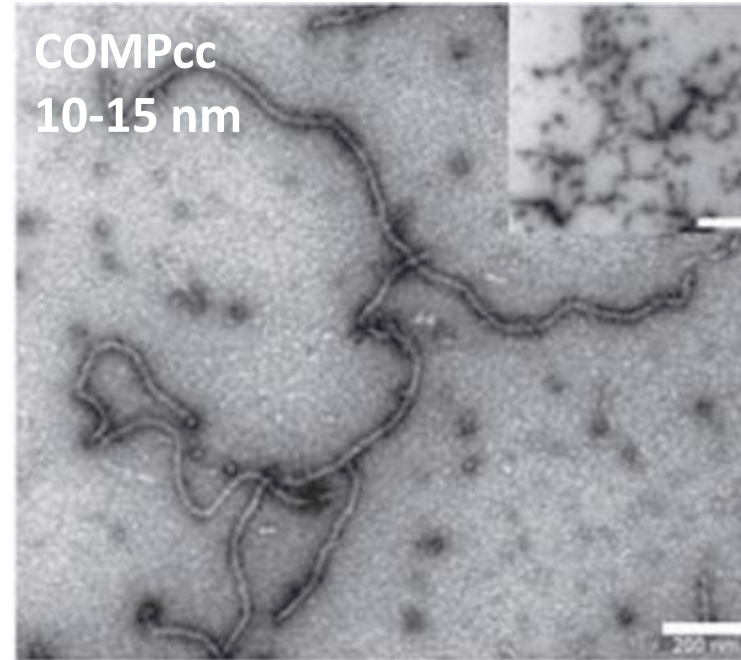
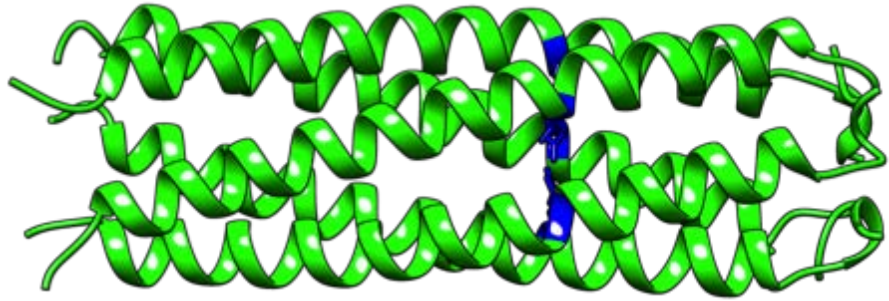
- COMP's N-terminal domain maintains a coiled-coil structure

Cartilage Oligomeric Matrix Protein, Coiled-coil Domain (COMPcc)



- Green = protein COMPcc
- Grey = small molecule cargo

COMPcc Fibers

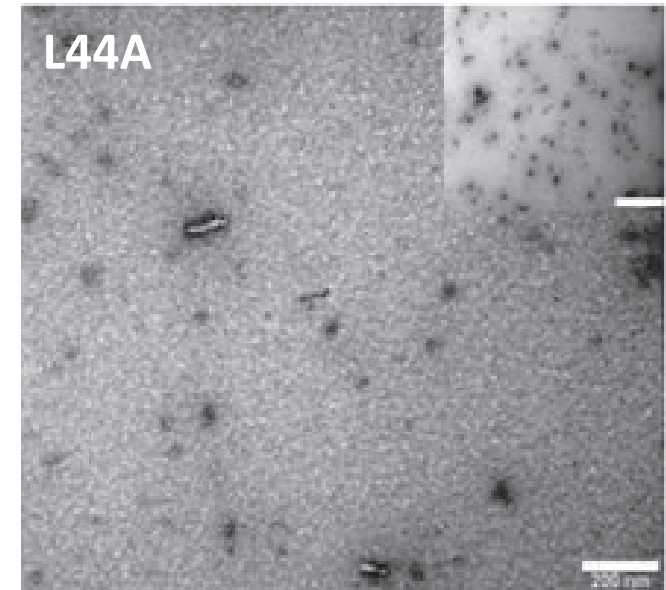
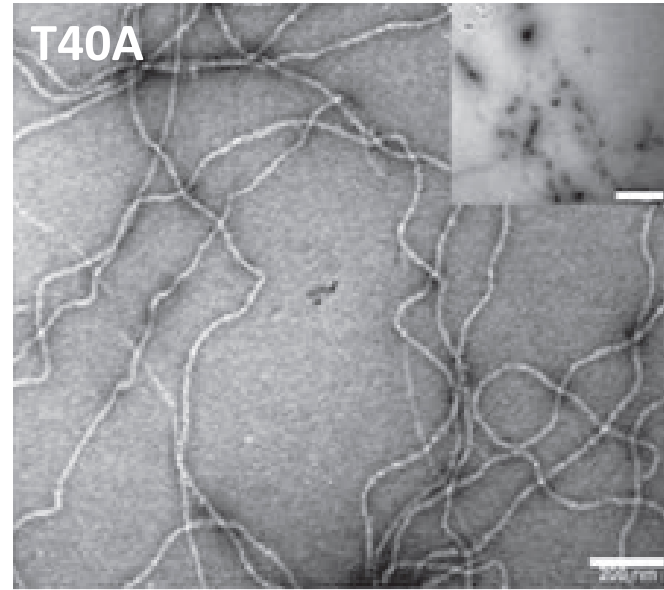
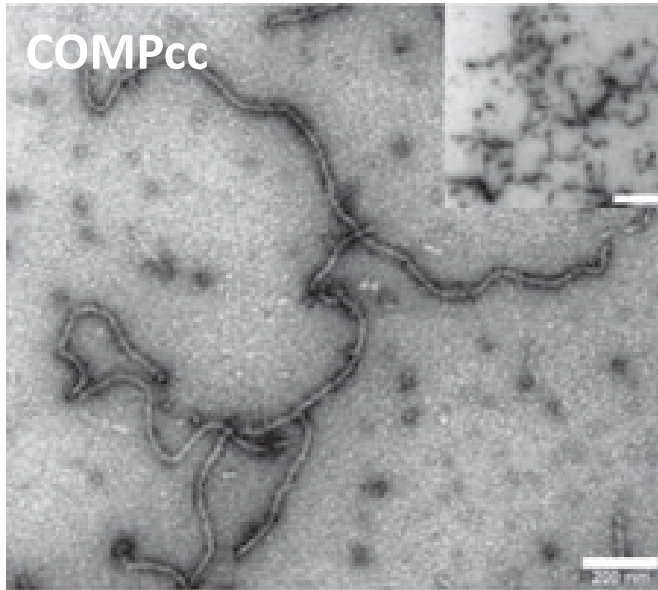


COMPcc Single-residue Mutants

COMPcc: MRGSHHHHHHGSGDL APQMLRE LQETNAA LQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

T40A: MRGSHHHHHHGSGDL APQMLRE LQEANAA LQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

L44A: MRGSHHHHHHGSGDL APQMLRE LQETNAA AQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

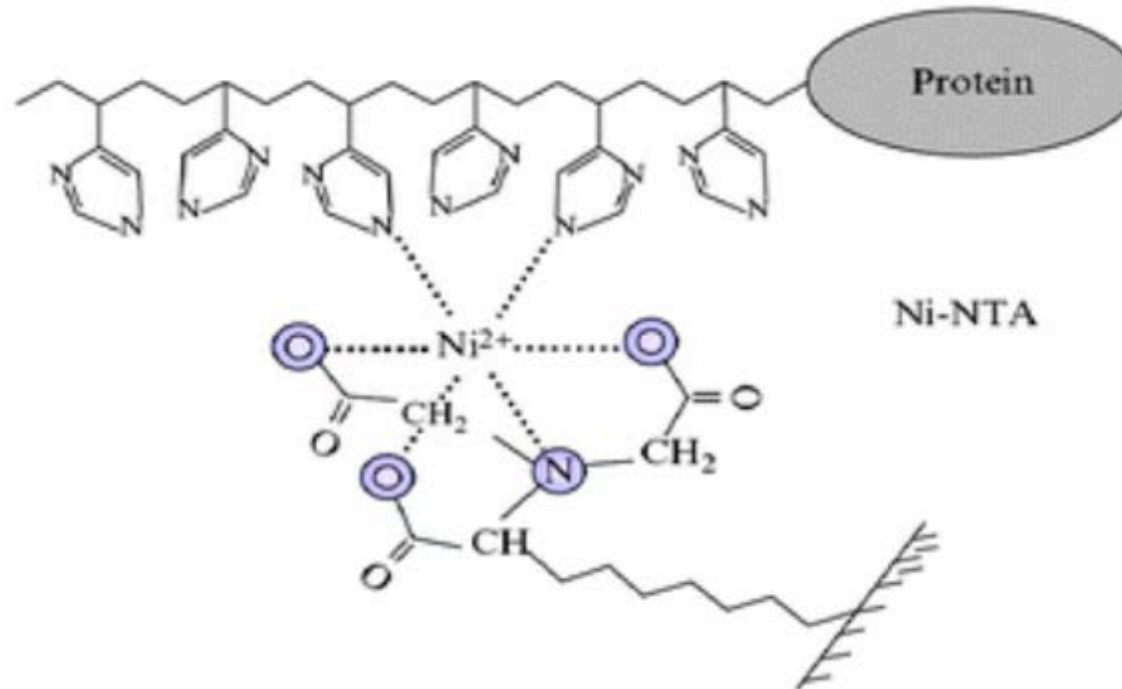


COMPcc Single-residue Mutants

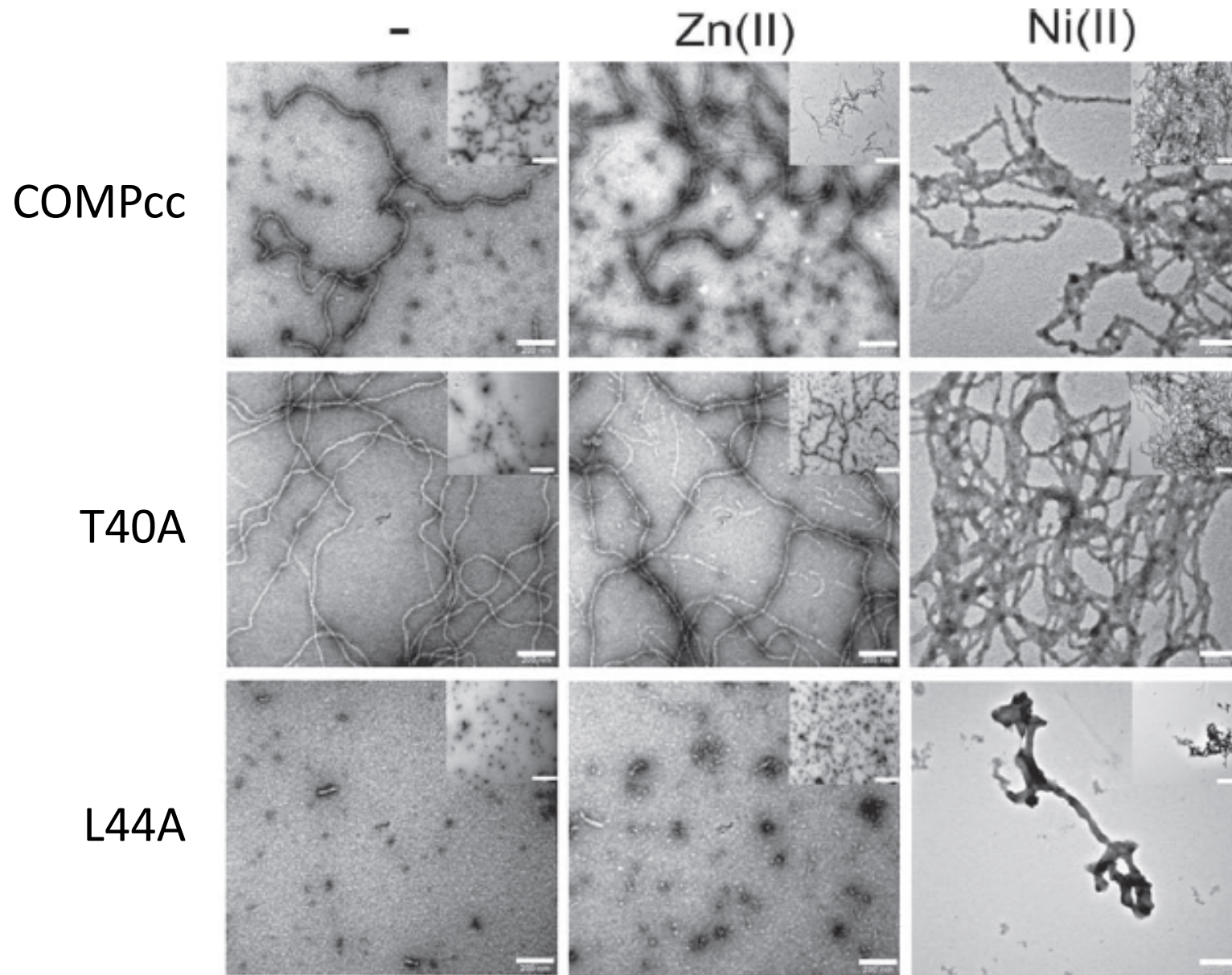
COMPcc: MRGSHHHHHHGSGDL APQMLRE LQETNAA LQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

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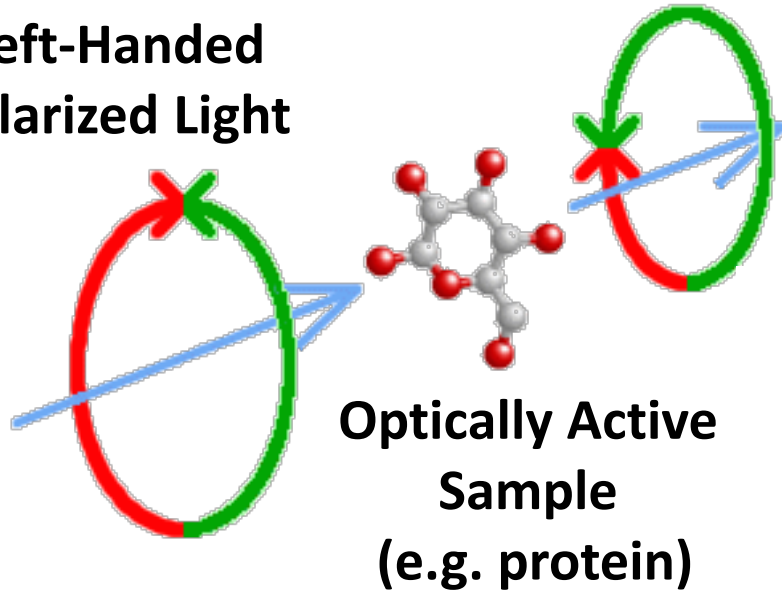
COMPcc Single-residue Mutants



Circular Dichroism Spectroscopy (CD): Structural Assessment

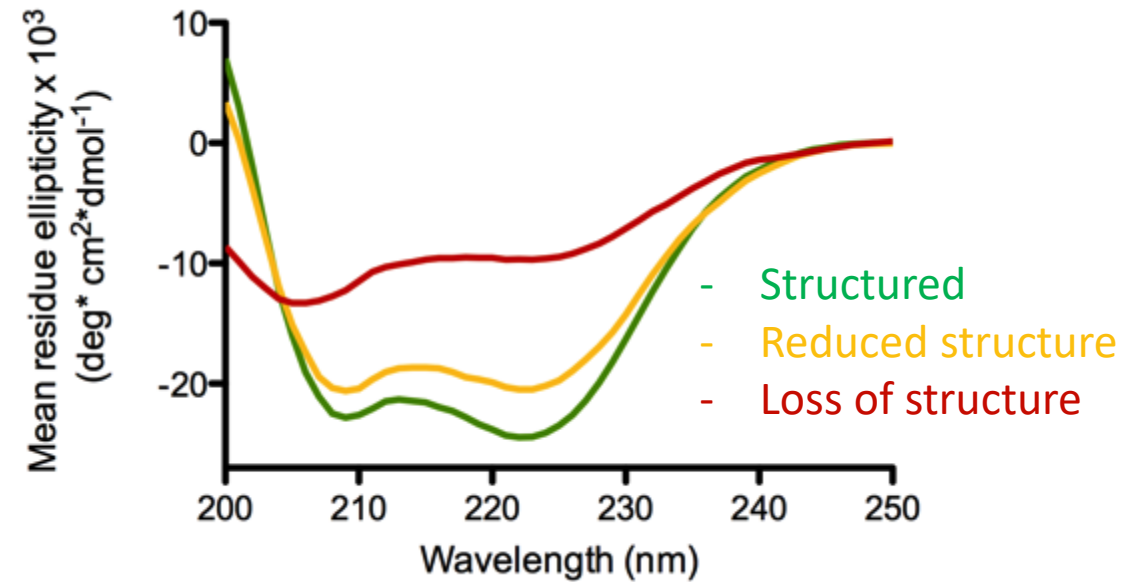
Right and Left-Handed
Circularly Polarized Light

Photon
Beam



Optically Active
Sample
(e.g. protein)

CD Signal

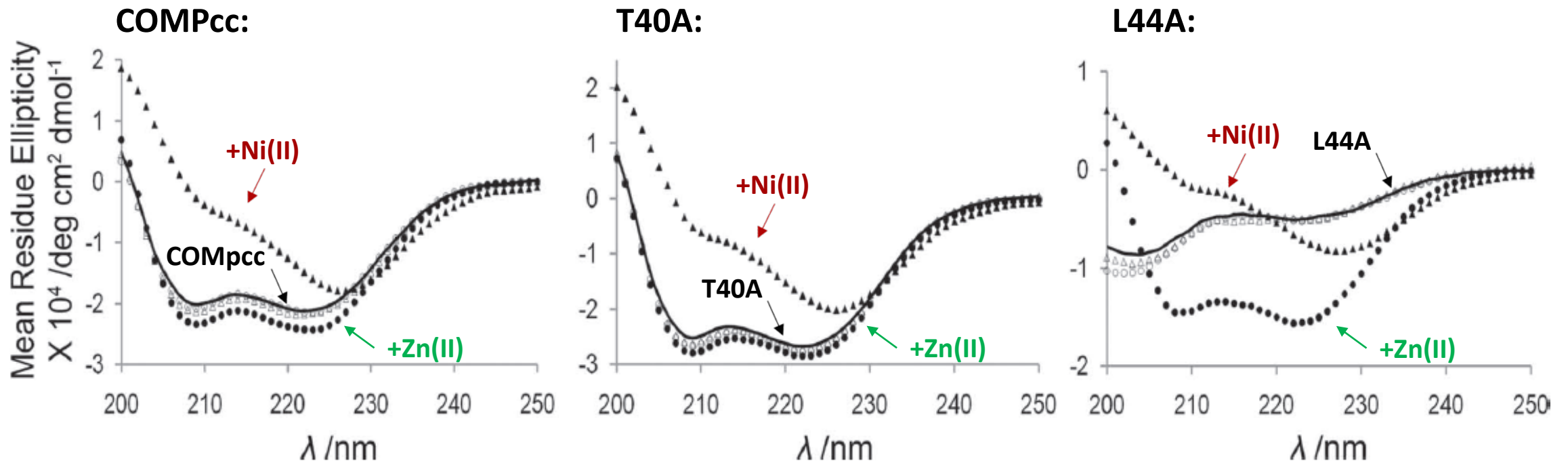


COMPcc Single-residue Mutants

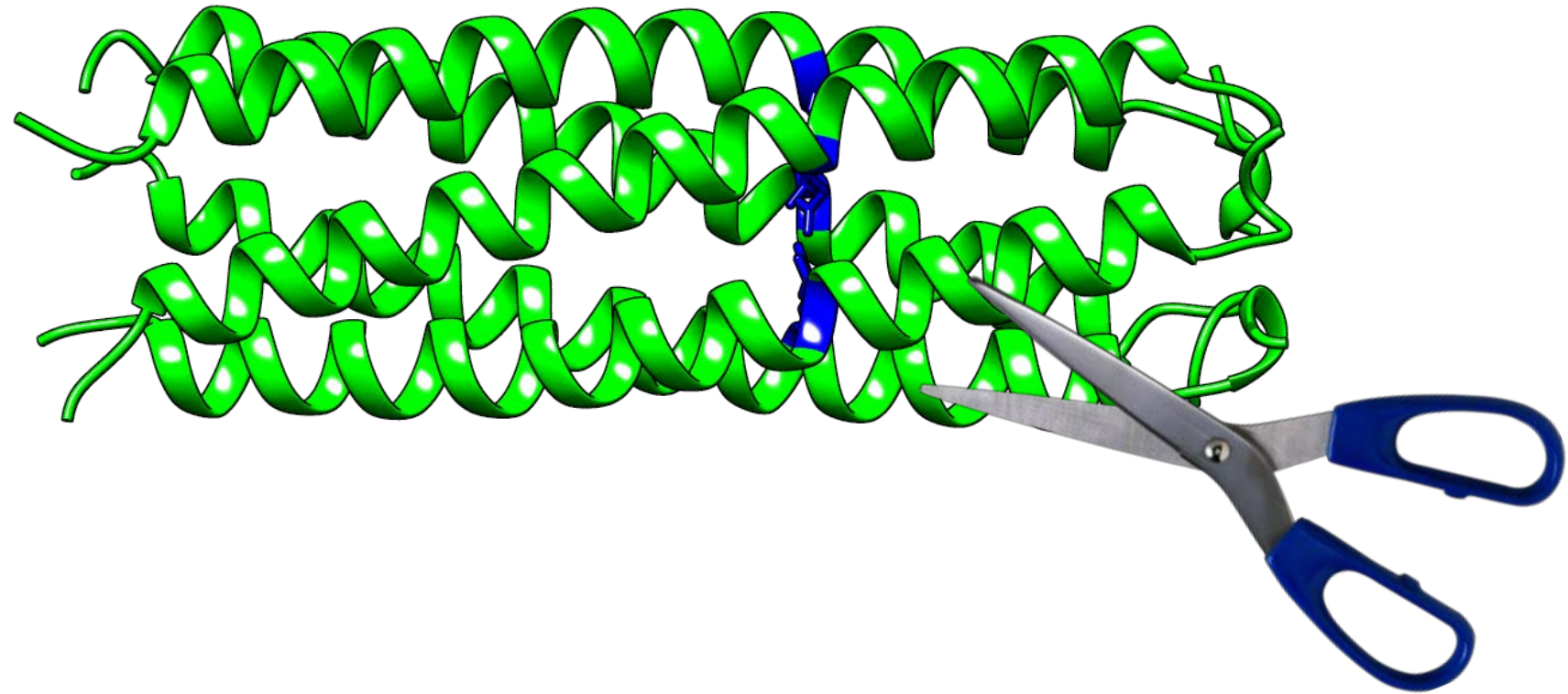
COMPcc: MRGSHHHHHHGSGDL APQMLRE LQETNAA LQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

T40A: MRGSHHHHHHGSGDL APQMLRE LQEANAA LQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

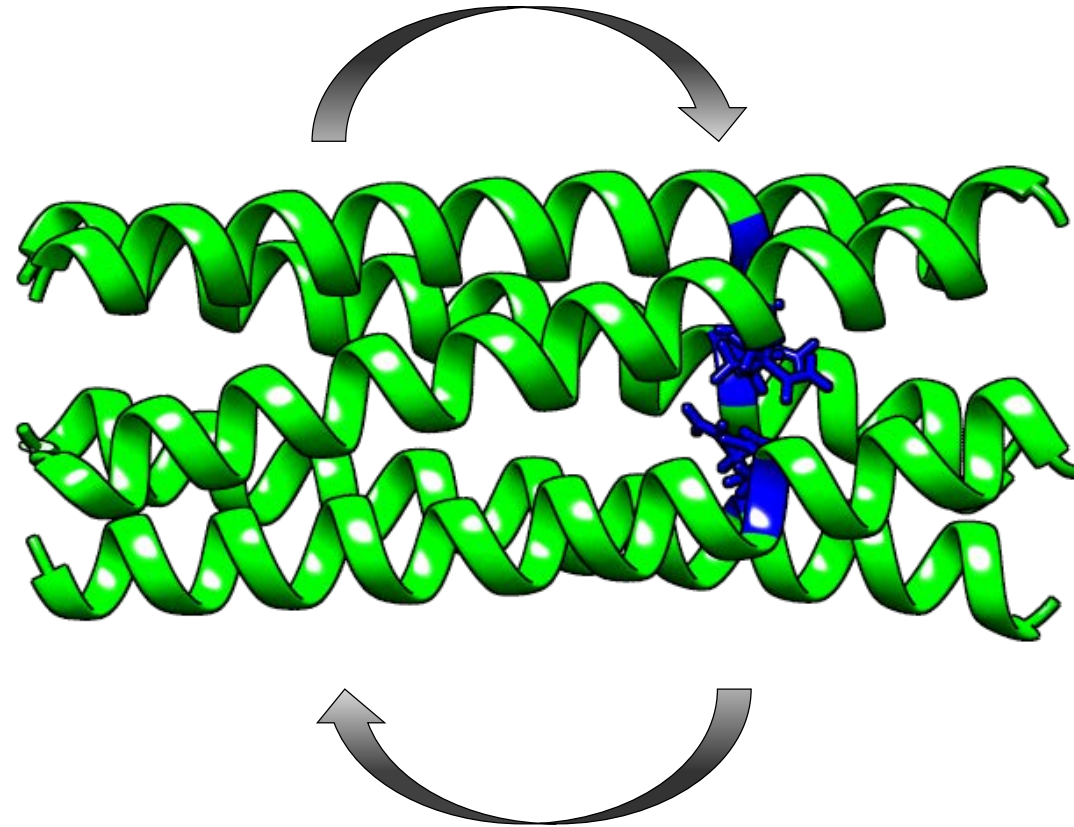
L44A: MRGSHHHHHHGSGDL APQMLRE LQETNAA AQDVREL LRQQVKE ITFLKNT VMESDAS GKLN



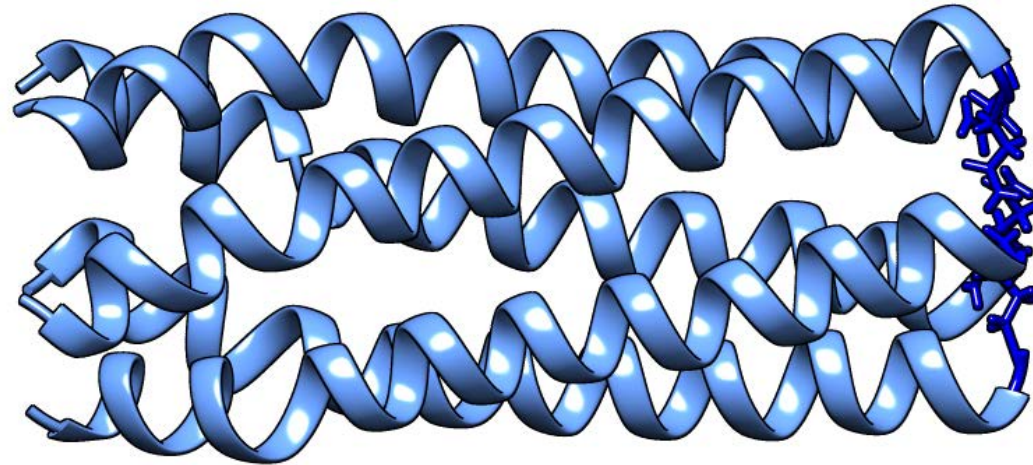
COMPcc Variants



COMPcc Variants

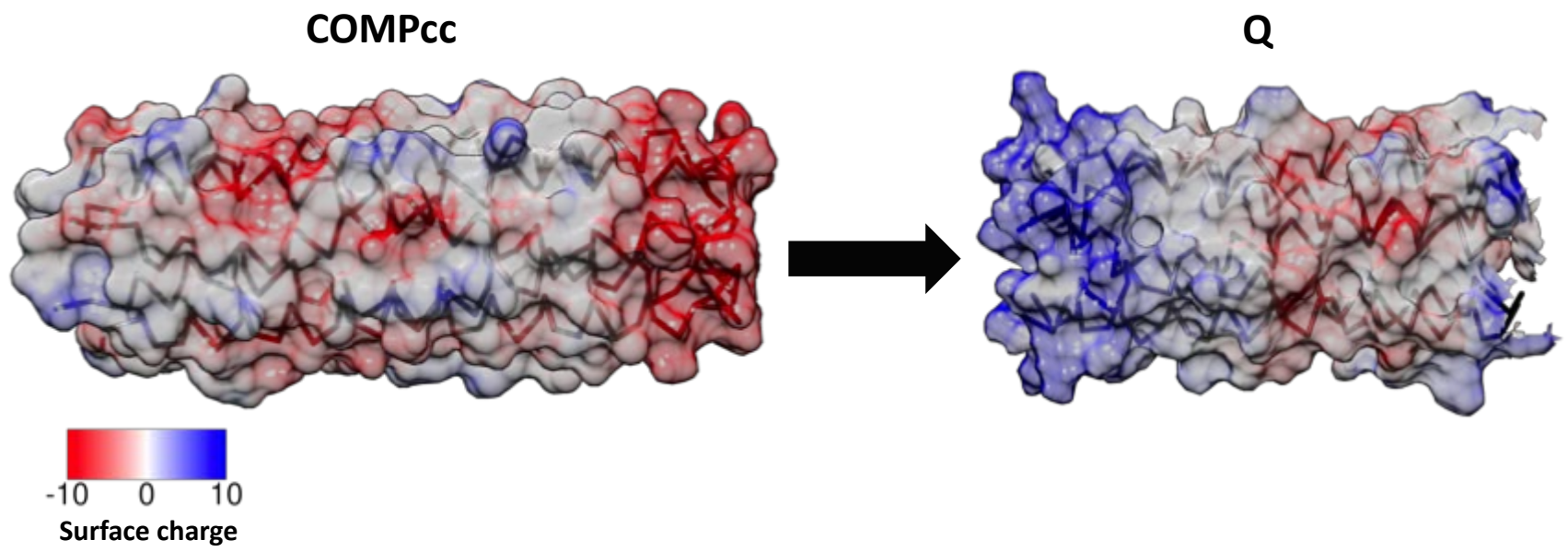


Domain swapped COMPcc: Q protein

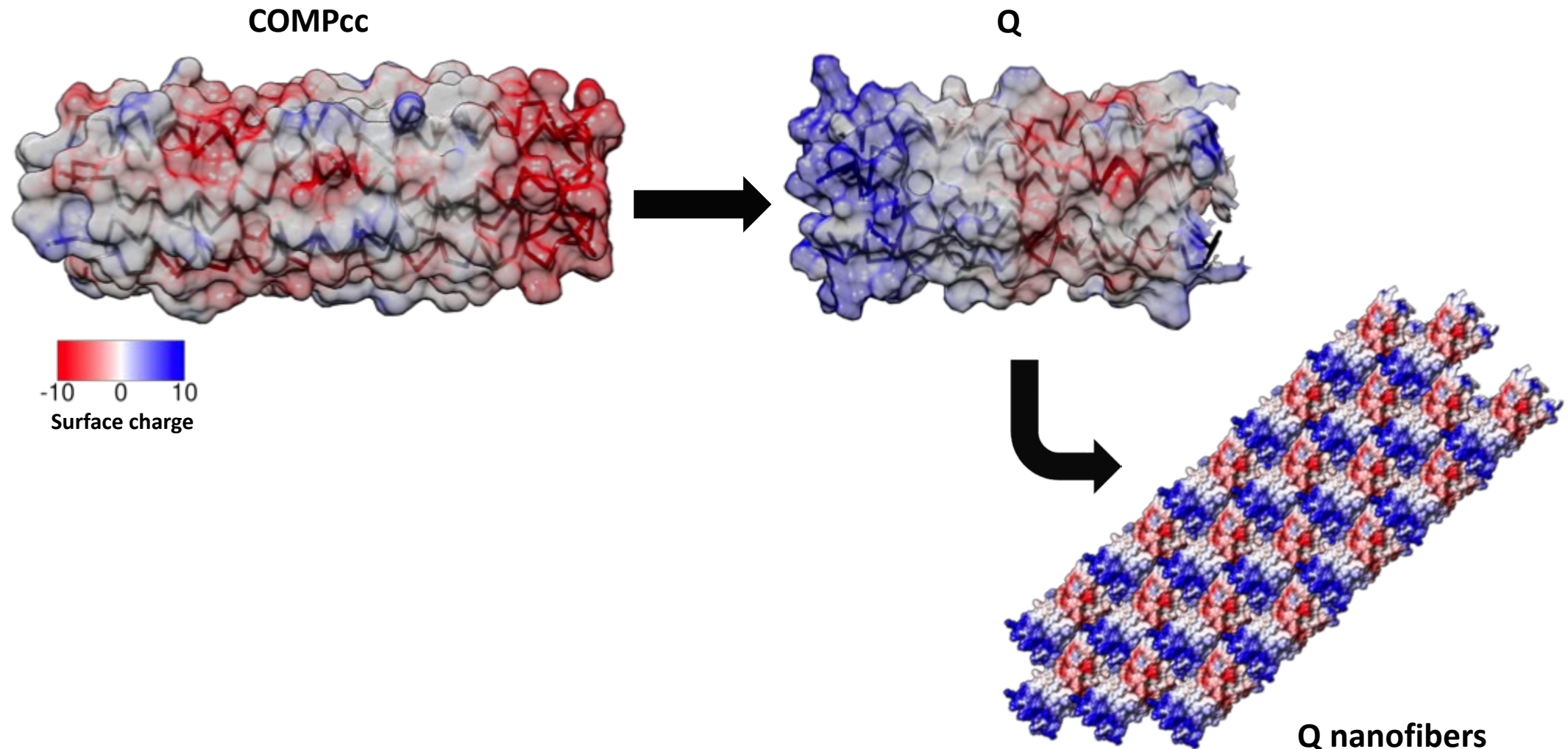


Q: MRGSHHHHHHHSIEGR VKE ITFLKNT APQMLRE LQETNAA LQDVREL LRQQSKL

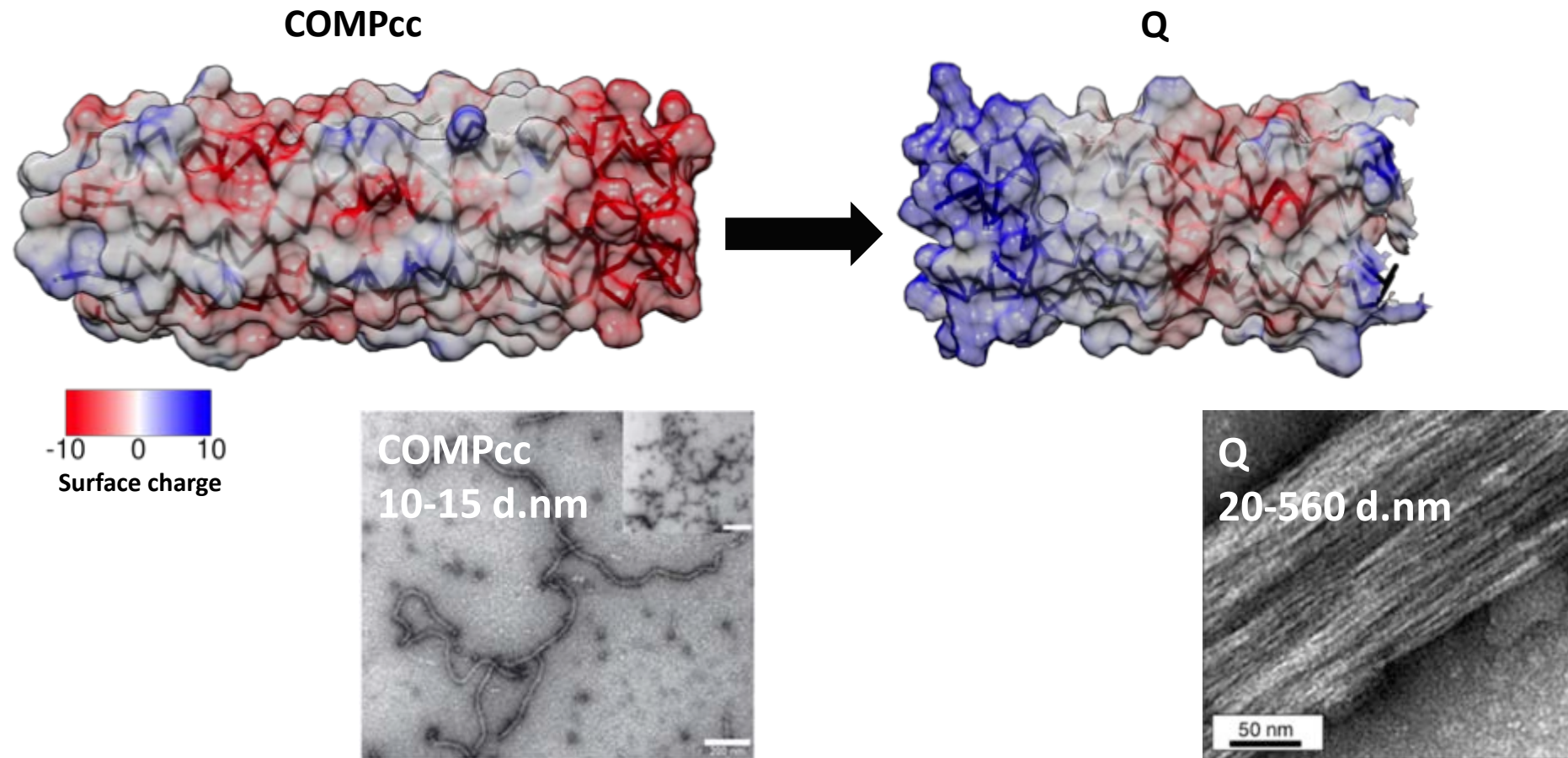
Domain swapped COMPcc: Q protein



Domain swapped COMPcc: Q protein



Domain swapped COMPcc: Q protein

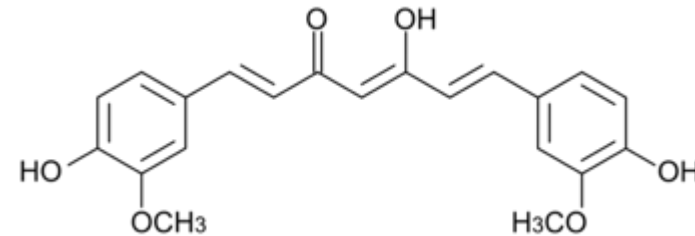


Small Drug Binding



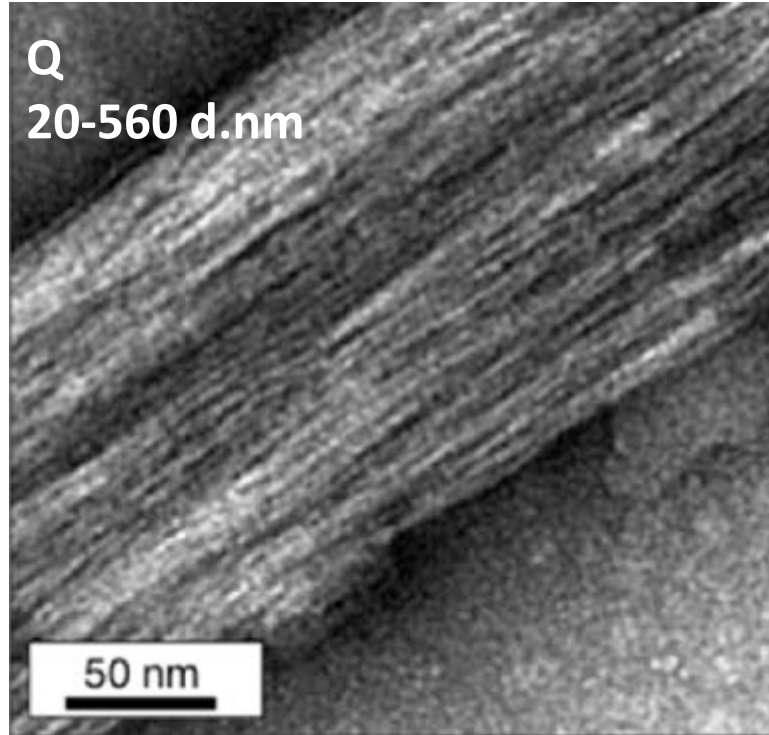
Curcumin: Derived from Turmeric

- Active ingredient in turmeric
- Has anti-cancer, anti-inflammatory, and anti-bacterial
- Absorbs at 420 nm
- Fluoresces at 530 nm when protein-bound

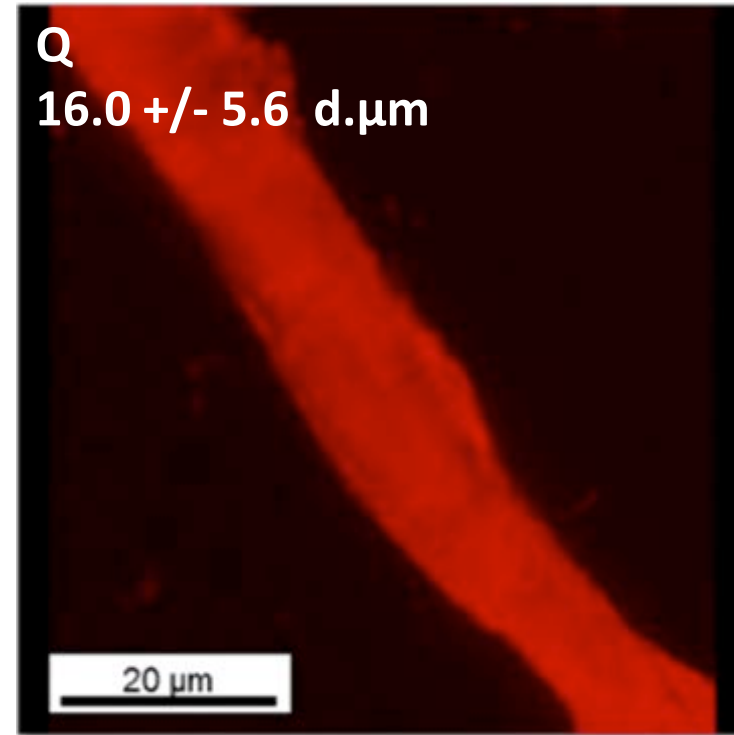
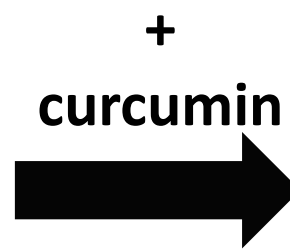


Domain swapped COMPcc: Q protein

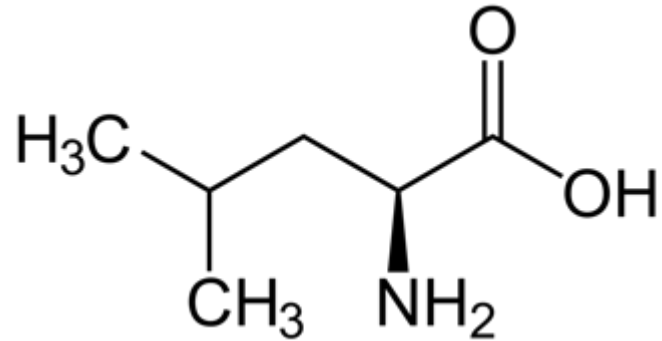
Small Drug Binding



**Nano-scale Fibers
(Nanofibers)**

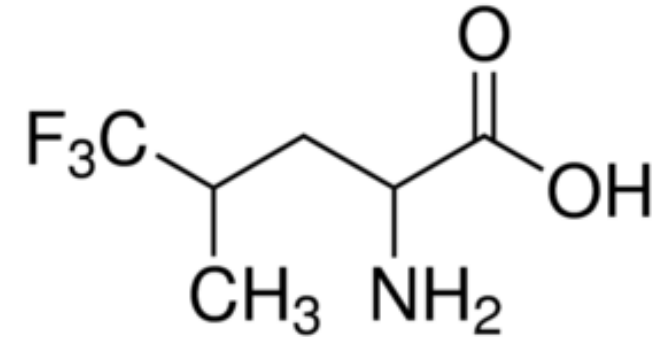


**Micron-scale Fibers
(Mesofibers)**



Leucine

- Naturally-occurring amino acid



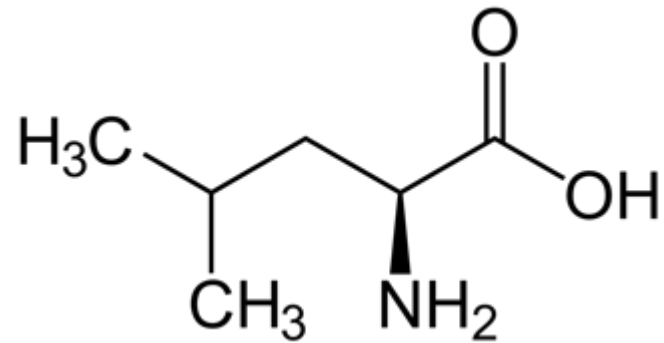
Trifluoroleucine

- Non-natural/ non-canonical amino acid
- Includes three fluorine atoms

Fluorine:

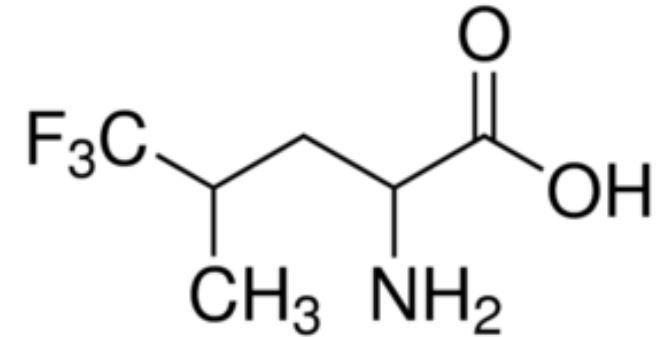
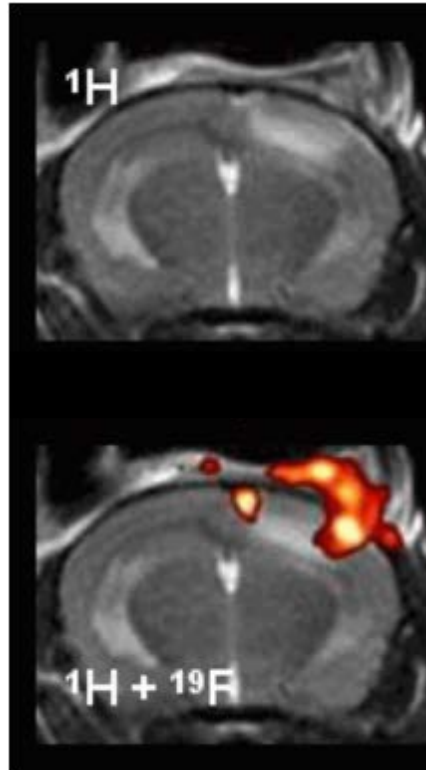
- Improves protein stability against heat and chemicals
- Fluorine MRI (limited fluorine in body → signal is specific to the construct)

Fluorinated Q protein



Leucine

- Naturally-occurring amino acid



Trifluoroleucine

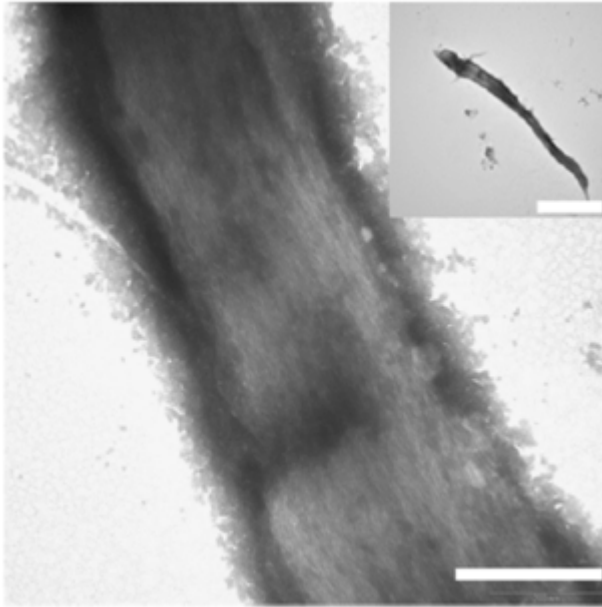
- Non-natural/ non-canonical amino acid
- Includes three fluorine atoms

Fluorine:

- Improves protein stability against heat and chemicals
- Fluorine MRI (limited fluorine in body → signal is specific to the construct)


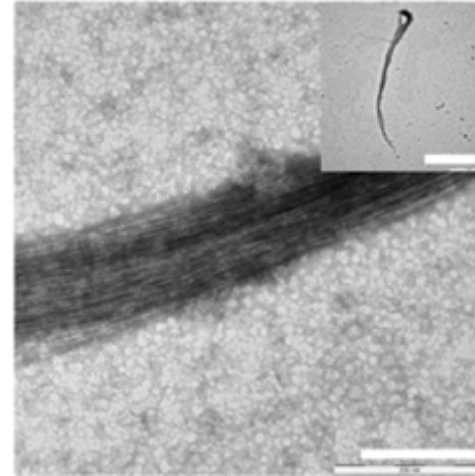
Fluorinated Q protein

Metal Binding by Q+TFL




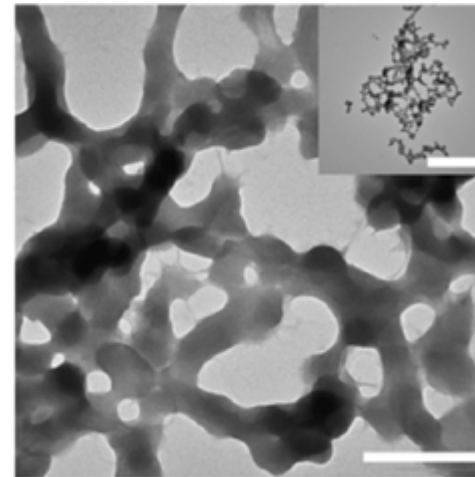
- TFL-incorporated Q fibers have increased thermostability and chemical stability

+
Zinc

- Zinc-stabilized fibers

+
Nickel

- Nickel disrupts fiber formation and yields aggregation

Protein Engineered Nanomaterials

- **Proteins as building blocks**
- **Engineered protein fibers**

Any Questions?

Objectives

- Proteins as building blocks
- Engineered protein fibers
- **Engineered protein nanoparticles**

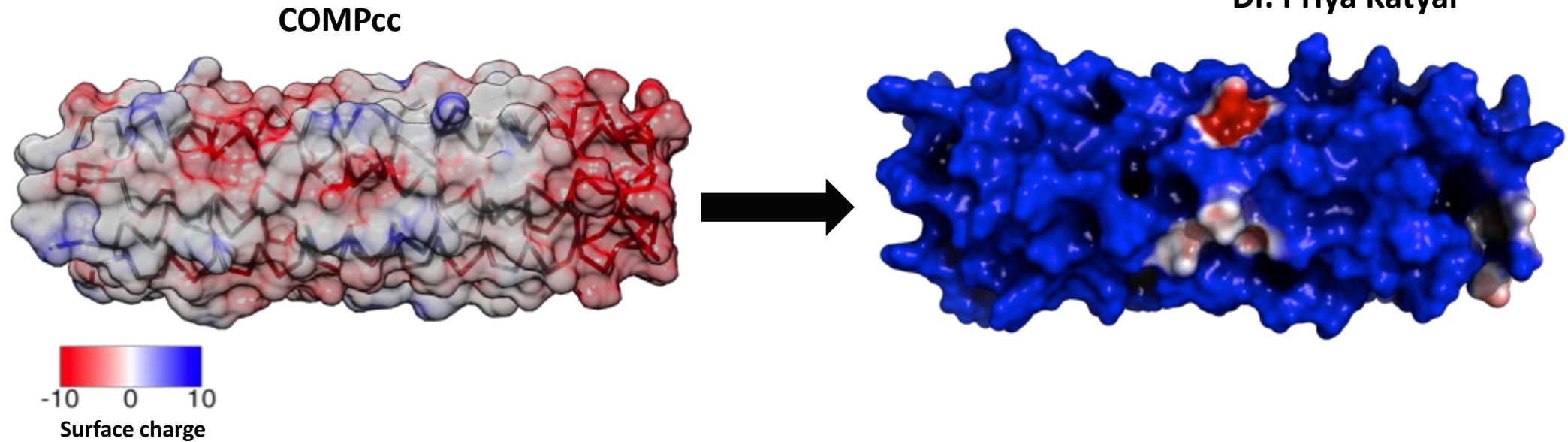


Dr. Priya Katyal

COMPcc Supercharged Protein (CSP)



Dr. Priya Katyal

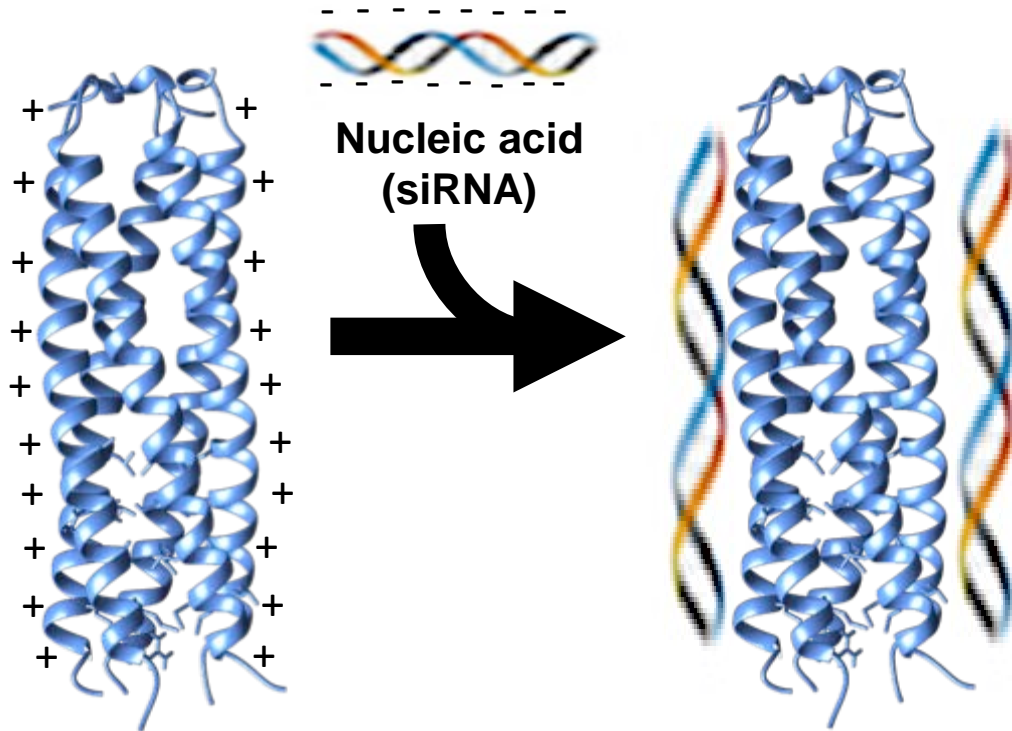


COMPcc: MRGSHHHHHHGSGDL APQMLRE LQETNAA LQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

CSP MRGSHHHHHHGSGRL **RP**QMLRE LQ**R**TNAA L**R**DVREL LRQQVKE IT**R**LKNT **VRRS**RAS GKLN

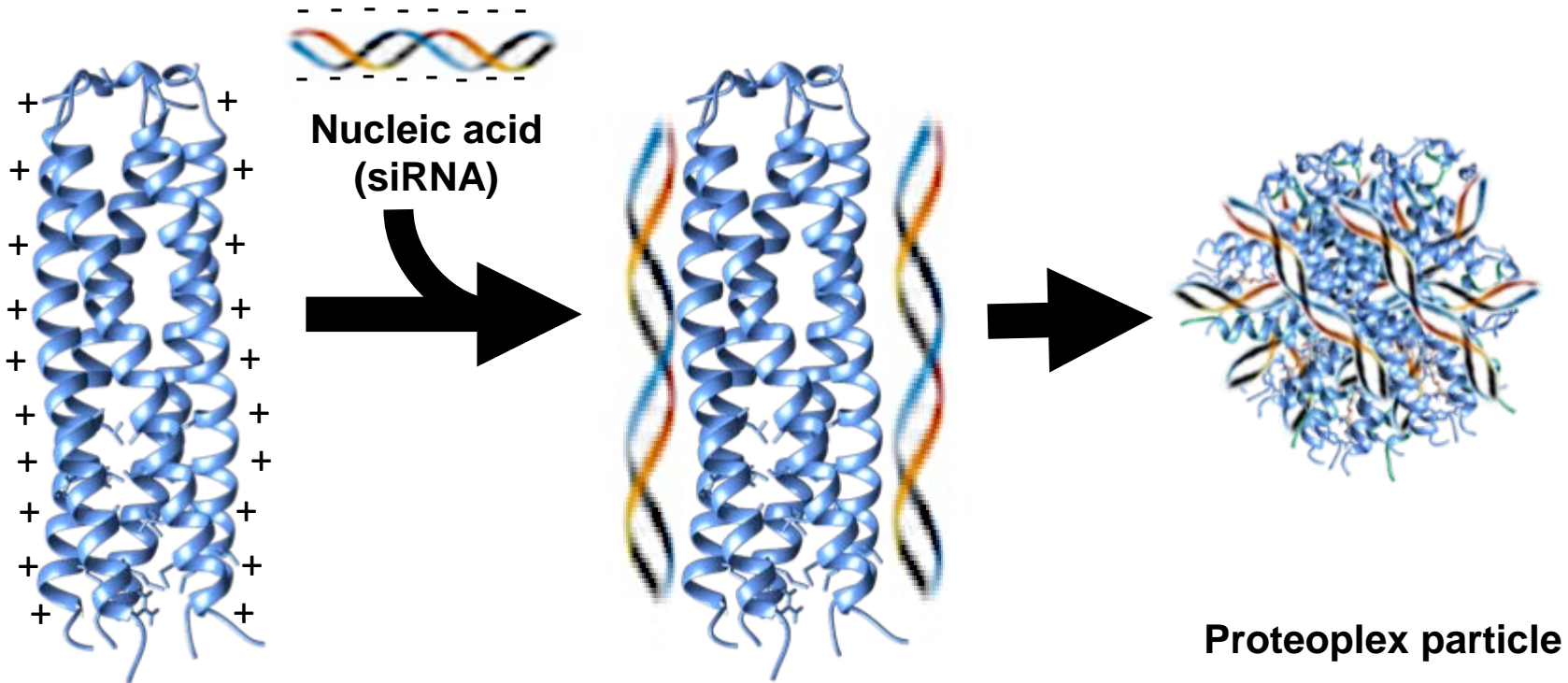
COMPcc Supercharged Protein (CSP)

Nucleic acid Binding



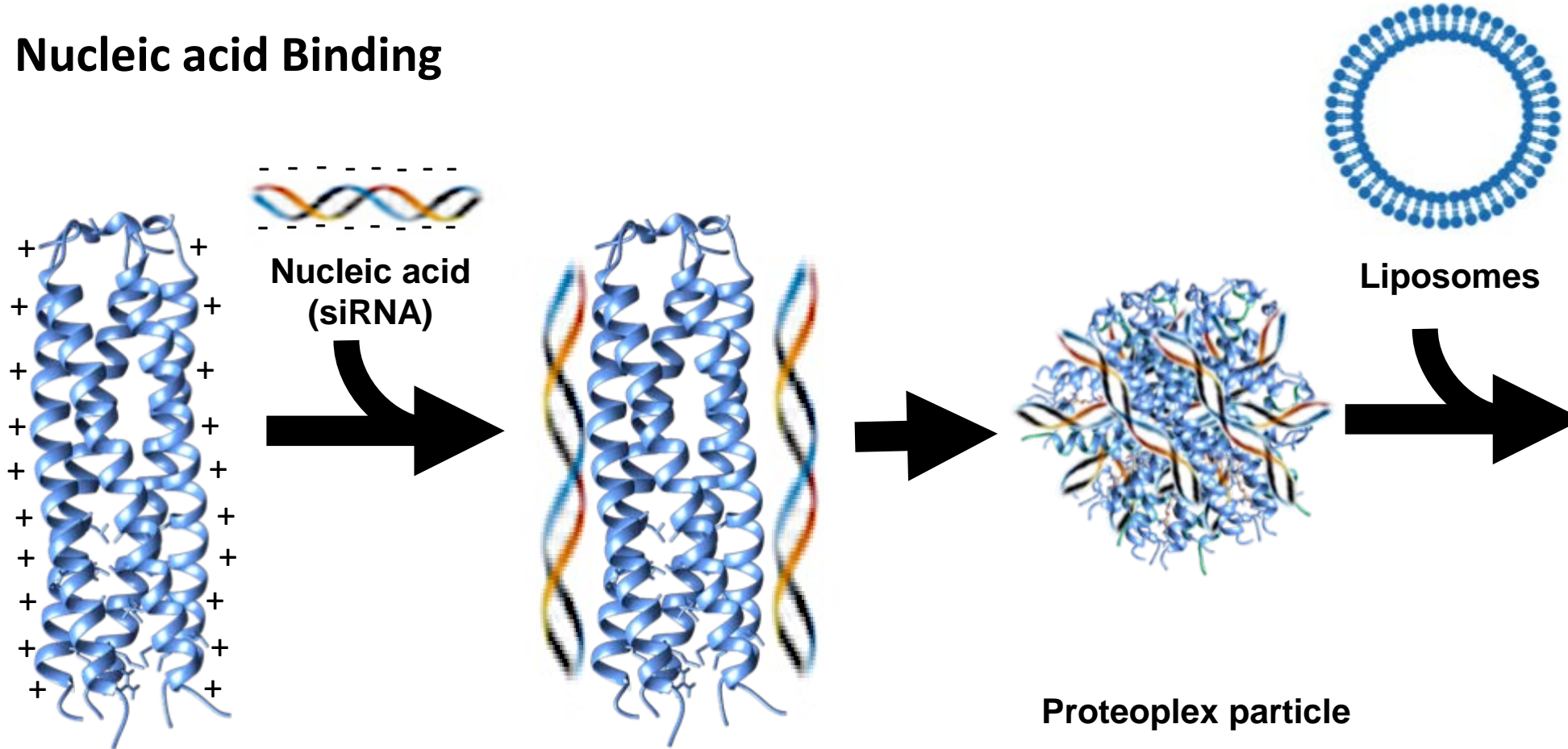
COMPcc Supercharged Protein (CSP)

Nucleic acid Binding



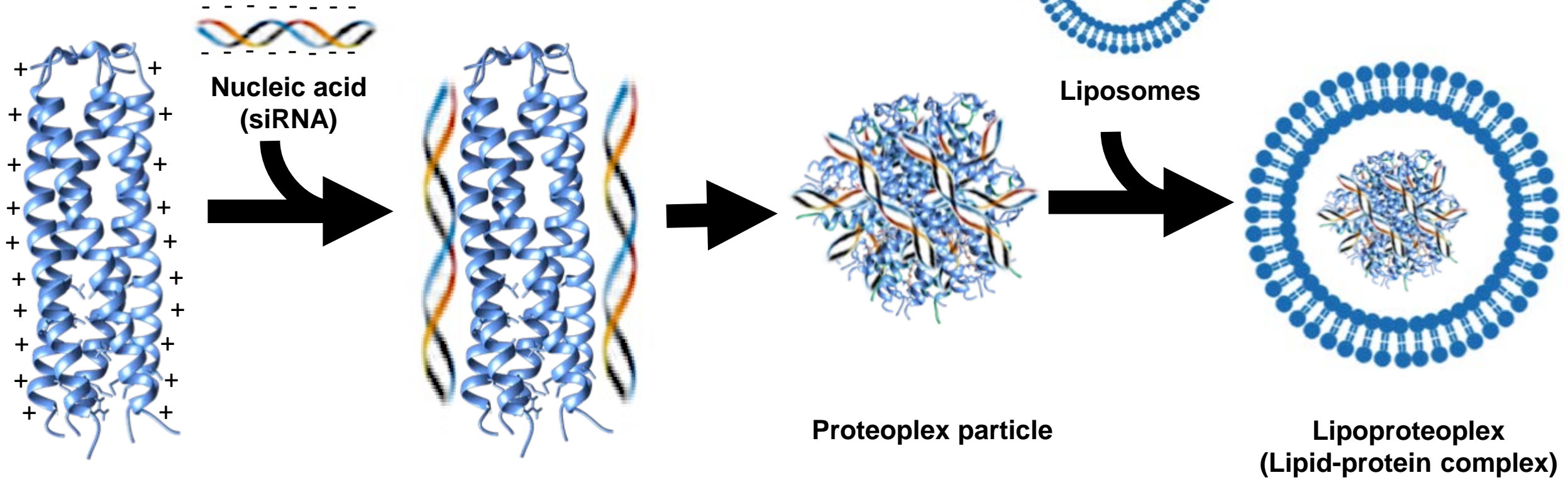
CSP-Lipid assemblies

Nucleic acid Binding



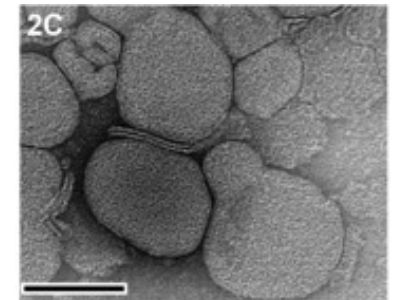
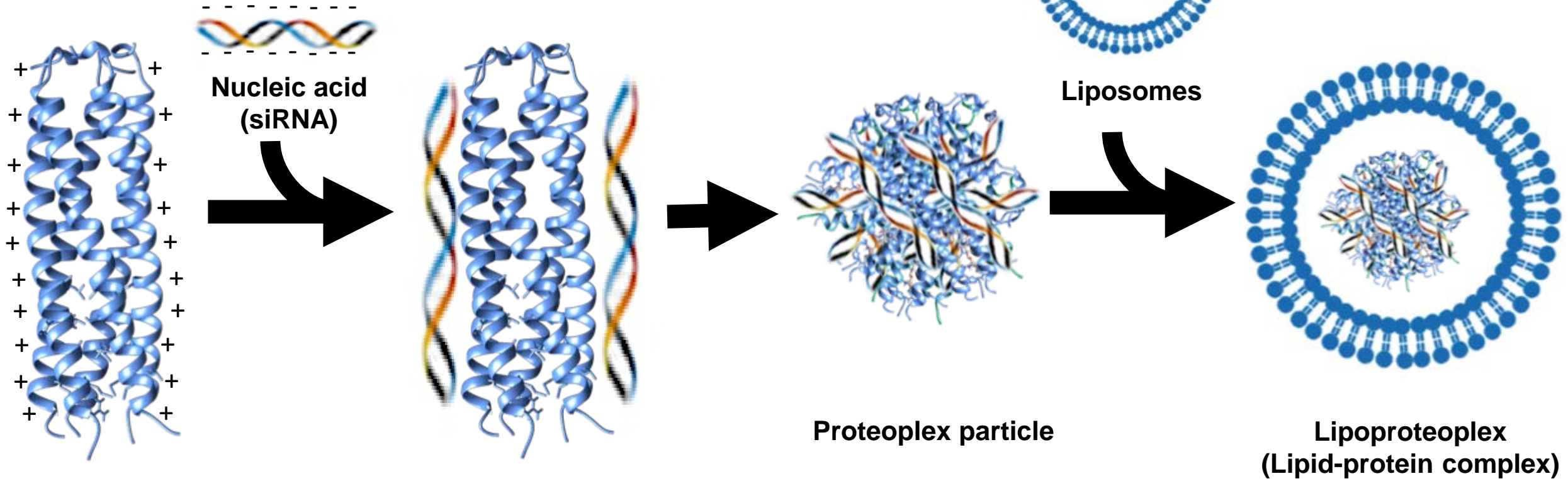
CSP-Lipid assemblies

Nucleic acid Binding

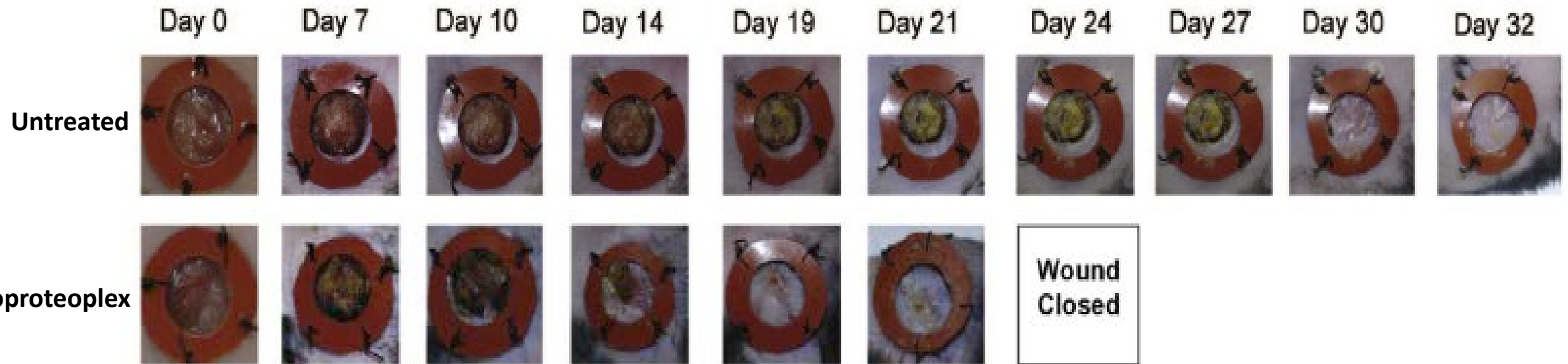


CSP-Lipid assemblies

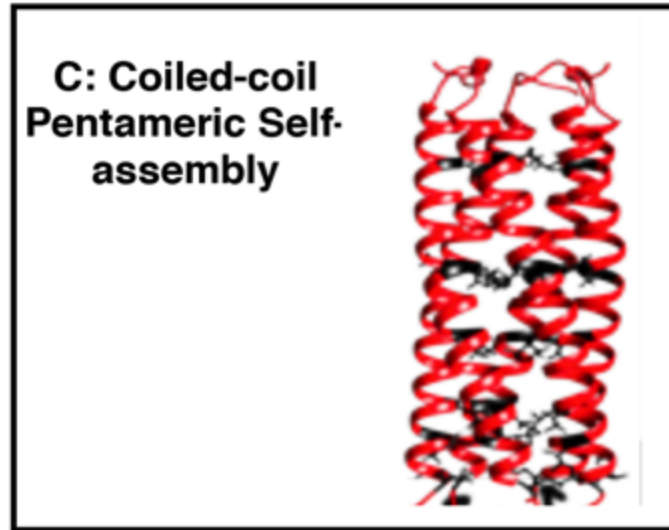
Nucleic acid Binding



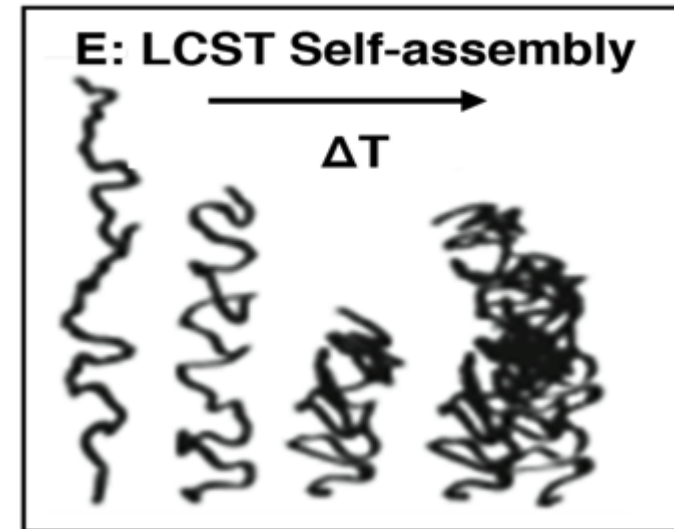
Diabetic Wound Healing



Topical lipoproteoplex-gene therapy accelerates diabetic wound closure

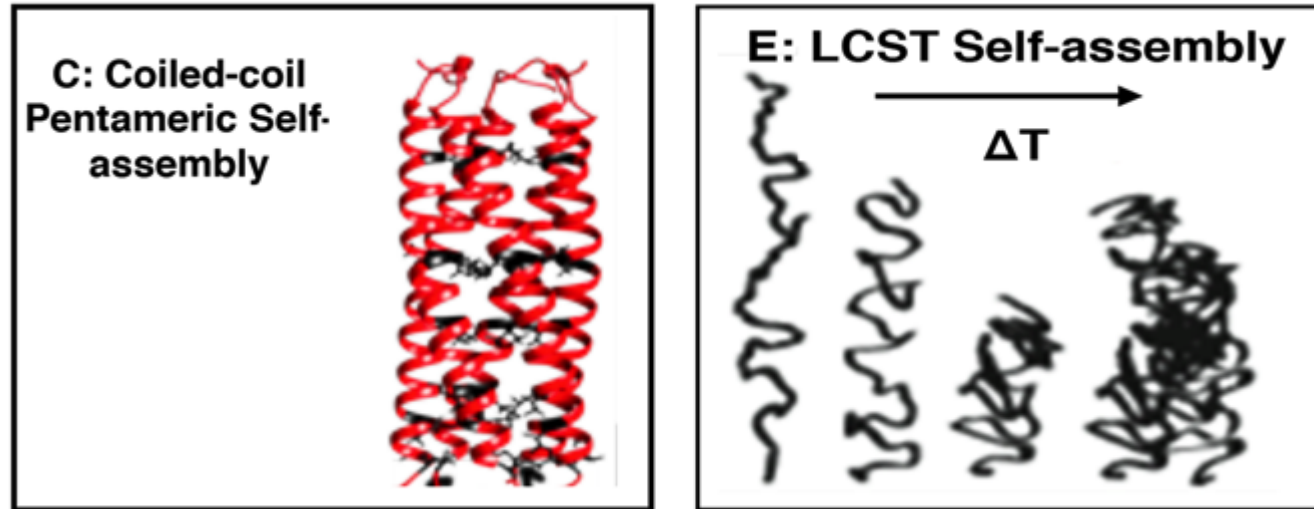


Cartilage Oligomeric Matrix Protein (COMPcc):
Homopentameric structure
Hydrophobic pore



Elastin-like-Polypeptide (ELP):
(VPGXG)_n Repeating units
Self assembles into helical *beta spiral*

COMPcc (C) and Elastin (E)

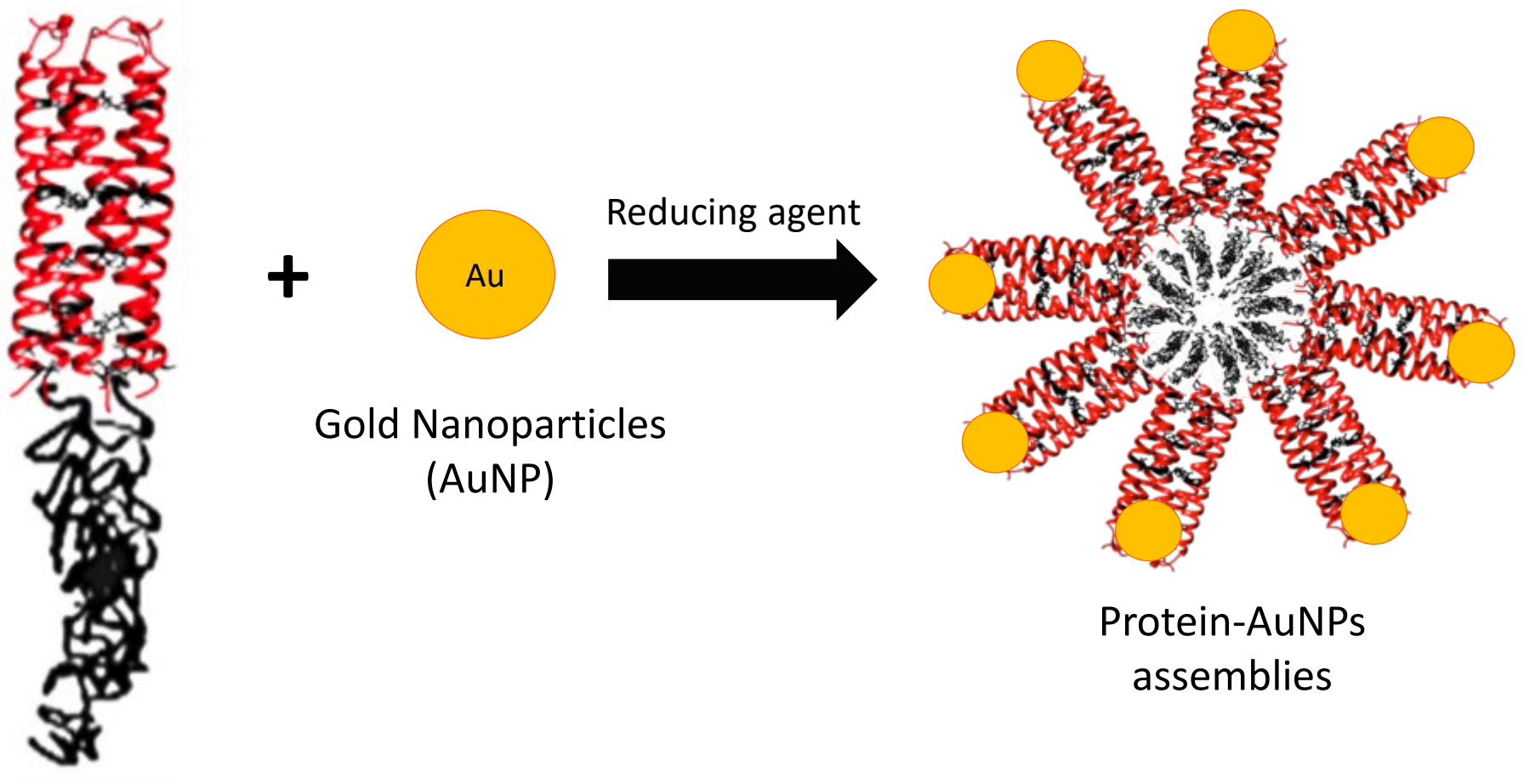


C: MRGSHHHHHHGSGDL APQMLRE LQETNAA LQDVREL LRQQVKE ITFLKNT VMESDAS GKLN

E: [(VPGVG)₂ VPGFG (VPGVG)₂]₅

COMPcc (C) and Elastin (E) fusion protein

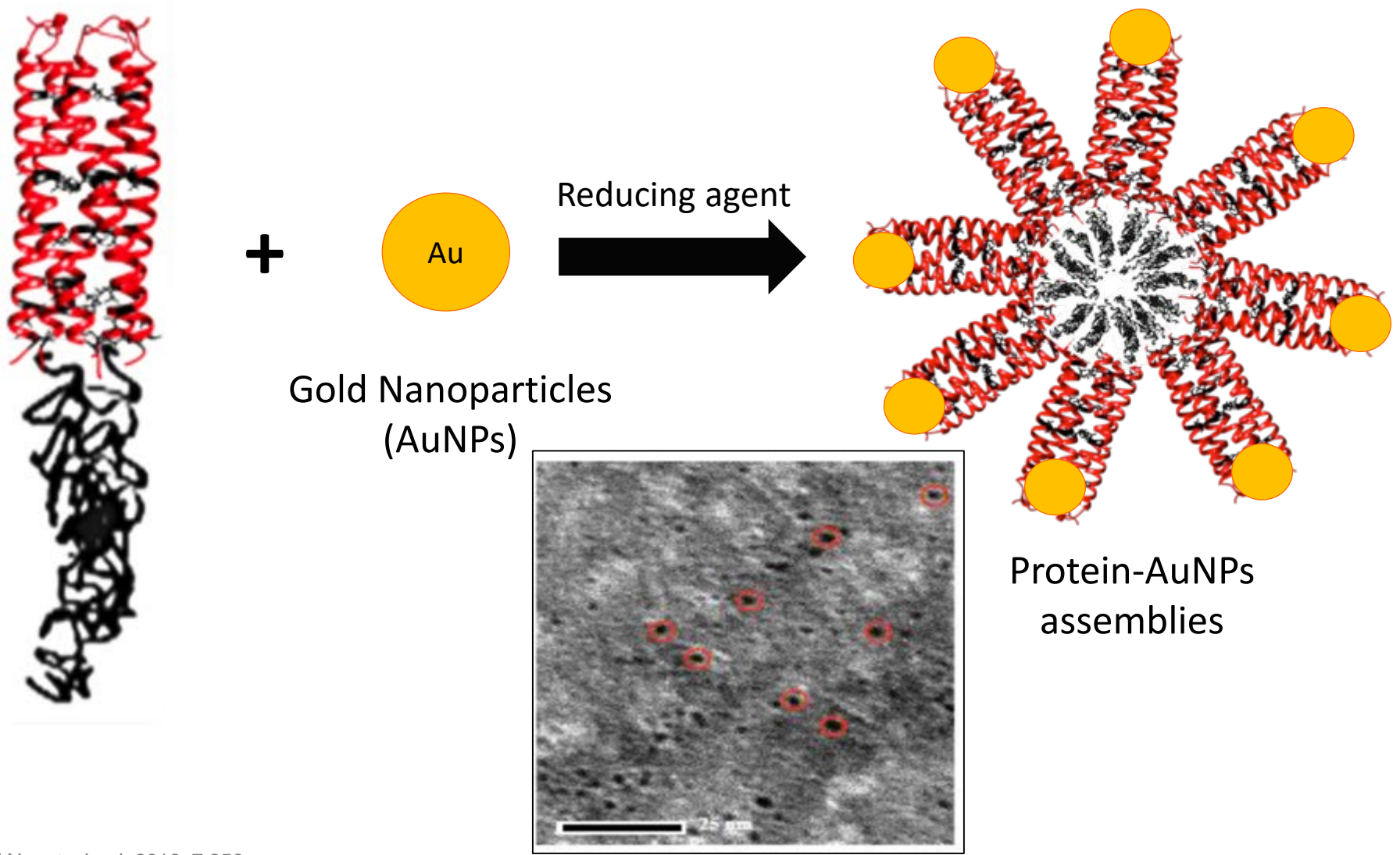
Gold Nanoparticle templation



CE: MRGSHHHHHHGSACELA(AT)₆AACG-C-LQA(AT)₆AVDKPIASSA-E-LEGS GTGGAKLN

COMPcc (C) and Elastin (E) fusion protein

Gold Nanoparticle templation



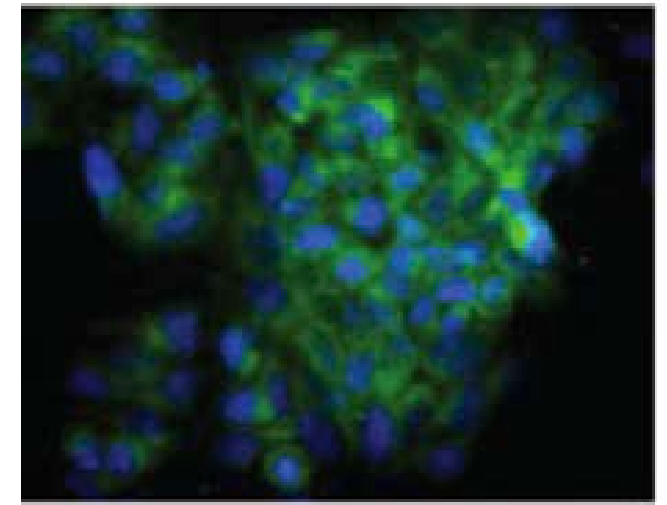
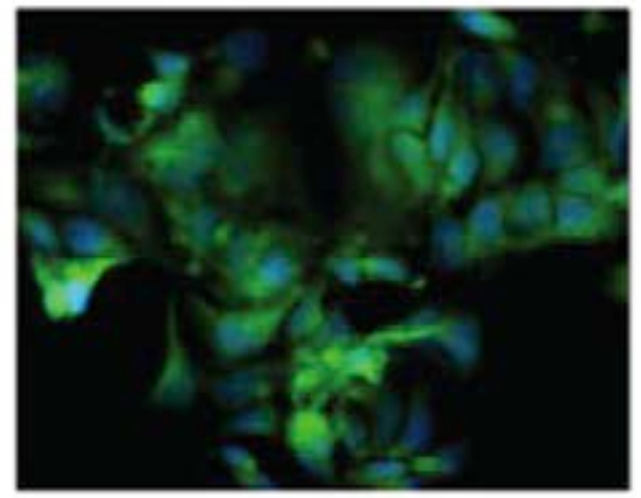
COMPcc (C) and Elastin (E) fusion protein Gold Nanoparticle and Curcumin (CCM) binding

MCF-7 cells with CE

CCM absorption at 420nm

MCF-7 cells with CE-AuNP

+CCM

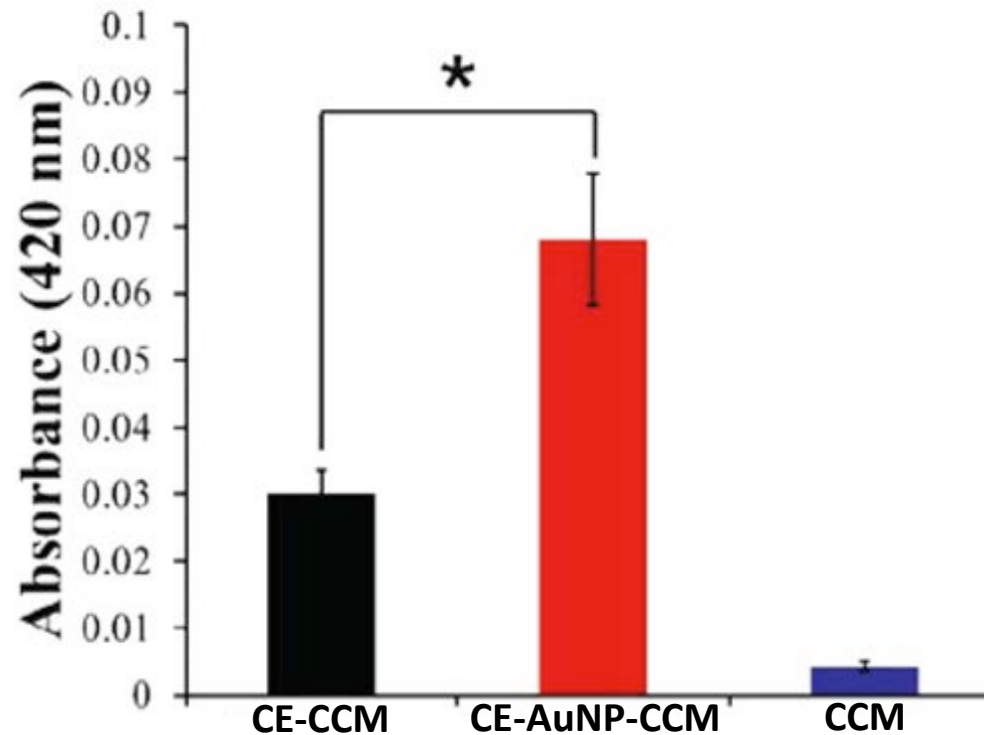
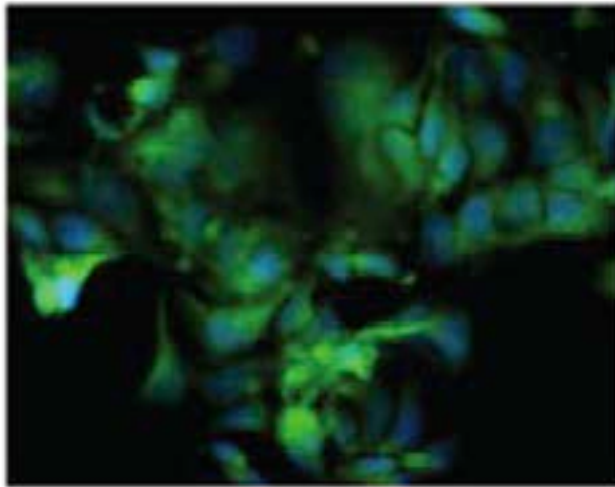


+CCM

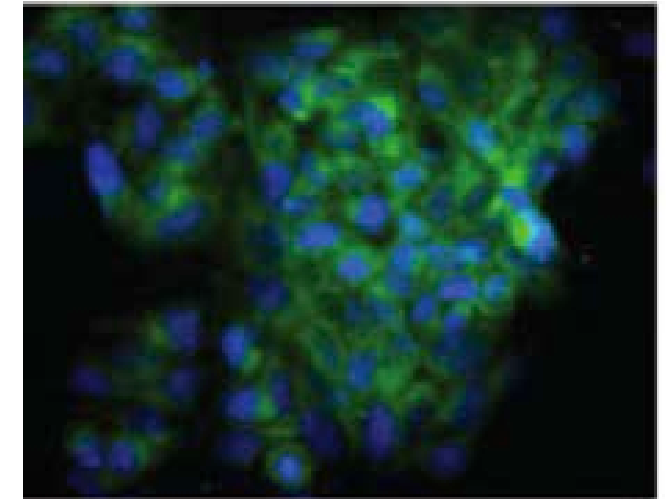
Uptake of Curcumin (CCM) by MCF-7 breast cancer cells

COMPcc (C) and Elastin (E) fusion protein Gold Nanoparticle and Curcumin uptake

MCF-7 cells with CE

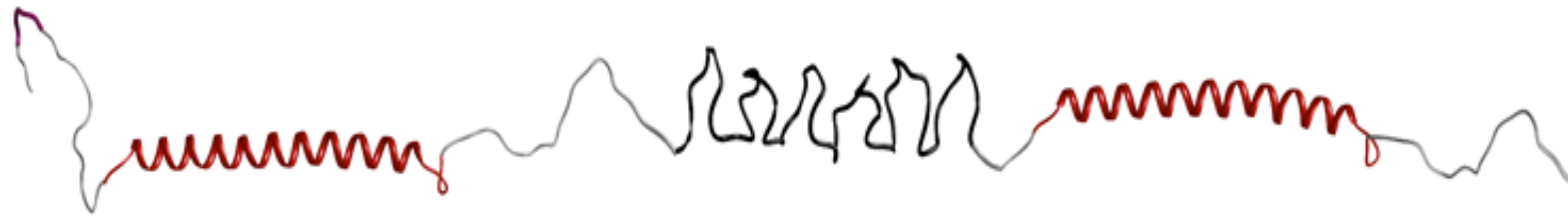


MCF-7 cells with CE-AuNP



Uptake of Curcumin (CCM) by MCF-7 breast cancer cells

COMPcc (C) and Elastin (E) triblock: CEC

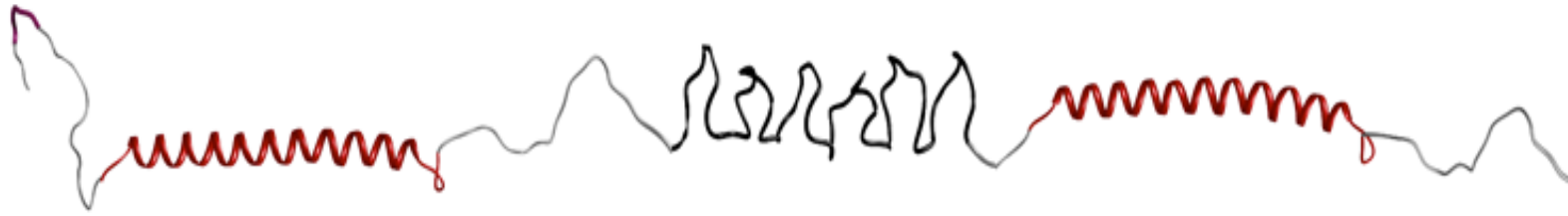


COMP (C)

Elastin (E)

COMP (C)

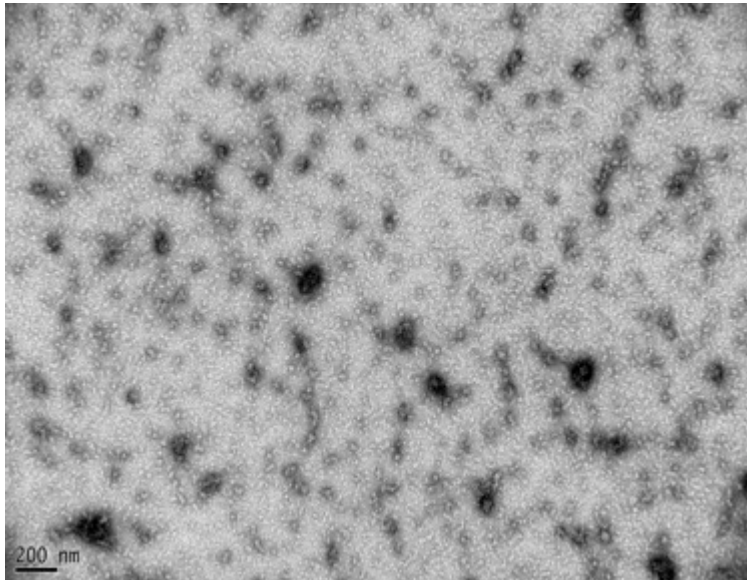
COMPcc (C) and Elastin (E) triblock: CEC



COMP (C)

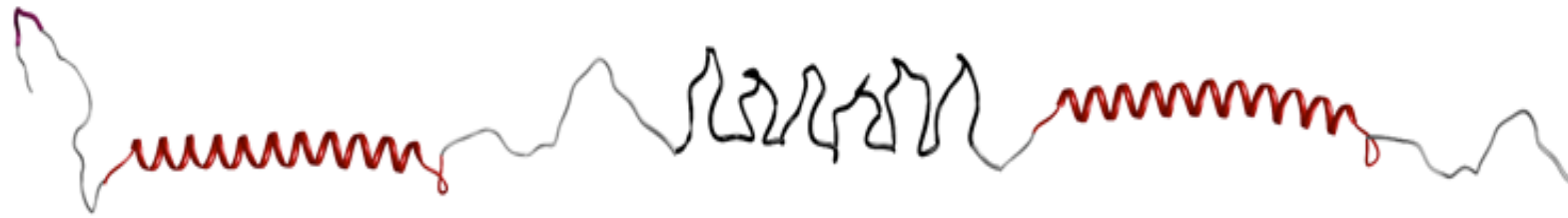
Elastin (E)

COMP (C)



Nanoparticles formation

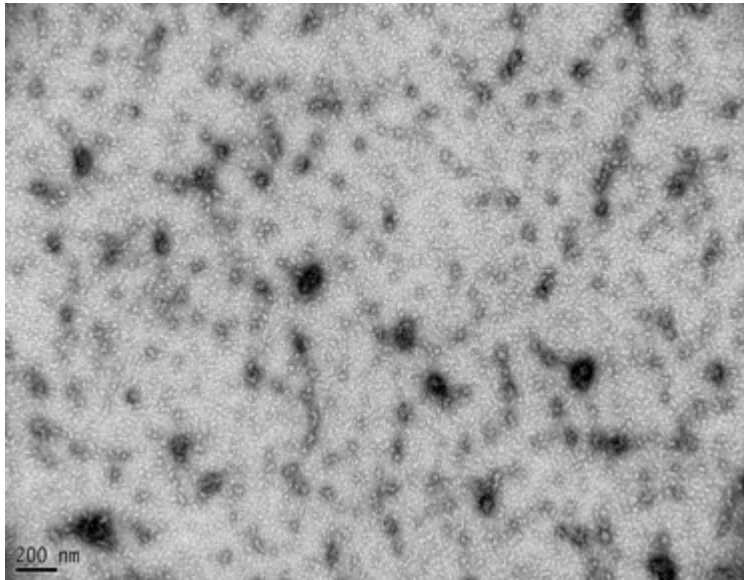
COMPcc (C) and Elastin (E) triblock: CEC



COMP (C)

Elastin (E)

COMP (C)



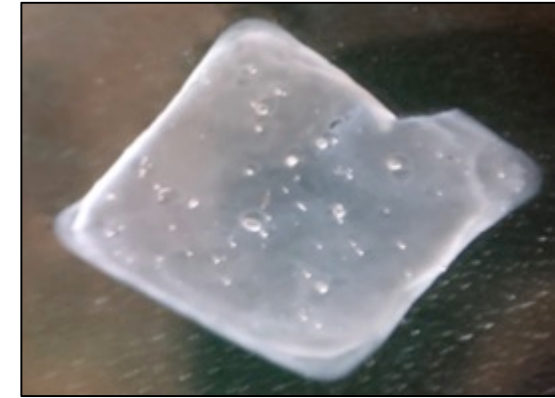
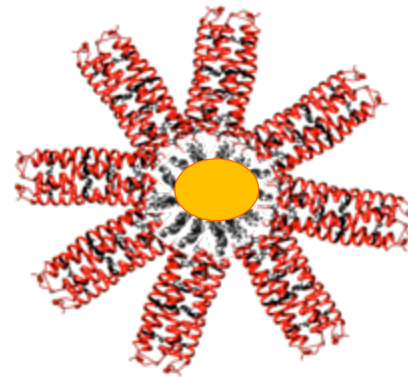
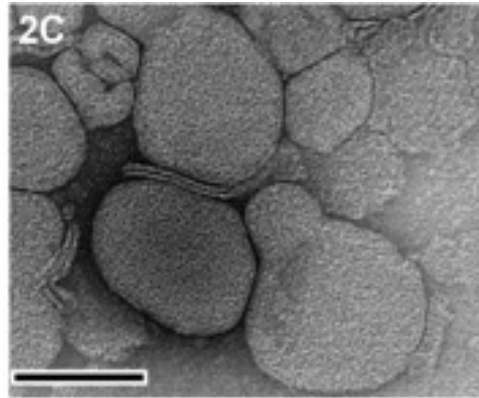
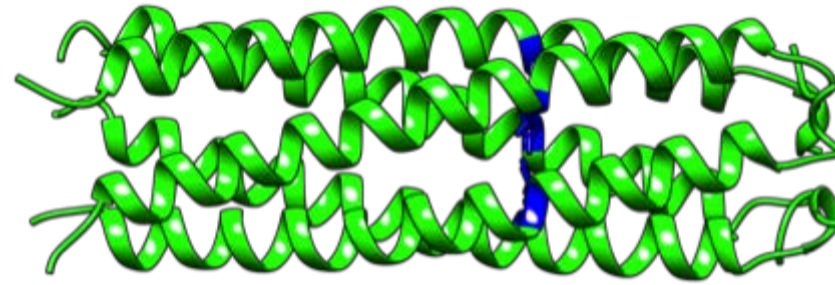
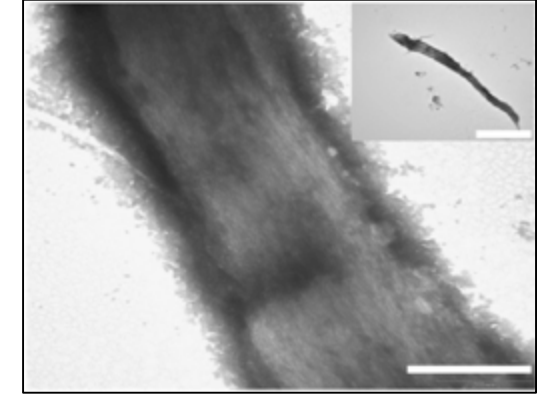
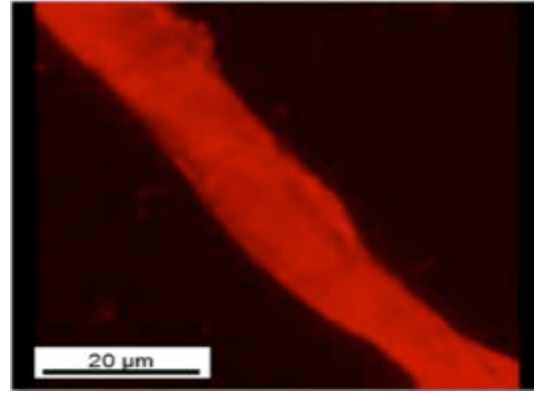
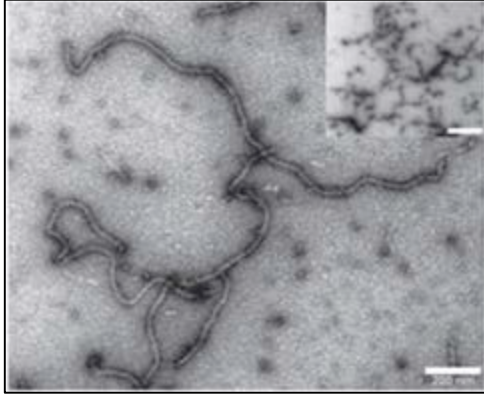
Nanoparticles formation



Gel formation

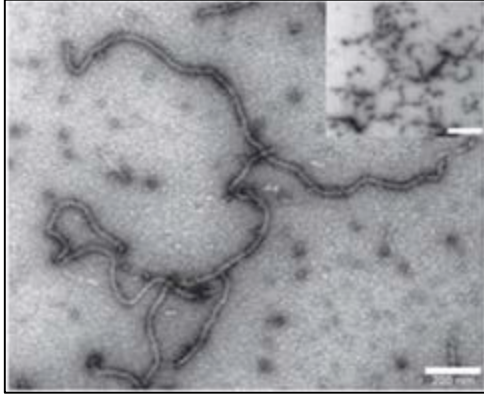
Summary

Smart Biomaterials

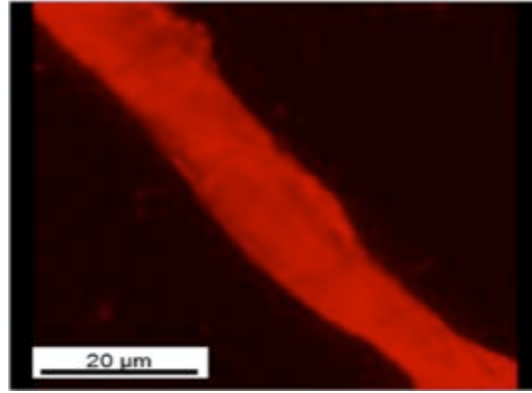


Summary

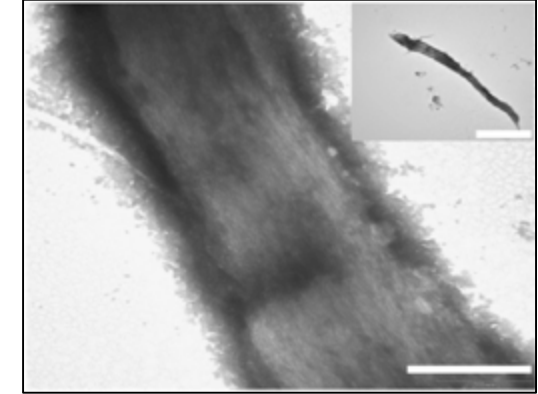
Applications



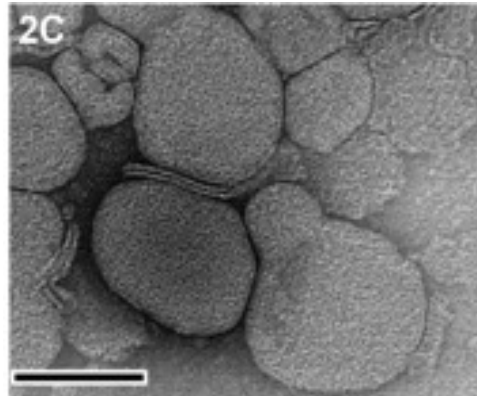
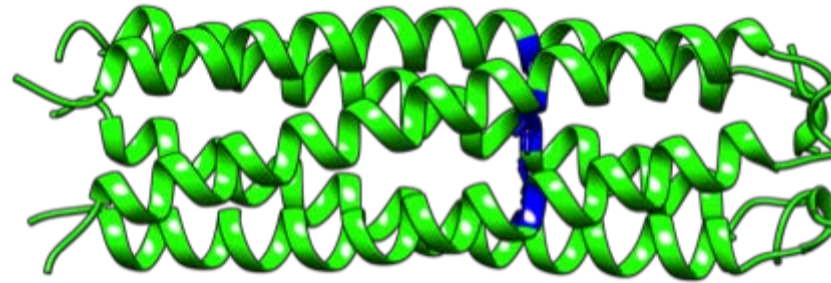
Osteoarthritis



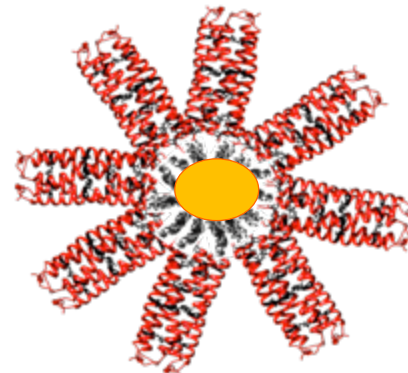
Iron templation



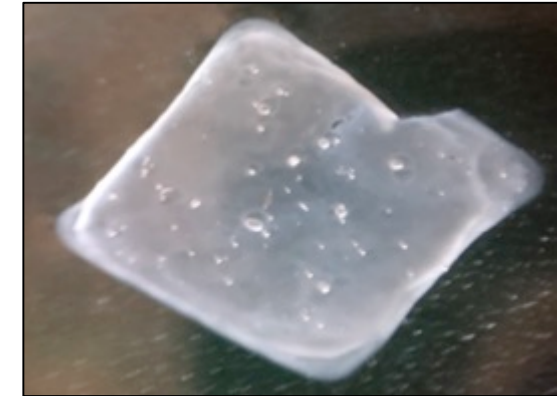
Fluorine imaging



Gene delivery



Breast cancer



Biomimicry

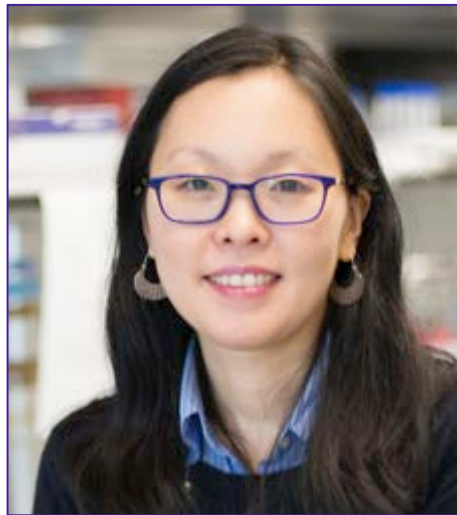
Protein Engineered Nanomaterials

Thank You!



Protein Engineered Nanomaterials

Thank You!



Dr. Jin Kim Montclare



Lindsay K. Hill



Dr. Priya Katyal

Email: montclare@nyu.edu