U.S. DEPARTMENT OF ENERGY | Office of Science

Basic Energy Sciences

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Microtubule Mimicry: Toward Biomolecular Self-Assembly in Synthetic Materials

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DOE/BES Division of Materials Sciences and Engineering
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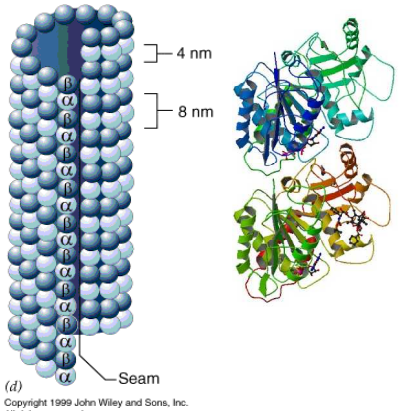
Microtubules and Motor Proteins: Dynamic Biomolecular



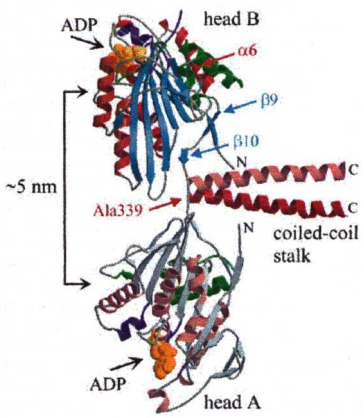
Microtubules (MTs)

Polar protein filaments (~25 nm diameter)

Polymerized from α-tubulin/β-tubulin dimers

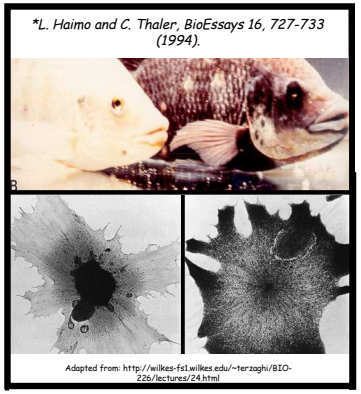
Dynamically polymerize and depolymerize.

Highly specific interactions with motor proteins (kinesins and dyneins).

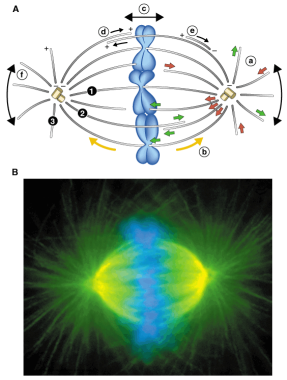


Kinesin motor protein

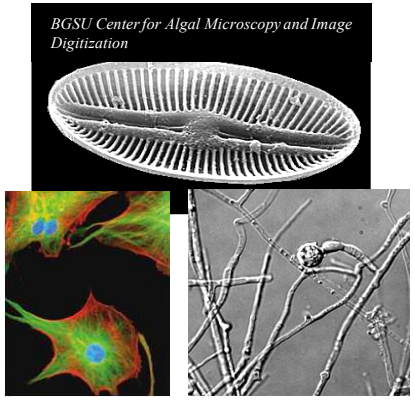
Video Credits: "Inner Life of Cell"
Conceptualized by *Dr. Alain Viel Ph.D.*, and *Dr. Robert Lue Ph.D.*, Molecular and Cellular Biology, Harvard University
Animated by *John Lieber* of **XVIVO**, Inc.
Funded by the *Howard Hughes Medical Institute*



Adaptive reorganization of pigment granules in melanocytes



Chromosome positioning and separation during cell splitting



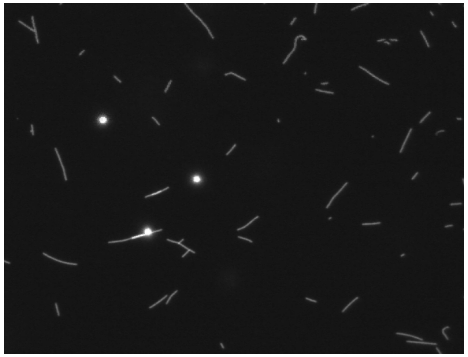
http://probes.invitrogen.com/

Trafficking of vesicles and macromolecule building blocks

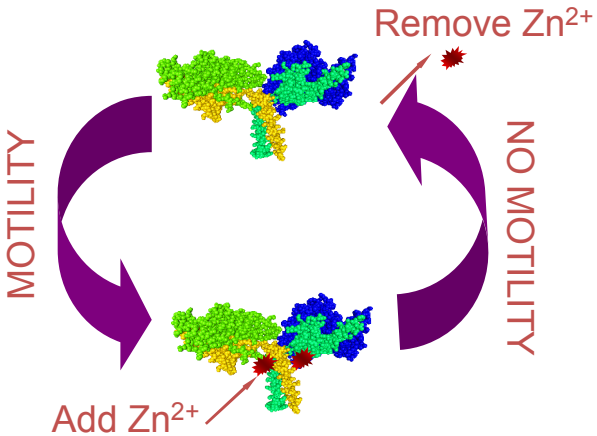
How can we utilize or mimic the form and function of these dynamic, functionally diverse biomolecular materials?

Active Assembly of Dynamic and Adaptable Materials

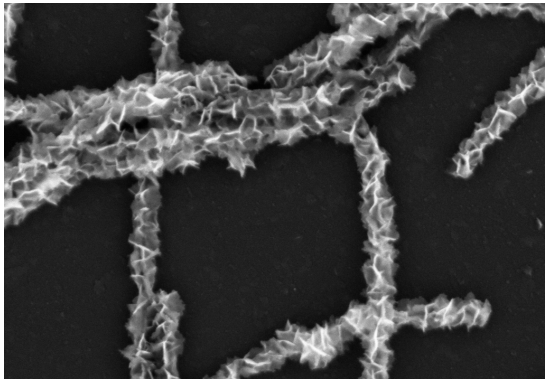
Biomolecular cargo transport



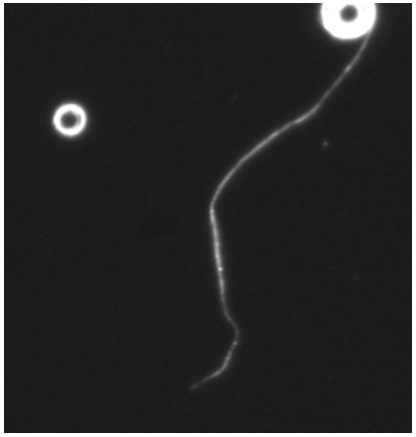
Kinesin “shackles”



Nanoscale templates

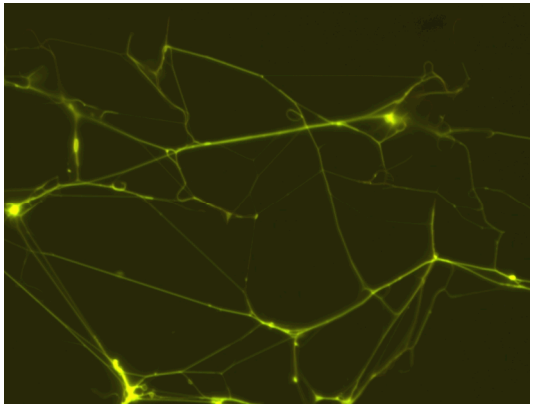


Non-equilibrium assemblies



3D-Microtubule Assemblies

Kinesin-based lipid tubule formation



See George Bachand’s Poster

George Bachand, Marlene Bachand, Erik Spoerke, Haiqing Liu, Darryl Sasaki

Artificial Microtubules

Key Characteristics of Microtubules:

1-Dimensional Nanostructures

Assembled from nanoscale building blocks

Building block chemistry and form directs assembled architecture

Dynamic, Programmable Assembly (chemical and thermal)

Secondary Assembly (MT organization)

Biomolecular Polarity (α-β asymmetry)

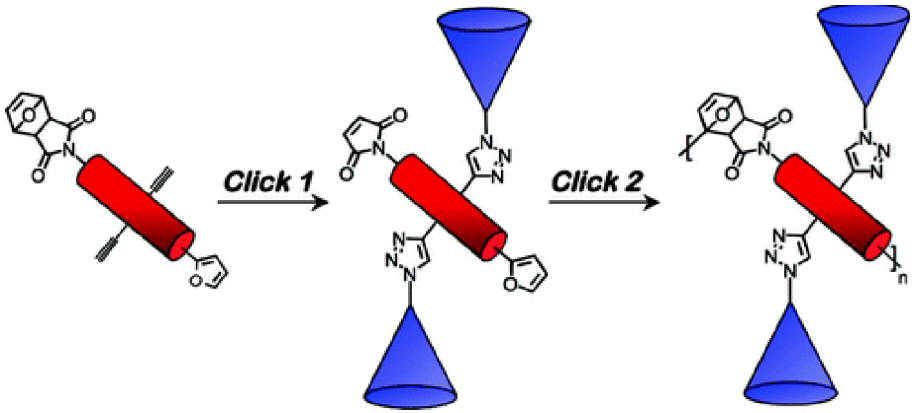
Motor Protein Interactions

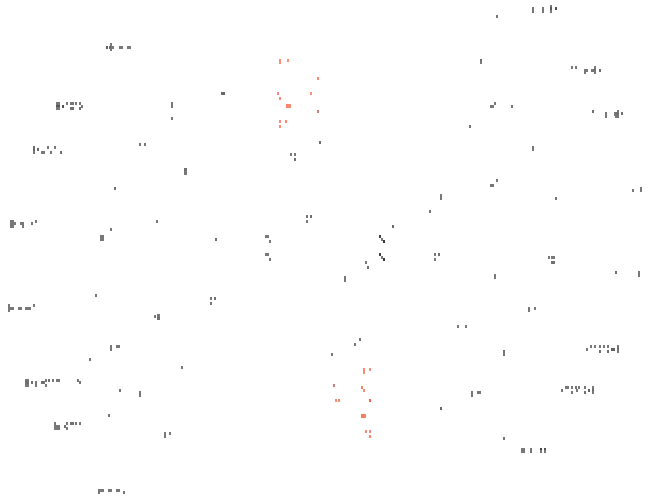
Thermally-Reversible Dendronized Polymers

Synthetic objective: Create linear assemblies of dendrons, polymerized together using thermally-reversible "Click" chemistry.

Based on Sequential Huisgen 1,3-Dipolar Cycloaddition and Diels-Alder Reactions

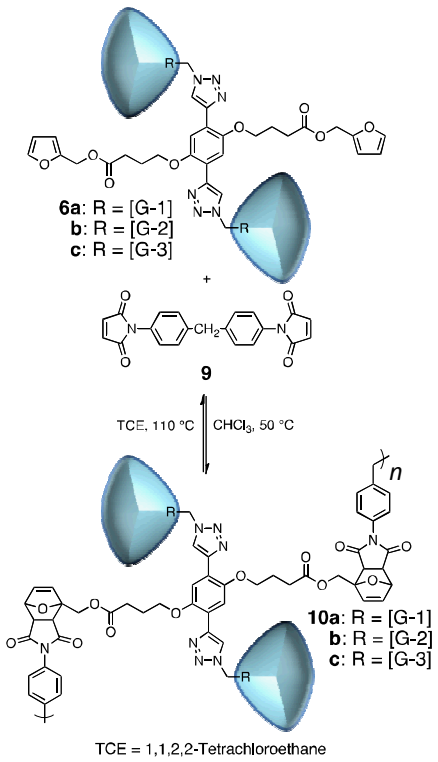
Forward reaction occurs at temperatures up to ~55 °C, with the reverse occurring above ~65 °C
(Anthracene – maleimide: 250 – 300 °C; Dicyclopentadiene: ~215 °C)

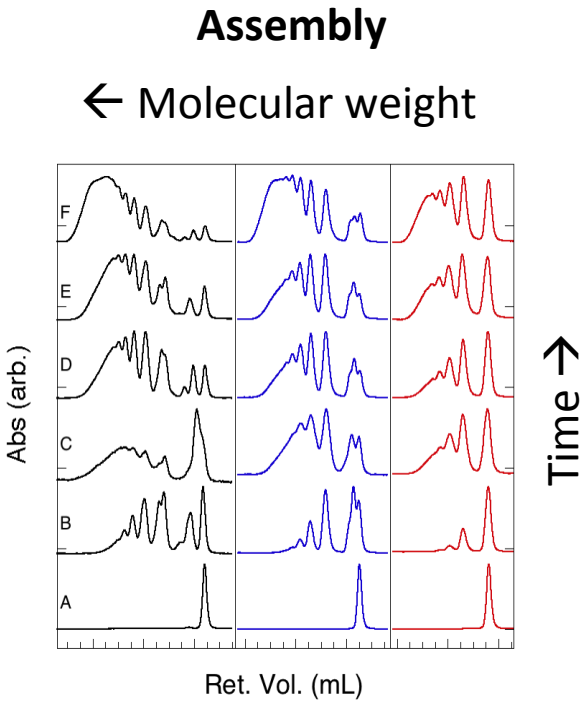


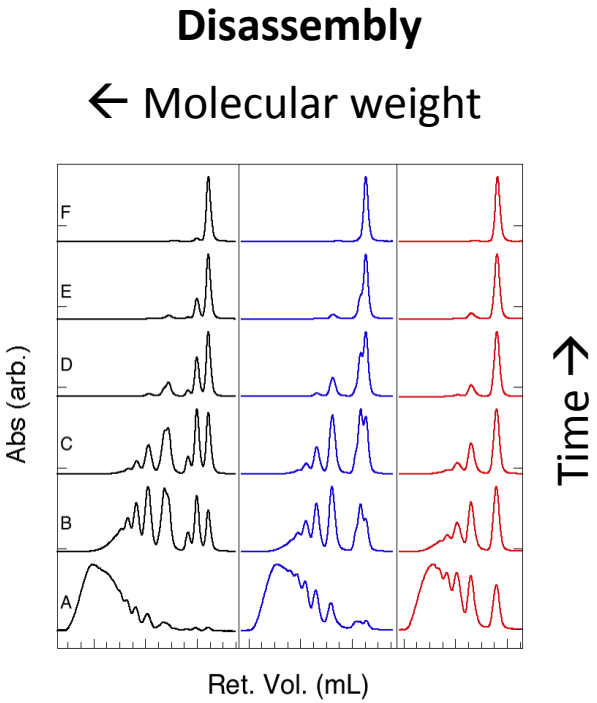


James McElhanon, Nathan Polakse*, Dominick V. McGrath*
*University of Arizona

Tracking Polymerization







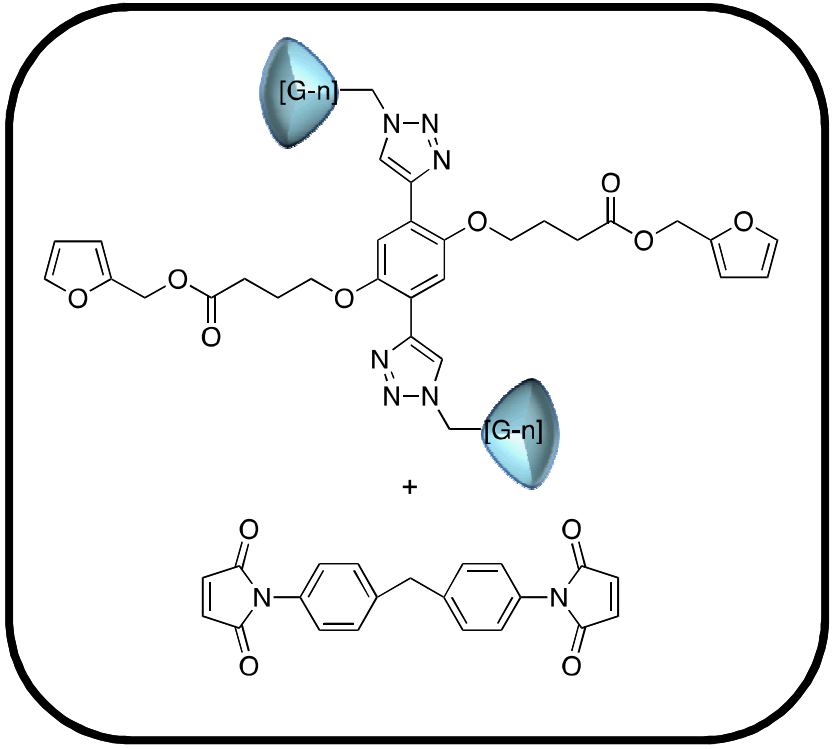
Chromatography (GPC) shows the thermally reversible polymerization of dendritic building blocks.

reveals the kinetics and extent of both polymerization and depolymerization processes.

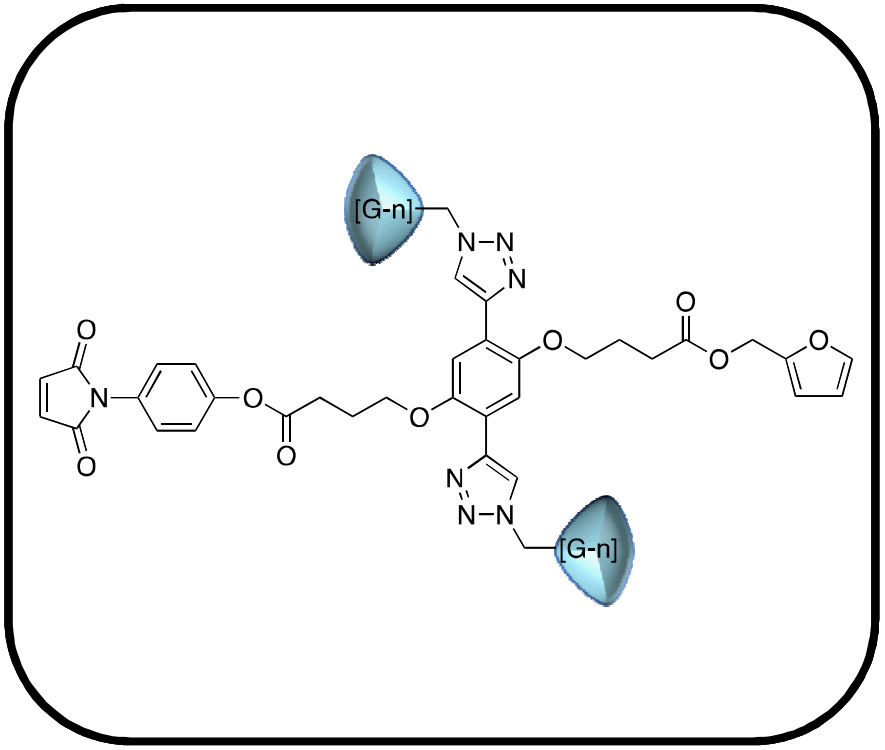
Polaske, McGrath, McElhanon, Macromolecules (2010)

Asymmetric Building Blocks

AA-BB System

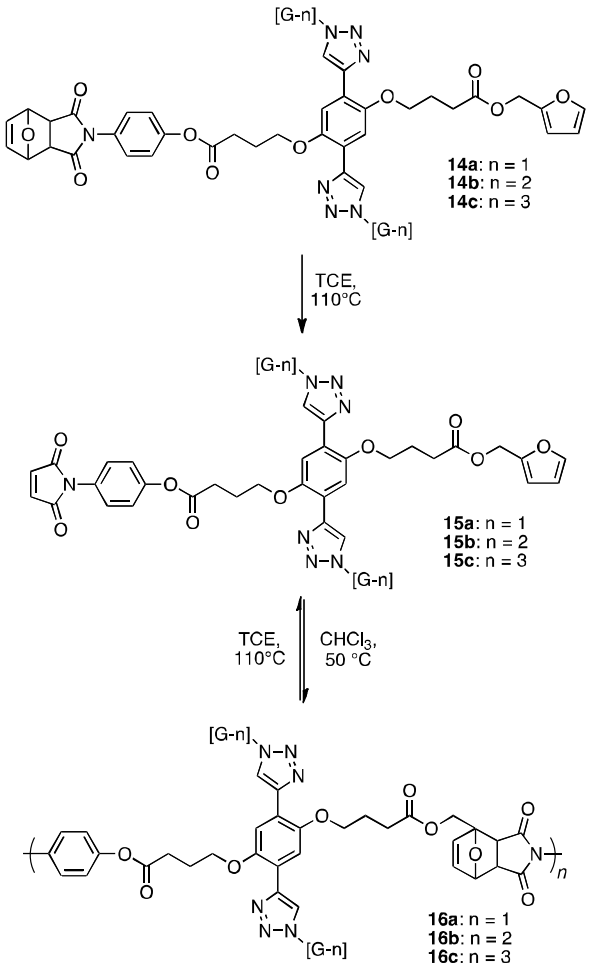


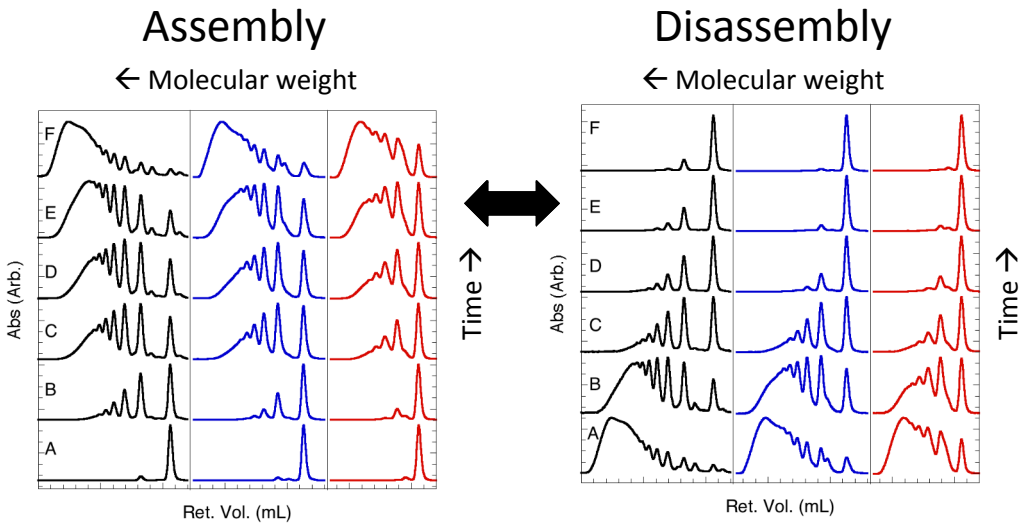
AB System



Asymmetric dendrimers introduce polarity mimicking MT biochemical polarity

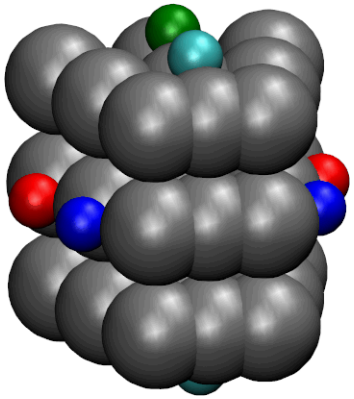
Cyclable Thermal Assembly of AB-Dendronized Polymers





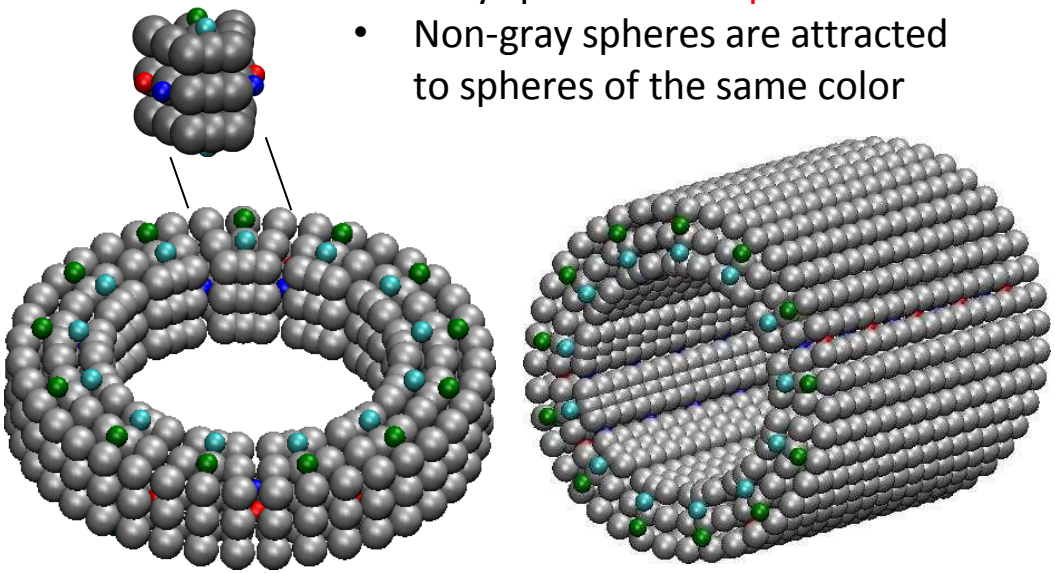
Asymmetric building blocks show promising, reversible assembly.

Theoretical Design Rules for Microtubule Mimics



Molecular Dynamics simulations were performed to probe the influences of monomer shapes as well as strength and geometric distribution of interaction potentials on each monomer.

- Gray spheres are repulsive
- Non-gray spheres are attracted to spheres of the same color



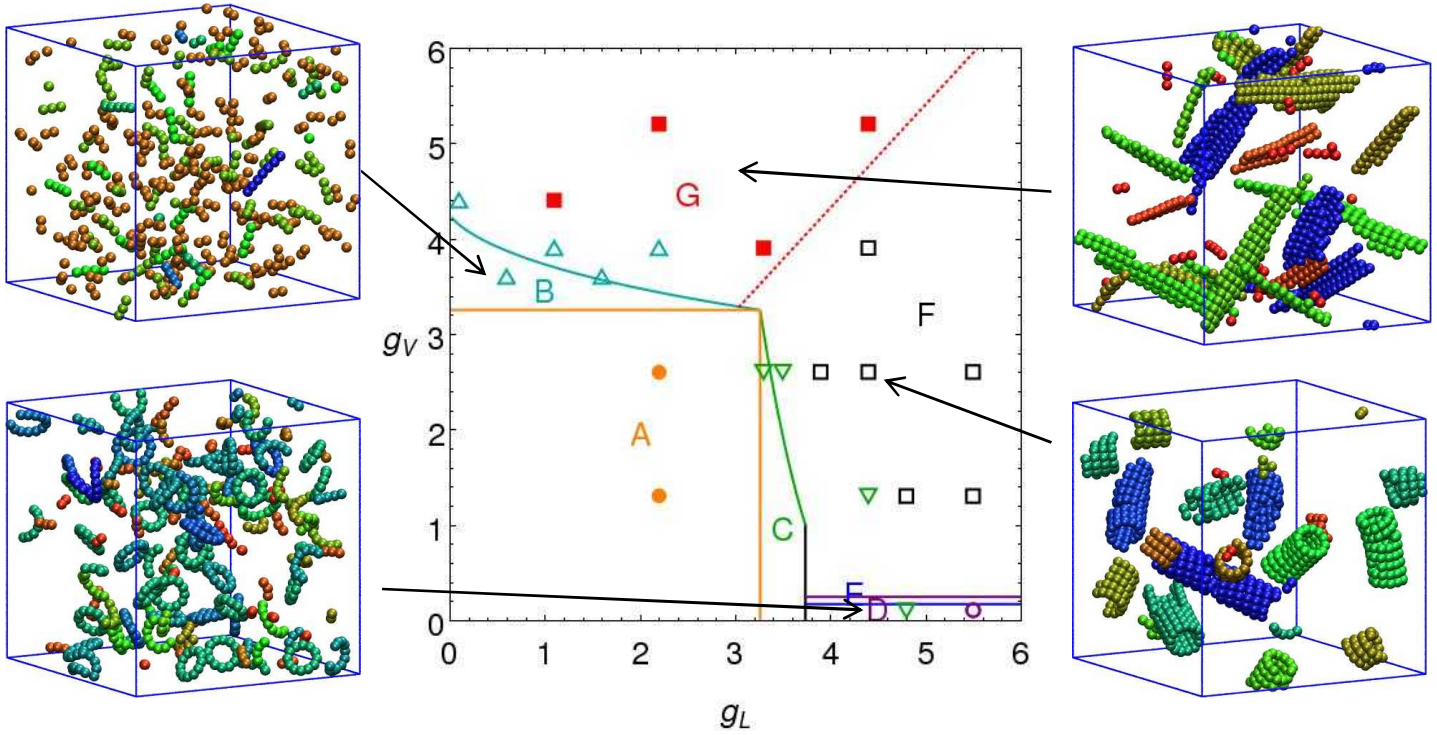
Asymmetric assembles into rings (13 monomers), and those rings would stack to form tubes.

Asymmetry is critical!

Mark Stevens, Shengfeng Cheng

Simulating Supramolecular Assembly

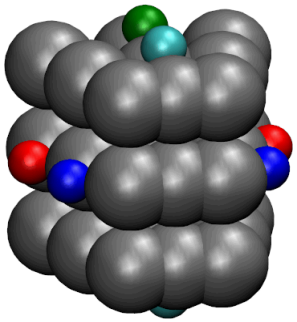
Tuning the lateral and vertical interaction parameters drives the assembly of filaments, rings, and tubes.

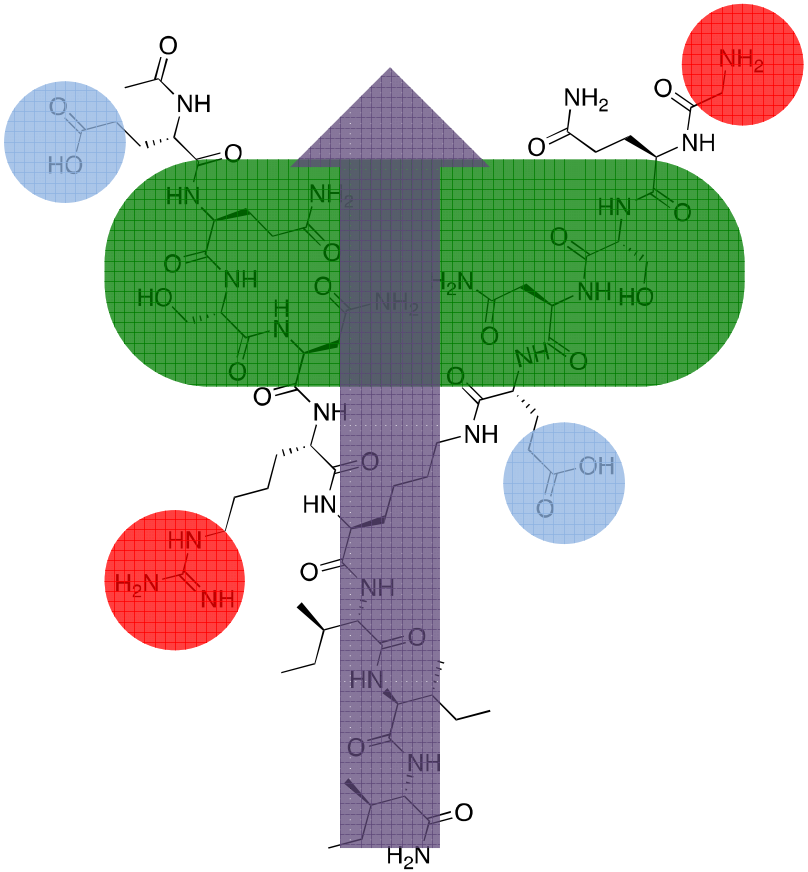


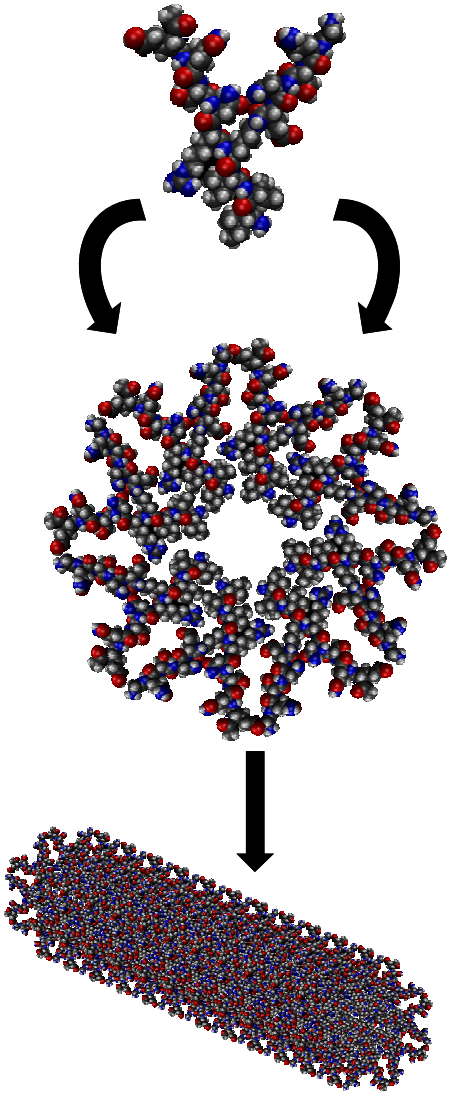
A = No assembly
B = Filaments
C = Partial Rings
D = Full Rings
E = Rings, short tubes
F = Full tubes
G = Curved sheets, helical tubes

Simulation-Inspired Peptide Wedges

Asymmetric branched peptides can be engineered with tunable handles to mimic simulated wedge particles.

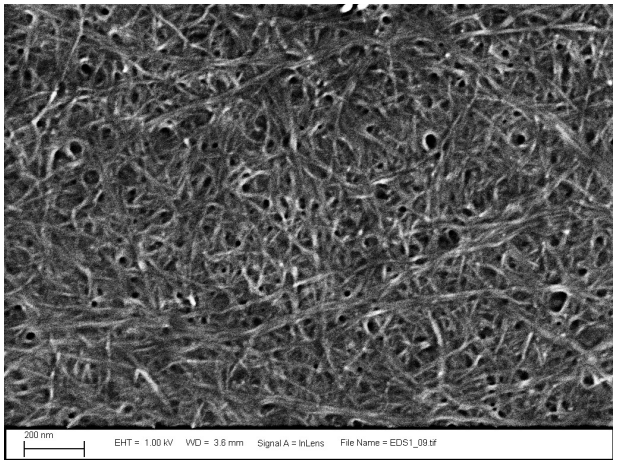




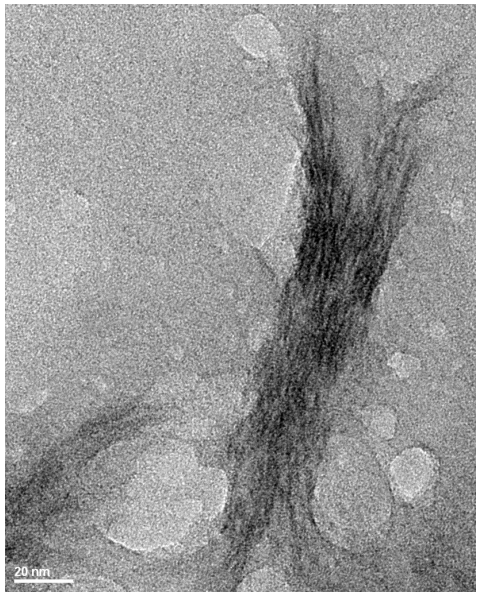


Asymmetric Wedge Assembly

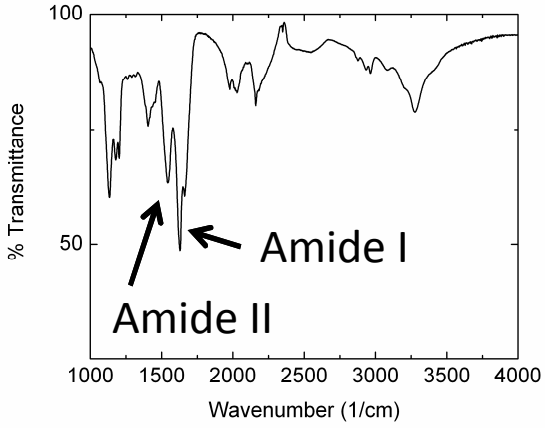
Peptide assembled into a self-supporting gel at ~10 mg/mL at neutral pH in water.

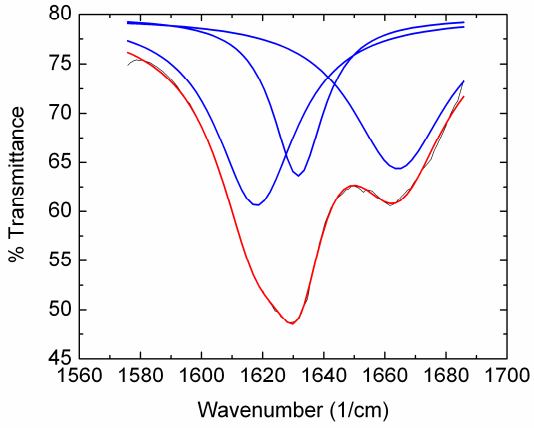


Scanning electron micrograph (SEM) shows the gel is composed of self-assembled nanofibers.



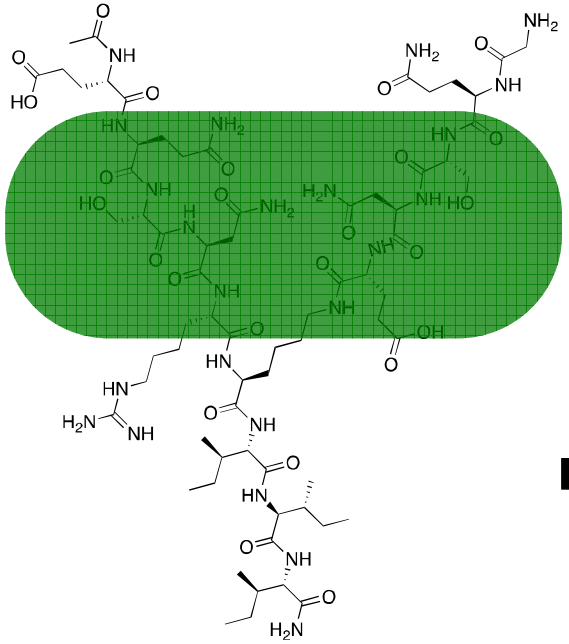
Transmission electron micrograph (TEM) confirms fiber formation and indicates nanofiber bundling.



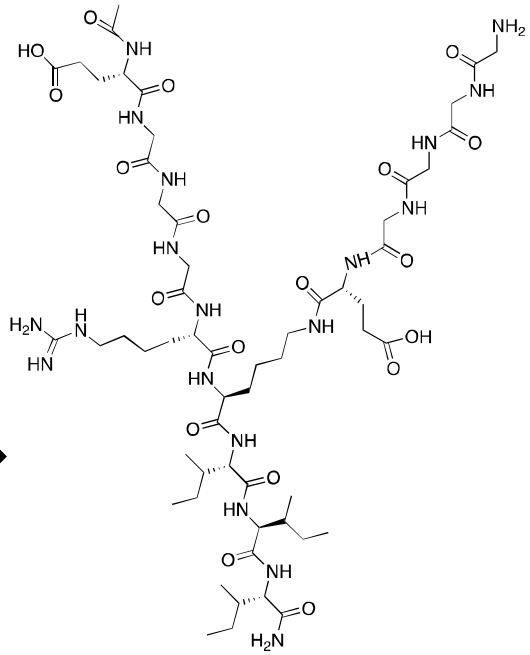


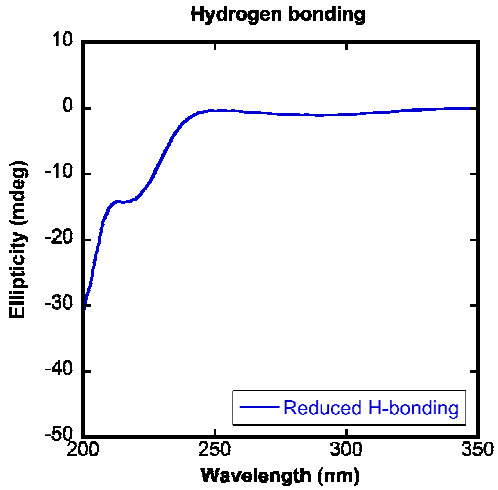
Deconvolution of the Amide I FTIR band reveals secondary structure (β-sheet, disordered)

Modifying Hydrogen Bonding



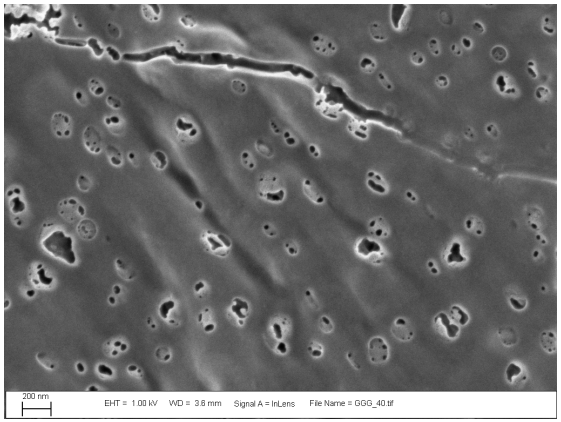
Replacing Hydrogen Bonding section of the peptide with glycines causes dramatic change in assembly.





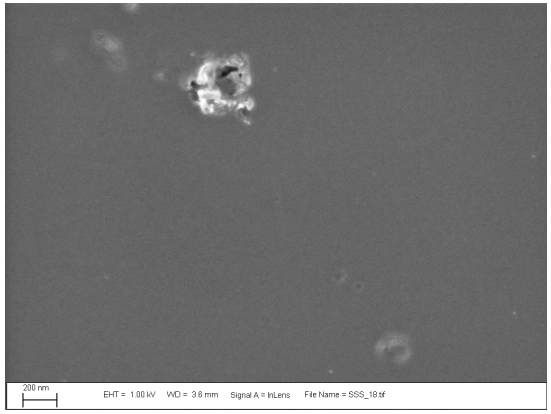
Circular dichroism shows molecular disorder (left).

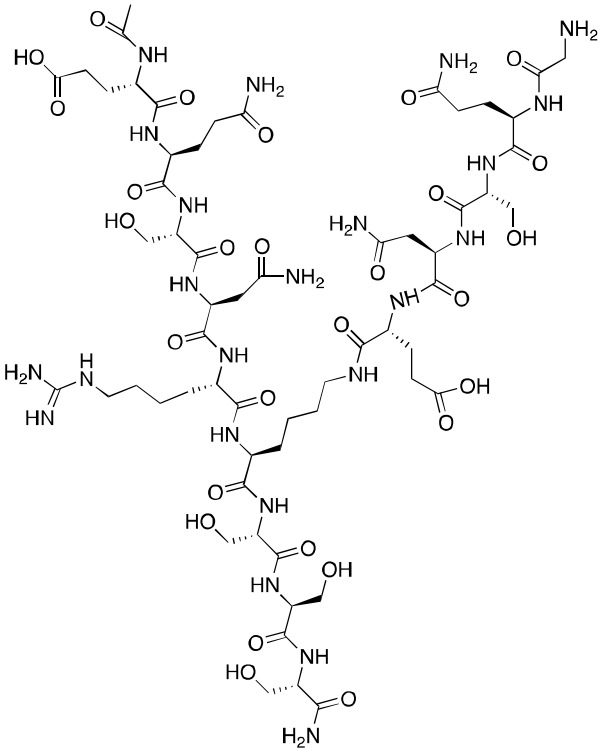
SEM shows no fiber formation (right).

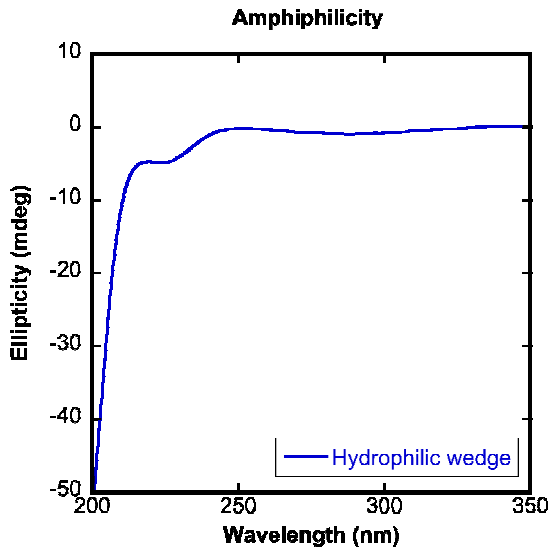


Modifying Amphiphilicity

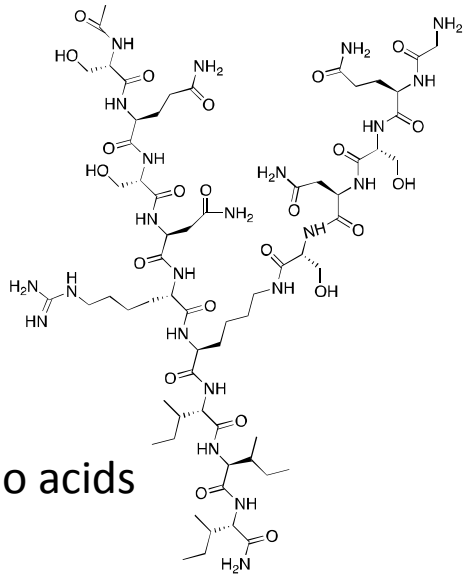
Removing the hydrophobic isoleucine residues from the Wedge also produces supramolecular disorder (by CD, below) and prevents nanofiber formation (SEM, above).



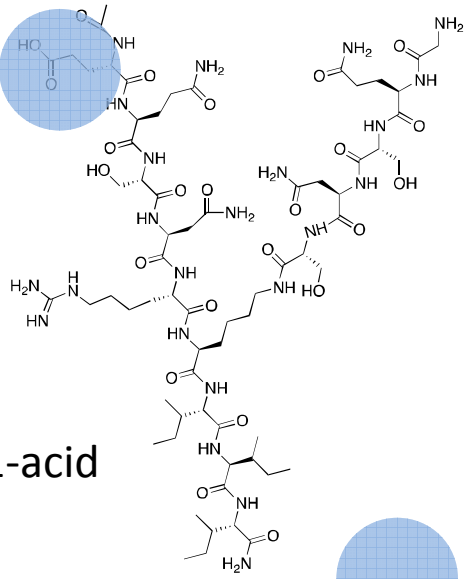




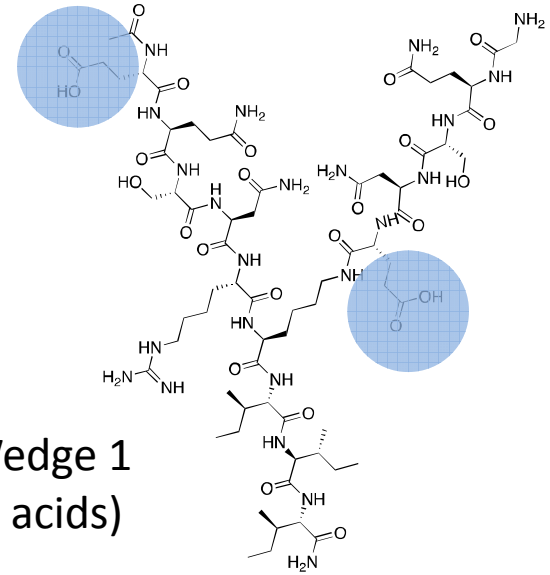
Modifying Wedge Charge



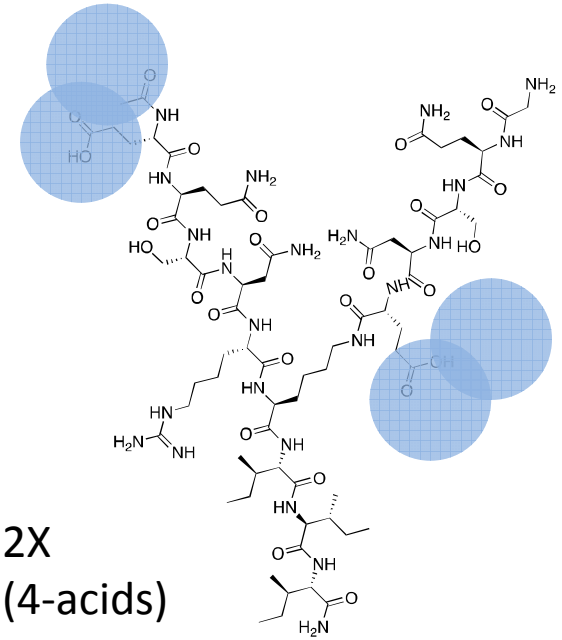
No acids



1-acid

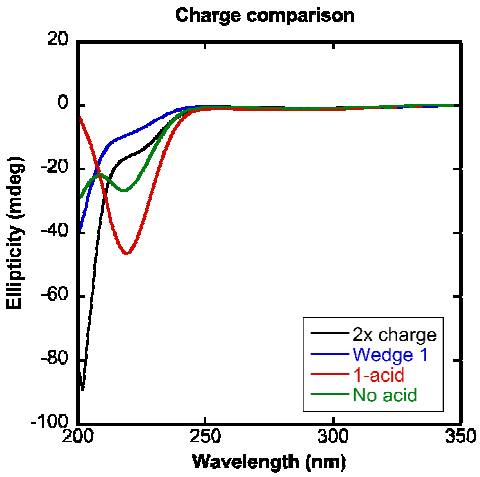


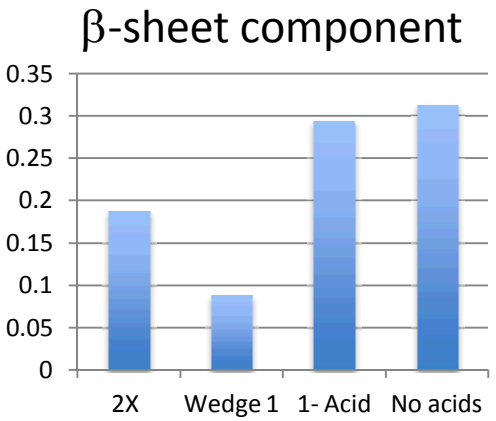
Wedge 1
(2 acids)

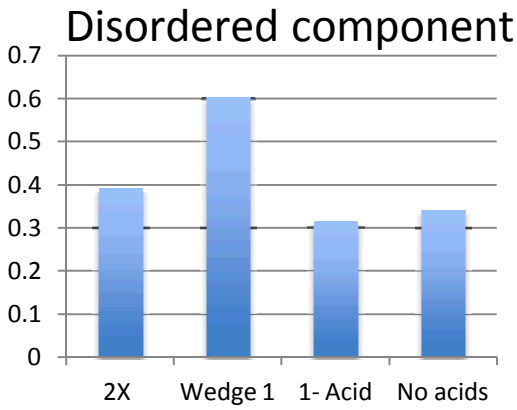


2X
(4-acids)

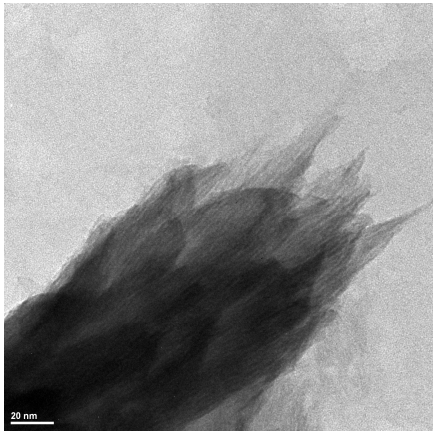
Self-Assembly of Charged Wedges

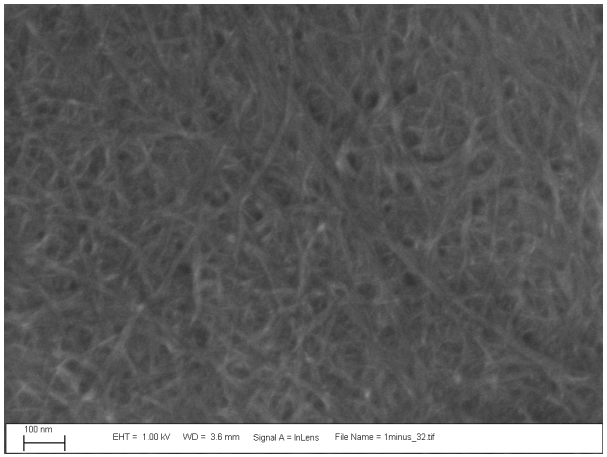






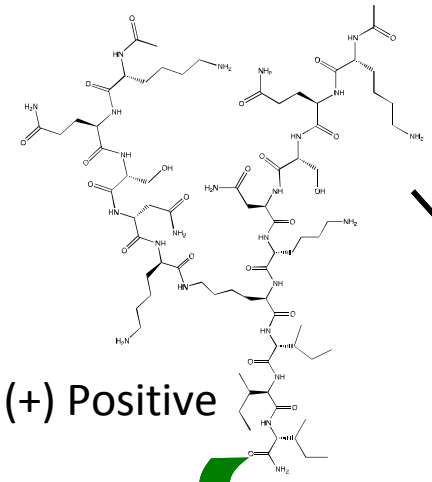
Variation in electrostatic charge has a strong influence on assembly and molecular order.



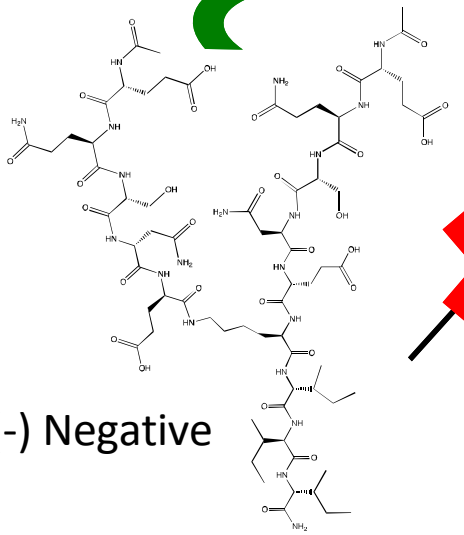


TEM (left) and SEM (right) show strong fiber formation in wedges with strong β-sheet character

Programmable Assembly: Wedge Dimers



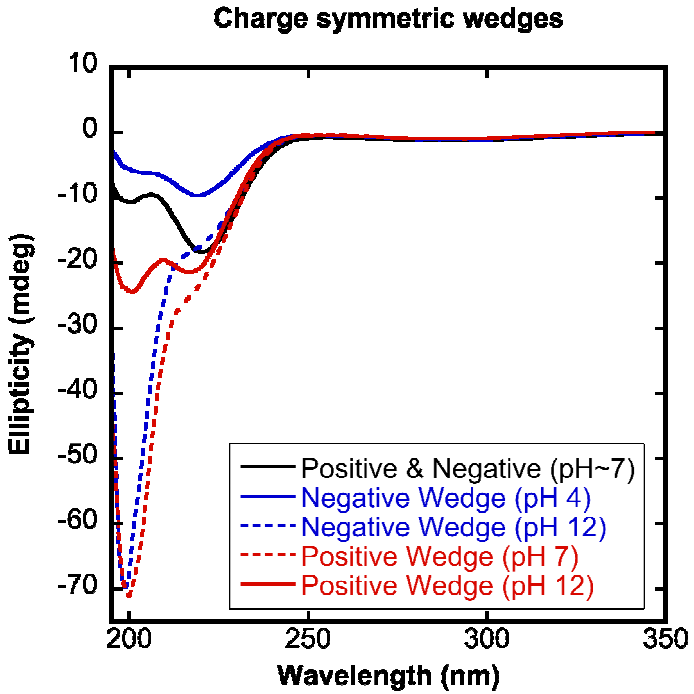
(+) Positive

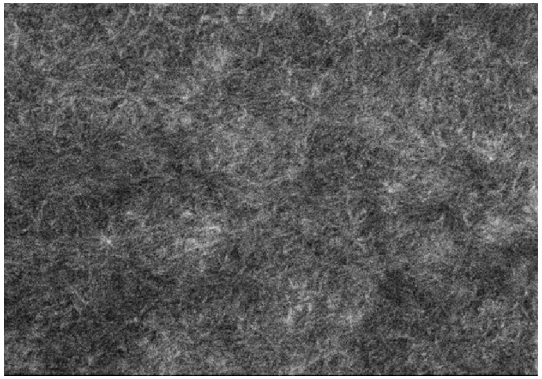


(-) Negative

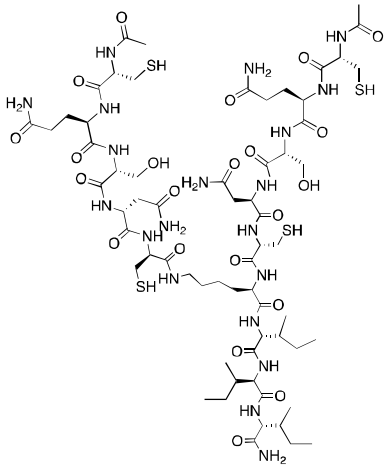
Charge-symmetric wedges will not assemble independently at neutral pH

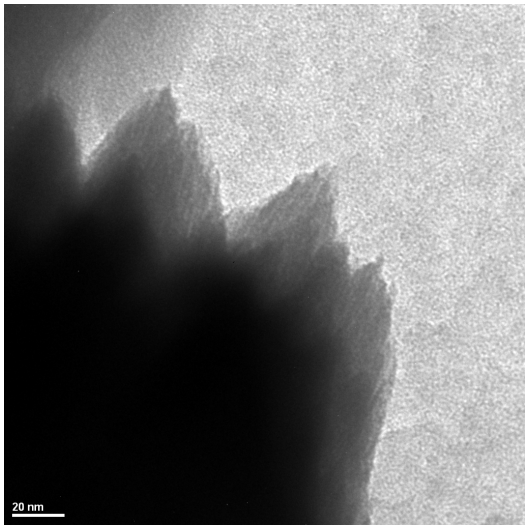
When mixed, the charged wedges cooperatively assemble to form nanofibers with strong β-sheet character.



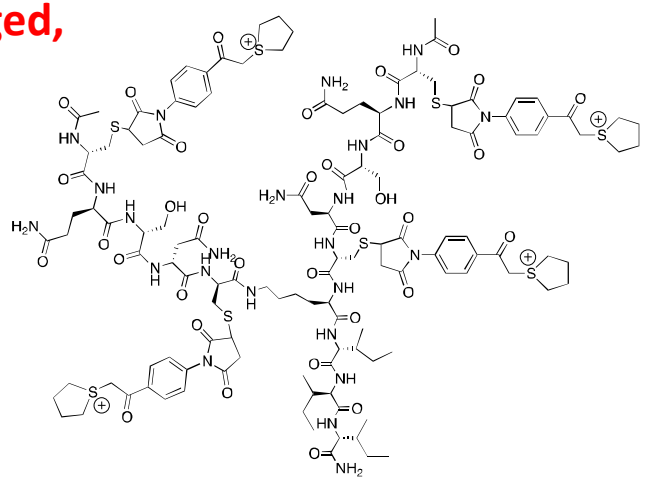


Photoswitchable Assembly

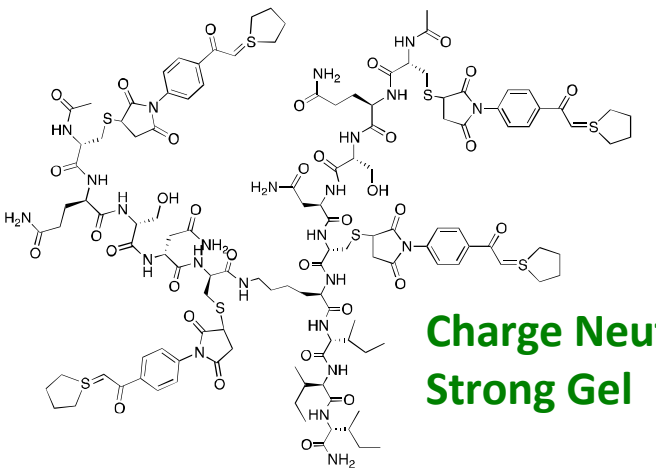




+ Charged, No Gel



UV



Charge Neutral Strong Gel

Future Directions:

Dendronized polymers:

Increase linear character of polymers

Adapt for aqueous assembly

Molecular modeling:

Explore form and function of Wedge peptides

Peptide Assembly:

Extended characterization of Wedge Assemblies (e.g. NMR, Cryo TEM)

Investigate influences of shape, size, and molecular rigidity in the Wedges.

Thank you

Basic Energy Sciences

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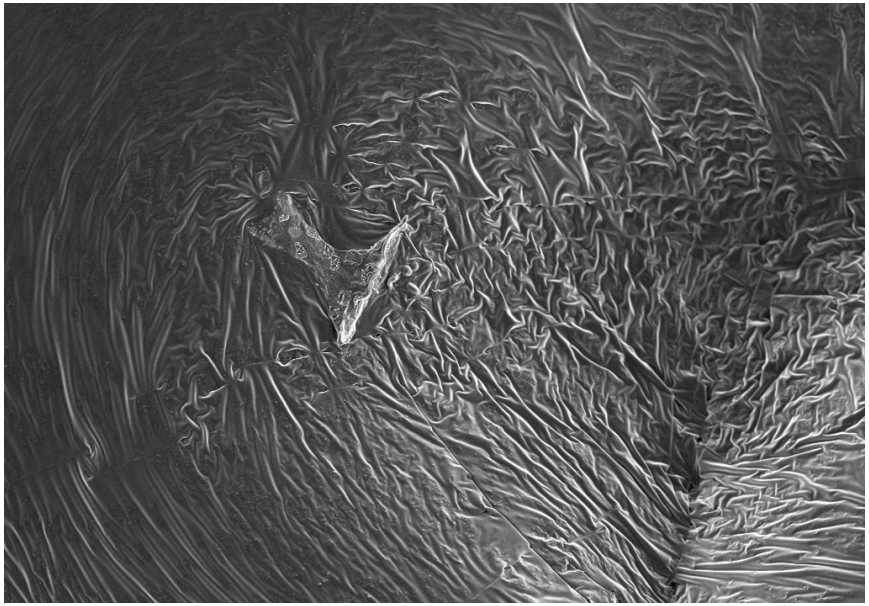

A “summer dress” made from wedge-nanofiber fabric in a micro-whirlpool

Artificial Microtubules
Jim McElhanon
Mark Stevens
Dara Gough
Shengfeng Cheng
Nathan Polaske (UA)
Dominick McGrath (UA)
Jill Wheeler
David Wheeler

Active Assembly
Bruce Bunker
George Bachand*
Darryl Sasaki
Marlene Bachand
Nathan Bouxsein

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* Poster: Dynamic Self-Assembly of Composite Nanomaterials