# Microtubule-Inspired Self-Assembly of Multifunctional Peptides

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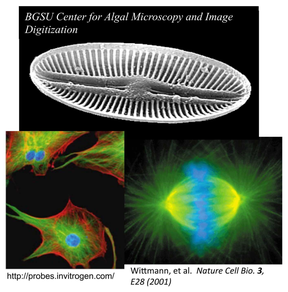
## Background and Motivation

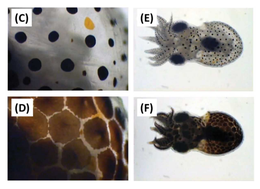



http://probes.invitrogen.com/

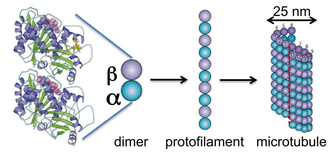
Wittmann, et al. *Nature Cell Bio.* **3**, E28 (2001)

### Bio-inspiration:

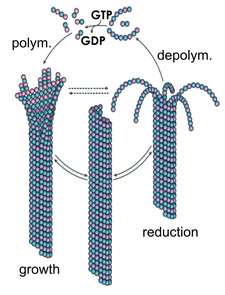
Microtubules are energy consuming protein assemblies that enable the dynamic, adaptable organization of nanomaterials within cells. We aim to extrapolate key aspects of these remarkable protein structures to synthetic self-assembling molecular materials.




E. Kreit, et al. *Journal of the Royal Society, Interface / the Royal Society* (2012)




Akhmanova, A.; Steinmetz, M.O. *Nat. Rev. Mol Cell. Bio.* **2008**, *9*, 309.
Nogales, E. *Annu. Rev. Biochem.* **2000**, *69*, 277.




### Target Microtubule Characteristics:

- Self-assembly from nanoscale building blocks
- Cooperative α−β dimer asymmetry
- Dynamic, programmable assembly
- Cooperative molecular interactions
- Controlled nanostructure morphology
- Motility and transport

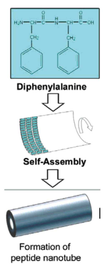
## Peptide Self-Assembly

### Modified Dipeptide Assembly

**Inspiration:** *Di(phenylalanine) dipeptides are known to self-assembly with crystalline order into tubules.*

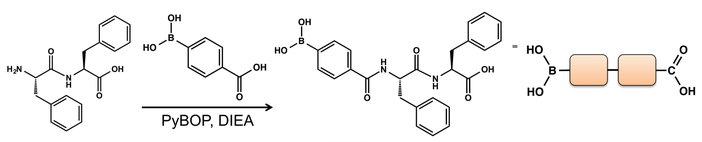
**Scientific Challenge:** *Can we modify this simple dimer building block for programmable self-assembly?*

**Technical Approach:** *Incorporate diol/polyol-reactive boronic acids into di(phenylalanine) chemistry.*



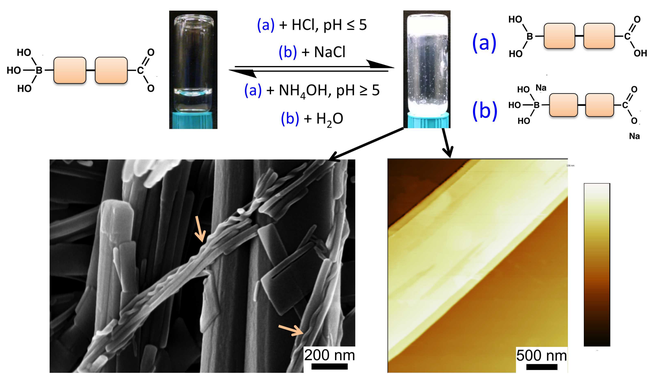

O. Carny, et al. *Nano Lett.* (2006) 5(6) 1594.

**Model boronic acid di(phenylalanine) dipeptide: BFF**



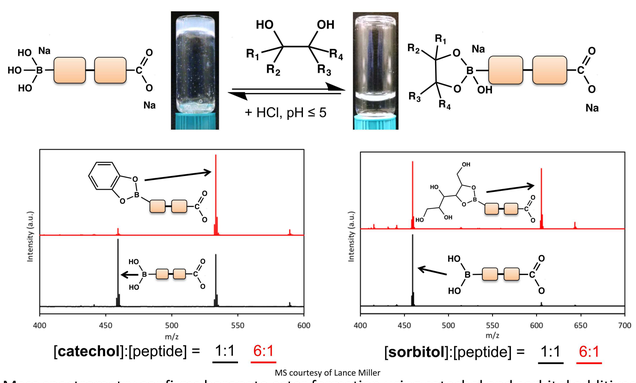

Jones, B.H., et al. *Chem. Comm.*., (2015) *In Revision*.
Jones, B.H. et al. *Tet. Lett.* (2015) In Review.

**BFF shows increased aqueous solubility, and reversibly forms nanoribbon gels through ΔpH or Δionic strength.**




Scanning electron (left) and atomic force (right) microscopies reveal assembled BFF ribbon morphologies.

**In addition, gel-sol transitions are uniquely triggered by addition of saccharides or polyols.**



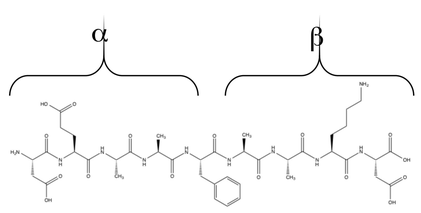

MS courtesy of Lance Miller

Mass spectrometry confirms boronate ester formation using catechol and sorbitol additives.

### MT-Inspired Functional Block Peptides

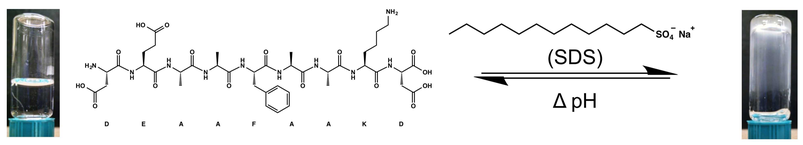
**Inspiration:** *MTs assemble from dimers, through interactions with secondary biomolecules (e.g., GTP).*

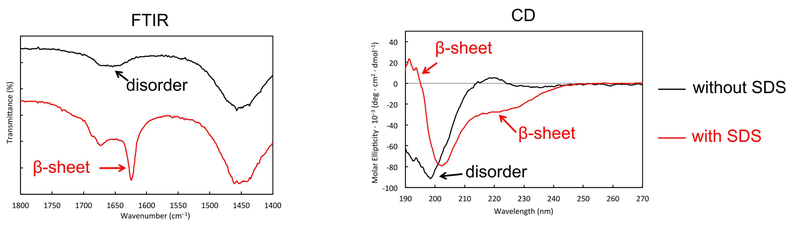
**Scientific Challenge:** *Can we create a peptide analog that follows this assembly motif?*



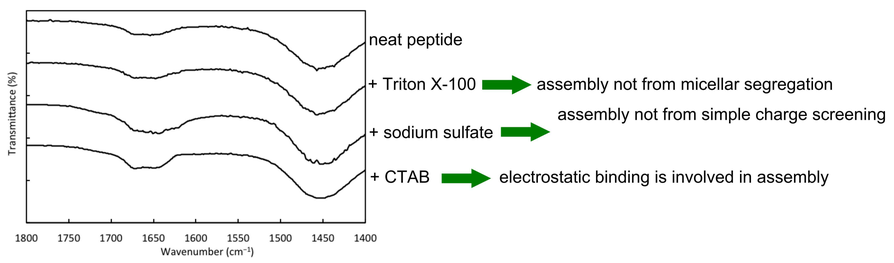

**Technical Approach:** *Create a "bola" peptide with an enzymatically cleavable linkage, that assembles through interactions with secondary molecular interactions.*

**Small amounts of surfactant can induce ordered secondary structure and hydrogelation of otherwise unstructured peptides.**

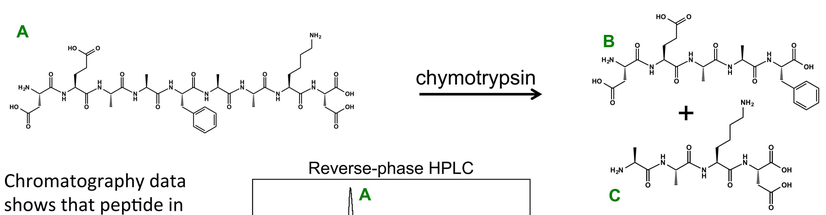


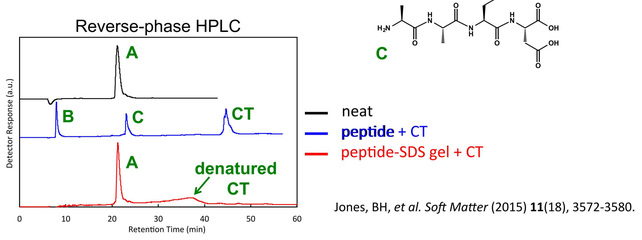

**FTIR and Circular Dichroism (CD) spectroscopically confirm changes in peptide secondary structure resulting from SDS/peptide interactions and confirm combined electrostatic/amphiphilic influence of SDS on peptide assembly.**




**SDS not only induces self-assembly, but also stabilizes the peptide against enzymatic degradation.**




Chromatography data shows that peptide in SDS/peptide gels does not cleave when exposed to chymotrypsin. Moreover, the chymotrypsin is evidently denatured by the SDS.

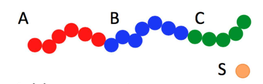



Jones, BH, *et al. Soft Matter* (2015) **11**(18), 3572-3580.

### Simulation-Inspired Triblock Assemblies

**Inspiration:** *Tubulin assembly is regulated through cooperative intra- and intermolecular interactions.*

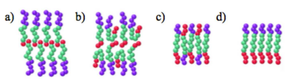
**Scientific Challenge:** *Can theory identify a multicomponent peptide building block capable of controlled assembly? Can we synthesize a simulation-inspired peptide that assembles as predicted?*




Variable parameters:

- Block size (e.g., $N_A$, $N_B$, $N_C$)
- Relative block size (e.g., $f_A = N_A/(N_A+N_C)$)
- Block parameters ($\chi_{AB}$, $\chi_{AC}$, $\chi_{BC}$, $\chi_{AS}$, $\chi_{BS}$, $\chi_{CS}$)

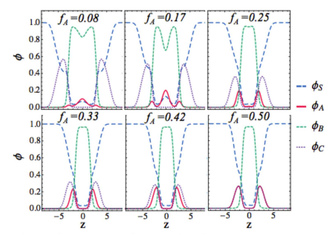
**Technical Approach:** *Combine self-consistent field theory (SCFT) and peptide chemistry to create a novel self-assembling triblock system.*



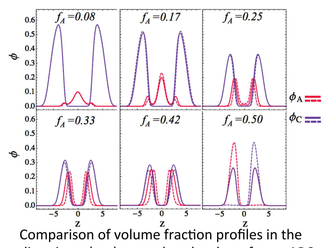

Schematic of SCFT sheet structures: a) bilayer; b) doubly mixed bilayer; c) mixed monolayer; and d) segregated monolayer

**Volume fraction profiles identify ABC molecular arrangements and block associations in an assembled sheet morphology.**

Varying interaction asymmetry between A and C blocks affects molecular arrangement in sheets.

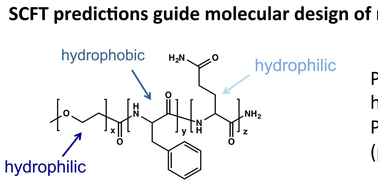



Volume fraction profiles in the direction z (nm) normal to the sheet for an ABC triblock with $\chi_{AC} = 0$.




Comparison of volume fraction profiles in the direction z (nm) normal to the sheet for an ABC triblock with $\chi_{AC} = 0$ (solid) and $\chi_{AC} = 3.5$ (dashed).

C.L. Ting, et al. *Soft Matter* (2016) DOI: 10.1039/C5SM01447F




Determined volume fraction profiles can be used to assemble a predictive phase diagram of sheet morphology as a function of interaction asymmetry $\chi_{AC}$ and molecular asymmetry $f_A$.

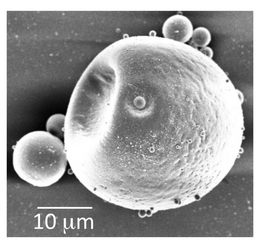
Asymmetric monolayers are likely most desirable for vesicle/tubule formation.

**SCFT predictions guide molecular design of new triblock peptide.**



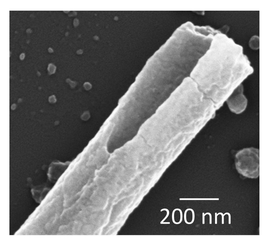

Peptide design incorporates dissimilar hydrophilic blocks (polyglutamine and PEG) , flanking a hydrophobic core (polyphenylalanine).

Triblock molecules were synthesized with block volume fractions approximating ratios predicted by SCFT to form asymmetric monolayers. Dissolved in polar organic solvents and precipitated/assembled in water, the simulation-inspired ABC triblock molecule forms spherical vesicles (below, left), consistent with expectation based on SCFT predictions.




The versatility of peptide chemistry allows systematic variation of triblock composition and interaction parameters.

Initial variations in peptide composition, such as increasing the size of the glutamine block, result in a shift in assembly that produces tubular structures (right).




## Future Plans

***Looking forward, we plan to emphasize how developing alternative, bio-inspired synthetic systems can be used to manipulate the organization, transport, and function of biomolecular and synthetic nanomaterials.***

Controlled shape:


Alternative triggers for programmable assembly:

- Light
- Heat
- Secondary (Bio)molecules

Secondary interactions with assembled peptides to drive organization into larger, multi-dimensional structures and to facilitate unique supramolecular functions:

- Environmentally responsive, adaptive materials
- Non-equilibrium behaviors (e.g., alternative motility, triggered materials chemistry)


Mimic 3D MT assembly

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