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Experimental Characterization of Computationally Designed Self-assembling Peptide Bundles

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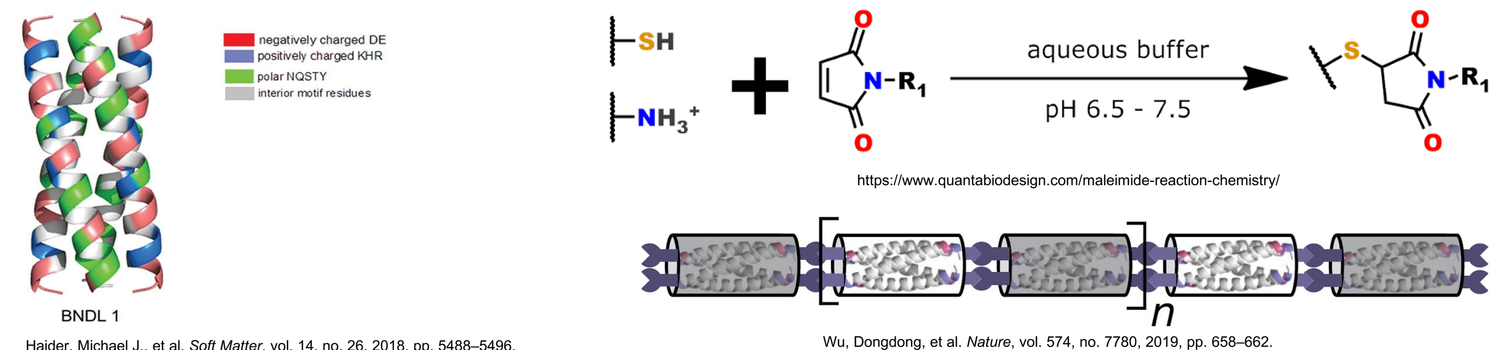
Introduction

- The 29-amino-acid-long peptide sequence computationally designed in the Saven Lab, named BNDL1, forms homotetrameric, antiparallel, α-helical bundle monomers
- These bundles can be linked into rigid rods, utilizing the thiol-maleimide reaction to efficiently link bundles into long polymers of bundles



Haider, Michael J., et al. *Soft Matter*, vol. 14, no. 26, 2018, pp. 5488–5496.

Wu, Dongdong, et al. *Nature*, vol. 574, no. 7780, 2019, pp. 658–662.

- This project aims to use variants ("mutants") of the 29-residue long sequence to generate and probe hypotheses regarding structure formation
- Variants ("mutants") of the BNDL1 sequence have been identified: I4A, I4V, E28M.
- Molecular simulations suggest that the E28M mutation is expected to stabilize the structure by protecting the bundles from water solvation.
- Mutating the isoleucine to a smaller hydrophobic amino acid (I4A, I4V) is conjectured to destabilize structure because the cavity between monomers in the bundle is wider in the mutant, allowing for more water molecules to enter this space
- We hypothesize that, in their cross-linked polymeric form, the designed variants will exhibit distinct structure and assembly properties
- I conducted experimental characterization of the I4A variant with circular dichroism (CD) spectroscopy to study its secondary structure at various temperatures, melting temperature, and reversibility of thermal denaturing
- Rod formation has not been successfully executed yet due to complications in the purification of the I4A-maleimide peptide
- As alternatives to the original plan, we reattempted bundle linkage through adding 1,4-bismaleimidobutane (BMB) cross linkers between I4A-Cysteine bundles

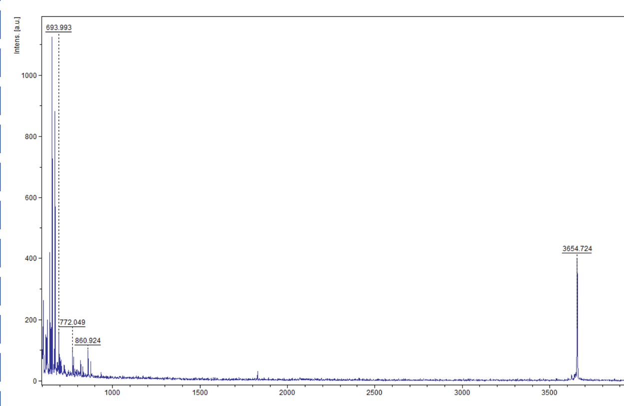
Methods and Materials

- The following two peptides were ordered from Genscript, 25mg each with >95% purity
  I4A-Mal: **Mal**-DEE**A**RRMAEEIRQMAERIQQMAEQIYQEA
  I4A-Cys: **C**DEE**A**RRMAEEIRQMAERIQQMAEQIYQEA
- The I4A-Mal peptide had problems during purification according to Genscript, so we received the crude peptide. Size exclusion chromatography was performed on the crude I4A-Mal using the HiLoad 16/60 Superdex 75 pg column.
- CHCA matrix was used with the peptide program on MALDI
- All circular dichroism spectroscopy measurements were taken on an Aviv spectrometer. Sample solutions were prepared at 0.1 mM concentrations in a quartz cuvette with 1 mm path length. 100mM phosphate, 100mM NaCl, pH 7.0 buffer was used to dissolve the peptide. For wavelength scans, the ellipticity were recorded from 190-260nm with a wavelength step of 1nm, averaging time of 15 seconds, and 3 scans to average. For T-melt scans, the ellipticity at 222 nm was measured as a function of temperature, with a bandwidth of 1nm, temperature step of 2C, and reverse temperature wait time of 60 minutes. All spectra were adjusted based on peptide concentration and buffer background values. All data was processed and reported in terms of mean residue ellipticity.

Results and Discussion

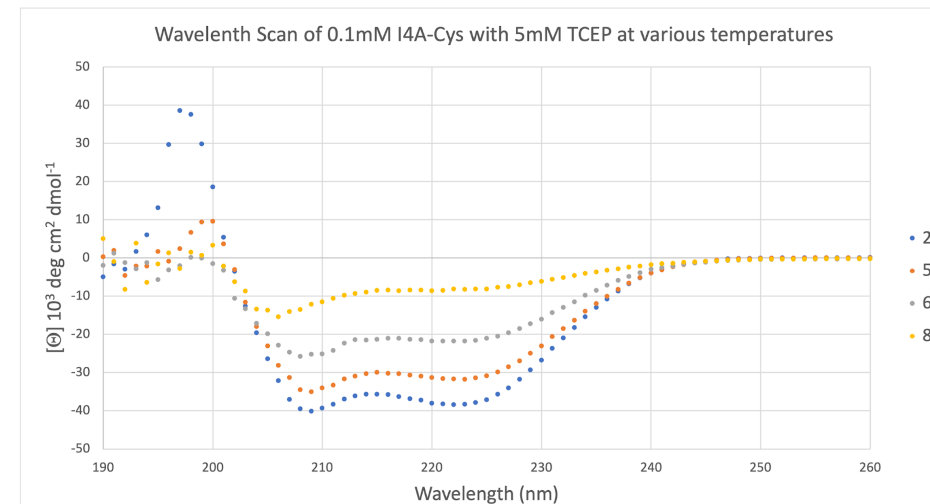
I4A-Cys

MALDI-TOF MS



- Theoretical mass: 3656.09
- Experimental mass: 3654.724

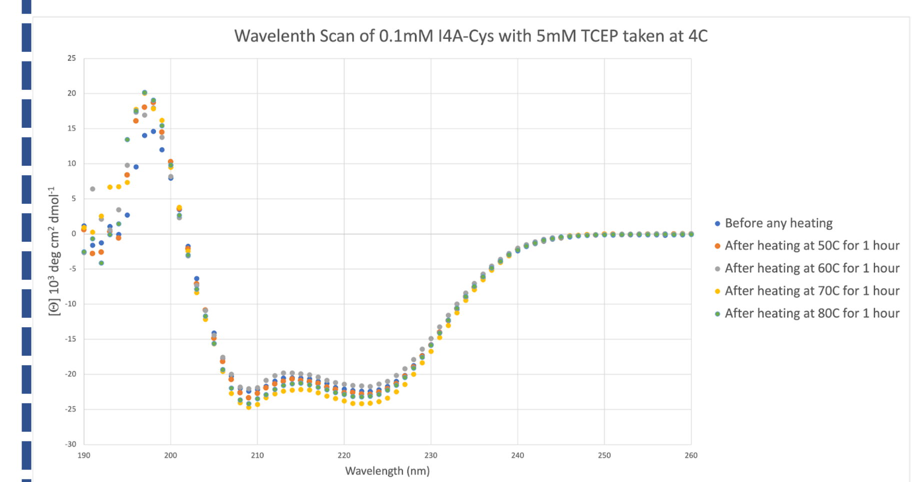
Circular Dichroism - Comparison of secondary structures and monitoring unfolding as a function of temperature



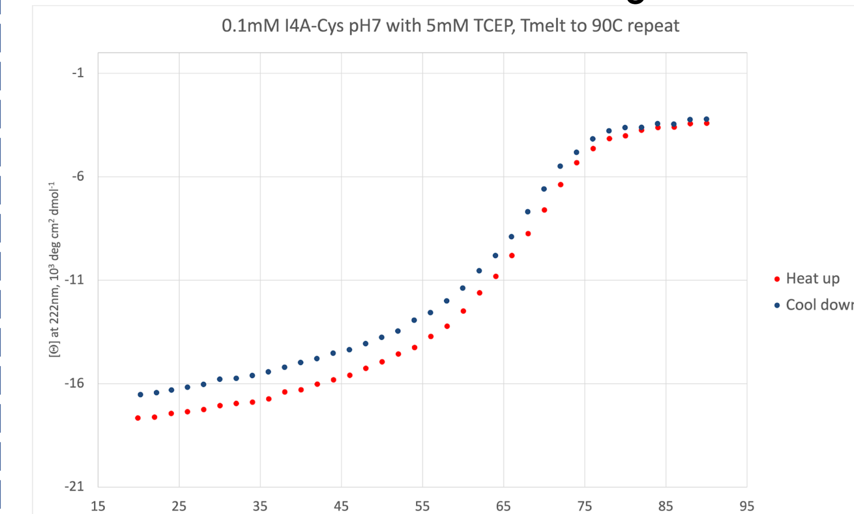
- Alpha-helical secondary structure
  - Maximum at ~193nm
  - Minima at ~222nm and ~208nm
- Coiled-coil structure

Temperature (C)	[Θ]222/[Θ]208
20	0.973
50	0.917
65	0.843

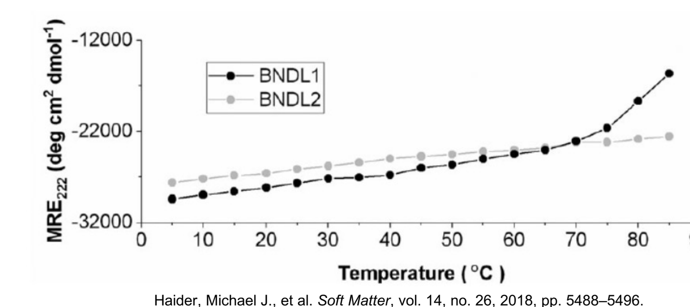
Circular Dichroism - Reversibility of thermal unfolding



Circular Dichroism - Assessing thermal stability with thermal melt scans



- Increase in MRE at around 55C → beginning of unfolding
- plateau around 75C → helices have mostly unfolded



Haider, Michael J., et al. *Soft Matter*, vol. 14, no. 26, 2018, pp. 5488–5496.

- Increase in MRE at around 70C → beginning of unfolding
- No plateau shown
- Higher melting temperature than I4A-Cys

Circular Dichroism – Curve fitting using an oligomeric, two-state model and the resulting melting temperature (T_m)

$$y(T) = \varphi(a_f T + b_f) + (1-\varphi)(a_u T + b_u)$$

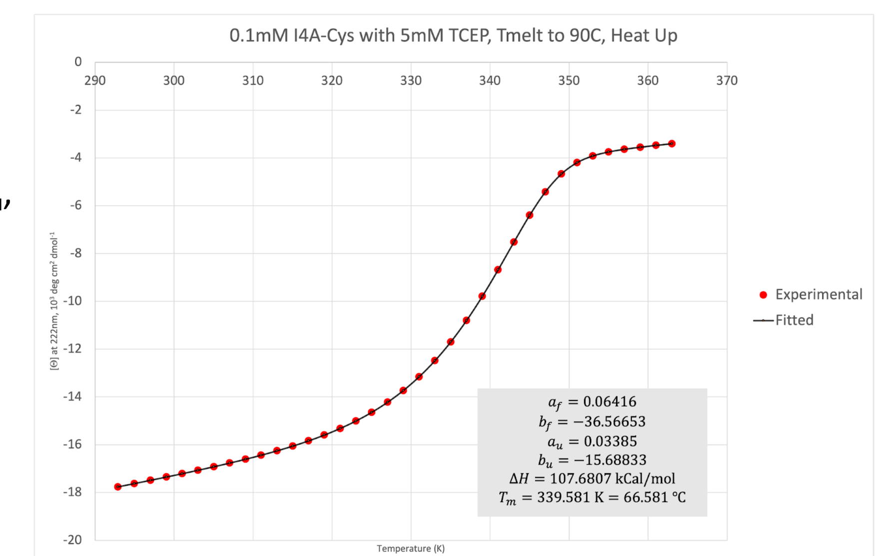
$$\varphi = 1 + \frac{1}{2}\sqrt{u-v} - \frac{1}{2}\sqrt{2\sqrt{u^2+uv+v^2} - (u-v)}$$

$$u = \sqrt[3]{\sqrt{\frac{e^{4x}}{128^2} + \frac{e^{3x}}{6^3}} + \frac{e^{2x}}{128}}$$

$$v = \sqrt[3]{\sqrt{\frac{e^{4x}}{128^2} + \frac{e^{3x}}{6^3}} + \frac{e^{2x}}{128}}$$

$$x = -\frac{\Delta H}{R}\left(\frac{1}{T} - \frac{1}{T_m}\right)$$

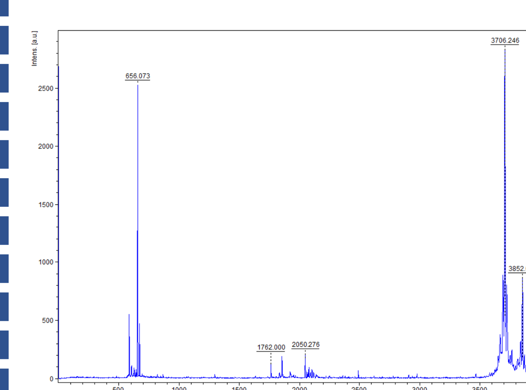
- 6 fitted parameters: a_f, a_u, b_f, b_u, ΔH, T_m
- y(T): molar ellipticity per residue (MRE) at 222nm
- T: temperature
- ϕ: fraction of the folded peptides
- a_f, a_u, b_f, b_u: slopes and intercepts



Böttcher, A.; Kowerko, D.; Sigel, R. K. O. *Biophys. Chem.* 2015, *202*, 32– 39

I4A-Mal

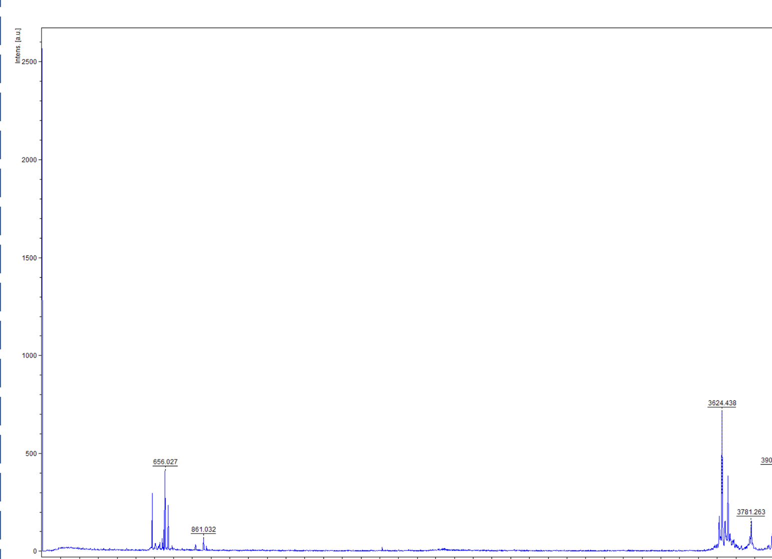
MALDI-TOF MS



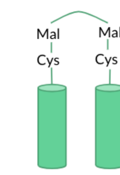
- After size exclusion chromatography
- Theoretical mass: 3704.07
- Experimental mass: 3706.246
- See several impurities
  - 1762.000
  - 2050.276

I4A-Cys reacted with 2eq BMB

MALDI-TOF MS



- Relevant masses
  - BMB: 248.23
  - I4A-Cys: 3656.09



- Peaks
  - 3624.438 (off by ~30 from I4A-Cys' theoretical mass)
  - 3781.263
  - 3906.981
- 3906.981 - 3656.09 = 250.891, which is close to the mass of BMB. Therefore, the 3906.981 peak could be attributed to the structure:
- Assuming the 3781.263 peak is z = 2, m = 7562.526. This is close to the mass of the structure on the left which has a mass of 3656.09*2+248.23 = 7560.41

Conclusion and Future Directions

- Our data shows that the I4A mutant of the BNDL1 peptide forms homotetrameric, antiparallel, α-helical bundles as hypothesized.
- The mutant also exhibits a lower melting temperature of 66C, indicating a lower structural stability compared to BNDL1.
- Future experiments could involve running the MALDI protein program on the I4A-Cys + BMB mixture, which can detect >7.5 kDa. We could also study the I4V and E28M mutants or utilize different click chemistry such as the azide-alkyne cycloaddition, as the maleimide turned out to be problematic during synthesis.

Acknowledgement

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