SAND2022-12783C

Computational design of collagen-like-peptides (CLP) for desired CLP triple helix melting transition and assembled structure

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CINT User Meeting
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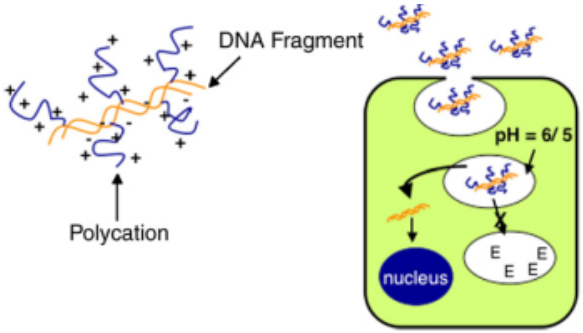
Why do we need thermoresponsive polypeptides?

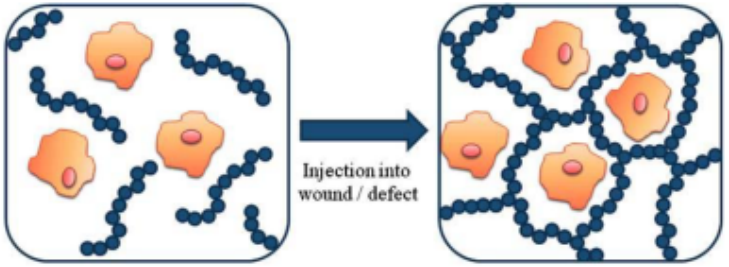
- Polypeptides can respond to stimuli such as pH, **temperature**, ionic strength, light, and/or chemical and biological stimuli.

Drug Delivery Applications



Schmaljohann, D. *Advanced Drug Delivery Reviews* 2006, *58*, 1655-1670.

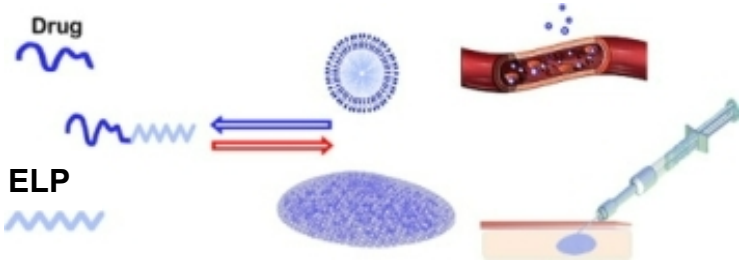
Tissue Engineering Applications



Ward, M.; Georgiou, T. *Polymers* 2011, *3*, 1215-1242.

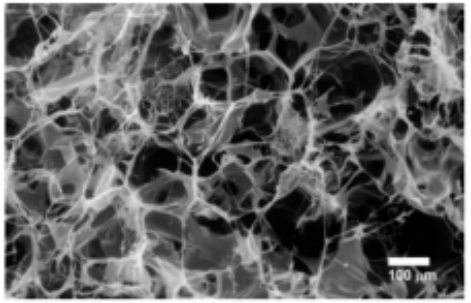
- The ***structure*** and ***thermal stability*** of thermoresponsive biomaterials determine their viability for applications in nanomedicine.

Stable nanocarriers at physiological temperatures



Rodríguez-Cabello, J. et al.. *Advanced Drug Delivery Reviews* 2016, *97*, 85-100.

Stable tissue engineering scaffolds at physiological temperatures



Glowacki, J.; Mizuno, S. *Biopolymers* 2008, *89*, 338-344.

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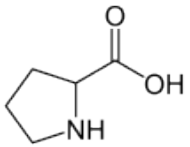
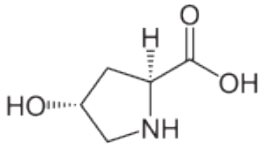
Thermoresponsive biopolymers: Collagen-like Peptides (CLP)

- Collagen-like peptides are biopolymers consisting of repeat units of (**X**-**Y**-**G**) amino acid triplets, where **X** and **Y** are usually proline (P) and hydroxyproline (O), respectively.

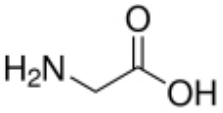
Proline (P)



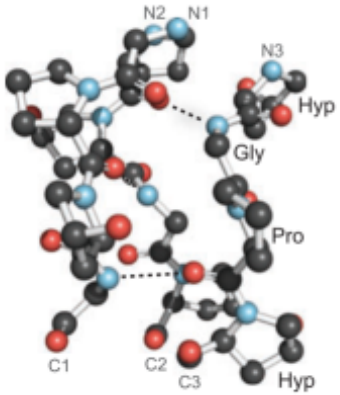
Hydroxyproline (O)

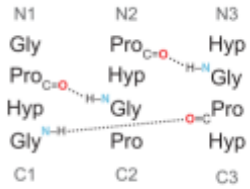


Glycine (G)



Bella, J. Biochemical Journal 2016, 473, 1001-1025.



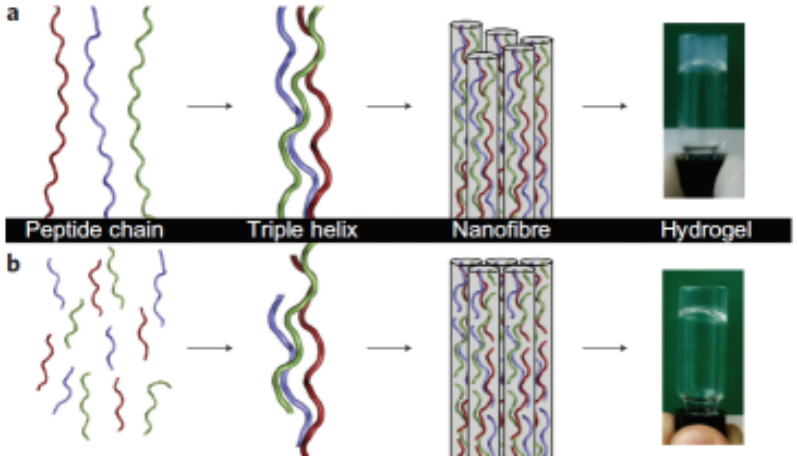


Shoulders, M.; Raines, R. Annual Review of Biochemistry 2009, 78, 929-958.

- CLP triple helix is stabilized **by inter-strand hydrogen bonds** involving the N-H hydrogen of glycine and the C=O oxygen of proline.

- CLP triple helices exhibit larger-scale assembly into fibrils and hydrogels.

O'Leary et al.. Nature Chemistry 2011, 3, 821-828.



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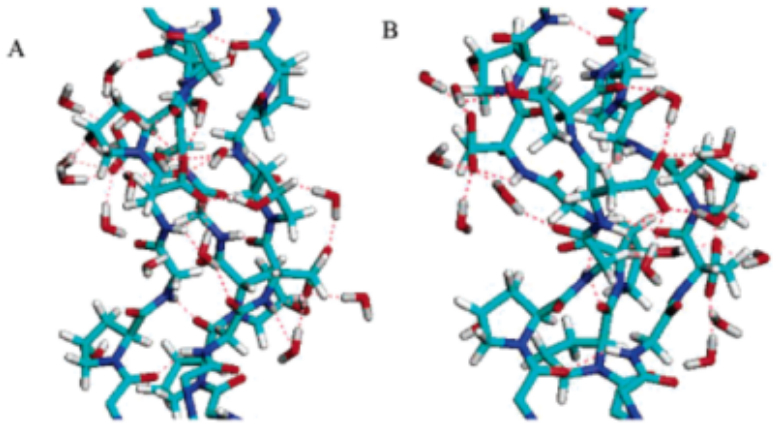
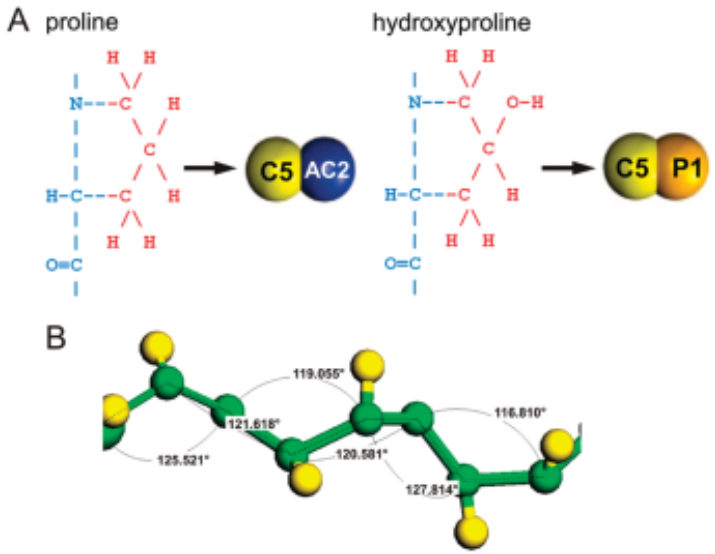
Past simulation models for biomacromolecules

Atomistic (AA) models



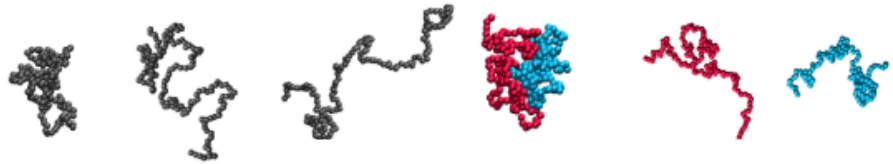
Raman et al. The Journal of Physical Chemistry B, 2006, 110, 20678-20685.

Coarse-grained (CG) models



Gautieri et al., Journal of Chemical Theory and Computation, 2010, 6, 1210-1218.

- AA models capture **hydrogen bonding** and **chain conformations** but are limited to **small length scales (Å-nm)** and **time scales (ns)**
- There is a need for CG models which capture **hierarchical assembly**, **phase transitions**, and **directional interactions**.



Dignon et al. *ACS Central Science* 2019, *5*, 821-830.

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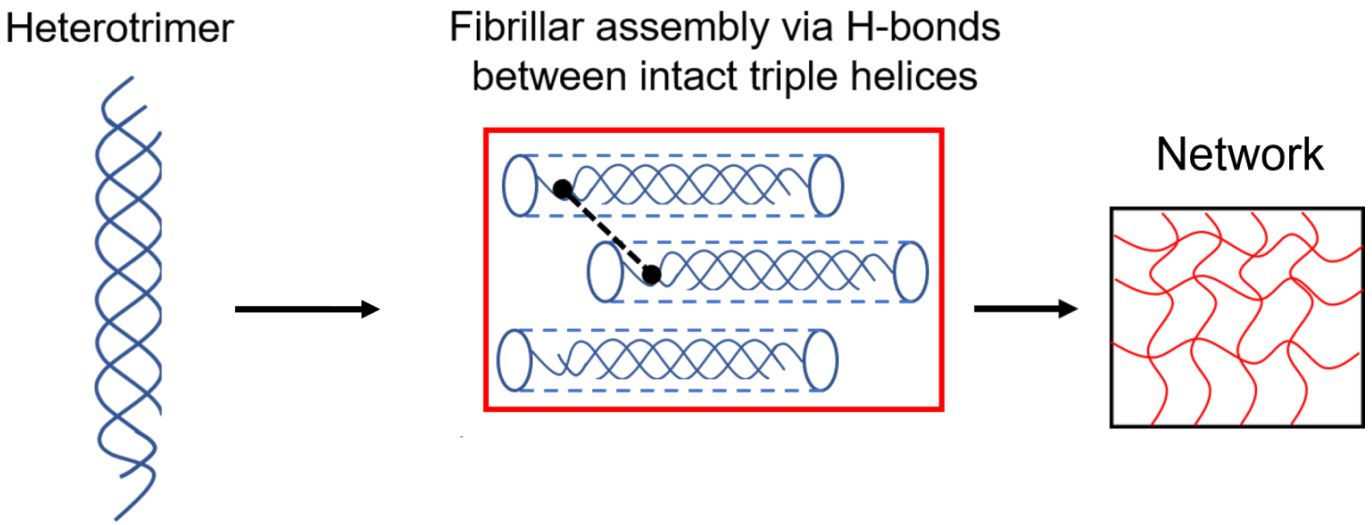
Overview

Goal: Use the recently developed coarse-grained model for CLP with molecular dynamics simulations **to design novel CLP heterotrimers with sticky ends and predict their hierarchical assembly.**



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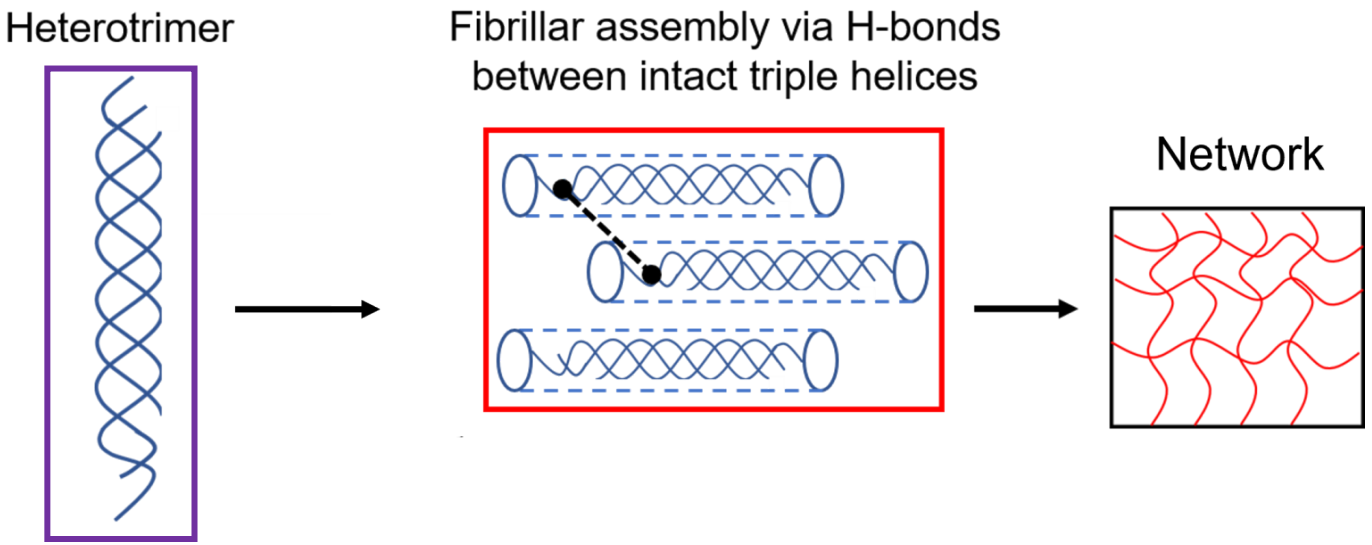

Objectives:

1. **To understand the impact of CLP heterotrimer design (length and number of sticky ends, (POG) repeats) on the melting transitions of CLP heterotrimeric triple helices**

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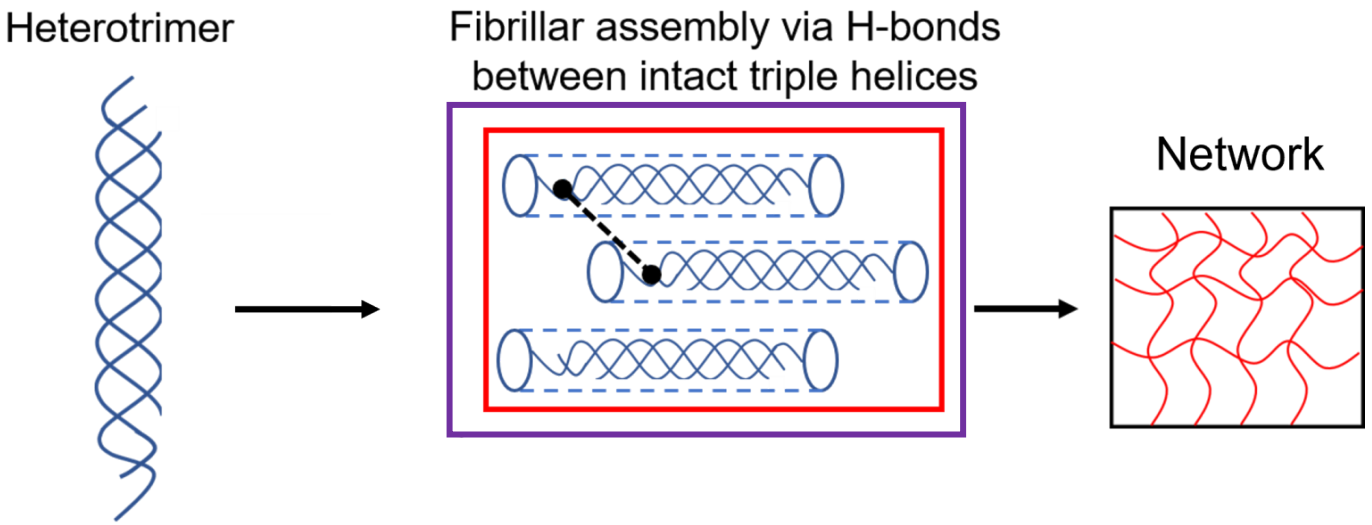
Overview

Goal: Use the recently developed coarse-grained model for CLP with molecular dynamics simulations **to design novel CLP heterotrimers with sticky ends and predict their hierarchical assembly.**



Objectives:

2. **To investigate the higher-order assembly of CLP heterotrimers as a function of CLP design**
   - **Assembly of intact CLP heterotrimers at low temperatures below T_m**

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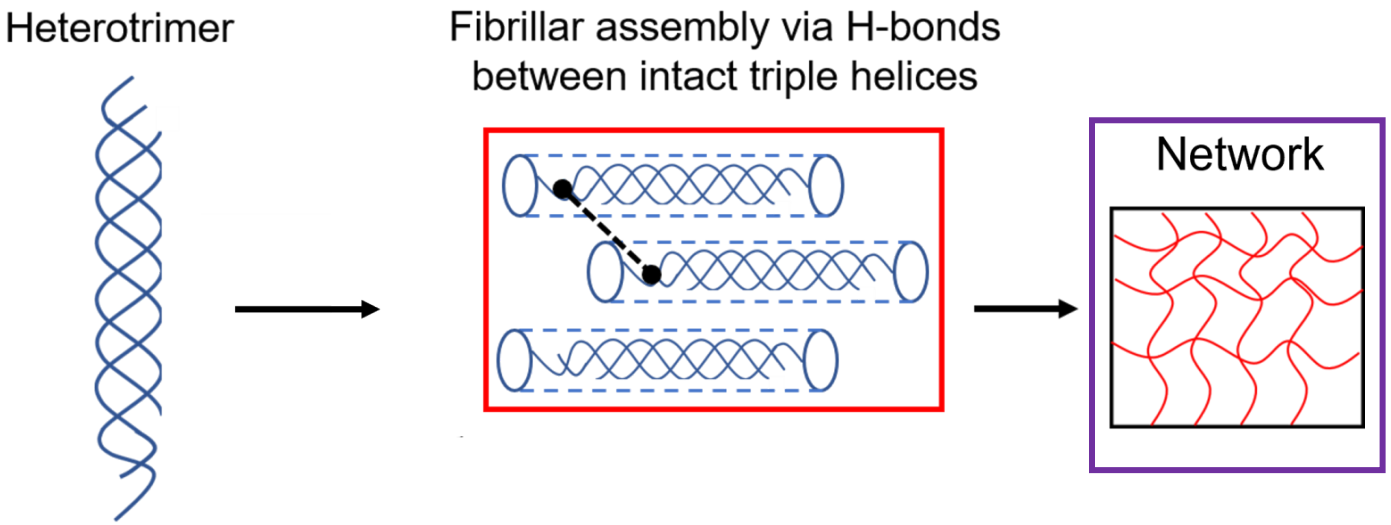
Overview

Goal: Use the recently developed coarse-grained model for CLP with molecular dynamics simulations **to design novel CLP heterotrimers with sticky ends and predict their hierarchical assembly.**



Objectives:

3. **To examine the impact of solution conditions (CLP concentration) on the formation of networks of CLP heterotrimers**

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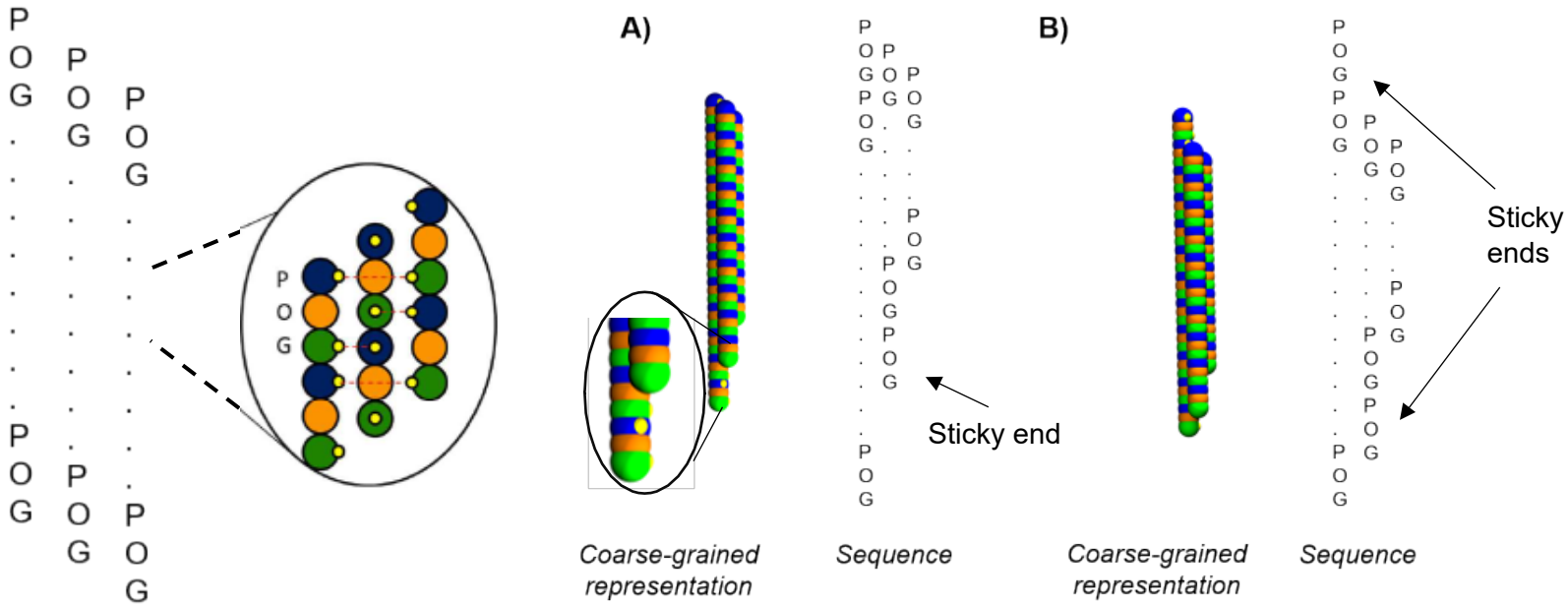
Impact of sequence length, composition, and dispersity on the melting and assembly of Collagen-like Peptides (CLP)

- Can we achieve hierarchical assembly (helix → fiber → network) by using **heterotrimeric** CLP triple helices with sticky ends?
  - **$(POG)_{10}$ family:** $(POG)_{10+\Delta}$-$(POG)_{10}$-$(POG)_{10-\Delta}$ with Δ = 2, 4, or 6
  - **$(POG)_{12}$ family:** $(POG)_{12+\Delta}$-$(POG)_{12}$-$(POG)_{12-\Delta}$ with Δ = 2, 4, or 6

Traditional homotrimeric CLP triple helix

CLP heterotrimers with "sticky ends"



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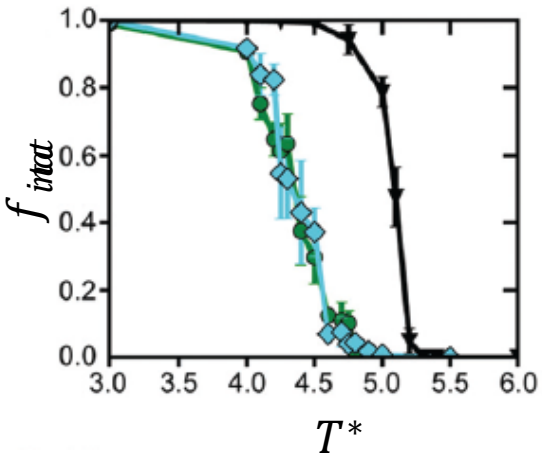
Coarse-grained model validation for CLP homotrimers

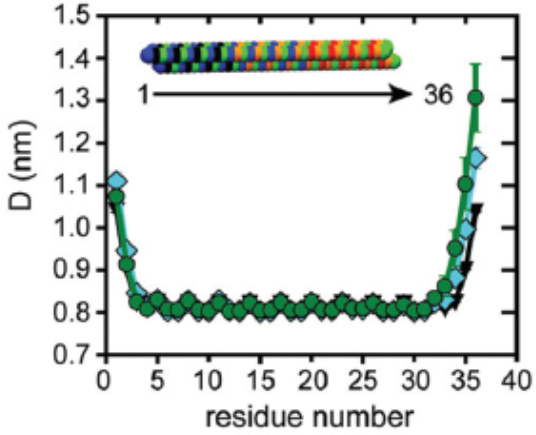
Experimental Melting Temperatures

Sequence		~T_m (°C)
$(POG)_{12}$	▼	65
$(PKG)_4(POG)_4(DOG)_4$	◇	20
$(PKG)_3(PKaG)(POG)_4(DOG)_4$	●	14

Computational Melting Curves



Triple Helical Diameters



- Incorporation of charged amino acids **destabilize the triple helices compared to uncharged, (POG) sequences**
- Reactive handles (allylyoxycarbonyl functionalized K, Ka) **further destabilize the triple helix compared to charged residues.**

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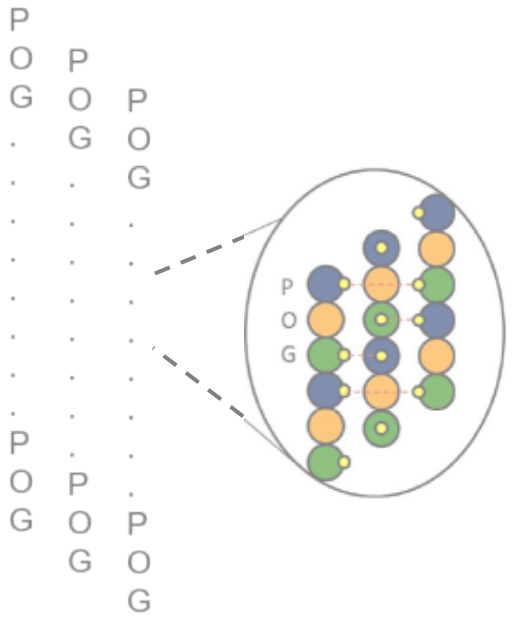
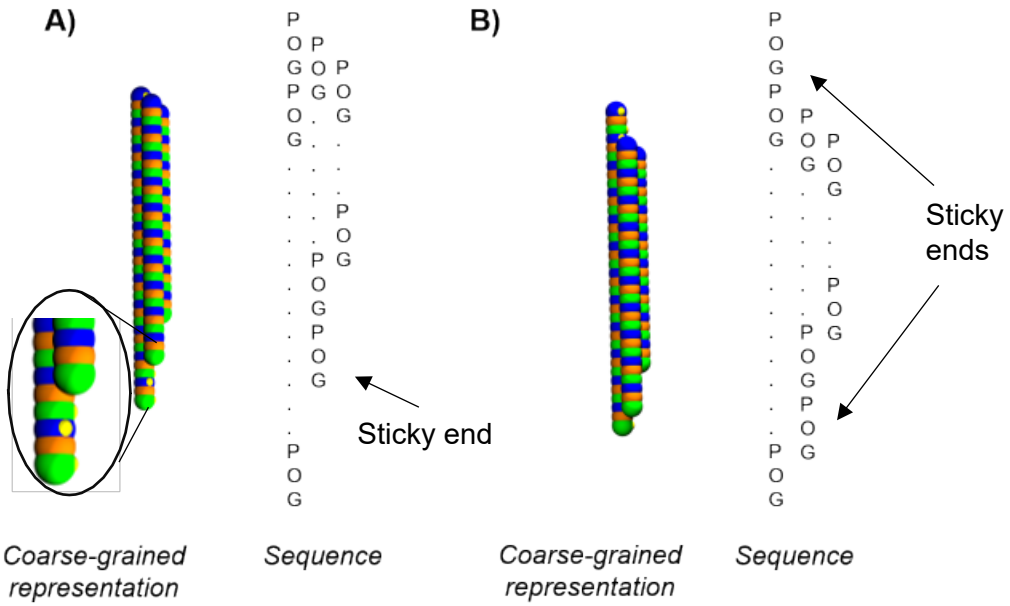
Hilderbrand, A.; **Taylor, P.A.**; Stanzione, F.; LaRue, M.; Guo, C.; Jayaraman, A.; Kloxin, A. *Soft Matter* 2021, *17*, 1985-1998.

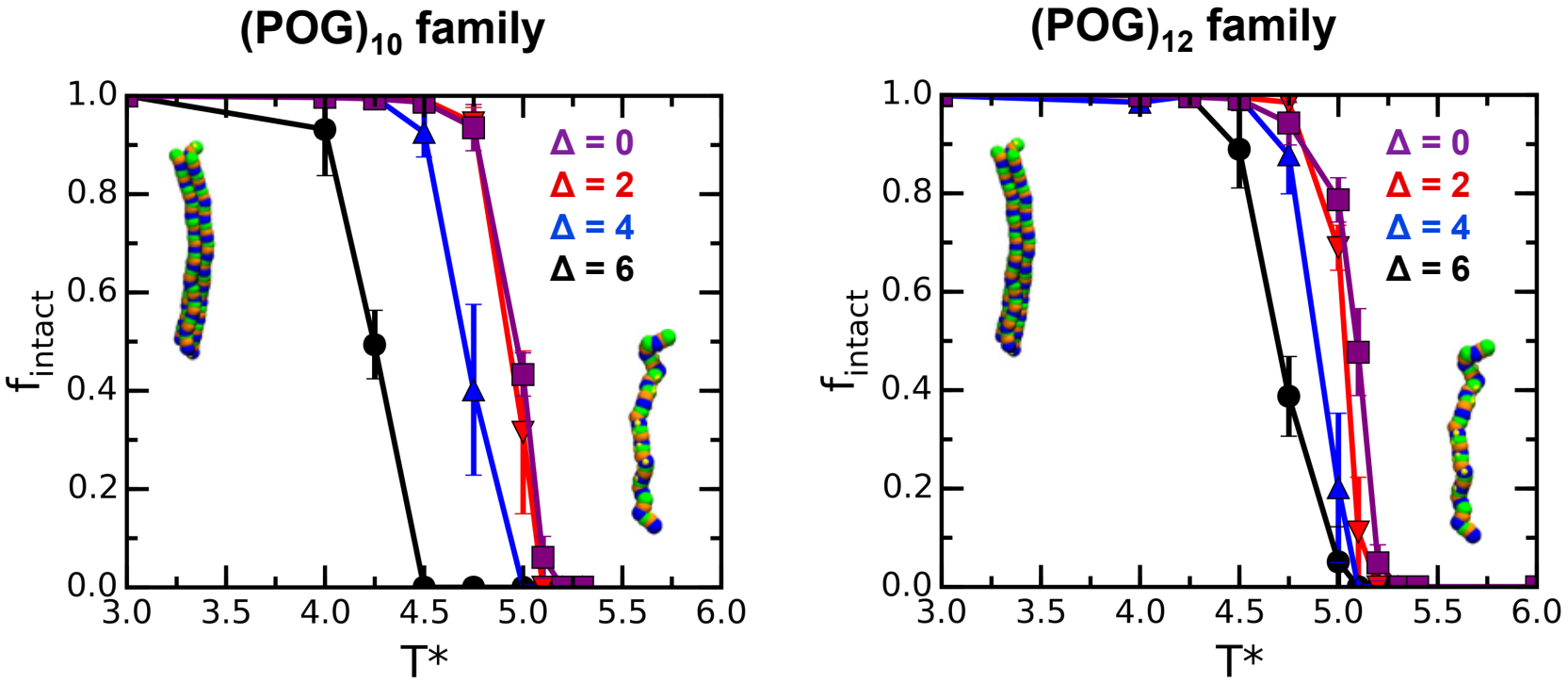
Traditional homotrimeric CLP triple helix



CLP heterotrimers with "sticky ends"



Effect of sticky end length (Δ) on the melting transitions of heterotrimeric CLP triple helices



** Data shown for a CLP concentration of 0.3 mM*

- Increasing sticky end length (Δ) decreases thermal stability (T_m) of the triple helix.
- Larger shift in T_m observed for $(POG)_{10}$ vs $(POG)_{12}$ family with increasing Δ.
- This behavior is found in both "One" and "Two" sticky ended CLP designs.

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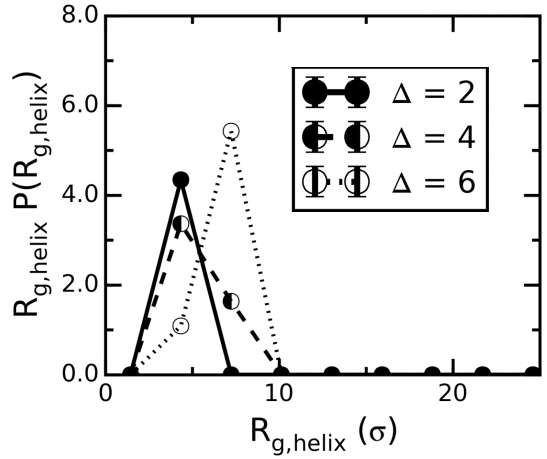
Taylor, P.A.; Kloxin, A.; Jayaraman, A . Soft Matter 2022, 18, 3177-3192.

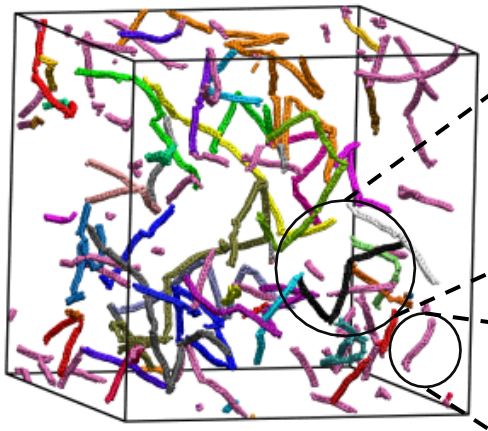
Impact of sticky end length (Δ) on CLP assembly: $(POG)_{12}$ family

CLP concentration: 1 mM

Free

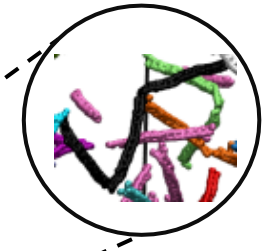
Assembled

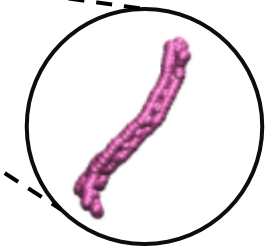


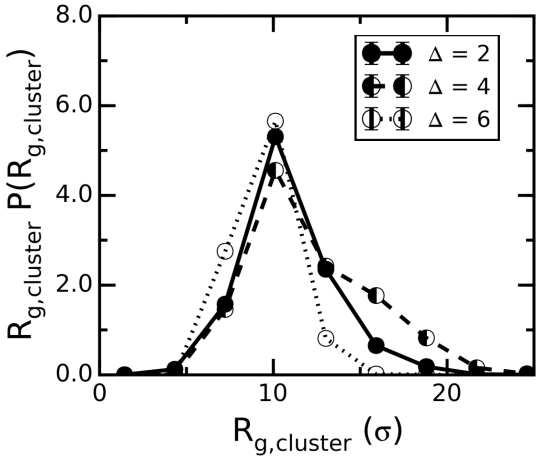


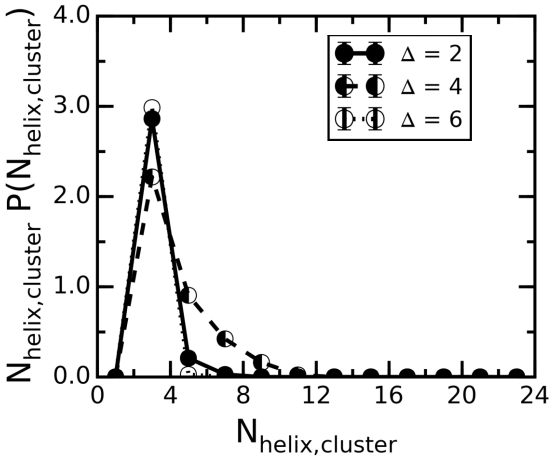
Assembled ($N \geq 2$)

Free ($N = 1$)









Longer sticky ends (Δ) have a **monotonic effect on triple helix sizes** ($R_{g,helix}$) but a **non-monotonic effect on larger CLP clusters** for a one sticky ended design.

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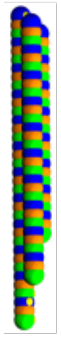
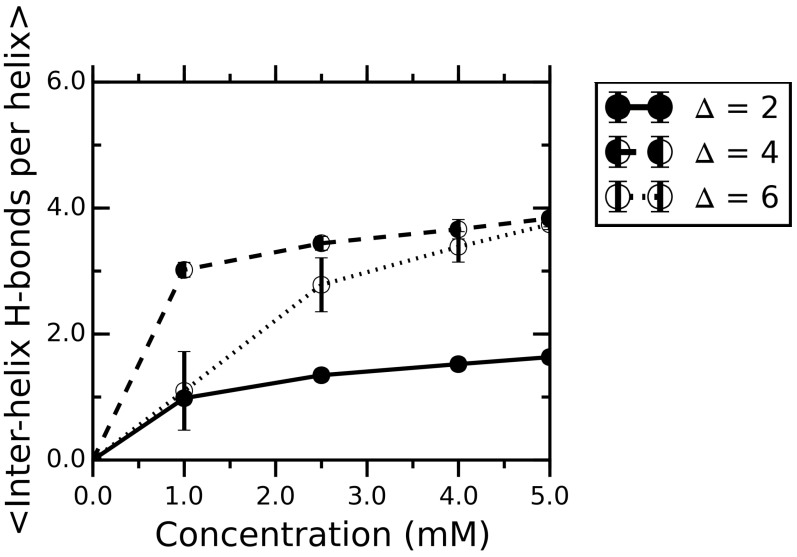
Taylor, P.A.; Kloxin, A.; Jayaraman, A . Soft Matter 2022, 18, 3177-3192.

Effect of CLP design and concentration on inter-helix hydrogen bonding: $(POG)_{12}$ family

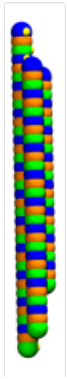


One sticky end

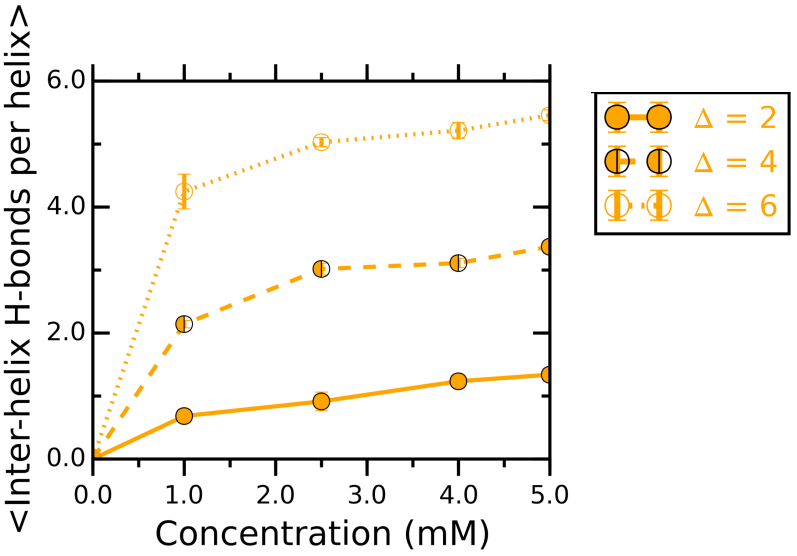


One sticky ended design

- Non-monotonic effect of sticky end length on inter-helix hydrogen bonds.



Two sticky ends



Two sticky ended design

- Increasing sticky end length increases inter-helix hydrogen bonds.

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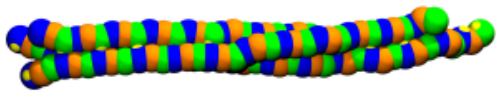
Effect of sticky end length (Δ) on triple helix conformations: $(POG)_{12}$ family at 0 mM

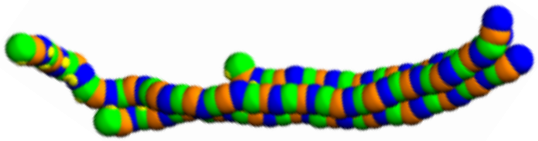
Proline
Hydroxyproline
Glycine

One sticky ended design

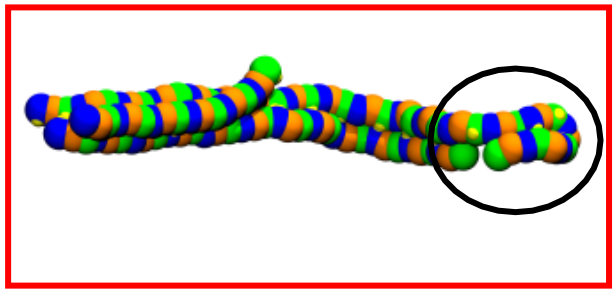
(Δ = 2)



(Δ = 4)

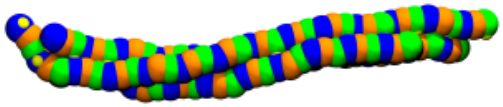


(Δ = 6)

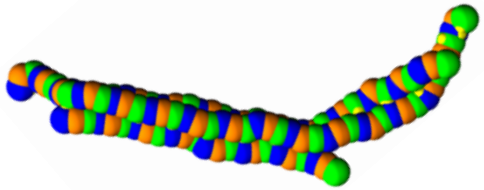


Two sticky ended design

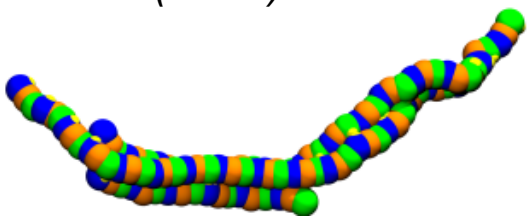
(Δ = 2)



(Δ = 4)



(Δ = 6)



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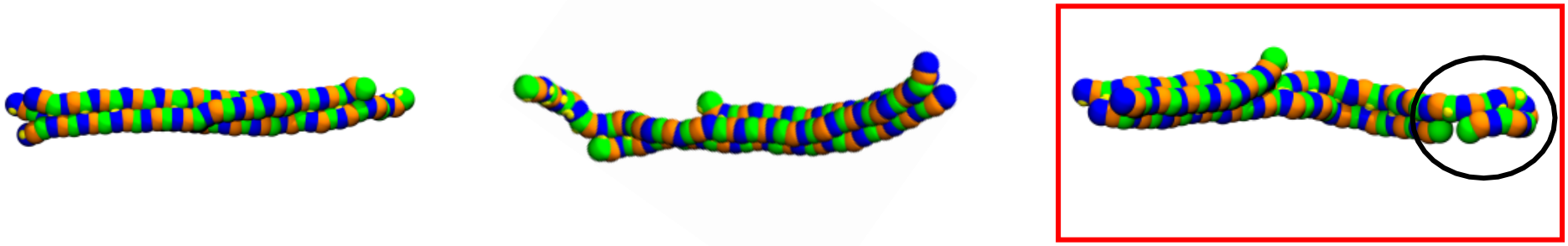
Effect of sticky end length (Δ) on triple helix conformations: $(POG)_{12}$ family at 0 mM

Proline
Hydroxyproline
Glycine

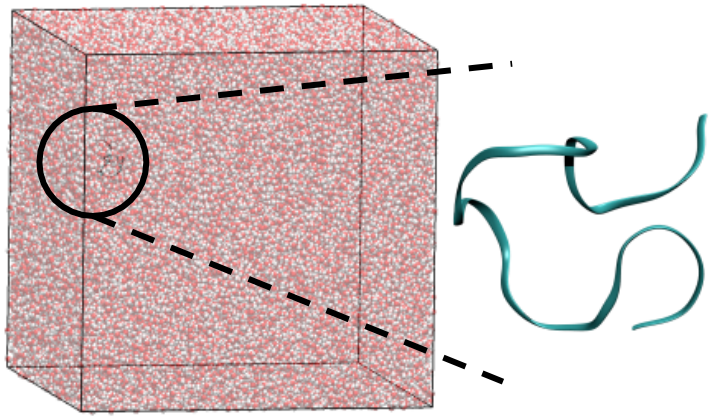
One sticky ended design

(Δ = 2) *(Δ = 4)* *(Δ = 6)*

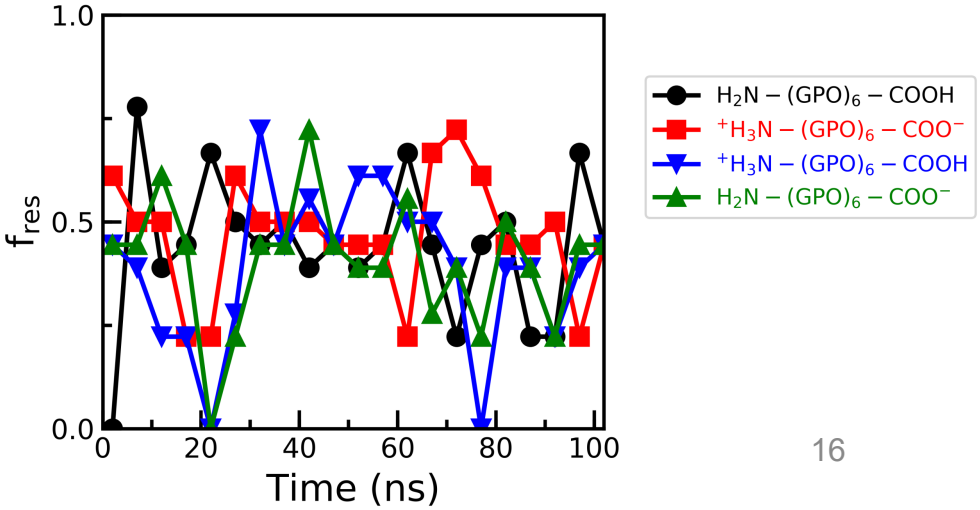


Confirmation of loops/turn structures using atomistic simulations

H_2N-$(GPO)_6$-COOH

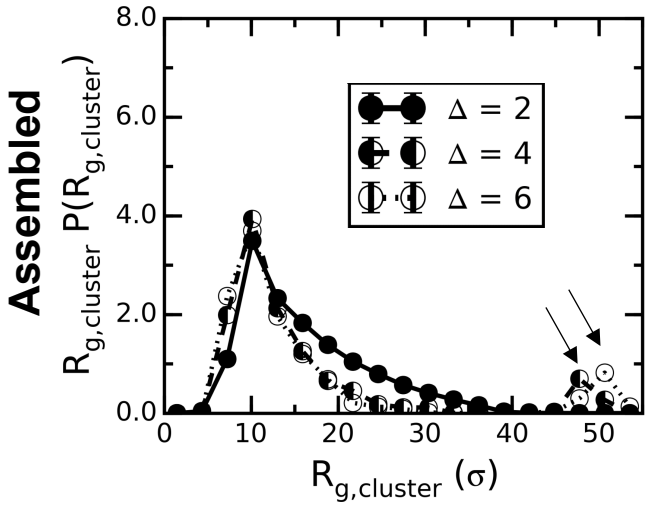


f_{res}: fraction of amino acids participating in turn structures

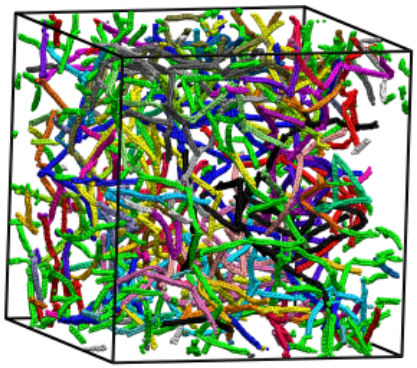


Effect of sticky end length on CLP assembly: $(POG)_{12}$ family

CLP concentration: 20 mM

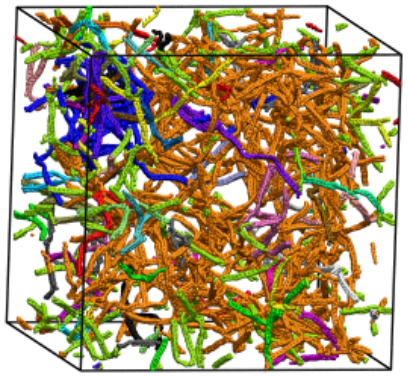


Δ = 2



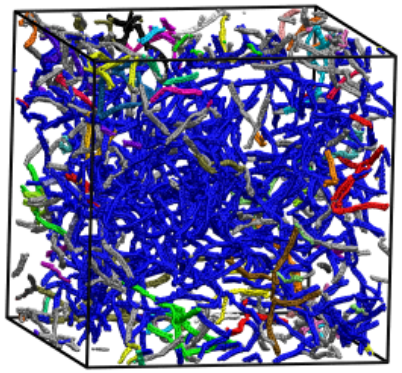
Not percolated

Δ = 4



Percolated

Δ = 6



Percolated

- Percolated networks are observed for long sticky end lengths, Δ.
  - Minimum sticky end length to observe networks: Δ = 4

Taylor, P.A.; Kloxin, A.; Jayaraman, A . Soft Matter 2022, 18, 3177-3192.

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Conclusions

Computational design of self-assembling CLPs with sticky ends into fibrillar structures and supramolecular networks

1. CLP design (**length of sticky ends, Δ, and (POG) family**) can be used to tailor the melting transitions of CLP heterotrimers
   1. Long sticky ends (Δ) result in a lower T_m
   2. Fewer (POG) repeat units lead to a larger reduction in T_m with increasing Δ.

2. Sticky end length (**Δ**) impacts CLP assembly at multiple lengths scales
   - Nonmonotonic effect of Δ on $R_{g,cluster}$ for a **1-sticky ended design** at low CLP concentrations for larger clusters

3. Percolated networks of CLP heterotrimers are observed for **long sticky ends (Δ≥ 4).**

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