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De novo design of collagen-like peptides for cell-based technologies

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Background

Native collagen, a structural protein of 300 kDa, folds into triple helix self-assembling into microns long fibres forming extracellular matrices (ECMs) (Figure 1). This ability to form hierarchical structures from the molecule up has inspired the development of collagen-like peptides (CLPs), typically of 3 kDa, to mimic the native collagen and ECMs. CLPs form collagen like fibres, but also nanoscale spheres, which is particularly the case for sequences with aromatic amino acids at their termini, and ring-like particles. All these assemblies are underpinned by the triple helix formation, which emphasises the potential of CLPs to broaden a range of collagen-like materials and their applications.

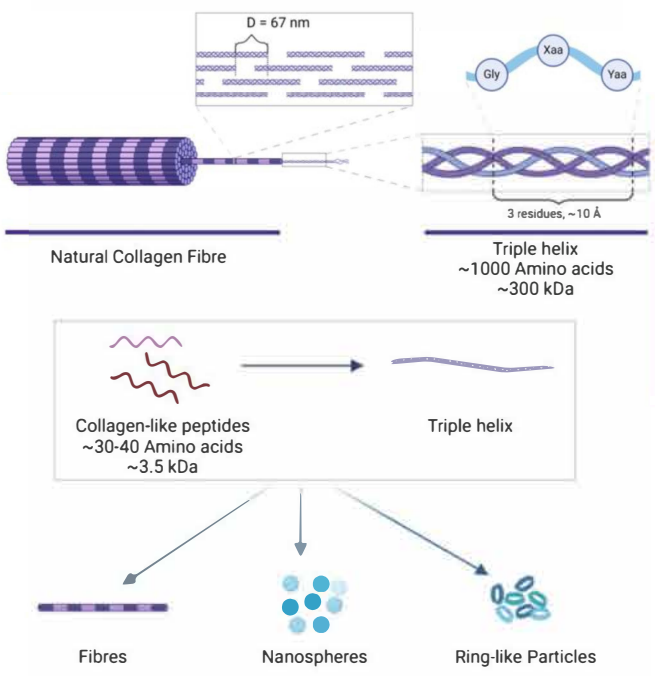


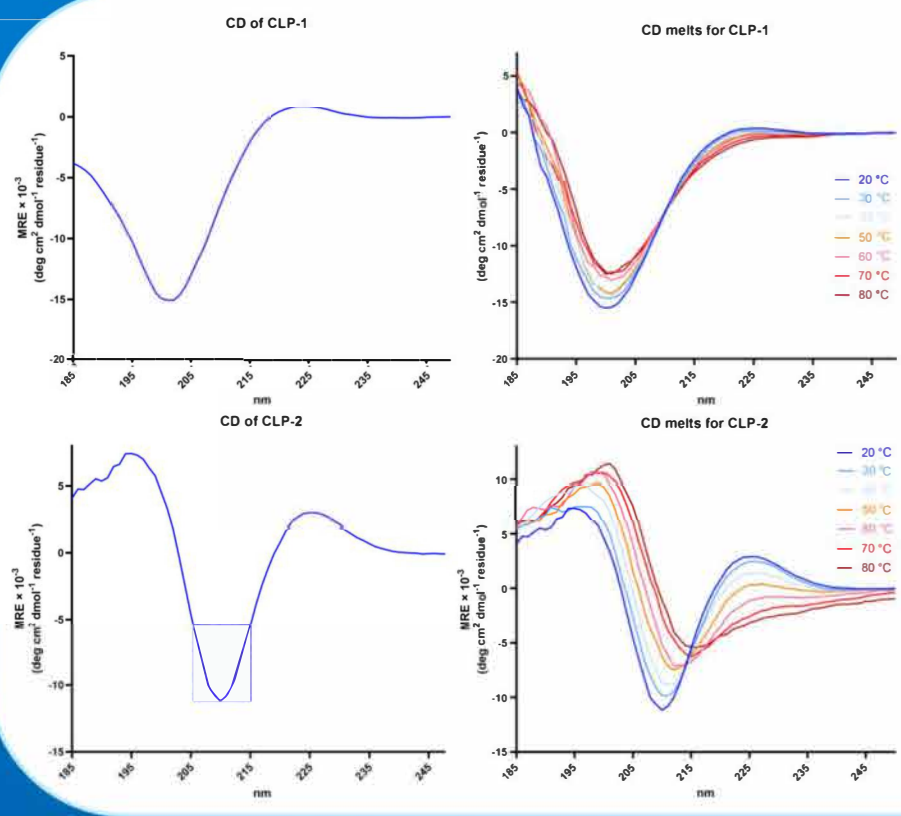
Figure 1: *Representation of collagen's hierarchical architecture and of CLPs' higher order structures. The artwork has been created with www.biorender.com.*

Challenges

Collagen-like peptides tend to assemble into both fibres and particles. Here we show that shorter sequences can form both structures simultaneously. However:

- Can shorter CLPs maintain polyproline II helical structure and fibre formation?
- What is the shortest peptide sequence required to form a triple helix and assemble into higher-order structures?
- Does the density and abundance of fibre formation depend on the sequence length of CLPs?
- Is the fibre formation necessary for gelation?
- What is the length cut-off of CLPs required to form fibres and particles or particles only?

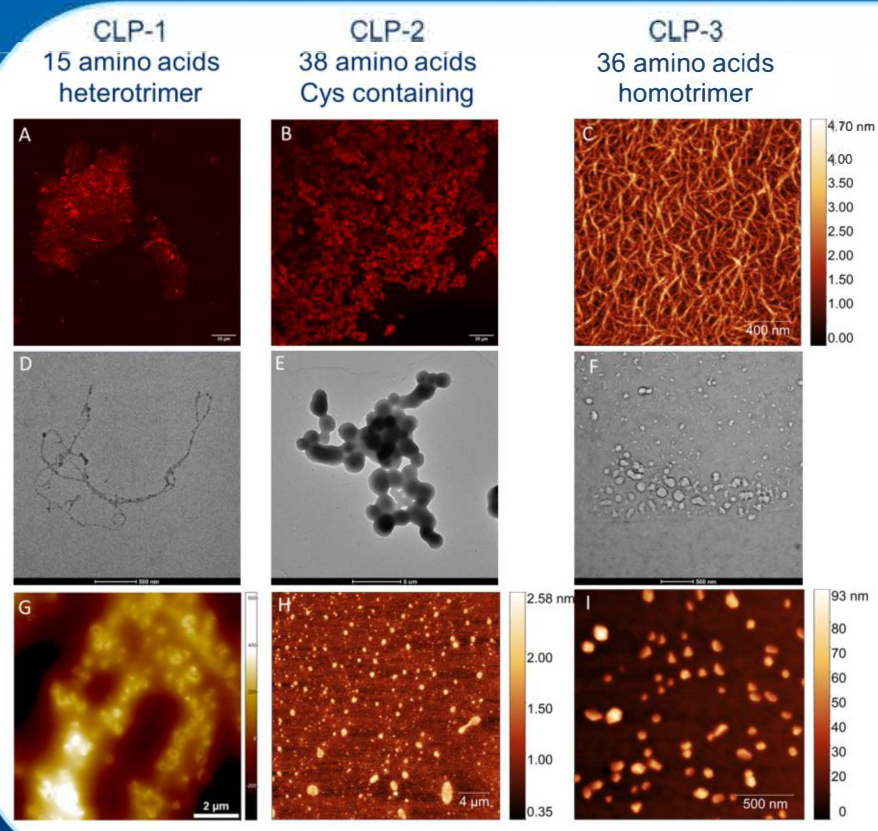
CLPs fold as a collagen triple helix



Circular Dichroism spectra show **polyproline II** conformations, characteristic of the triple helix, for **CLP-1** and CLP-2, with expected **maximum at 225 nm** and a strong **minimum at 200 nm**.

Upon thermal denaturation the CLPs undergo a fully reversable conformational transition with a single **iso-dichroic point** around 215 nm.

CLPs assemble into fibre and spherical structures



Confocal, electron and atomic-force micrographs revealed that **CLP-2**, a peptide with N-terminus cysteine, self-assembles into **nanospheres** approximately 1 µm in size whereas **CLP-1** and **CLP-3**, formed both **spherical** and **fibrous structures**.

Figure 2: *The observed higher ordered CLP structures. A) CLP-1 and B) CLP-2 confocal images, CLPs stained with Congo Red; D) CLP-1, E) CLP-2 and F) CLP-3 electron micrographs, with samples stained with uranyl acetate (2% w/v); G) CLP-1, H) CLP-2, C) and F) CLP-3 atomic force micrographs.*

Conclusion

- Fibre formation is not dominant in collagen-like peptides that are 15 amino acids long.
- Cysteine promotes the assembly into spherical particles via disulfide bridges.
- Both particle and fibre formation are present in short and medium-length CLPs.
- The longer the collagen sequence, the more predominant fibre formation is over particle formation.

Future Work

- Investigate the use of CLP particles for cell transfection applications.
- Determine the conditions under which both fibres and particles co-form.
- Develop CLP-based hydrogels or integrate CLPs into existing hydrogels to study their cell-ECM cues.

References

1. Yu, L. T. et al. Hollow Octadecameric Self-Assembly of Collagen-like Peptides. J. Am. Chem. Soc. 145, 5285–5296 (2023).
2. Sun, X. et al. Peptide-triggered self-assembly of collagen mimetic peptides into nanospheres by Cejas, M. A. et al. Thrombogenic collagen-mimetic peptides: Self-assembly of triple helix-based fibrils driven by hydrophobic interactions. Proc. Natl. Acad. Sci. U. S. A. 105, 8513–8518 (2008).
3. O'Leary, L. E. R., Fallas, J. A., Bakota, E. L., Kang, M. K. & Hartgerink, J. D. Multi-hierarchical self-assembly of a collagen mimetic peptide from triple helix to nanofibre and hydrogel. Nat. Chem. 3, 821–828 (2011).
4. Noble, J. E. et al. Folding-Mediated DNA Delivery by α-Helical Amphipathic Peptides. ACS Biomater. Sci. Eng. 9, 2584–2595 (2023).

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