Context-dependent challenges in short peptide self-assembly

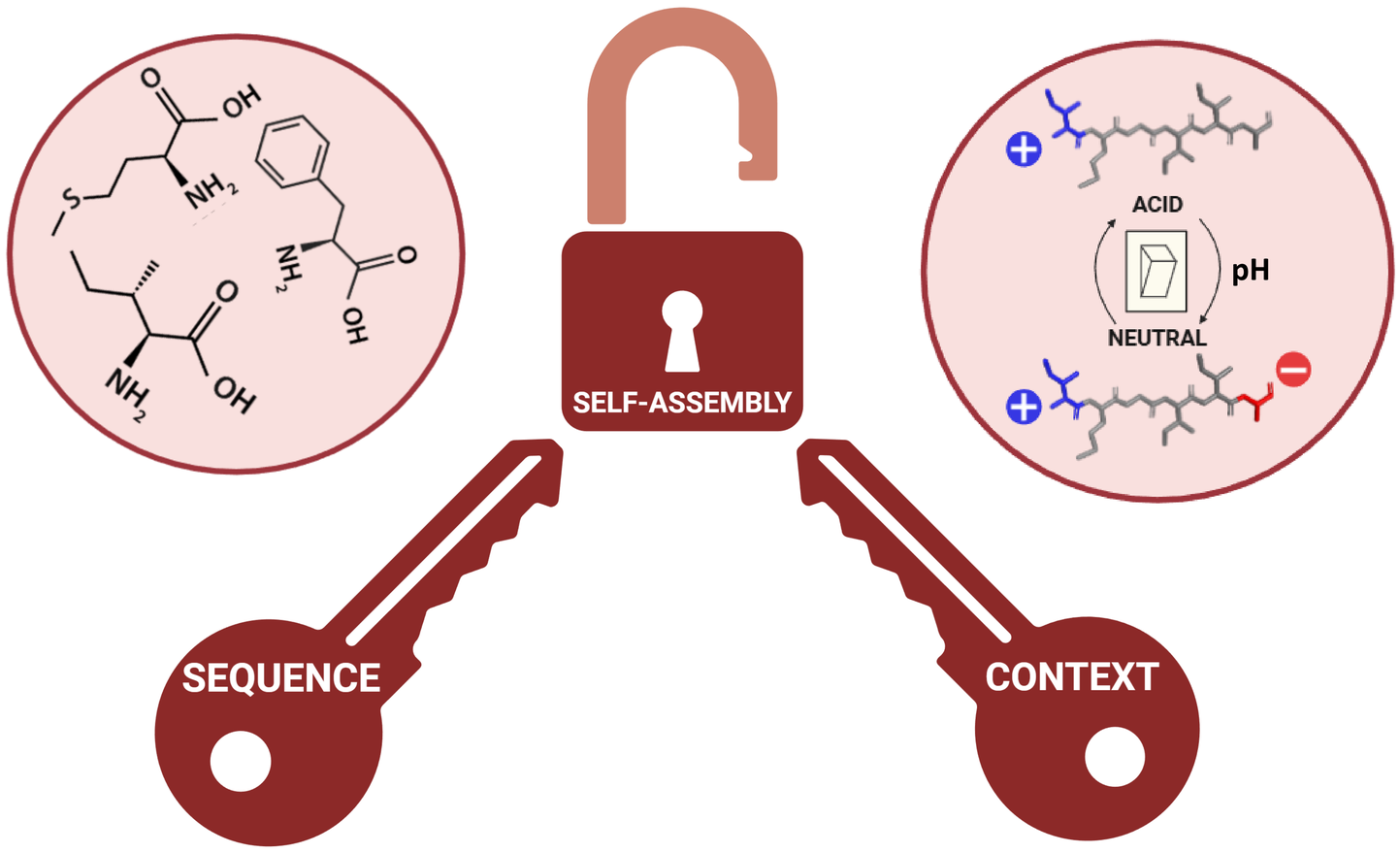
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- **IMGIIA** and **FMGIIF** hexapeptides with increasing aromaticity were assessed for their self-assembly propensity
- by introducing F instead of I at N-terminus and A at C-terminus
- design: **AI generated sequences**[1]



- peptides can form supramolecular assemblies guided by **sequence** and **experimental context**[2]
- **pH** and **concentration** influence the formation of nanostructures

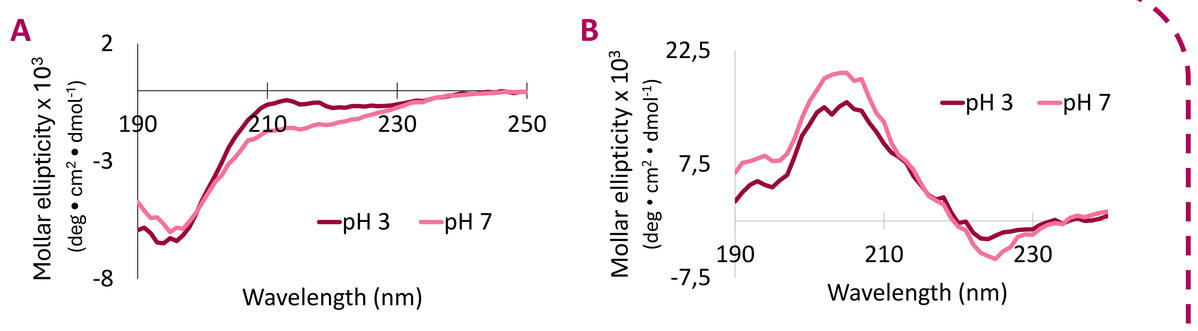


Figure 1. Sequence influences the formation of ordered supramolecular assemblies. Sequence-dependent secondary structure formation revealed by CD spectroscopy. **(A)** IMGIIA displays predominantly random coil characteristics, whereas **(B)** FMGIIF shows signatures of antiparallel β-sheet assembly.

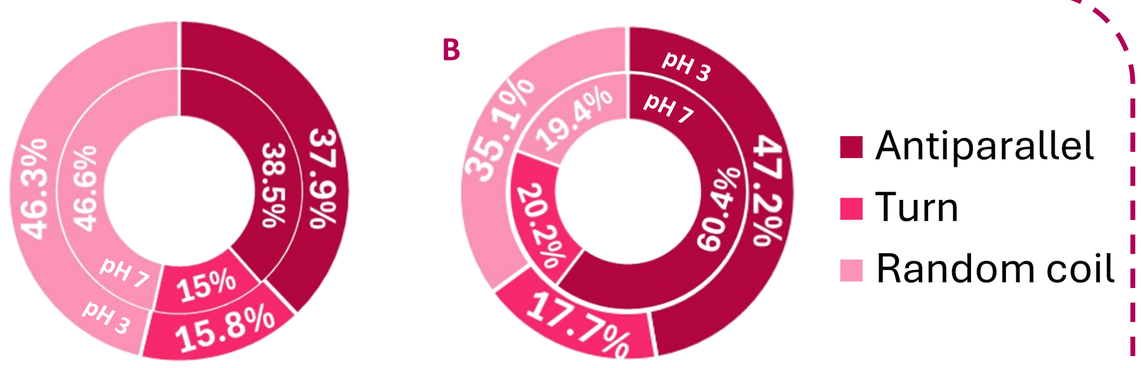


Figure 2. pH-dependent secondary structure-like behavior of IMGIIA and FMGIIF. BeStSel analysis of CD spectra at pH 3 and 7. **(A)** IMGIIA shows no major change in structural composition. **(B)** FMGIIF undergoes a pH-driven shift toward dominant antiparallel β-sheet content.

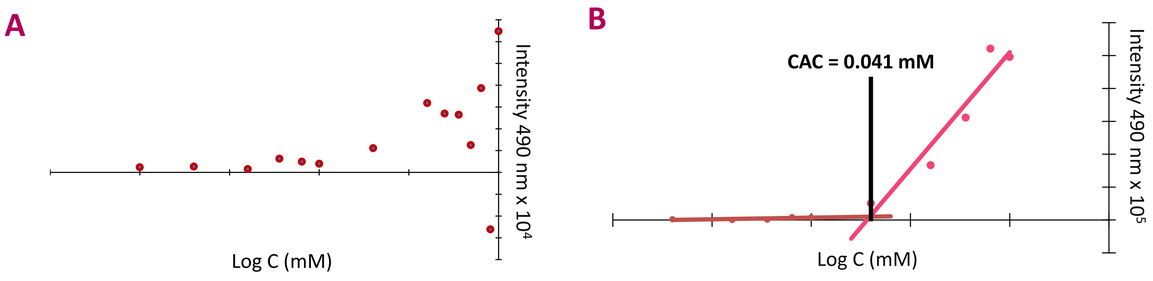


Figure 3. ThT assay reveals β-sheet-rich assembly in FMGIIF. Critical aggregation concentration (CAC) was determined by thioflavin T (ThT) assay. **(A)** IMGIIA shows no clear ThT fluorescence increase, indicating absence of assembly into β-sheet-rich structures. **(B)** FMGIIF exhibits a clear ThT response and a lower CAC, consistent with assembly into β-sheet-rich structures.

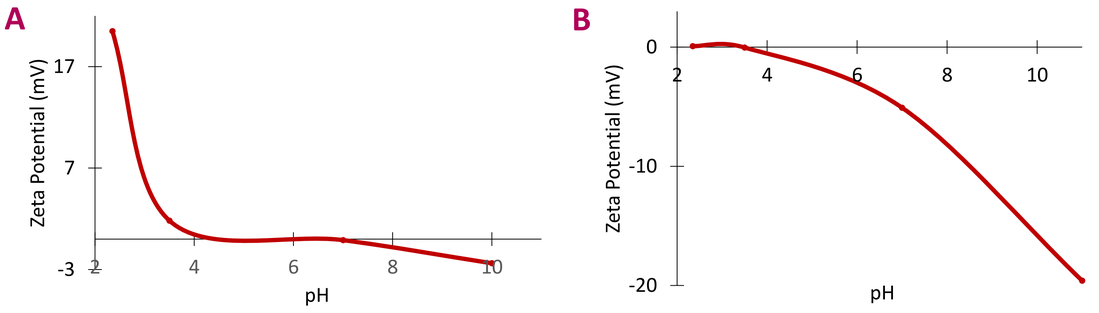


Figure 4. pH modulates surface charge. Change in peptide surface charge by pH revealed through zeta potential measurements. The contrasting electrostatic responses of **(A)** IMGIIA and **(B)** FMGIIF demonstrate how experimental context governs intermolecular interactions in peptide self-assembly.

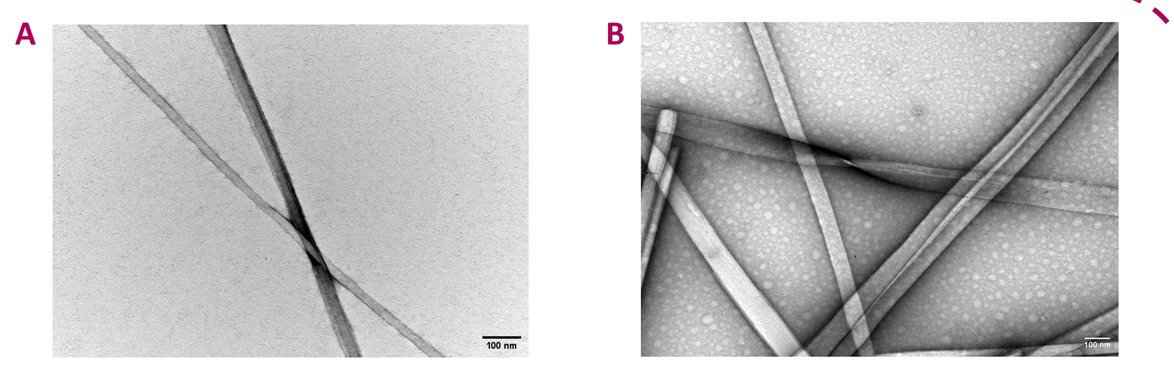


Figure 5. IMGIIA and FMGIIF self-assemble into nanoscale supramolecular structures. TEM images showing the formation of supramolecular structures by **(A)** IMGIIA and **(B)** FMGIIF under the investigated conditions (c = 1 mM, pH = 7).

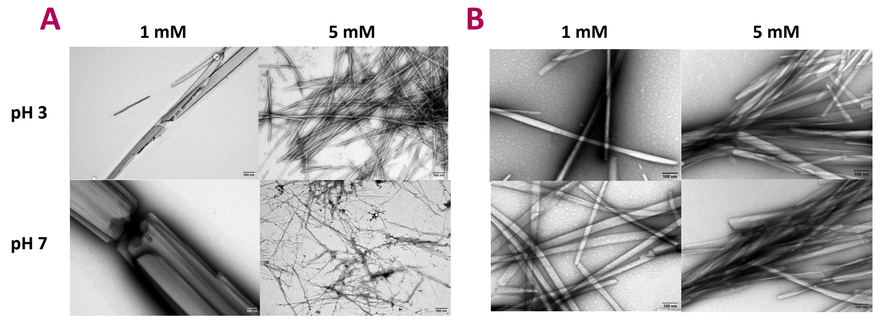


Figure 6. Higher peptide concentration promotes more closely associated supramolecular fibers. (A) IMGIIA and **(B)** FMGIIF were examined at pH 3 and 7 and at 1 and 5 mM. Denser fibrous networks are observed at higher concentration, especially for FMGIIF.

Sequence sets the self-assembly propensity, _context_ selects the outcome.

REFERENCES

[1] M. Njirjak, L. Žužić, M. Babić, P. Janković, E. Otović, D. Kalafatovic and G. Mauša, Nat. Mach. Intell., 2024, 6, 1487–1500

[2] E. Dražić, D. Jelušić, P. Janković Bevandić, G. Mauša and D. Kalafatovic, ACS Nano, 2025, 19, 20295–20320