Peptide Self-assembly Beyond Known Design Principles: Sequence and Context Interplay

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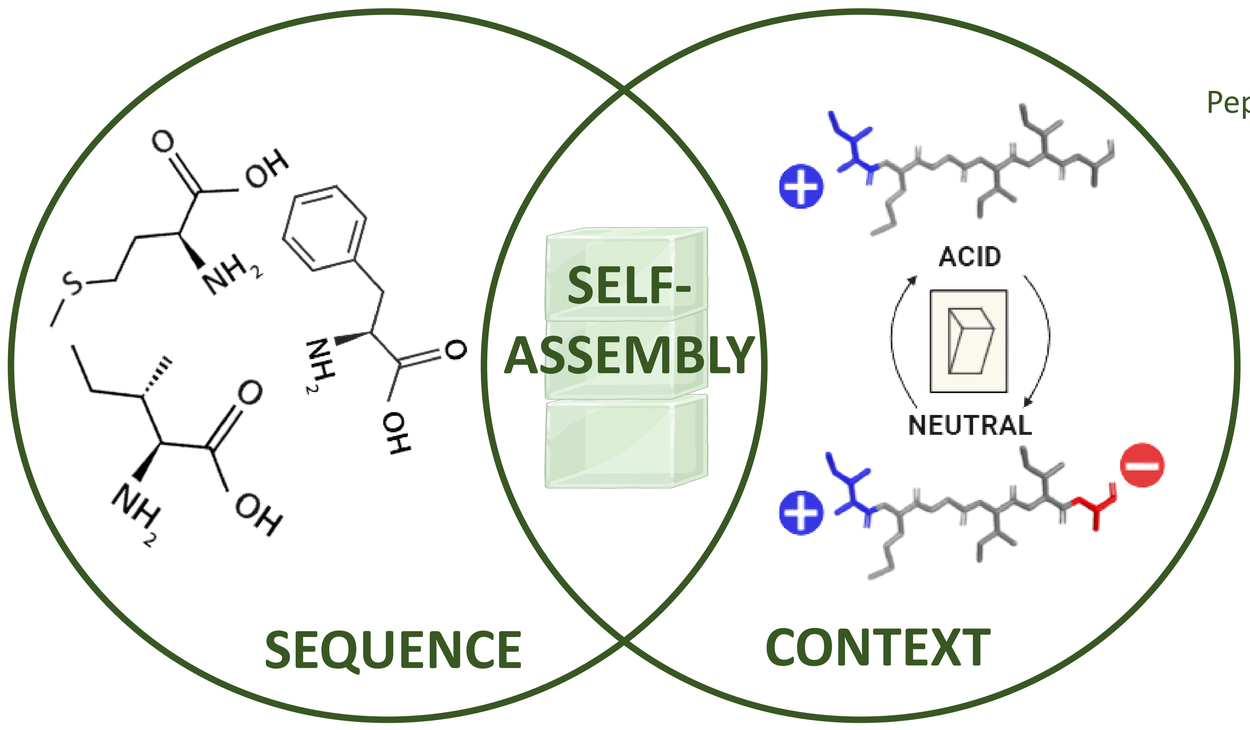
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AI generated hexapeptides with increasing aromaticity were assessed for their self-assembly propensity.[1]

Can sequence-assembly relationship in AI-generated hexapeptides reveal new design principles?



Peptides can form supramolecular assemblies guided by **sequence** and **experimental context.**[2]

Is self-assembly determined by sequence alone or changing conditions can redirect the outcome?

Sequence Matters: Self-Assembly of an Unconventional Hydrophobic Peptide

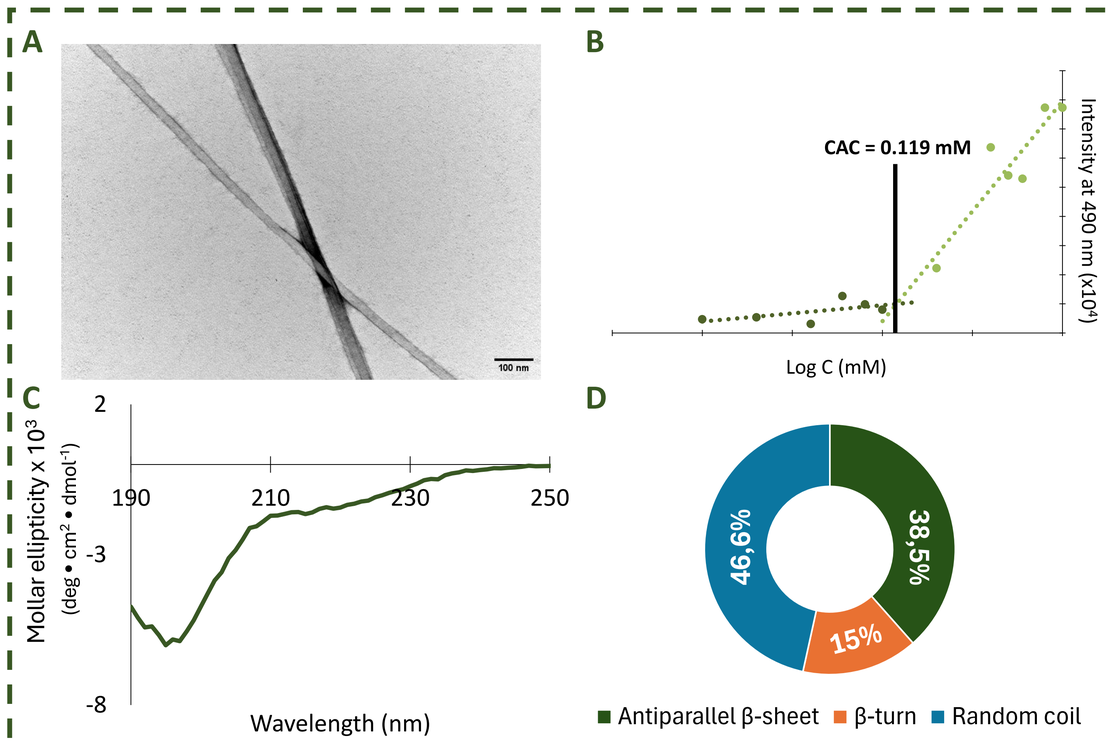


Figure 1. Despite lacking typical amphiphilic design, IMGIIA self-assembles into elongated fibrillar structures **(A)**. Concentration-dependent increase in ThT fluorescence indicates β-sheet-like assembly propensity **(B)**, while CD shows an overall random-coil-like organization **(C)**. BeStSel analysis shows a mix of random coil and antiparallel β-sheet content **(D)**, suggesting the mixed supramolecular organization of IMGIIA.

More Aromatic, More Ordered: Aromaticity Drives Assembly Toward β-sheet-like Organization

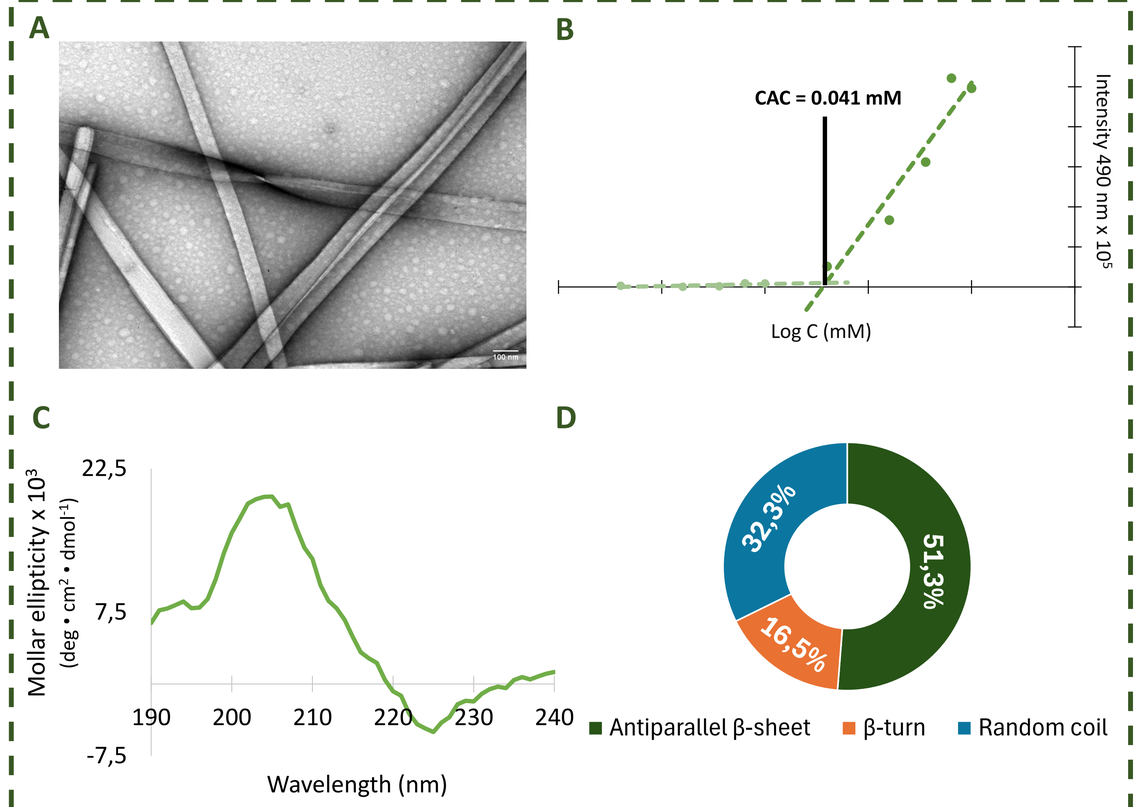


Figure 2. Introducing aromaticity by replacing the terminal I and A residues with F in FMGIIF changes the assembly behavior toward supramolecular order **(A)**. ThT fluorescence suggests the formation of fibrillar assemblies with CAC of 0.041 mM **(B)**, while CD confirms β-sheet-rich organization **(C)**, with antiparallel β-sheet content increasing from 38.5% in IMGIIA (Fig. 1) to 51.3% in FMGIIF **(D)**.

SELF-ASSEMBLY IS DRIVEN BY MORE THAN SEQUENCE ALONE: pH AND CONCENTRATION SHAPE THE SUPRAMOLECULAR OUTCOME

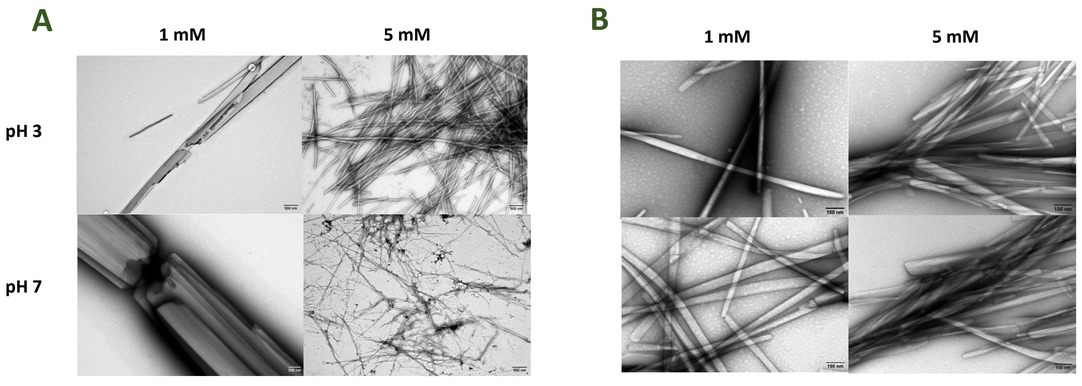


Figure 3. Both peptides self-assemble at pH 3 and pH 7, with fibrillar morphology influenced by concentration. **(A)** IMGIIA forms denser fiber networks at higher concentration at both pH values. **(B)** FMGIIF shows similar fibrillar assemblies at 5 mM at both pH values, while at 1 mM a denser fibril network is observed at pH 7. These results indicate that pH and concentration act together in defining the assembly outcome.

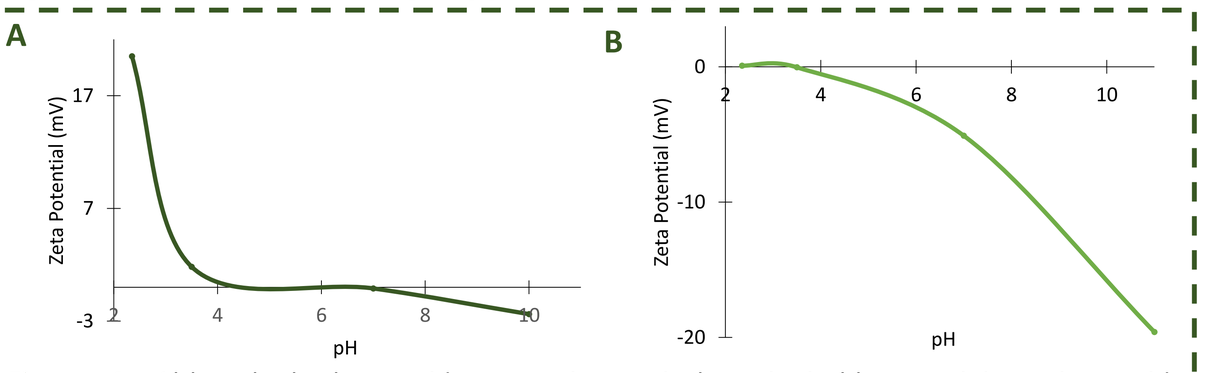


Figure 4. Although both peptides contain equivalent ionizable termini, amino acid substitutions at the N- and C-terminal positions may alter supramolecular packing and the surface exposure of these groups, explaining different pH-dependent zeta-potential profiles. **(A)** IMGIIA rapidly loses its positive surface charge between pH 2 and 4 and remains near neutral at higher pH. **(B)** FMGIIF gradually shifts toward a more negative surface charge with increasing pH.

SEQUENCE-CONTEXT INTERPLAY IS A KEY:
Sequence and experimental conditions (pH and concentration) together shape the self-assembly of IMGIIA and FMGIIF.

REFERENCES

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