Self-assembly control with hydrophobicity gradients in short peptide sequences

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MOTIVATION

We develop tunable biomaterials based on amphiphilic peptides that self-assemble into supramolecular peptide assemblies (**SPAs**) with potential applications in **tissue regeneration** and **drug delivery**[1,2]. However, the molecular mechanisms governing early aggregation events and their impact on final material properties remain poorly understood, limiting rational design. Here, we combine **fluorescence spectroscopy**, circular dichroism (**CD**), Fourier transform Infrared Spectroscopy (**FTIR)**, Atomic Force Microscopy (**AFM)**, and coarse-grained molecular dynamics (**CG-MD**) **simulations** to resolve self-assembly with enhanced molecular detail. We show that analyzed SPAs with different hydrophobic gradients form predominantly fibrous structures alongside other morphologies. Finally, we aim to incorporate positively charged peptides to provide antibacterial activity against pathogens such as *Pseudomonas aeruginosa* infection.

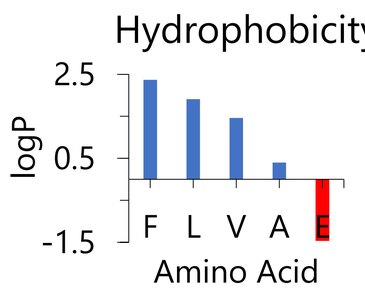
EXPERIMENTAL DEVELOPMENT

DESIGN

Hydrophobic Charged

$A_1\ A_2\ B_1\ B_2\ C_1\ C_2$ E E

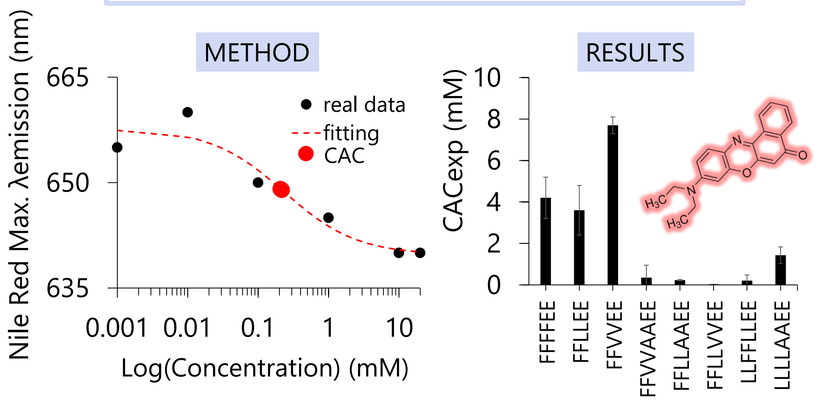
$A_1\ A_2\ B_1\ B_2$ E E



Design of the peptide amphiphiles analyzed with a hydrophobic gradient based on logP values

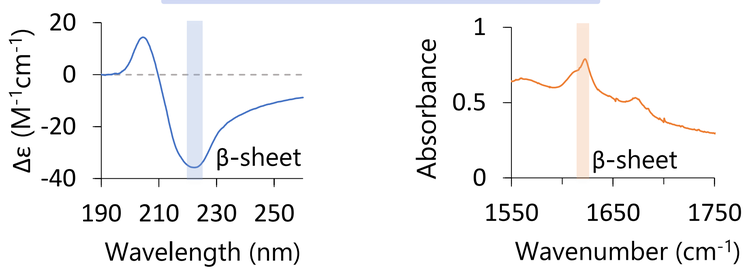
CHARACTERIZATION

Calculation of Critical Concentrations

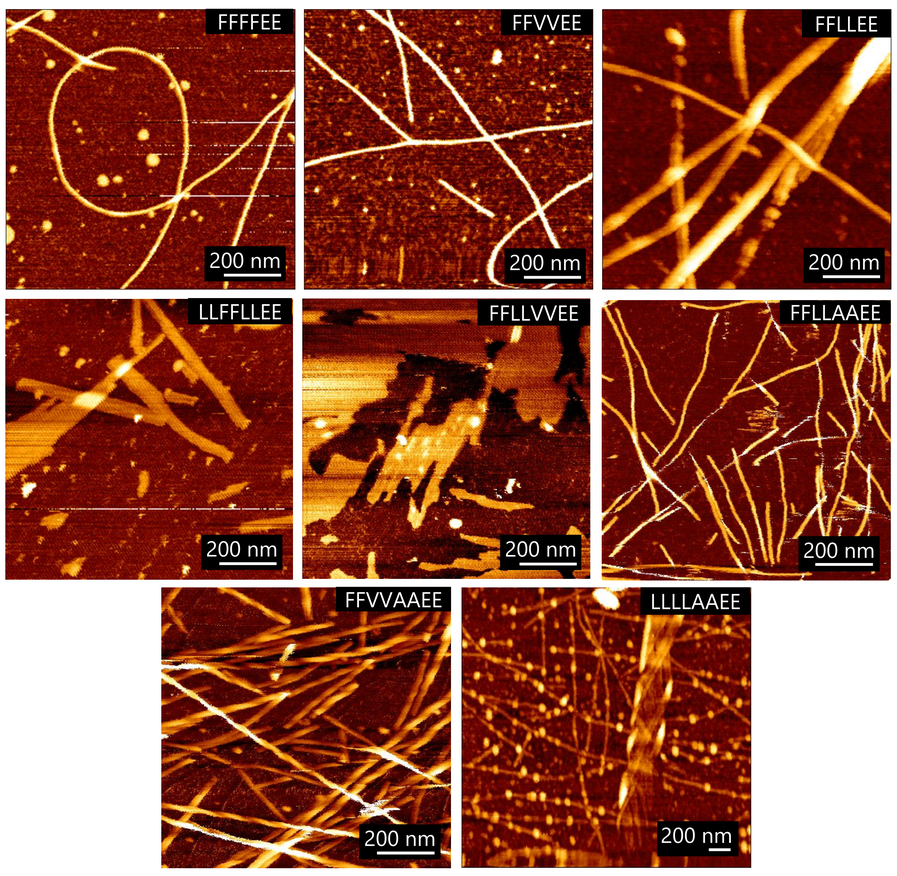


Critical Aggregation Concentrations (CAC) are extracted from the inflexion point (x_0) of sigmoidal fittings

Spectroscopy: CD & FTIR



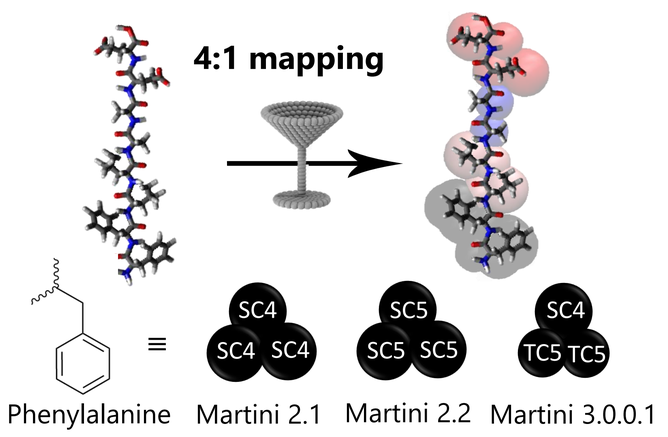
Microscopy: AFM at 10 mM



COMPUTATIONAL TOOL

CG-MD SIMULATIONS

Martini Force Field



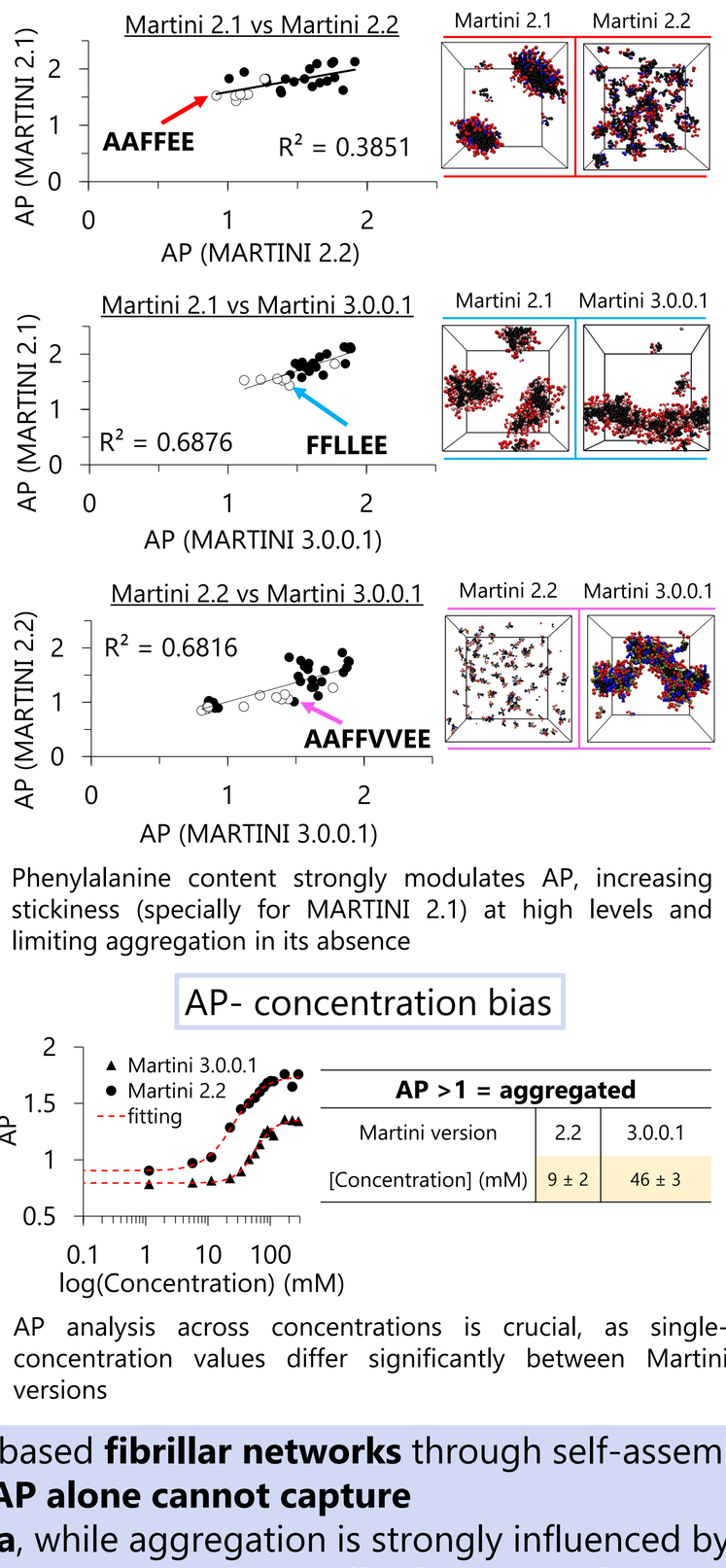
- Martini Force Field → Structure simplification
- Successive versions → Changes in bead types → impact on self-assembly [3]

SCREENING

AP across versions (11.3 mM)

8 AA
6 AA

AP = Aggregation Propensity



Phenylalanine content strongly modulates AP, increasing stickiness (specially for MARTINI 2.1) at high levels and limiting aggregation in its absence

AP- concentration bias

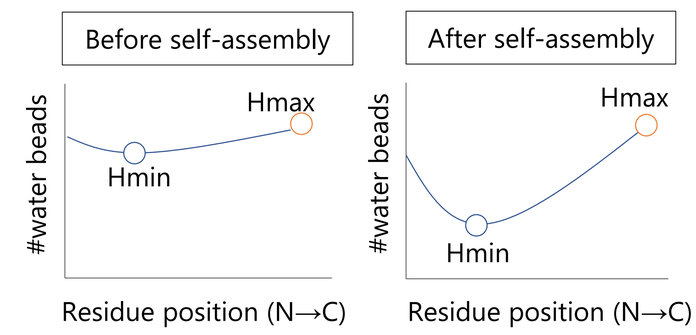
AP >1 = aggregated		
Martini version	2.2	3.0.0.1
[Concentration] (mM)	9 ± 2	46 ± 3

AP analysis across concentrations is crucial, as single-concentration values differ significantly between Martini versions

EXP/COMP COMBINED APPROACH

FRAMEWORK

AP quantifies molecular association and does not directly capture molecular organization → **Hydration as a molecular descriptor of self-assembly**

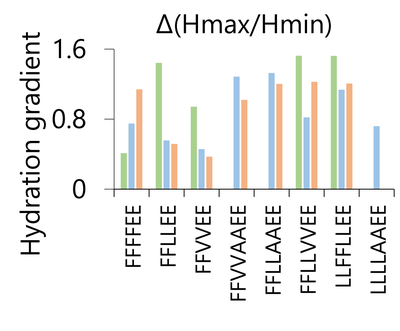


Hydration captures changes in the exposed surface of the peptides before and after self-assembly

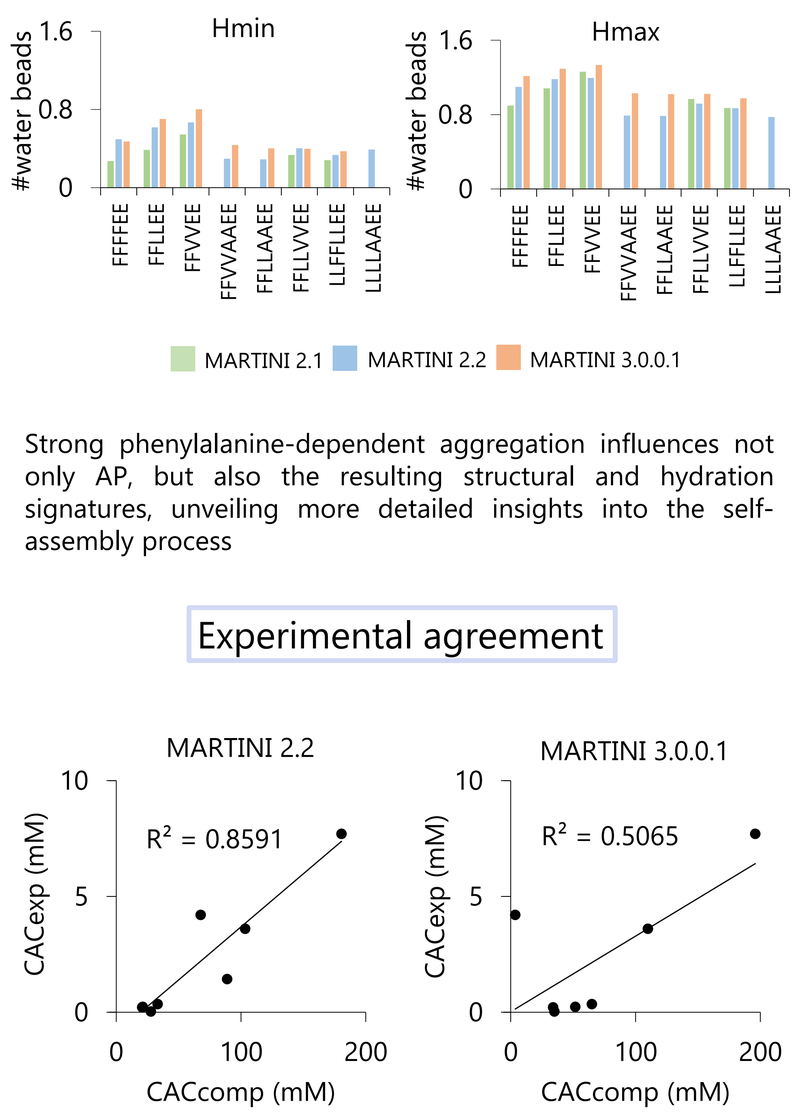
Δ(Hmax/Hmin)

$$\left(\frac{Hmax}{Hmin}\right)_A - \left(\frac{Hmax}{Hmin}\right)_B$$

A: After self-assembly
B: Before self-assembly



MOLECULAR RESOLUTION



Strong phenylalanine-dependent aggregation influences not only AP, but also the resulting structural and hydration signatures, unveiling more detailed insights into the self-assembly process

CORRLEATION

Experimental agreement

Martini 2.2 shows the best correlation with experimental data regarding aggregation onset, making it the most suitable model for an in-depth investigation of self-assembly in the systems analyzed

- Designed peptide amphiphiles form β-sheet–based **fibrillar networks** through self-assembly
- **Hydration reveals structural changes that AP alone cannot capture**
- **Martini 2.2 best matches experimental data**, while aggregation is strongly influenced by F-content and concentration:
  → **force-field shows a bias towards F-content**, but **experimentally there are self-assembling F-free sequences**
- Morphological analysis reveals nanofibers and nanosheet-like structures, with **hexapeptides displaying the smallest widths**

REFERENCES

1. Álvarez, Z., et al., Science 2021, 374, 848-856
2. Álvarez, Z., et al., Cell Stem Cell 2022, 30, 1-20
3. Marrink, S. J., et al., WIREs Comput. Mol. Sci. 2023, 13, e1620

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