A comparative study between phenylglycine- and phenylalanine-derived peptide hydrogels

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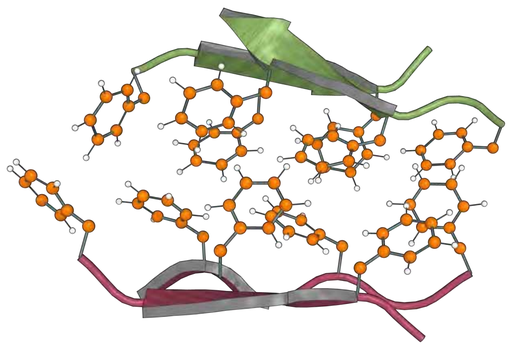
INTRODUCTION

Peptide hydrogels are soft materials which, due to their biocompatibility, are excellent candidates for the development of **controlled drug delivery** matrices and wound healing applications. The properties of the gel materials are directly linked to the peptide sequence. However, minor alterations in the sequence can entail major differences in the **assembly mode** and hence material characteristics. Many of the well-known hydrogelators self-assemble solely by non-covalent interactions; it is known that hydrophobic effects, π-π stacking, ionic interactions and hydrogen bonding play major roles. Nevertheless, the extent and tunability of each of these interactions in the self-assembly process is not fully understood. To further elucidate the role of **aromatic interactions** in this process, the amino acids **phenylalanine (Phe)** and **phenylglycine (Phg)** were interchanged in a short amphipathic peptide hydrogelator with sequence H-FQFQFK-NH_2 [1-2]. This substitution resulted in four new hydrogelators in which the aryl rings are oriented differently [3]. The novel soft materials were **characterized at different levels** and additionally, **atomic models** were obtained of the stacking modes by molecular dynamics (MD) simulations.

SCOPE



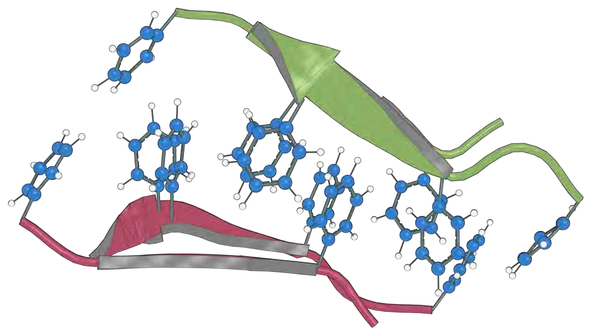
SBL-HG-063

Principally T-shaped π-π stacking

MD

Code	Sequence
SBL-HG-063	H-Phe-Gln-Phe-Gln-Phe-Lys-NH_2
SBL-HG-085	H-**Phg**-Gln-**Phg**-Gln-**Phg**-Lys-NH_2
SBL-HG-086	H-**Phg**-Gln-Phe-Gln-Phe-Lys-NH_2
SBL-HG-087	H-Phe-Gln-**Phg**-Gln-Phe-Lys-NH_2
SBL-HG-084	H-Phe-Gln-Phe-Gln-**Phg**-Lys-NH_2

MD



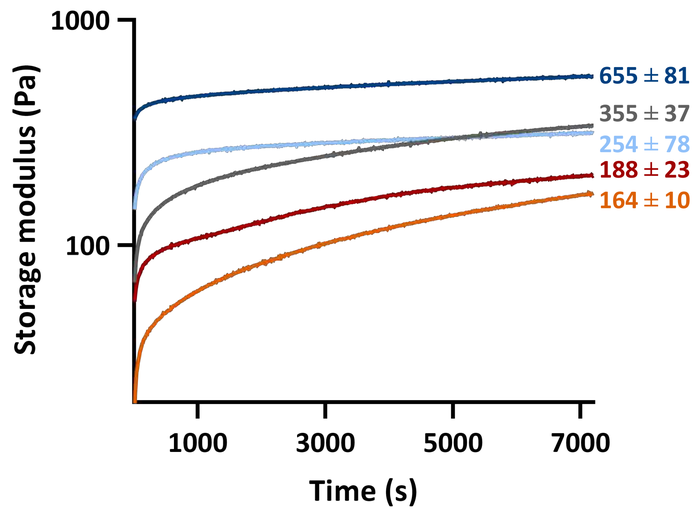
SBL-HG-085

Principally parallel-slipped π-π stacking

SPPS conditions: Rink amide AM resin – Coupling with Fmoc-AA/COMU/DMP in DMF, 45 min – Fmoc-deprotection with 20% 4-Me-piperidine in DMF, 20 min – Cleavage with TFA/TIS/H_2O (95:2.5:2.5 v/v/v), 3 h.

DYNAMIC RHEOLOGY

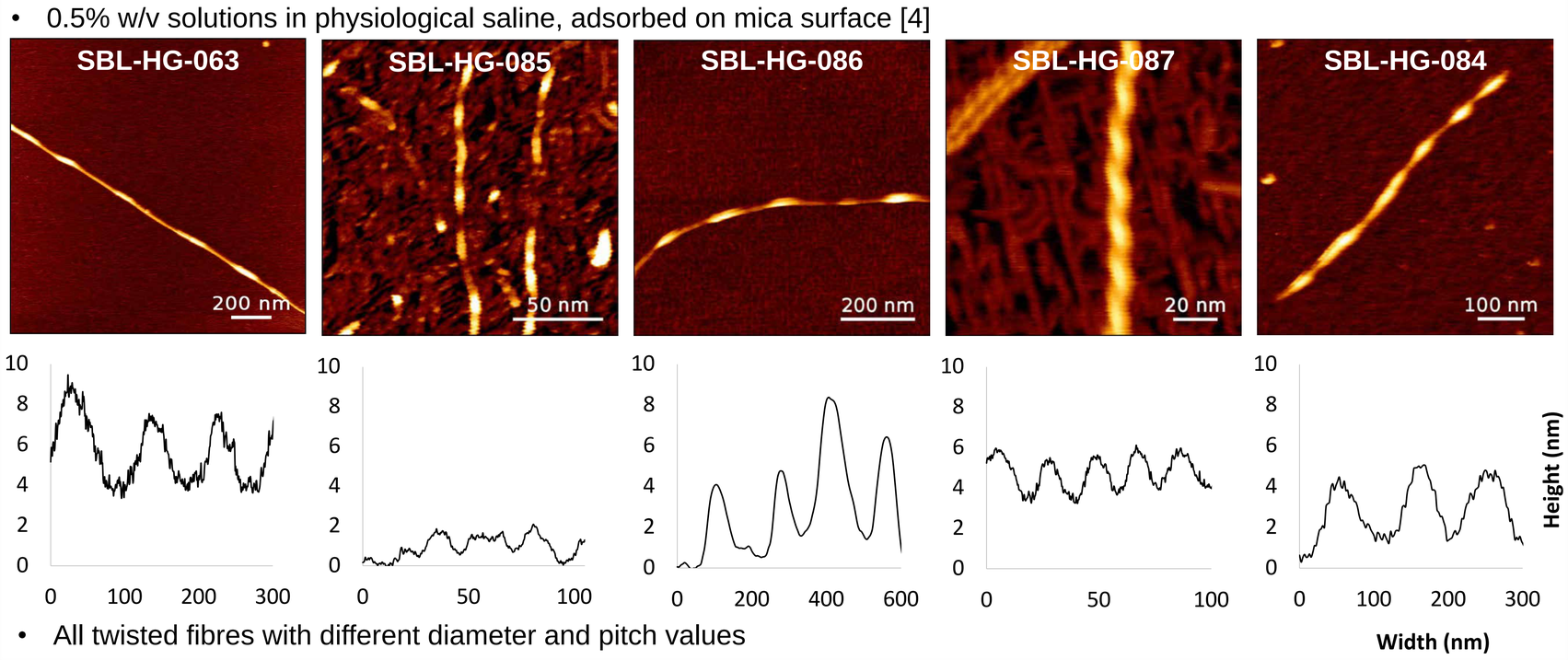
- 2% w/v gels in physiological saline



- 3-fold increase in gel strength for **SBL-HG-085**
- Thixotropic materials
- **SBL-HG-085** more stable upon injection

ATOMIC FORCE MICROSCOPY

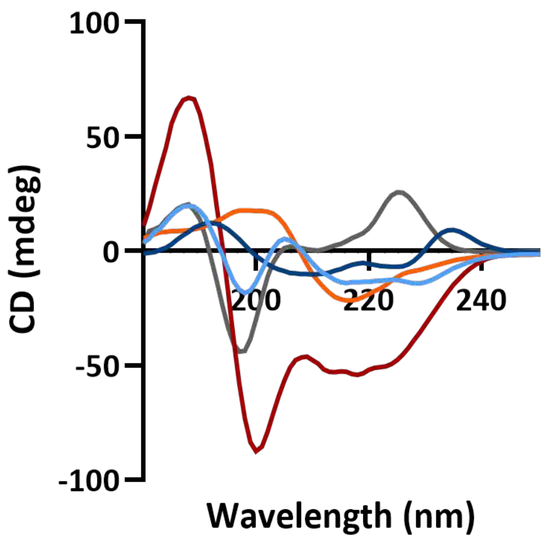
- 0.5% w/v solutions in physiological saline, adsorbed on mica surface [4]



- All twisted fibres with different diameter and pitch values

CIRCULAR DICHROISM

- 2% w/v gels in physiological saline

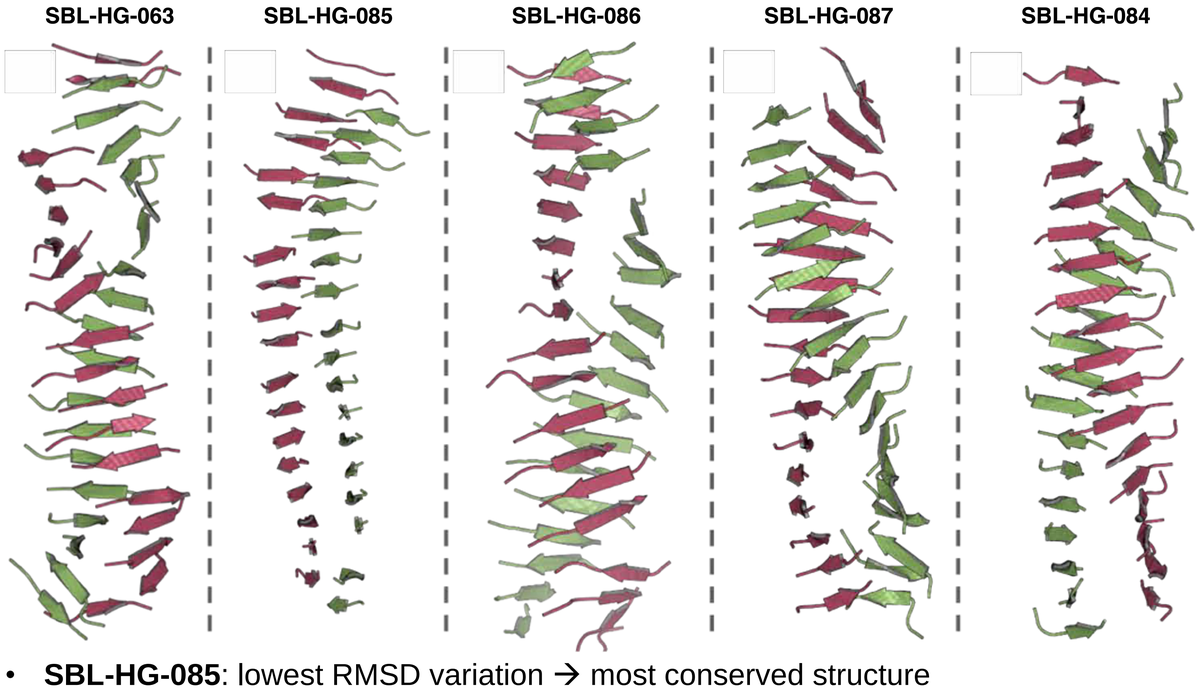


- Red-shifted β-sheet profile
- Indication of aromatic clustering
- Clear nanoribbons for **SBL-HG-087**

MOLECULAR DYNAMICS SIMULATIONS

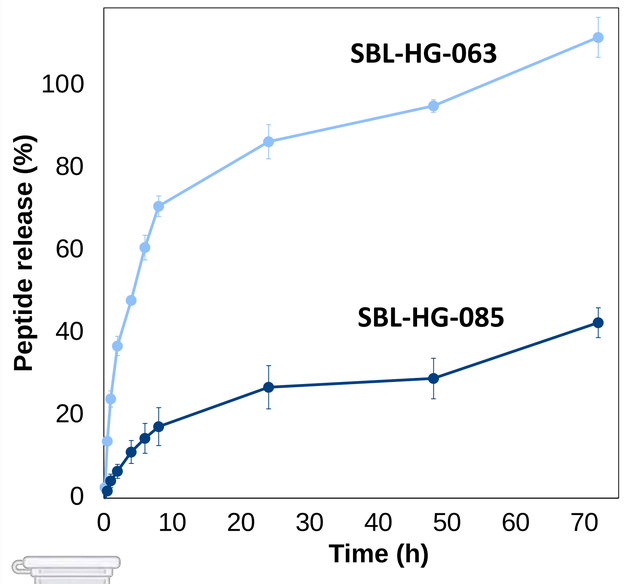
A. V. Cunha

- Antiparallel β-sheet double layer of 36 peptide strands
- 100 ns all-atom MD simulation (GROMACS)



- **SBL-HG-085**: lowest RMSD variation → most conserved structure
- High correlation between computed and experimental infrared spectra

HYDROGEL STABILITY



- *In vitro* release experiment
- 37°C, saline supernatants
- Phg-rich hydrogel remains more intact than Phe-rich hydrogel

CONCLUSION

The substitution of Phe with Phg in a previously described peptide hydrogelator led to the formation of four **new soft biomaterials**. The aromatic side chain of Phg reduced the side chain flexibility of the peptide sequence, leading to a different π-π stacking orientation during self-assembly. One of the studied hydrogels (**SBL-HG-085**) showed a three-fold increase in gel strength and more rapid recovery after disruption. Based on atomic force microscopy experiments, this system contained the smallest, yet most interacting fibres. Additionally, the Phg-rich hydrogel remained more intact during *in vitro* release studies. The antiparallel β-sheet double layer assembly was subjected to MD simulations and the assembled model was validated by means of calculated and experimental IR spectroscopy. Overall, **reducing the aromatic flexibility stabilized the assemblies** by modified **π-π stacking** interactions.

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