Design of fluorinated peptide-based hydrogels for sustained drug release application

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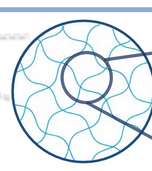
https://doi.org/10.17952/37EPS.2024.P2318

Introduction

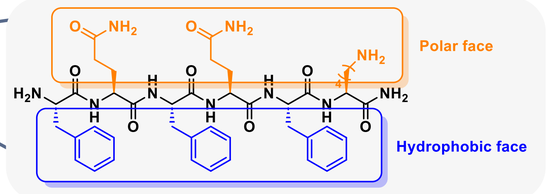
- **Amphipathic peptides** alternating hydrophobic and hydrophilic amino acids have shown to form **self-assembled hydrogels.**[1]
- Alternative system for **chronic pain treatment** as **extended-release drug delivery platforms** of pharmaceutical cargoes when injected subcutaneously.
- Subcutaneous injections can **increase patient compliance** by limiting the number of injections required for therapeutic efficiency. The drug release window is **limited to 3 to 4 days** and has to be extended for optimal use in clinical setting.[2,3]

Thus, two strategies are considered to incorporate fluorinated amino acids into the consensus sequence and provide access to a new class of injectable controlled-delivery systems that incorporates favorable properties of fluorine.

Peptide-based Hydrogelator Design[2,3]



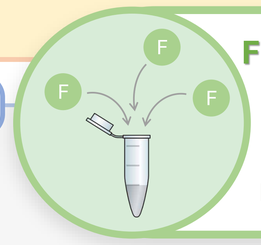
Gelation:
2%w/v in PBS



H-FQFQFK-NH_2

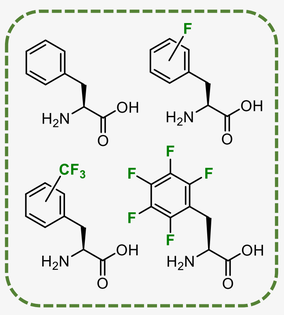
→ How to increase drug release properties of the system?

FLUORINE INTRODUCTION[4,5]



Increases the local hydrophobicity
Stabilizes and promotes secondary structures
Improves pharmacological properties

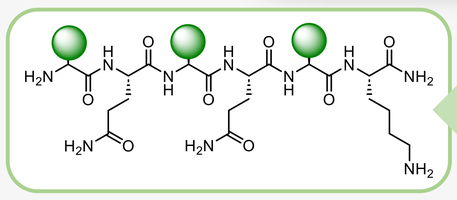
Strategy 1: Fluorinated hexapeptide Hydrogelators



(Fluorinated) amino acids

To investigate the impact of different fluorinated groups

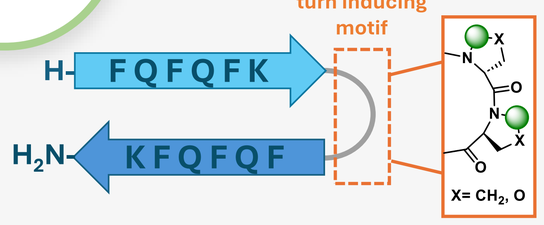
To investigate the importance of aromatics vs. hydrophobicity[7]

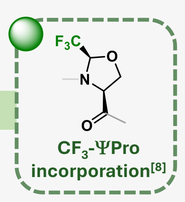


Reference
H-FQFQFK-NH_2

Strands and turn modulations

Strategy 2: β-hairpin Peptide Hydrogelators





To help the alignment between strands

To investigate conformational restriction with fluorinated amino acid

HYDROGEL MECHANICAL PROPERTIES (DYNAMIC RHEOMETRY)

Among **13 fluorinated hexamer** analogues synthesized, three formed the strongest hydrogels:

Hydrogel	*G'* (Pa)	*G'* (Pa)
H-FQFQFK-NH_2	1792 ± 236	162 ± 31
H-FQFQ(**oCF_3**)FK-NH_2	**13039** ± 8206	**921** ± 319
H-FQFQ(**F_5**)FK-NH_2	**16649** ± 1644	**1546** ± 205
H-FQFQ(**CF_3**)MK-NH_2	**33875** ± 4949	**1354** ± 403

HAIRPIN DESIGN
Higher gel rigidity (G')

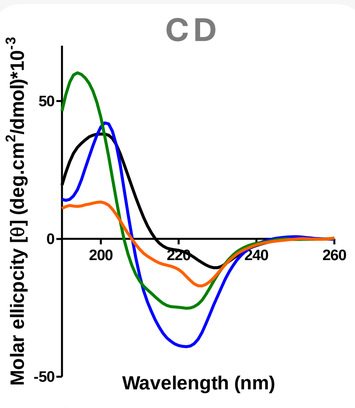
Among **14 analogues synthesized**, H-FQFQFK-pP-FQFQFK-NH_2 formed the strongest hydrogel:

Hydrogel	*G'* (Pa)	*G'* (Pa)
H-FQFQFK-pP-FQFQFK–NH_2	2557 ± 546	178 ± 82
H-FQFQFK-p(**CF_3ΨPro**)-FQFQFK–NH_2	**1094** ± 92	**107** ± 3
H-FQFQFK-(**DCF_3ΨPro**)P-FQFQFK–NH_2	**225** ± 117	**15** ± 8

HAIRPIN DESIGN
Higher gel rigidity (G')

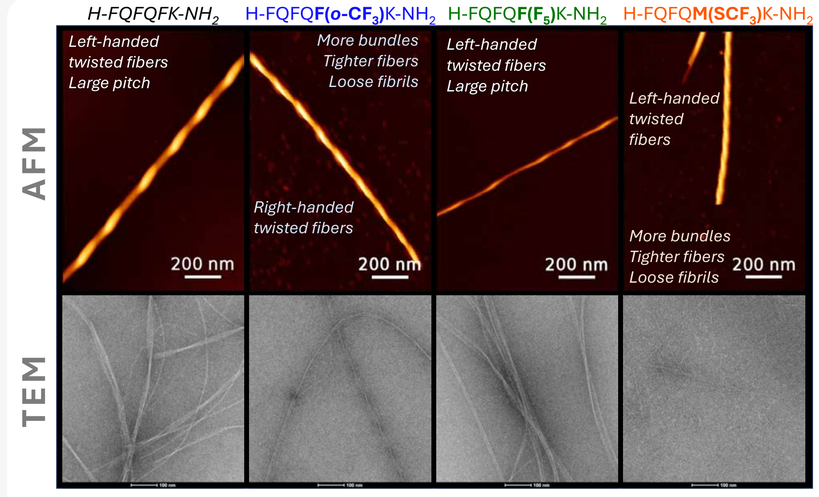
FLUORINATED TURN
CF_3ΨPro ↓ gel rigidity (G')

SECONDARY STRUCTURE (CIRCULAR DICHROISM) & FIBER NETWORK (AFM, TEM)

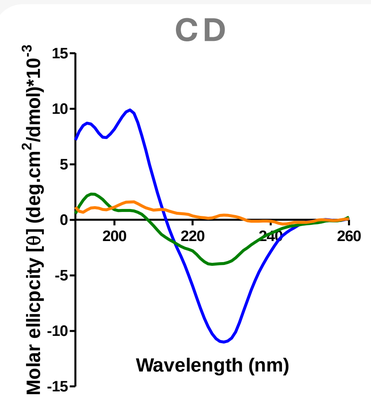


→ β-sheet signature

Fluorine

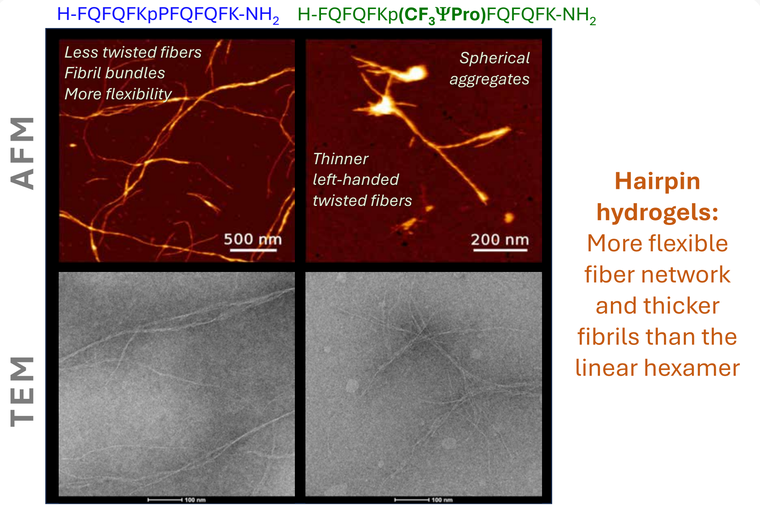


Fluorine incorporation: more bundled fibrils, tighter twisted fibers



→ β-sheet signature ↓ when *G'* ↓

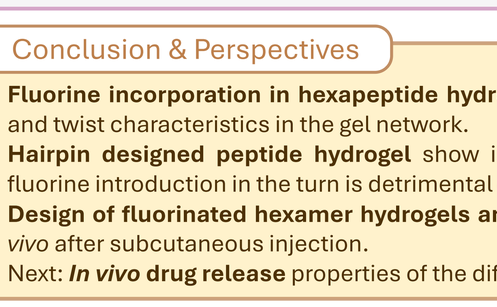
Fluorine



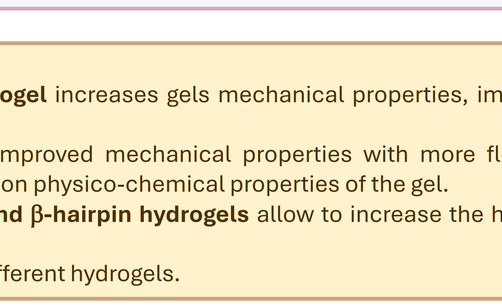
Hairpin hydrogels: More flexible fiber network and thicker fibrils than the linear hexamer

RELEASE KINETIC: *IN VITRO* DRUG RELEASE STUDY (ANALYTICAL RP-HPLC)

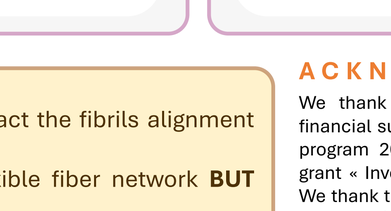
CARGO RELEASE



HYDROGELATOR RELEASE



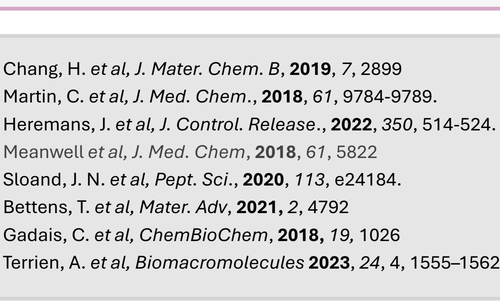
Similar cargo release but **fluorine increases the gel stability**



Cargo
opioid peptide
(Dmt-DArg-Phe-Phe-NH_2)
0.22%w/v loading in 2%w/v gel in PBS

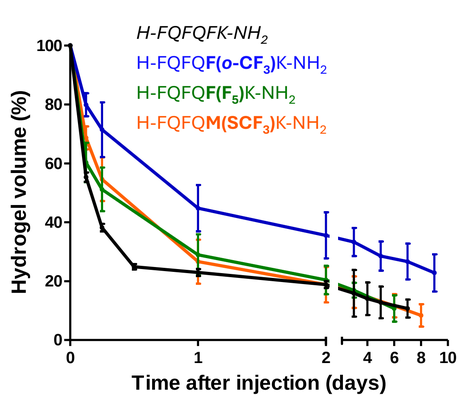
CARGO RELEASE

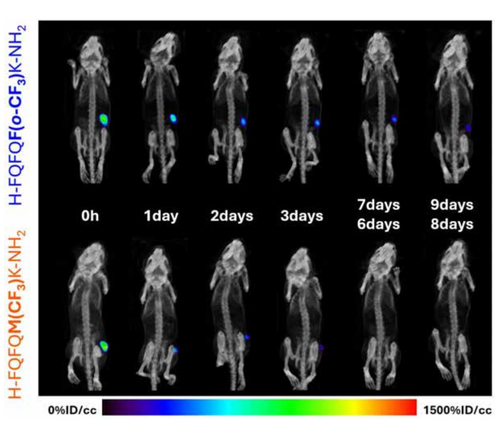
HYDROGELATOR RELEASE



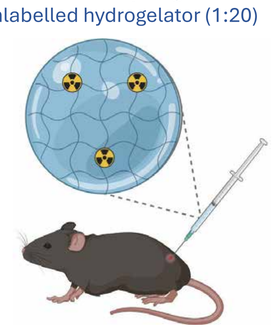
Similar cargo release but **hairpin design increases the gel stability**

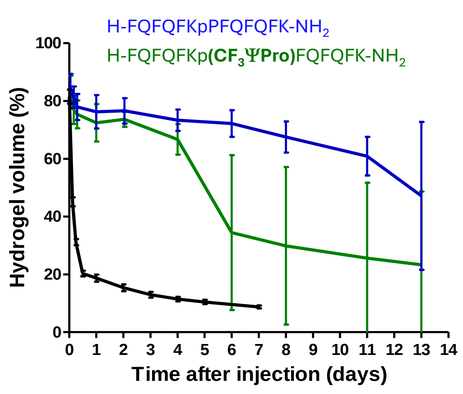
IN VIVO HYDROGEL STABILITY (SPECT/CT IMAGING)

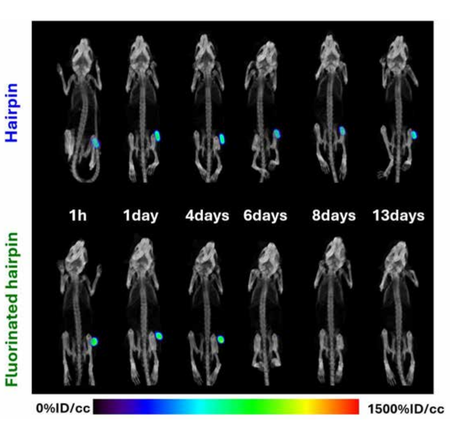




***In vivo* formulation:**
^{111}In-labelled-hydrogelator :
Unlabelled hydrogelator (1:20)







Fluorinated hydrogels display slower *in vivo* gel erosion → **Fluorine ↑ hydrogel residence time**

Slower *in vivo* gel erosion → **Hairpin design ↑ hydrogel residence time**

Conclusion & Perspectives

- **Fluorine incorporation in hexapeptide hydrogel** increases gels mechanical properties, impact the fibrils alignment and twist characteristics in the gel network.
- **Hairpin designed peptide hydrogel** show improved mechanical properties with more flexible fiber network **BUT** fluorine introduction in the turn is detrimental on physico-chemical properties of the gel.
- **Design of fluorinated hexamer hydrogels and β-hairpin hydrogels** allow to increase the hydrogel residence time *in vivo* after subcutaneous injection.
- Next: ***In vivo* drug release** properties of the different hydrogels.

ACKNOWLEDGEMENTS

We thank the CY Initiative of Excellence for their financial support through the EUTOPIA - PhD co-tutelle program 2020 (EXPLORE, 2020-166) and through the grant « Investissements d'Avenir » ANR-16-IDEX-0008). We thank the Research Council of VUB for the financial support through the Strategic Research Programme (SRP50 and SRP95). We thank the Research Foundation Flanders through FWO Hercules (OZR3939)

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