Supramolecular Upcycling of Protein Materials

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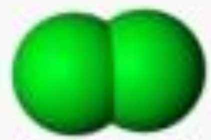
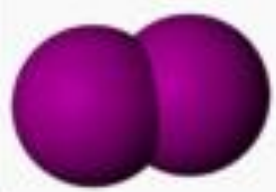
4. **International Institute for Sustainability with Knotted Chiral Meta Matter (WPI-SKCM2), Hiroshima University, Japan**

Workshop «Materiali da Proteine – Bioeconomy Day 2nd Ed.»
Politecnico di Milano, Italy - October 28, 2025

The Halogens...

The %’s of halogens in the rocks of Earth's crust are 0.06 F, 0.031 Cl, 0.00016 Br, and 0.00003 I. Seawater contains 536 mM chloride, 800 µM bromide, and 0.5 µM iodide anions.

halogen	molecule	structure	model	d(X–X) / pm (gas phase)	d(X–X) / pm (solid phase)
fluorine	F_2	F—F ⟷ 143 pm		143	149
chlorine	Cl_2	Cl—Cl ⟷ 199 pm		199	198
bromine	Br_2	Br—Br ⟷ 228 pm		228	227
iodine	I_2	I—I ⟷ 266 pm		266	272

19 F 9

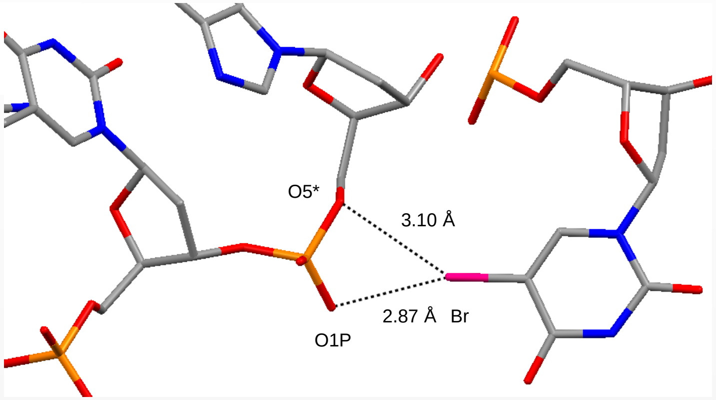
35 Cl 17

80 Br 35

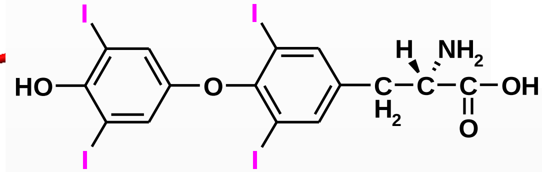
127 I 53

More than 4500 naturally occurring organohalogen compounds are known. Most natural organohalogens contain Cl (2300) or Br (2100), but a significant number contain I (120) or F (30).

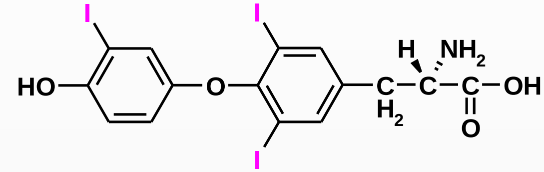
In vivo Functional Halogenation



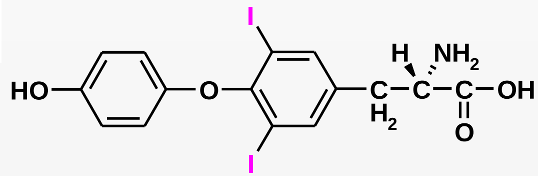
The short halogen bond between the bromine atom of a brominated uracil residue and the phosphate oxygens in a four-stranded DNA junction.



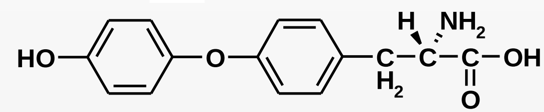
L-thyroxine (T4)
(71 ± 6%)



3,3',5-triiodo-L-thyronine (T3)
(62 ± 4%)

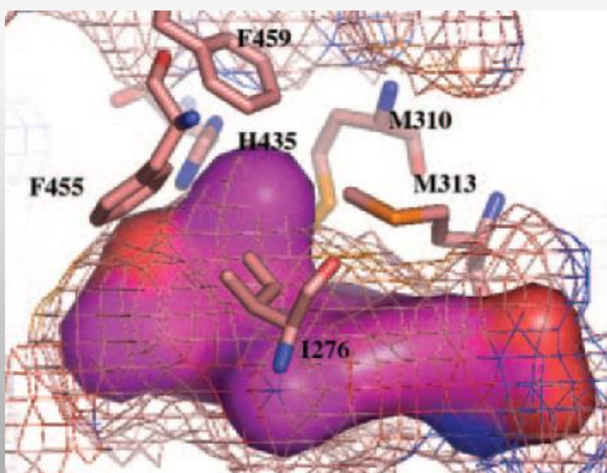


3,5-diiodo-L-thyronine (T2)
(15 ± 2%)



L-thyronine (T0)
(3 ± 1%)

Molecular structures of thyroxine derivatives and the binding activities of the RNA aptamer.

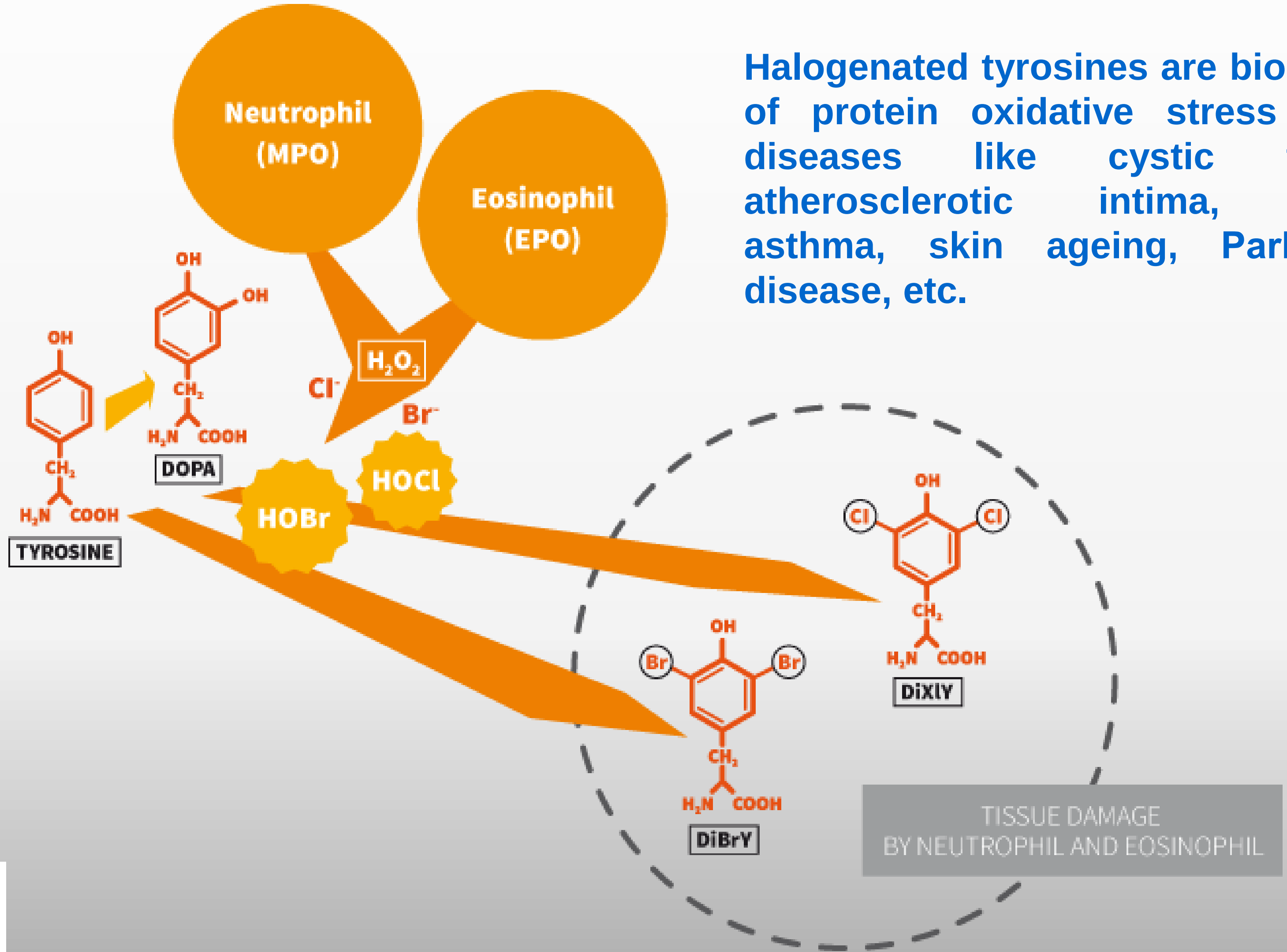


Pocket of the TR-T4 complex.

A. R. Voth, F. A. Hays, P. S. Ho *PNAS* 2007, *104*, 6188; D. Levésque, J.-D. Beaudoin, S. Roy, J.-P. Perreault *Biochem. J.* 2007, *403*, 129; B. Sandler, P. Webb, J. W. Apriletti, B. R. Huber, M. Togashi, S. T. Cunha Lima, S. Juric, S. Nilsson, R. Wagner, R. J. Fletterick, J. D. Baxter *J. Biol. Chem.* 2004, *279*, 55801.

Chem. Soc. Rev. 2011, *40*, 2267.

In vivo Pathological Protein Halogenation

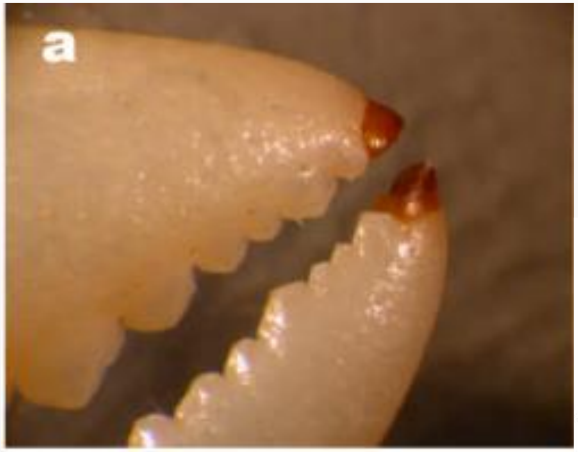


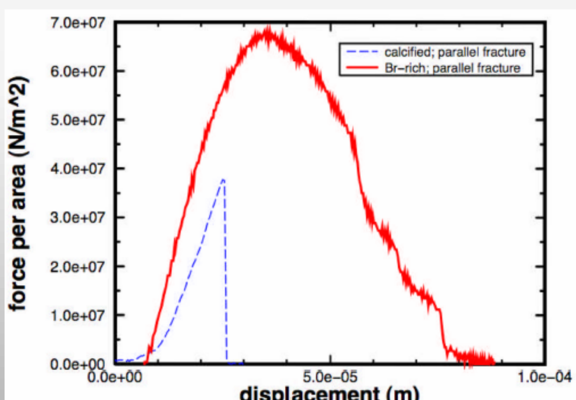
Halogenated tyrosines are biomarkers of protein oxidative stress related diseases like cystic fibrosis, atherosclerotic intima, sepsis, asthma, skin ageing, Parkinson's disease, etc.

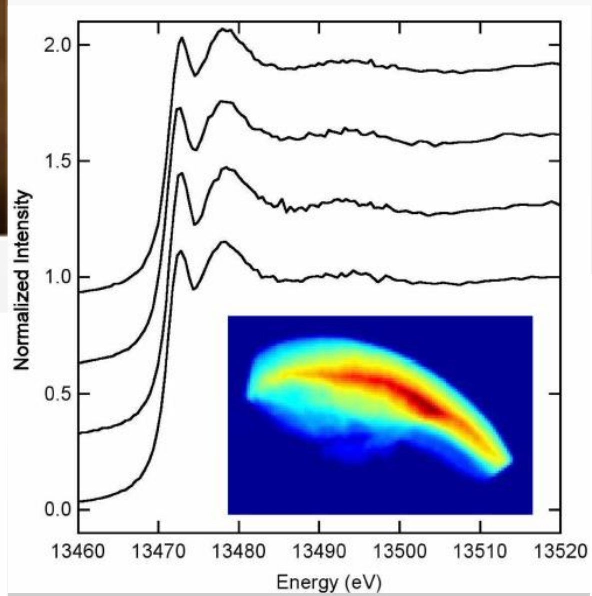
(Project web-site: www.foldalo.it)

Structural Role of *in-vivo* Halogenation

Invertebrates modify the mechanical properties and structures of their biomaterials either via calcification or by the introduction of heavy elements (Br, Zn, Cl, I).







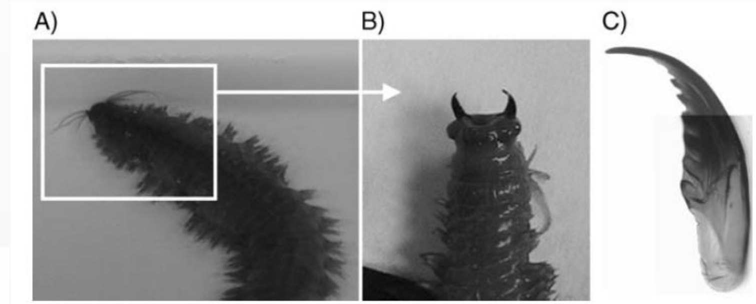
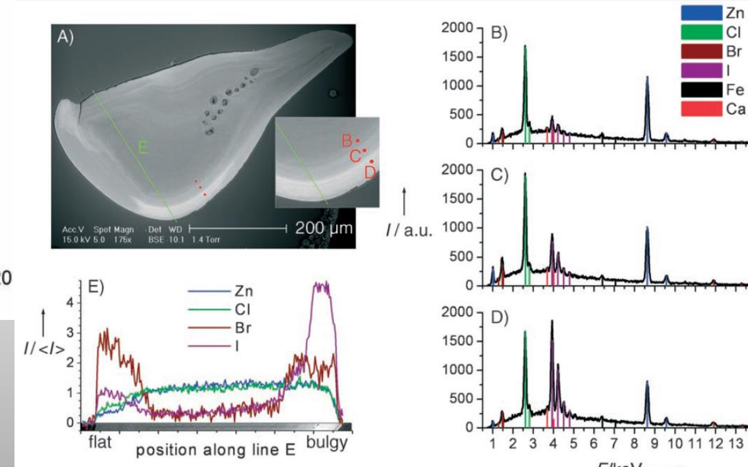


Figure 1. *N. virens* worm A) shown exploring, and B) with everted proboscis; the jaws are shown from the ventral side. C) A jaw extracted from the animal.



R. M. S. Schofield, *et al.*,
***J. Struct. Biol.* 2009, *166*, 272.**

H. Birkedal, *et al.*,
***ChemBioChem* 2006, *7*, 1392.**

The Halogen Bond is...

Chem. Eur. J. 2001, *7*, 2511 (Concept Article, 978 cit.)

Acc. Chem. Res. 2005, *38*, 386 (1764 citations)

Angew. Chem. Int. Ed. 2008, *47*, 6114 (Minireview, 1419 citations)

Science 2008, *321*, 918

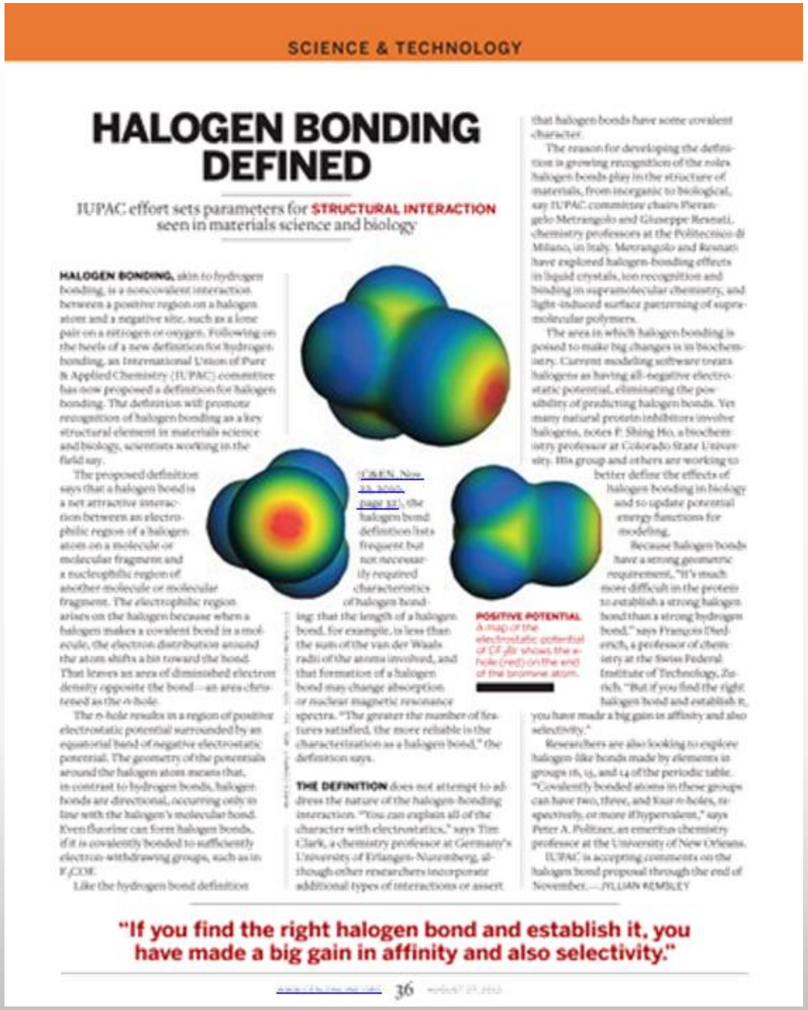
CrystEngComm 2009, *11*, 1187 (Highlight)

Nature Chem. 2011, *3*, 260

Chem. Eng. News 2012, *90*, 36 (27 August)

Pure Appl. Chem. 2013, *85*, 1711
(IUPAC project no. 2009-032-1-100, 1598 cit.)

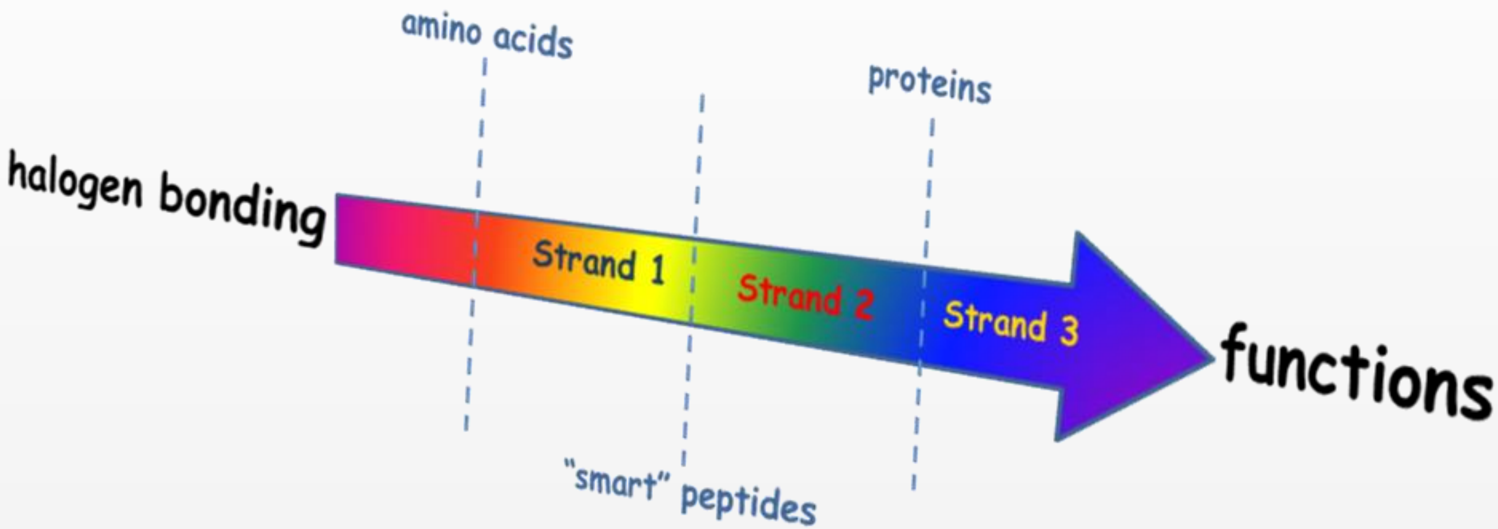
Chem. Rev. 2016, *116*, 2478 (3007 citations)



A halogen bond occurs when there is evidence of a net attractive interaction between an electrophilic region associated with a halogen atom in a molecular entity and a nucleophilic region in another, or the same, molecular entity.

Folding with Halogen Bonding (*FOLDHALO*)

ERC-StG-2012 (Consolidator), PE5-Supramolecular Chemistry



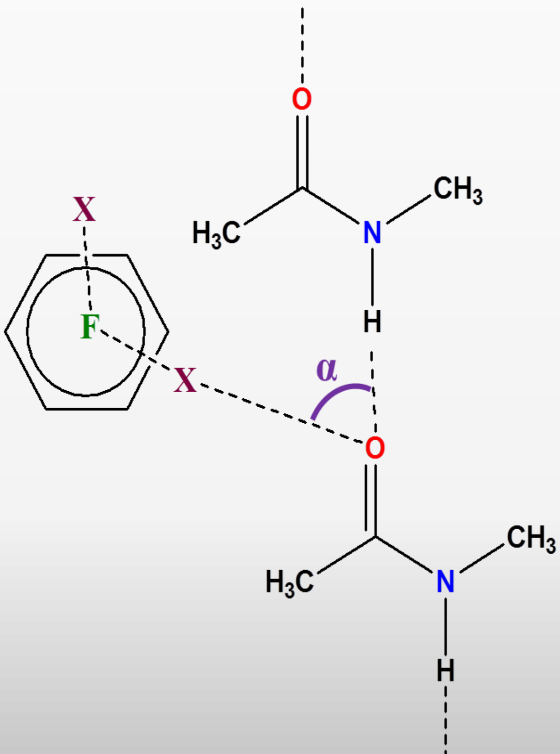
The overall goal of the FOLD*H*ALO project will be to fully elucidate the capabilities and properties of halogen atoms as recognition "sticky" sites in the context of biomolecules.

(Project web-site: www.foldalo.it)

Aalto University

Hydrogen and Halogen Bonds are (Bio)Orthogonal

Remarkable feature, common for **3a-3f**:
nearly orthogonal angle ∠ X···O···N



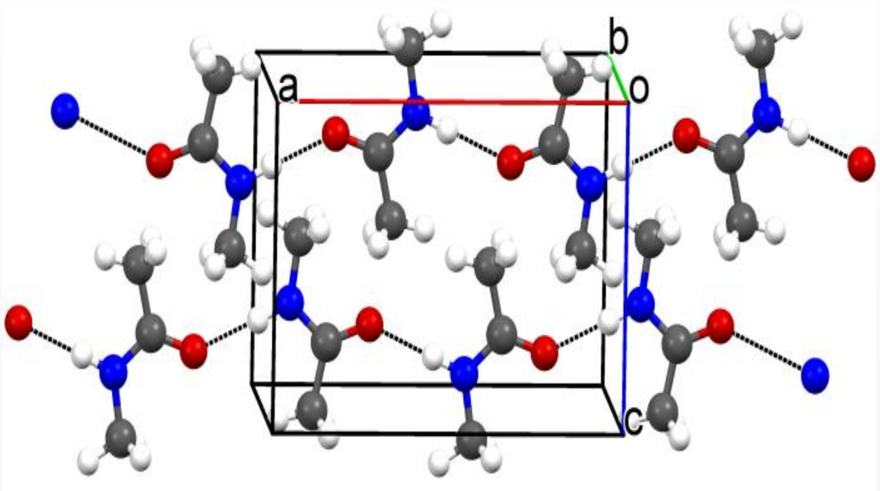
α (∠X···O···N) ≅ 90 °

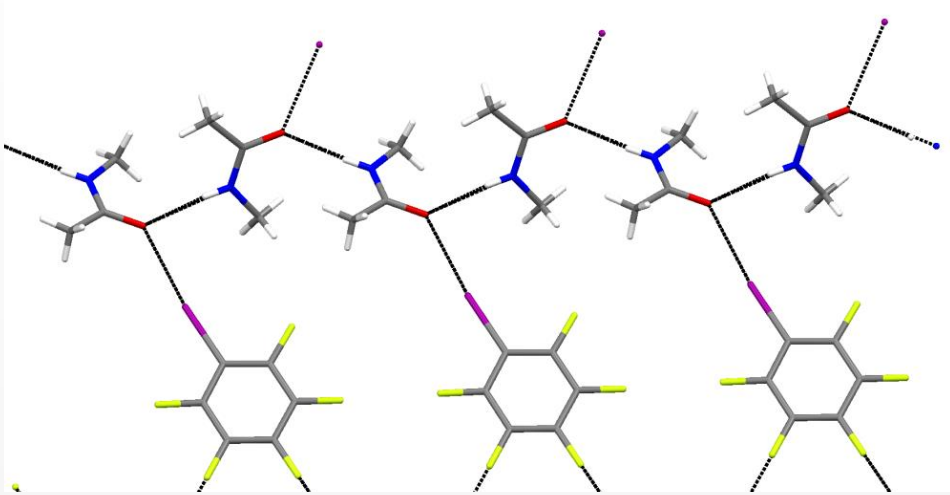
Adduct	α (∠X···O···N), °
3a	97.2
3b	91.9
3c	81.2
3d	82.8; 88.9[a]
3e	81.9
3f	78.9

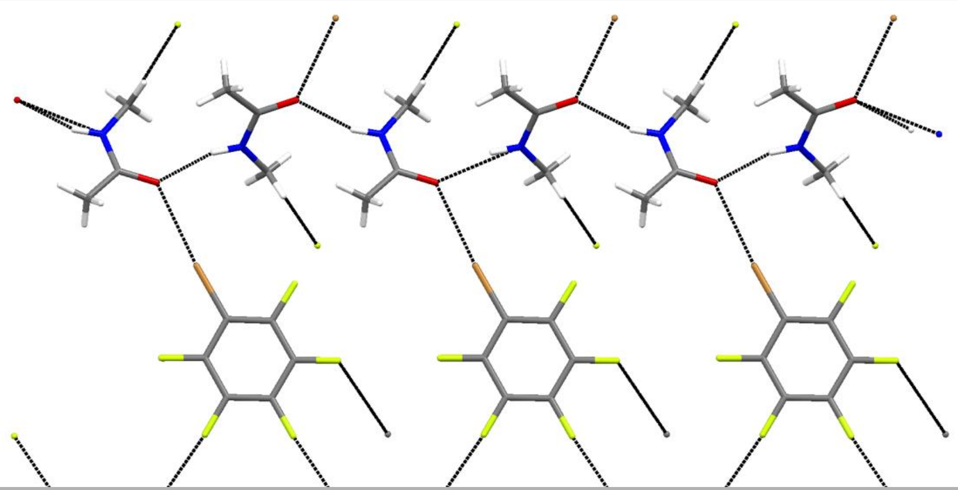
[a] two symmetry independent molecules

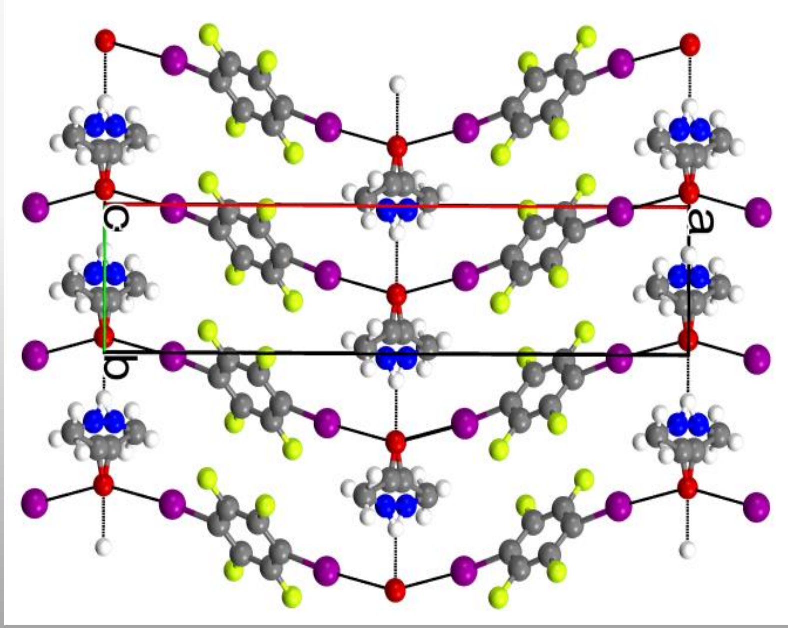
CrystEngComm **2014, *16*, 8102 (Hot Paper).**

Hydrogen and Halogen Bonds are (Bio)Orthogonal







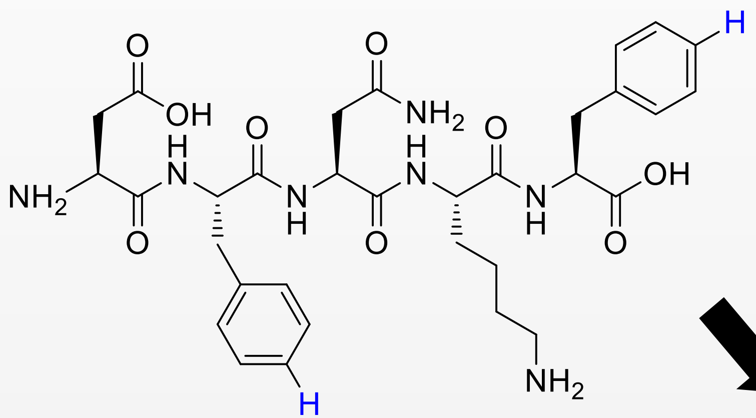


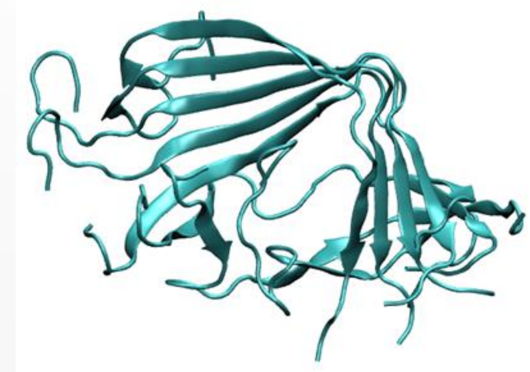
CrystEngComm 2014, *16*, 8102 (Hot Paper).

Single or Double Point Halogen Atom Mutations in a Natural Pentapeptide

Fibrillation of human calcitonin plays a role in thyroid medullar carcinoma

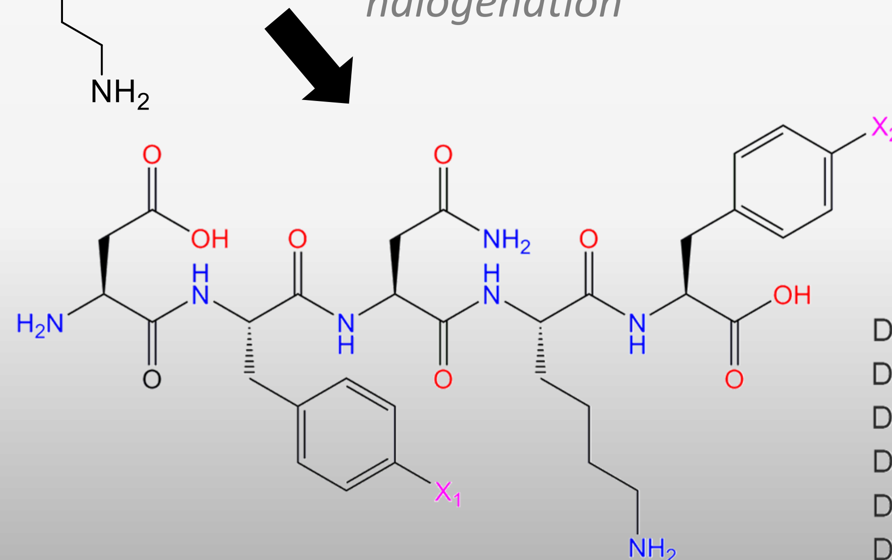
H_2N-CGNLSTCMLGTYTQ**DFNKF**HTFPQTAIGVGAP-COOH



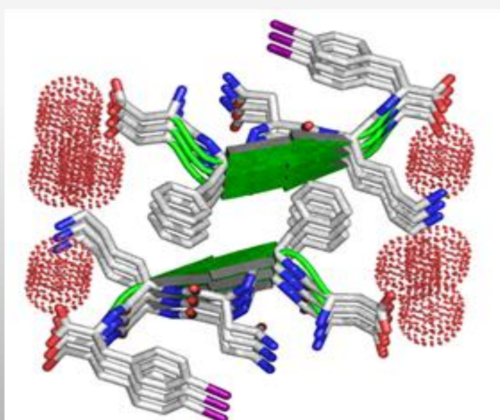


***J. Biol. Chem.* 2017, *292*, 7348.**

halogenation



		X_1	X_2
DFNKF	1	H	H
DFNKF(I)	2	H	I
DF(I)NKF	3	I	H
DF(I)NKF(I)	4	I	I
DF(Br)NKF(Br)	5	Br	Br
DF(Cl)NKF(Cl)	6	Cl	Cl

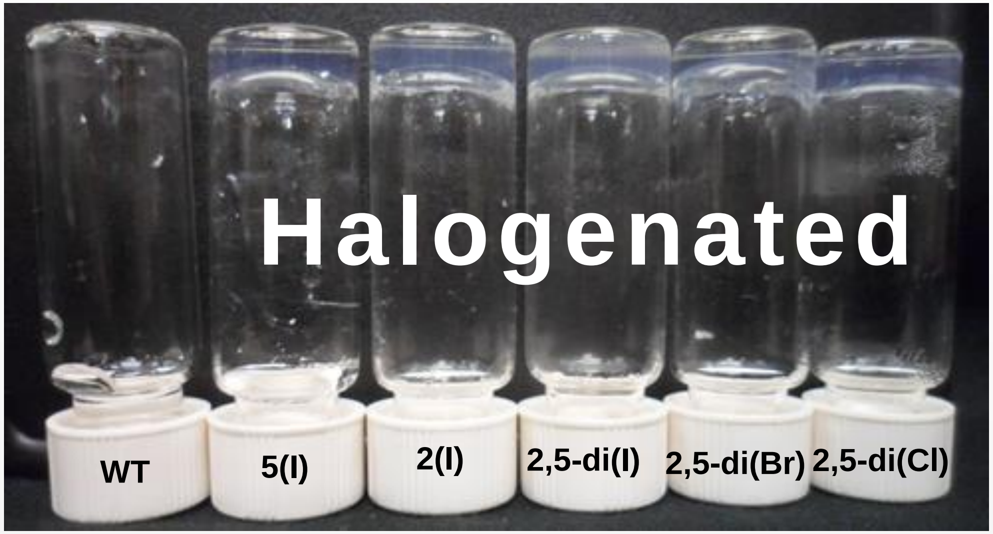


***Chem. Eur. J.* 2017, *23*, 2051.**
(VIP Paper and Front Cover).

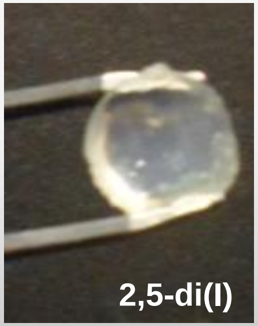
$hCT_{15\text{-}19}$ NH_2-Asp-Phe-Asn-Lys-Phe-COOH
1 2 3 4 5

Finding the best candidates

Hydrogel Formation Efficiency



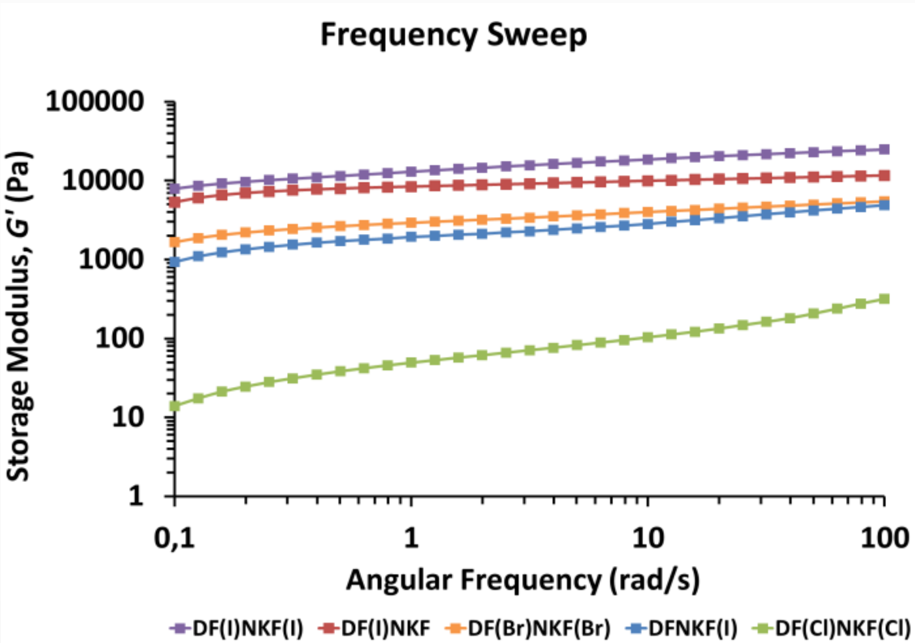
Peptide	Transition T (°C)
5(I)	64.7-69.0
2(I)	88.2-92.7
2,5-di(I)	114.0-119.0
2,5-di(Br)	92.1-96.4
2,5-di(Cl)	78.1-82.4

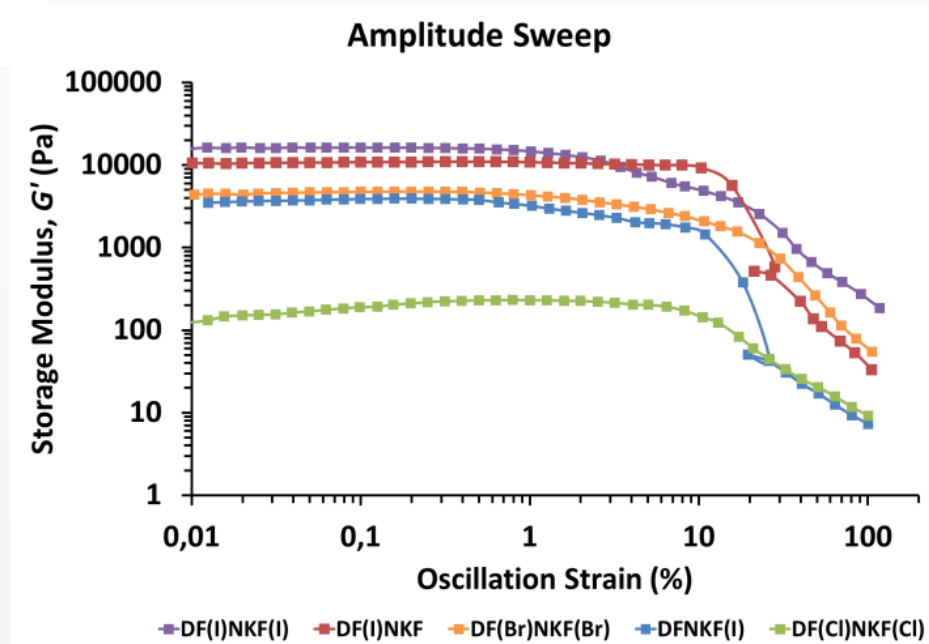


Peptide	Peptide Sequence	Minimum Critical Concentration (mM)		Time required for gelation
1	WT	75	5%	96h
2	5(I)	7.5	0.6%	96h
3	2(I)	2.5	0.2%	168h
4	2,5-di(I)	5*	0.5%	1h
5	2,5-di(Br)	8	0.7%	3h
6	2,5-di(Cl)	5	0.4%	360h

2,5-di(I) > 2,5-di(Br) ≥ 2(I) > 2,5-di(Cl) ≥ 5(I) >> WT

Oscillatory Rheology by Using the Ring-cast Method



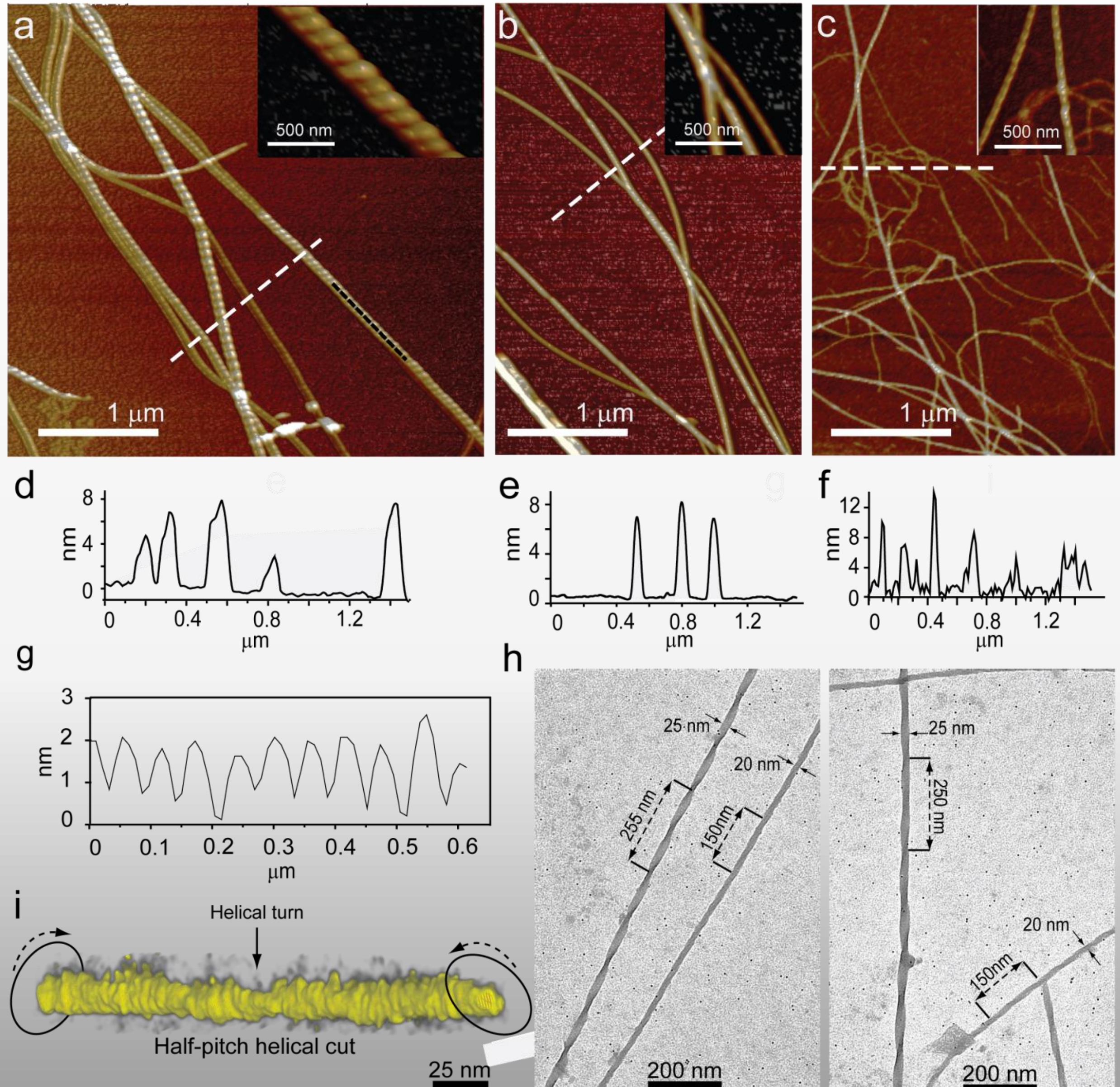


G' > 10^4 Pa

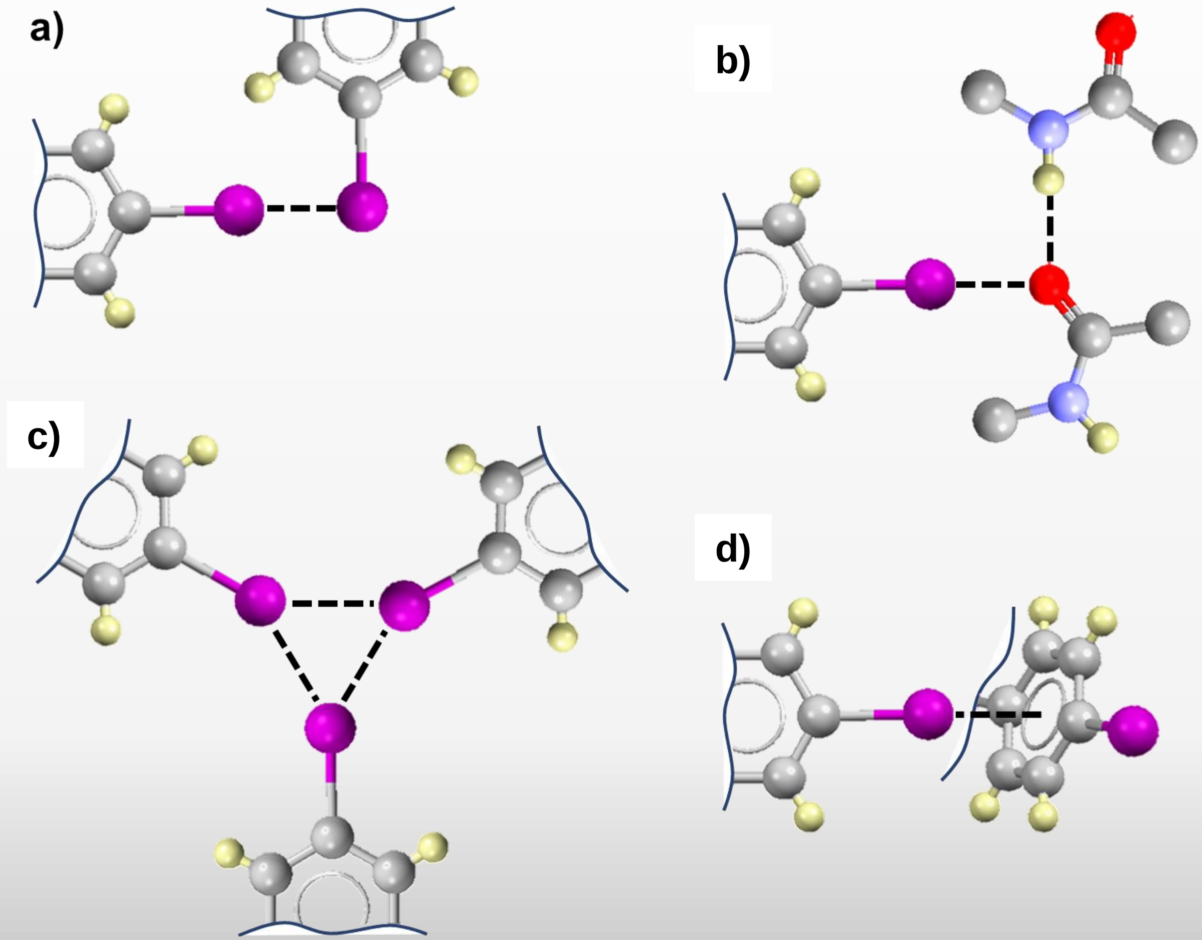
2,5-di(I) > 2(I) > 2,5-di(Br) > 5(I) > 2,5-di(Cl)

***Nature Comm.* 2015, 6:7574, DOI: *10.1038/ncomms8574*.**

Halogenated Fibers Self-Assemble into Helical Twisted Ribbon-like Superstructures

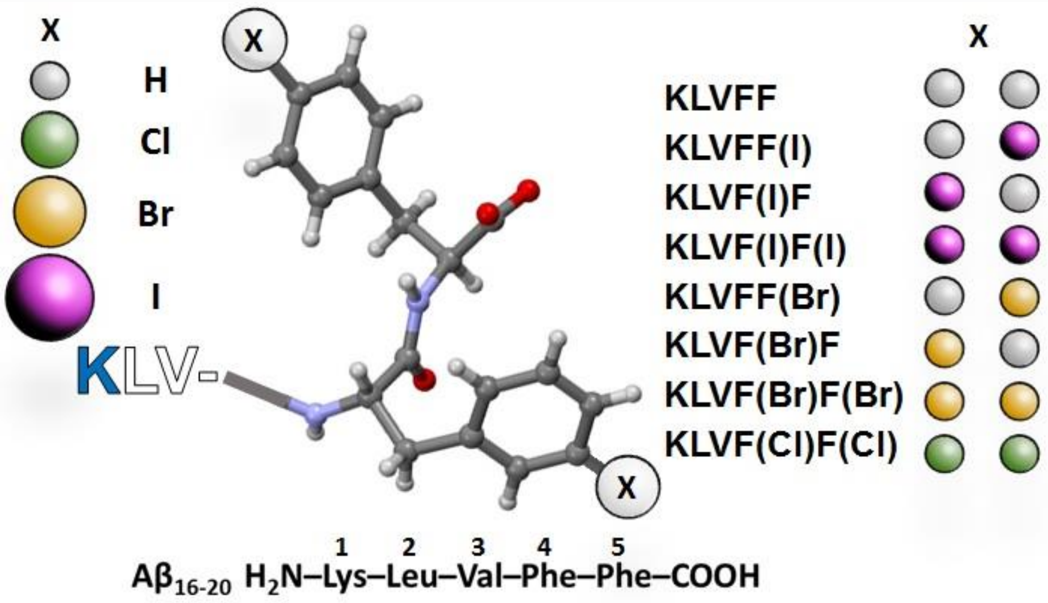


Potential Halogen Bonding Patterns Promoting Fibers Self-Assembly



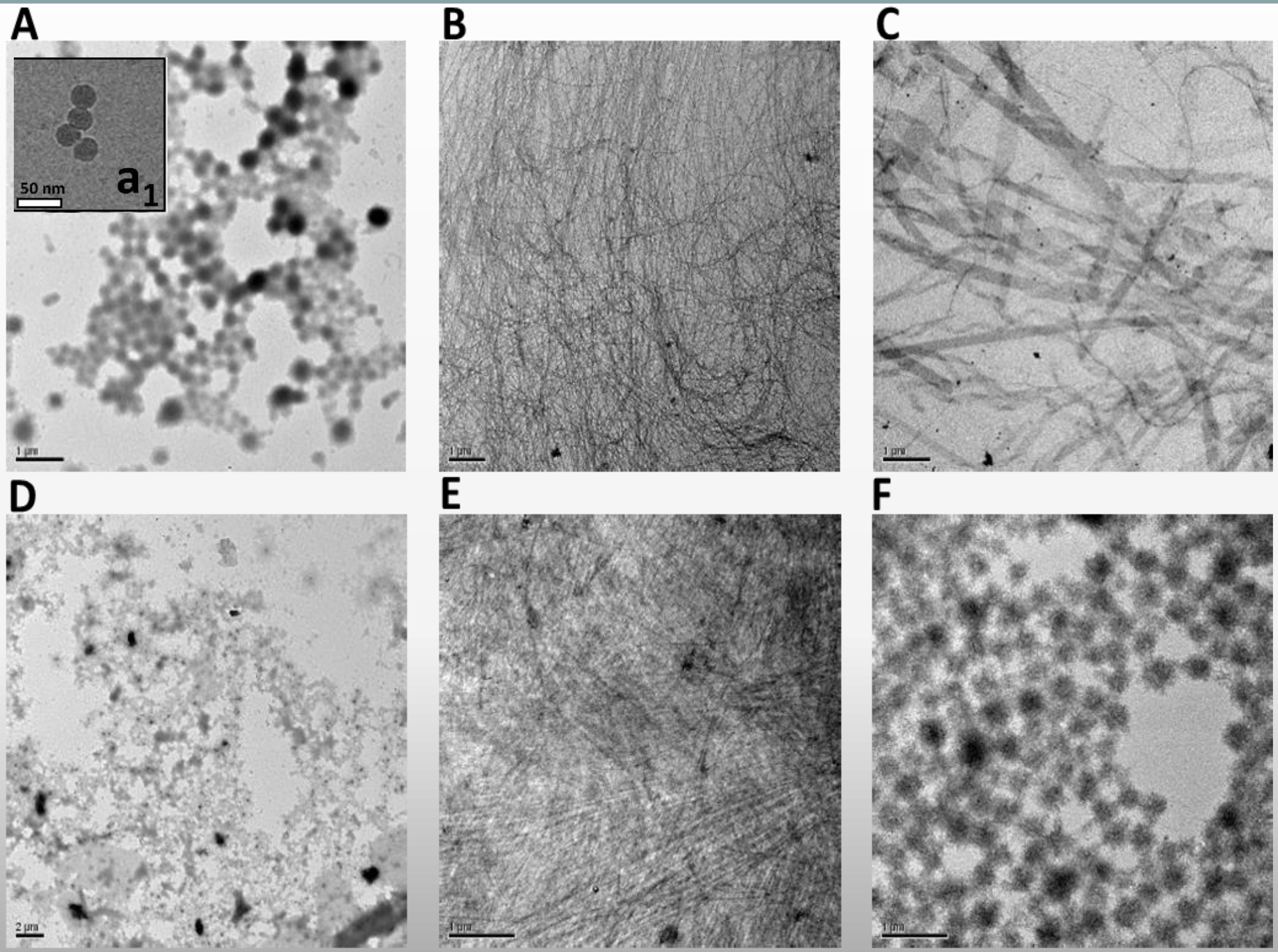
Interactions involving halogen atoms of peptide **2-6** as hydrogen bond acceptors may be ruled out since Cl and Br should give stronger interactions, and fibril formation effects, than I. Interactions involving the ionisable side chains of the peptides, Asp and Lys, may also be ruled out since the formation of these hydrogels is largely pH-independent. Finally, it is unlikely that aromatic $\pi\cdots\pi$ interactions are playing an important role in the hydrogel self-assembly here, since this would not explain the observed dependence on halogen polarizability. Halogen bonding therefore results the most likely interaction responsible for the observed fibril formation effects.

Nanostructure Control over Amyloid Self-Assembly



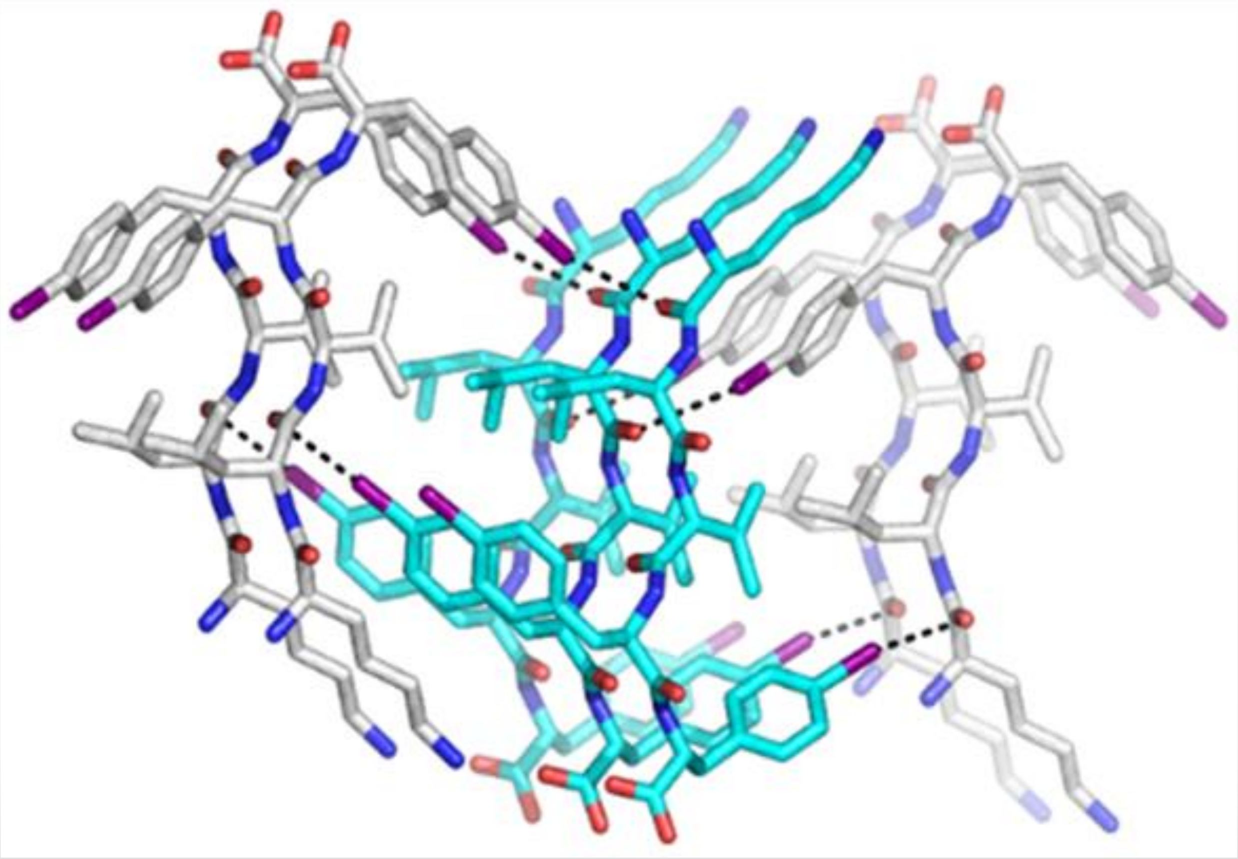
(15 mM Water Solutions)

Nanostructure Control over Amyloid Self-Assembly



Nanoscale 2017, *9*, 9805.

Nanostructure Control over Amyloid Self-Assembly

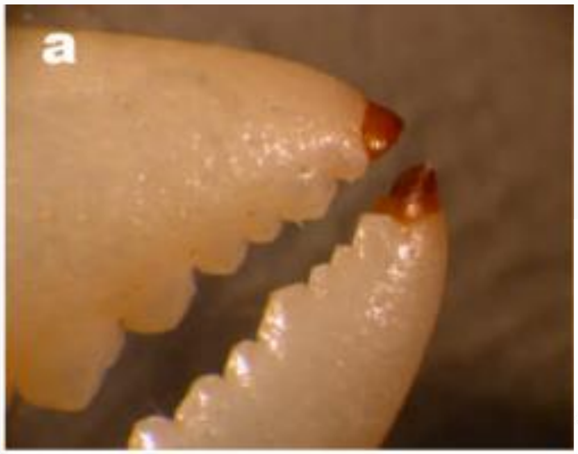


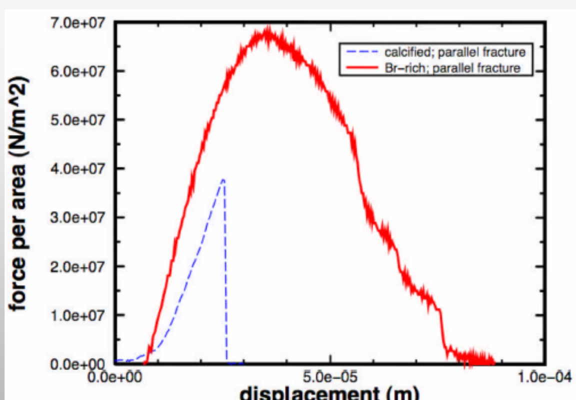
O_1···I_5 3.34(3) Å and O_2···I_4 3.43(3) Å, with C=O···I and O···I-C angles of 111(2)° and 103(2)°, and 165(1)° and 159(1)°, respectively. The O_2···I_4 halogen bond occurs orthogonally[27] to the O_2···N_3 hydrogen bond of the β-sheet (N···O distance 2.99(3) Å; N···O···I angle 88.5(8)°).

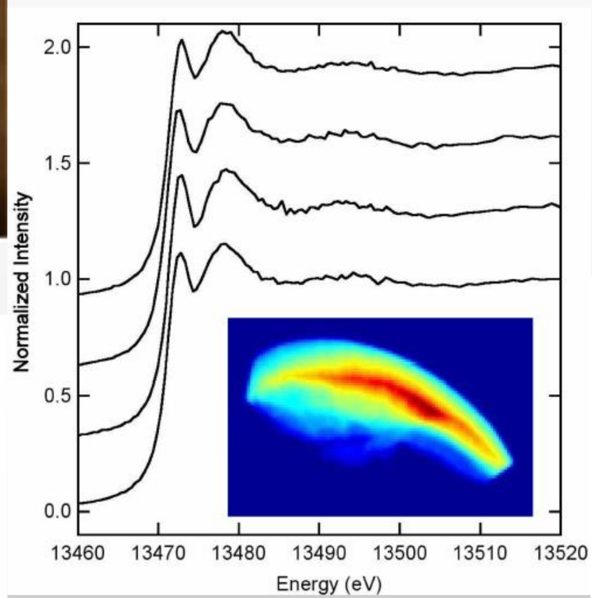
***Nanoscale* 2017, *9*, 9805.**

Functional Role of *in-vivo* Halogenation

Invertebrates modify the mechanical properties and structures of their biomaterials either via calcification or by the introduction of heavy elements (Br, Zn, Cl, I).







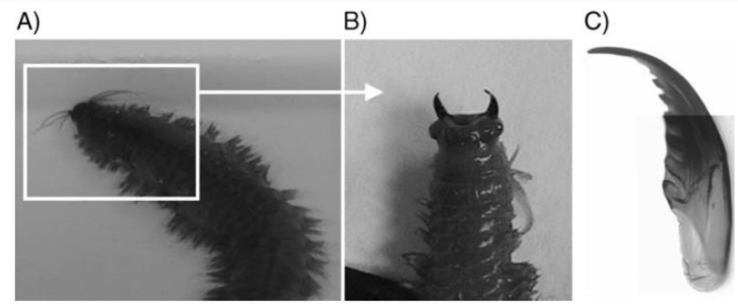
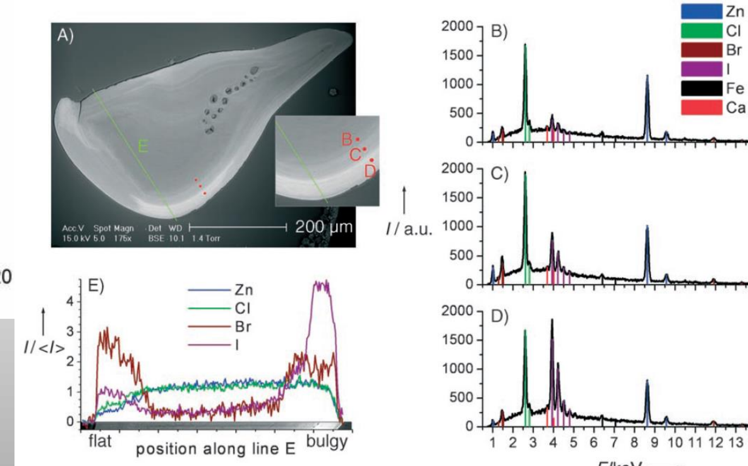


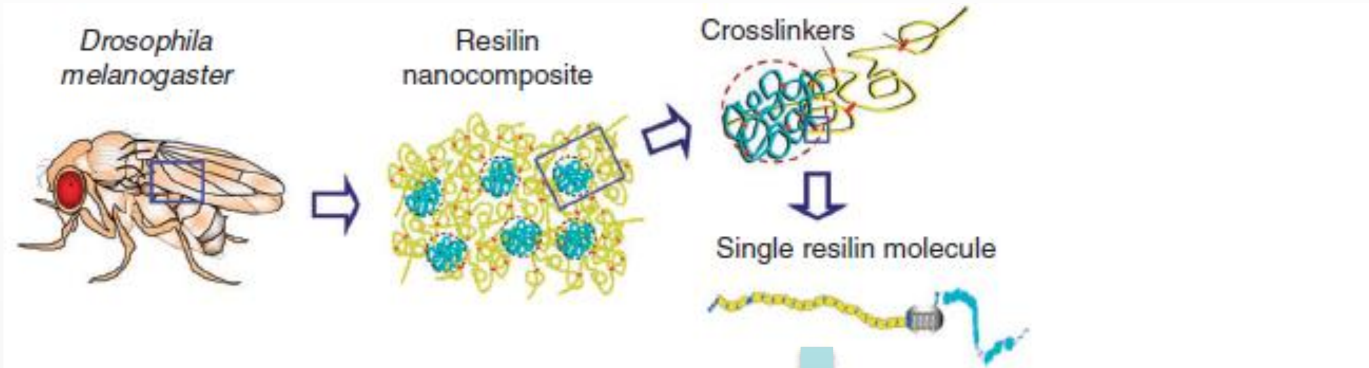
Figure 1. *N. virens* worm A) shown exploring, and B) with everted proboscis; the jaws are shown from the ventral side. C) A jaw extracted from the animal.



R. M. S. Schofield, *et al.*,
J. Struct. Biol. 2009, *166*, 272.

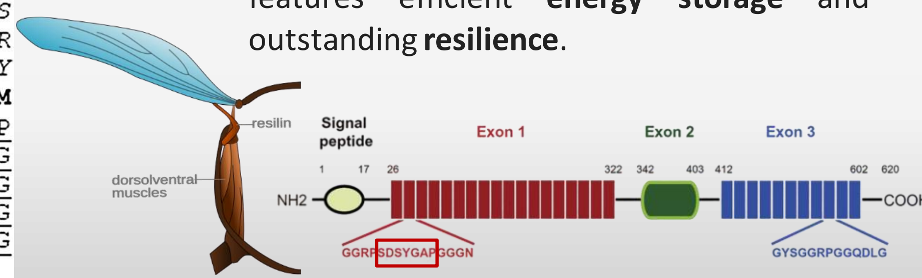
H. Birkedal, *et al.*,
ChemBioChem 2006, *7*, 1392.

Halogenation of Elastomeric Peptides




```
1     MVRPEPPVNS YLPPSDSYGA PGQSGPGGRP SDSYGAPGGG NGGRPSDSYG
51    APGQGQGQGQ GQGGYAGKPS DTYGAPGGGN GNGGRPSSSY GAPGGGNGGR
101   PSDTYGAPGG GNGGRPSDTY GAPGGGGNGN GGRPSSSYGA PGQGQGNGNG
151   GRSSSSYGAP GGGNGGRPSD TYGAPGGGNG GRPSDTYGAP GGGNNGGRPS
201   SSYGAPGGGN GGRPSDTYGA PGGGNGNGSG GRPSSSYGAP GQGQGGFGGR
251   PSDSYGAPGQ NQKPSDSYGA PGSGNGNGGR PSSSYGAPGS GPGGRPSDSY
301   GPPASGSGAG GAGGSGPGGA DYDNDEPAKY EFNYQVEDAP SGLSFGHSEM
351   RDGDFTTGQY NVLLPDGRKQ IVEYEADQQG YRPQIRYEGD ANDGSGPSGP
401   GGPGGQNLGA DGYSSGRPGN GNGNGNGGYS GGRPGGQDLG PSGYSGGRPG
451   GQDLGAGGYS NGKPGGQDLG PGGYSGGRPG GQDLGRDGYS GGRPGGQDLG
501   ASGYSNGRPG GNGNGGSDGG RVIIGGRVIG GQDGGDQGYS GGRPGGQDLG
551   RDGYSSGRPG GRPGGNGQDS QDGQGYSSGR PGQGGRNGFG PGGQNGDNDG
601   SGYRY
```

Resilin is a **native elastomeric protein** which features efficient **energy storage** and outstanding **resilience**.

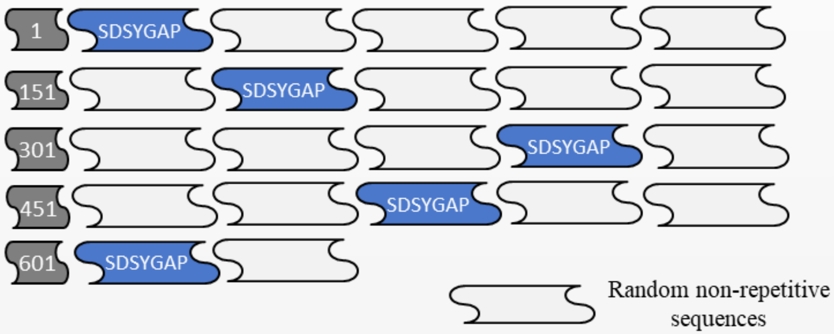


Resilin sequence ~ 600 amino acids

Halogenation of Elastomeric Peptides

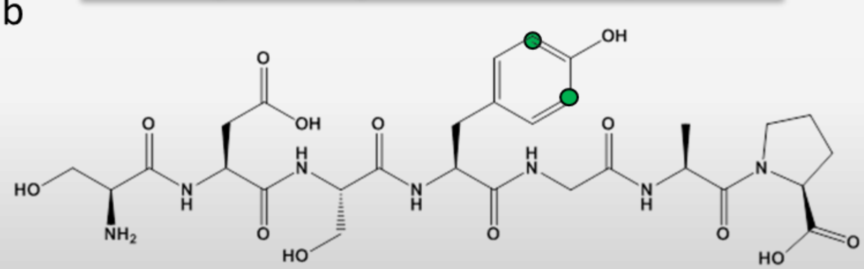
Small peptide fragment from full-length resilin - SDSYGAP

a



Engineered sequence – SDSY(3,5-Br)GAP

b



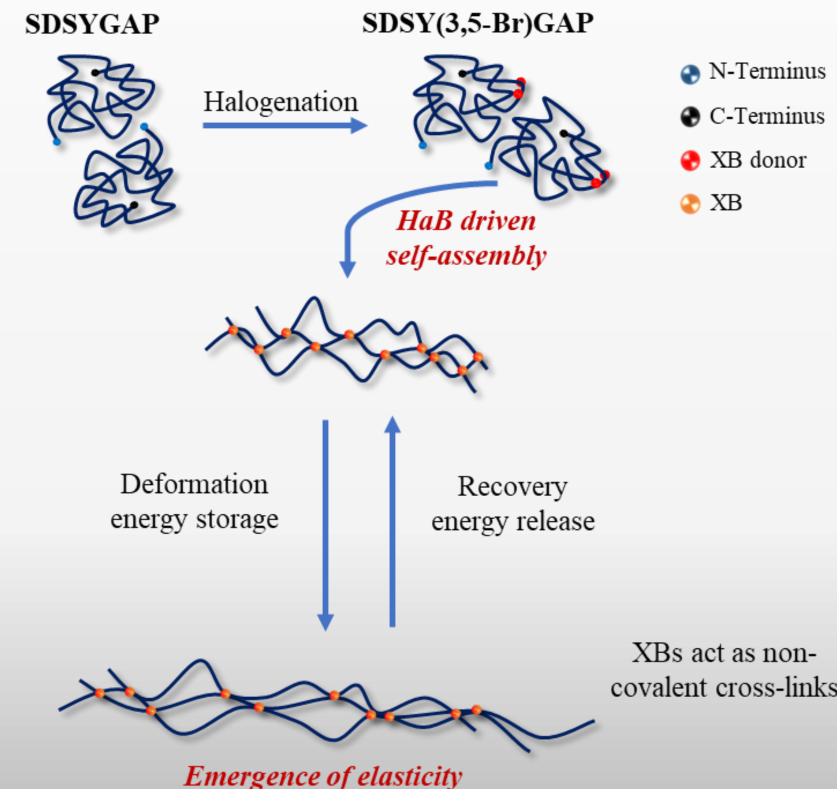
Halogenation target

Res-Br H_2N-S-D-S-Y(3,5-Br)-G-A-P-COOH

Res-Wt H_2N-S-D-S-Y-G-A-P-COOH

Self-assembly and elastomeric properties upon halogenation

c

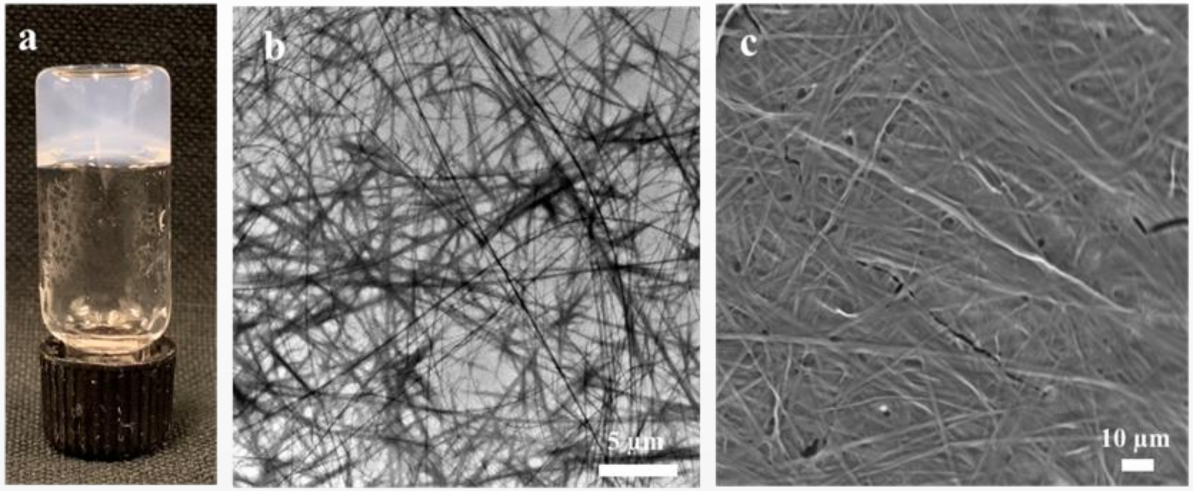


Small 2022, *18*, 2200807.

Halogenation of Elastomeric Peptides

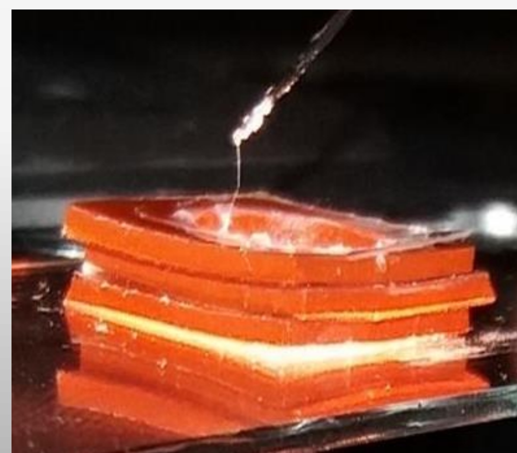
SWITCH 2 PRODUCT

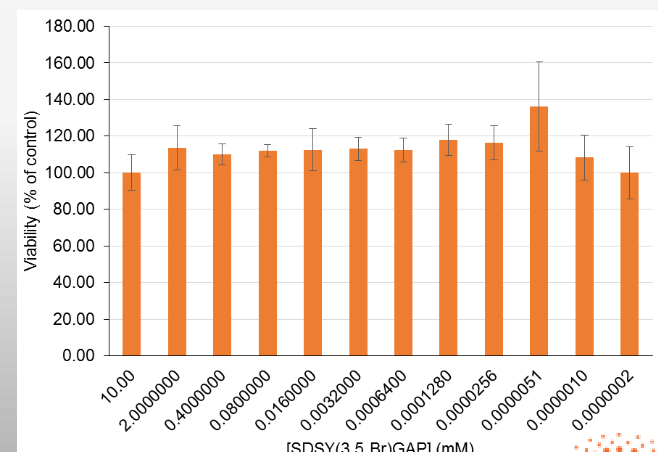
MINIRES



H_2N-S-D-S-Y-G-A-P-COOH

WO2019116342A1





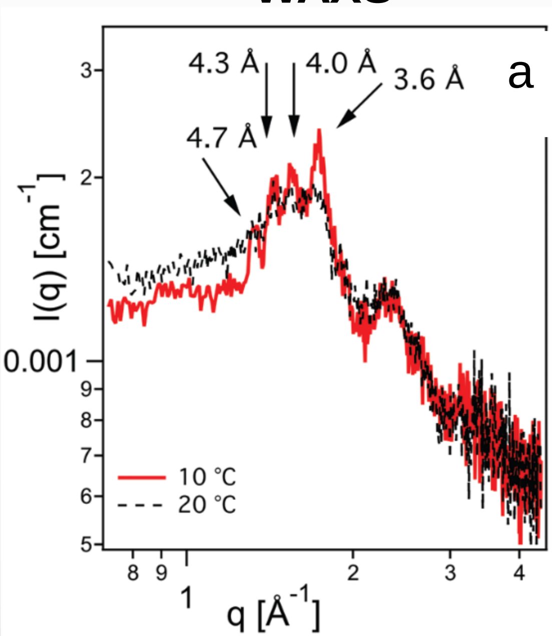
In collaboration with Markus Linder, Aalto University, Helsinki, Finland.

PoC2017 "A Minimalist Peptide Elastomer" – Switch2Product Innovation Challenge 2017 Champion

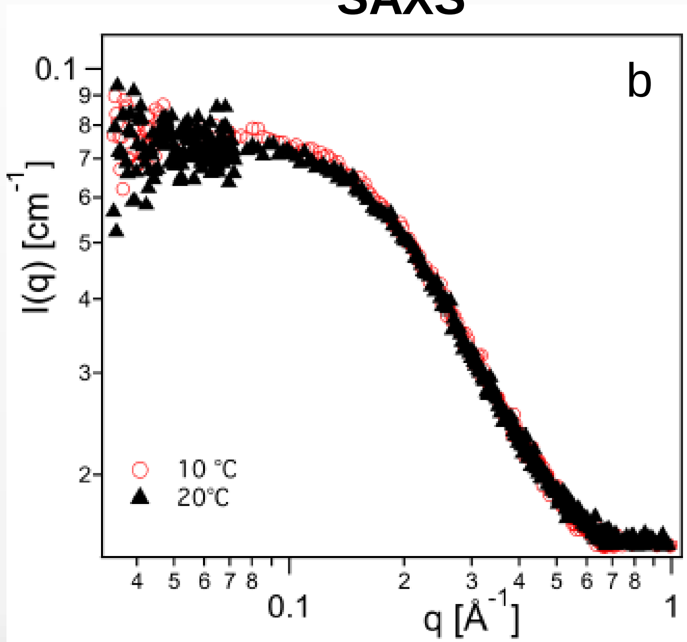
Halogenation of Elastomeric Peptides

200 mM **Res-Br** hydrogels and **Res-Wt** solutions

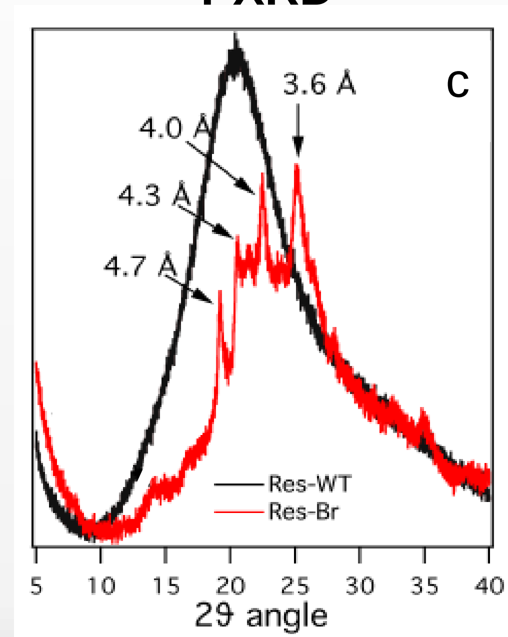
WAXS



SAXS



PXRD



q values among 1-4 Å^{-1}
Four distinct peaks correlated with a structural order <u>typical of an amyloidal cross-β architecture</u>

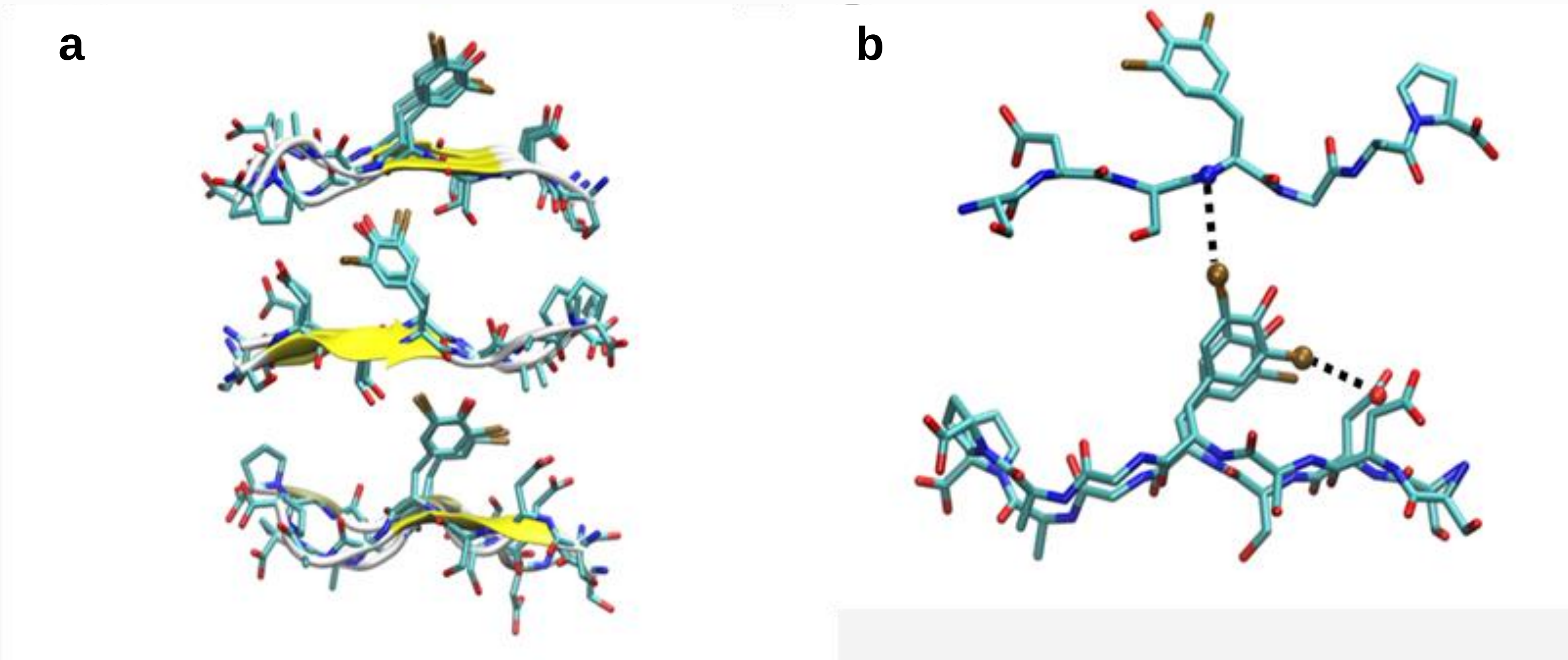
The scattering profile of the wild-type sequence is typical of <u>unstructured</u> proteins

Shows **diffraction peaks** consistent with a **cross-β architecture typical of amyloid materials**

Small **2022, *18*, 2200807.**

in collaboration with Pierre Panine - Xenocs SAS - Grenoble, France

Halogenation of Elastomeric Peptides



a. In the PAN (Parallel, Antiparallel, C-to-N) configuration, **parallel β-sheets** of Res-Br are stabilized by hydrogen bonds, in addition to **intermolecular π···π stacking between paired di-bromo-Tyr rings**

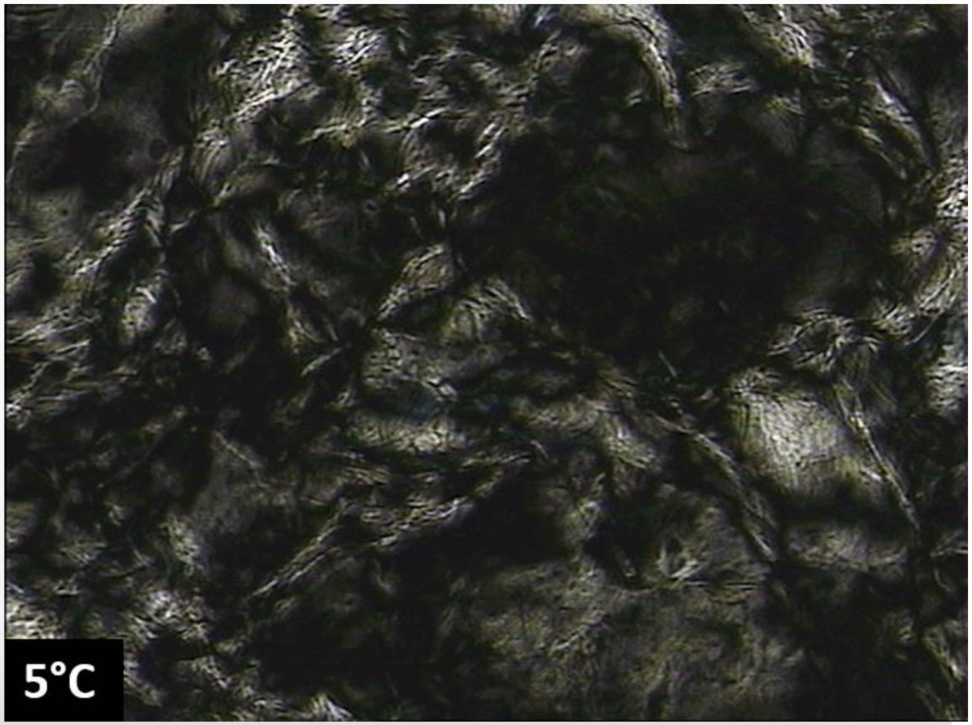
b. Bromine atoms exert a key role in strengthening peptide self-assembly thanks to XBs.

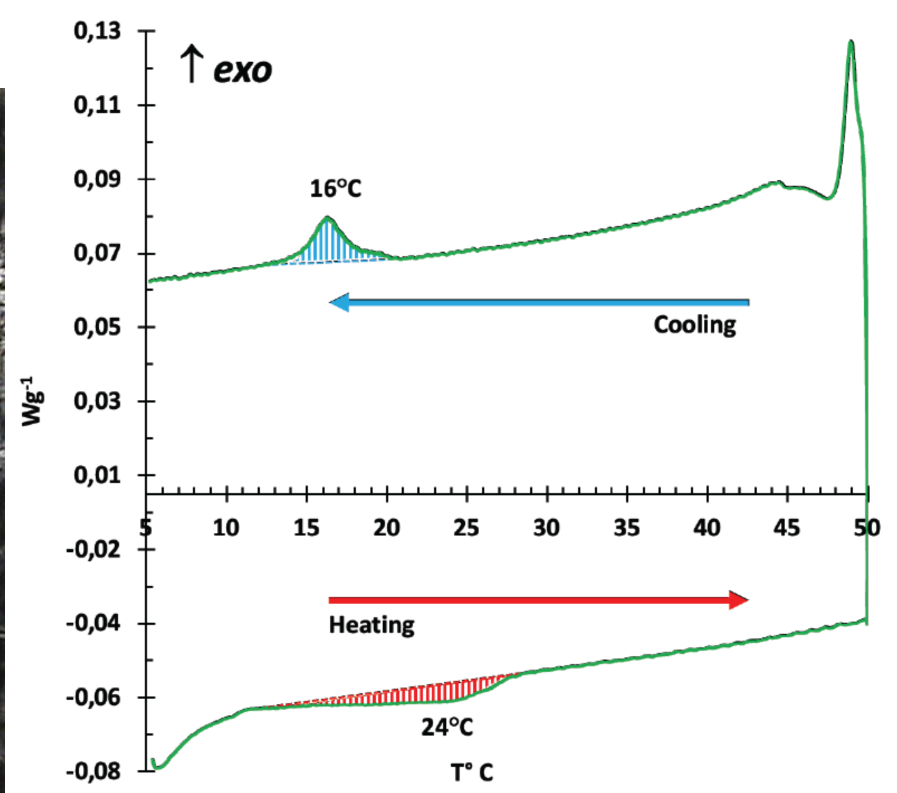
Small **2022**, *18*, 2200807.

in collaboration with Alfonso Gautieri of DEIB, Politecnico di Milano

Halogenation of Elastomeric Peptides

Heating from 5°C to 32°C





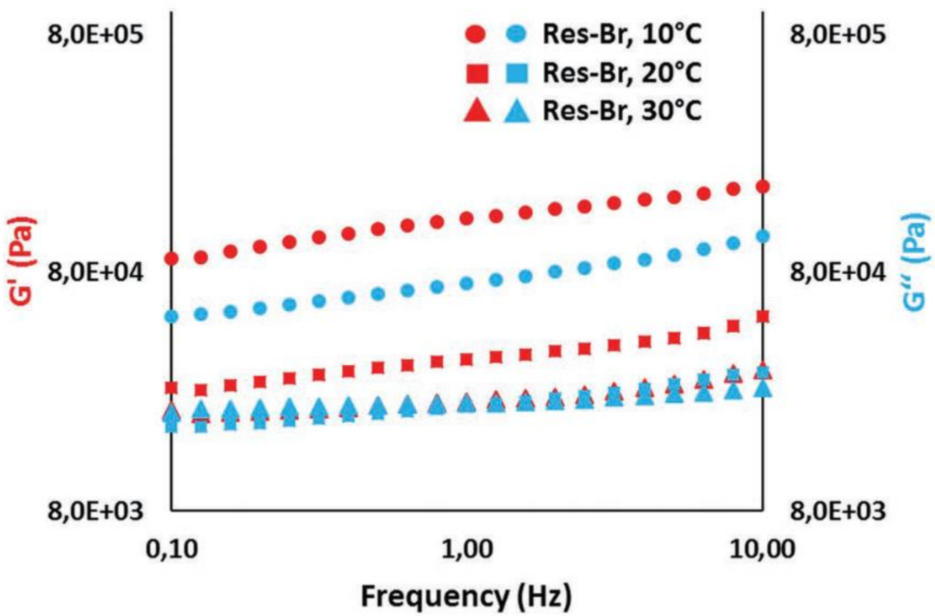
Polarized optical microscopy (POM):
The birefringent self-assembled peptide network progressively dissolves with increasing temperature

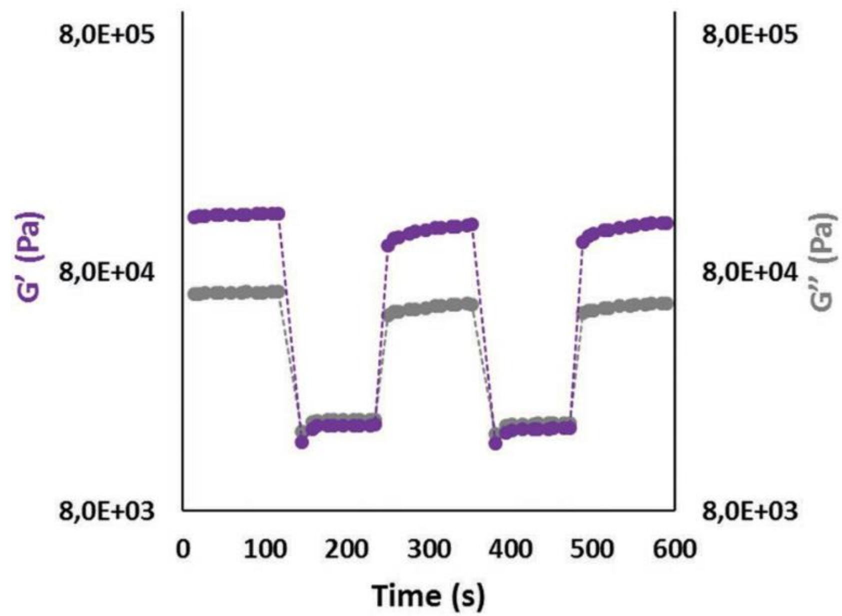
Differential Scanning Calorimetry (DSC):
Endotermic transition close to room temperature (24°C), consistent with **peptide solubilization** hence **disassembly of the supramolecular architecture**. Formation of the gel network is **thermoreversible**, with an exothermic signal in the range 18-15 °C

Small **2022**, *18*, 2200807.

Halogenation of Elastomeric Peptides

200 mM gel of brominated peptide; analyzed at controlled temperature



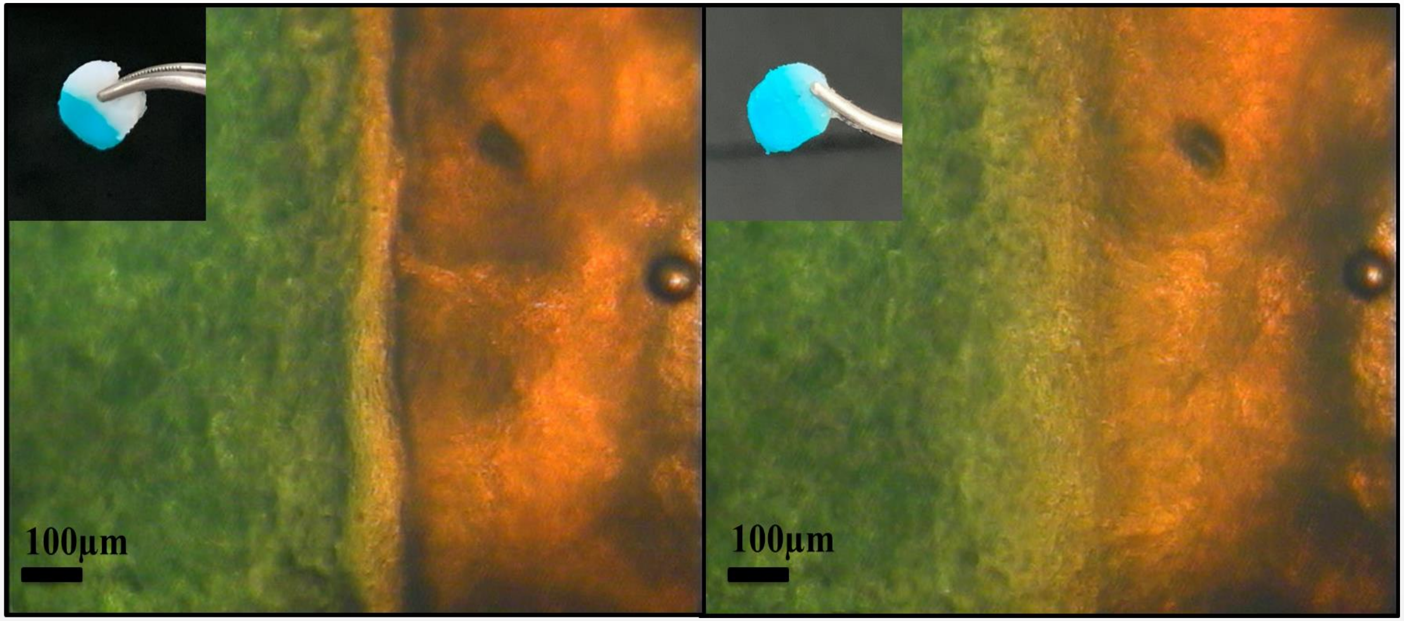


- **Predominance of elastic component (G' > G'')** for the tested hydrogels
- The **elastic modulus decreases with temperature**
- Step test to describe the regeneration of the material after deformation; **recovery interval there is a rapid and almost total recovery of the structure**

In collaboration with Antonio Grande - DAER - *Politecnico di Milano*

Small **2022**, *18*, 2200807.

Halogenation of Elastomeric Peptides



The microscopy images show the <u>initial (left) and final (right) stage</u> of the healing process, with the **disappearance of the joint between the two pieces of hydrogel**. The overall process takes **5 minutes**.

The insets show the macroscopic appearance of the **joint pieces of gel at the initial stage (left) and 24 h later (right)**. <u>*After healing, diffusion of the blue dye occurs in the whole sample.*</u>

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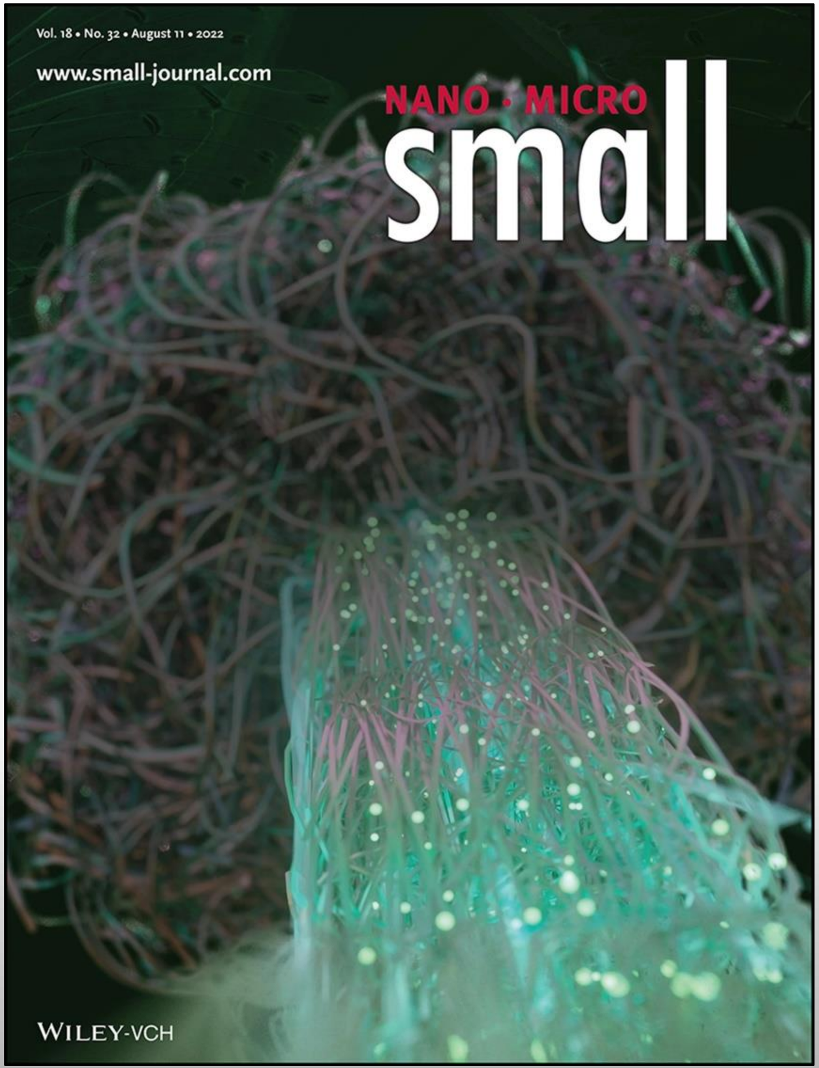
Acknowledgements

A. Pizzi



L. Sori

A. Pizzi, L. Sori, *et. al.*,
***Small* 2022, *18*, 2200807.**

A resilin-derived heptapeptide

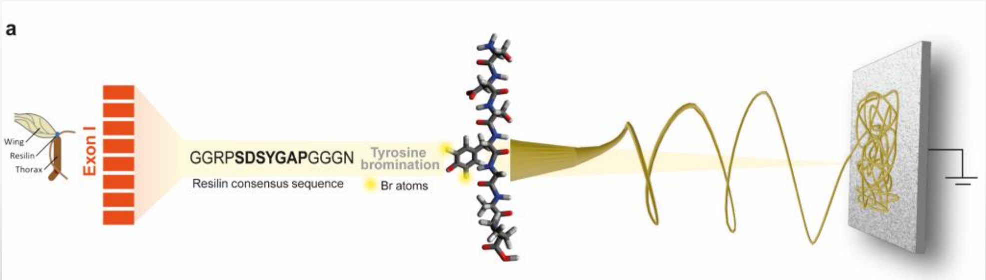
E. Marelli

Resilin is a **native elastomeric protein** which features efficient **energy storage** and outstanding **resilience**.

Self-assembly process
relying on peptide molecular interactions

Small, **2022**, 18, 2200807

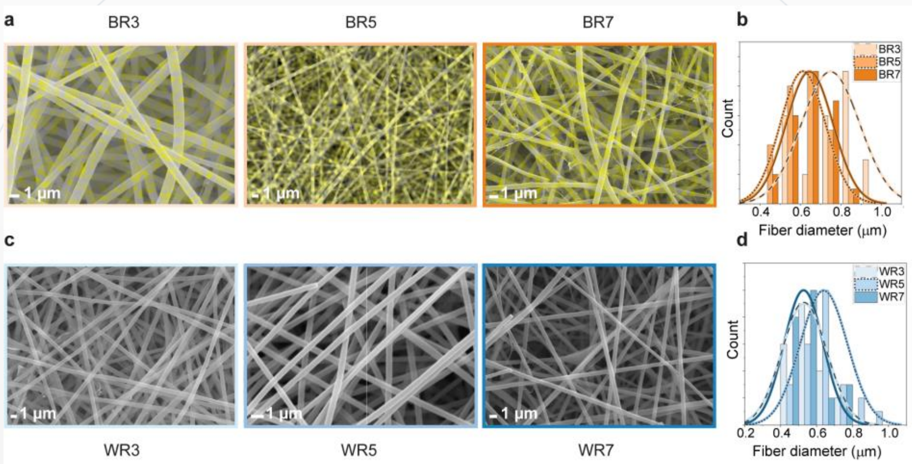
Directed assembly can be imposed through external constraints forming macroscopic materials



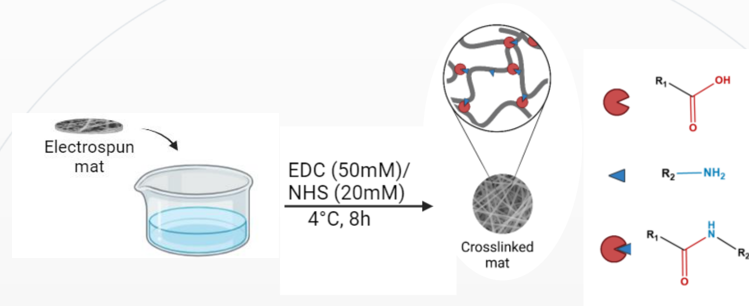
Use of **electrospinning** to direct the assembly of SDSY(3,5Br)GAP into nanofibers with higher control over material microstructure.

Halogenation of Elastomeric Peptides

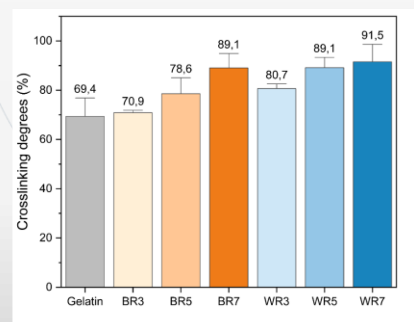
Electrospinning and crosslinking procedures



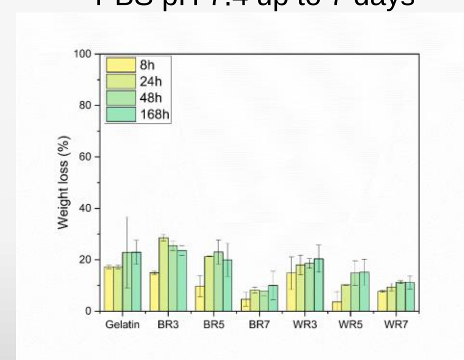
- Concentration 15% *m/v*-30% *m/v*
- Solvent: CH_3COOH:H_2O 1:1
- Voltage: 12-15kV
- Flow rate: 254 µL/h
- Distance from collector: 12 cm



Crosslinking degrees evaluated *via* TNBS



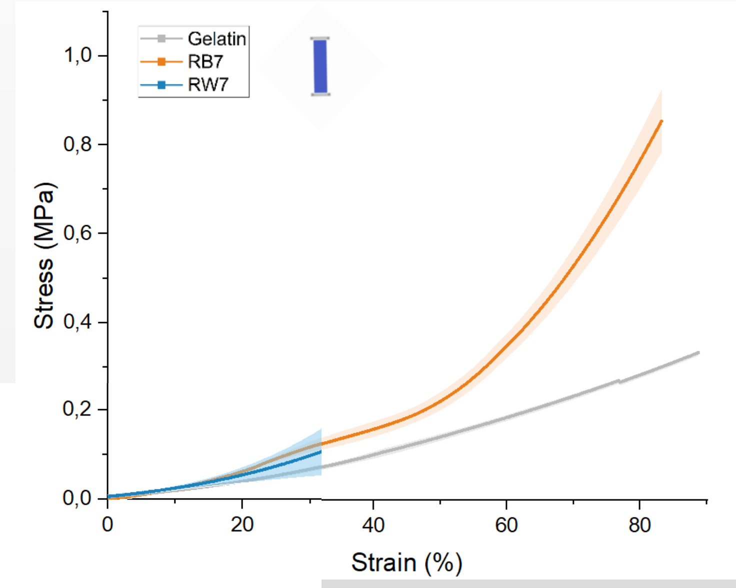
Scaffold stability evaluated in PBS pH 7.4 up to 7 days



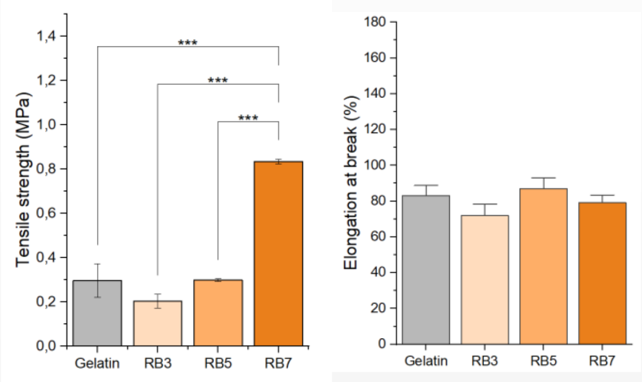
Adv. Funct. Mater.* 2025, *in press.

Static mechanical properties

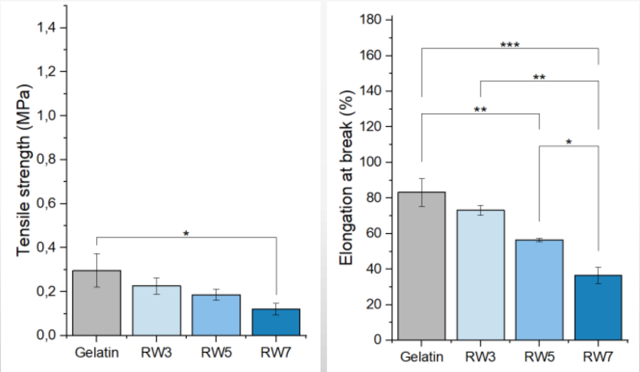
The mechanical properties of the matrices were tested *via* **tensile testing** after hydration in milliQ for one hour.



- BR7 **strain-hardening behaviour**



- WR7 **brittle** and prone to fracture



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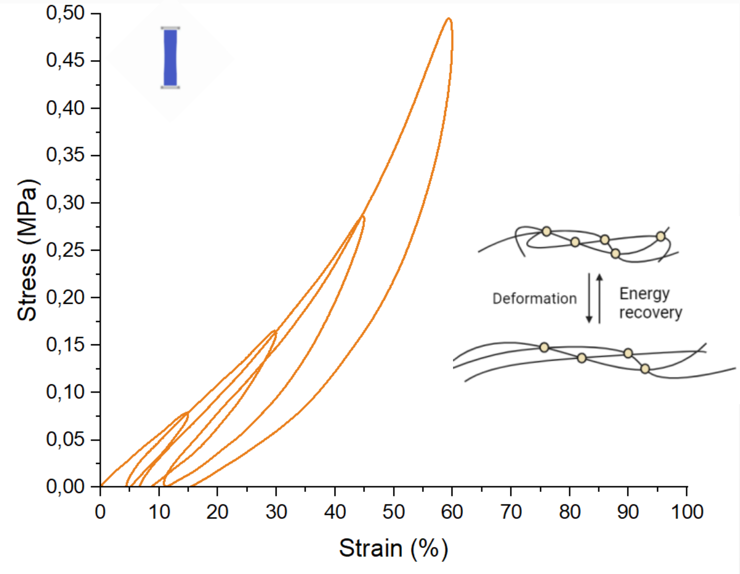
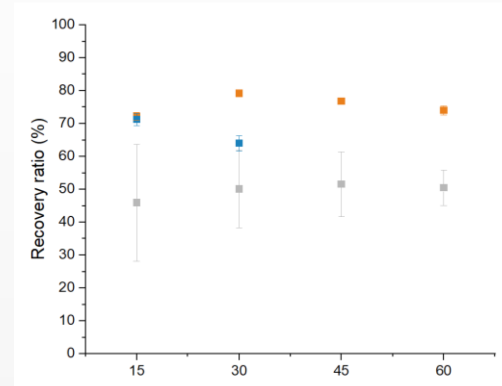
Halogenation of Elastomeric Peptides

Step-cycle mechanical properties



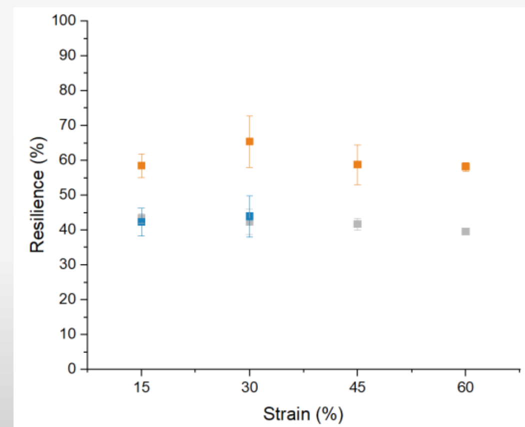
Recovery ratio

$$\frac{\varepsilon_{max} - \varepsilon_{fin}}{\varepsilon_{max}}$$



Resilience

$$\frac{Area_{unloading\ curve}}{Area_{loading\ curve}}$$



- Good recovery ratio
- Relatively low hysteresis
- Resilience>60%

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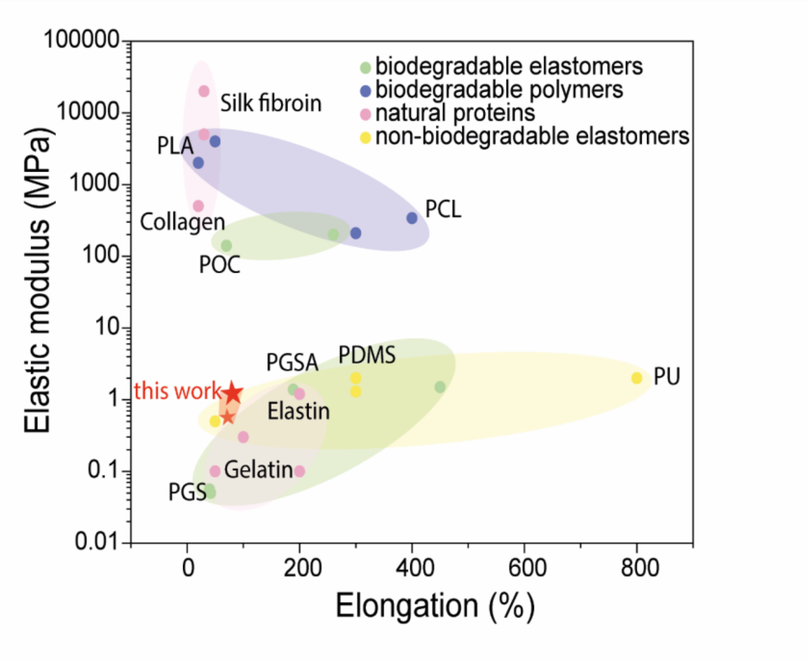
Halogenation of Elastomeric Peptides

Step-cycle mechanical properties



BR7 scaffolds as a promising alternative to conventional bioelastomers

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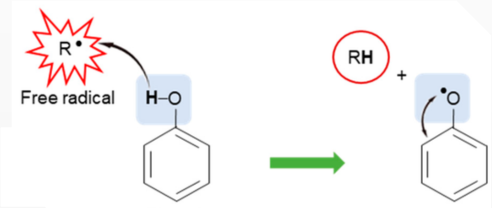
Halogenation of Elastomeric Peptides

Functional properties

Antioxidant properties

SDSYGAP



Tyrosine residues are usually associated with antioxidant activity, primarily due to their phenolic structure

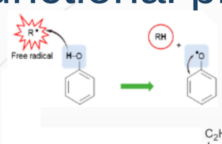
Adv. Funct. Mater. ***2025**, in press.*

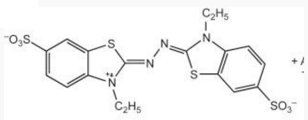
Halogenation of Elastomeric Peptides

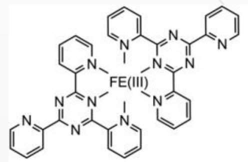
Functional properties

Antioxidant properties

SDSYGAP



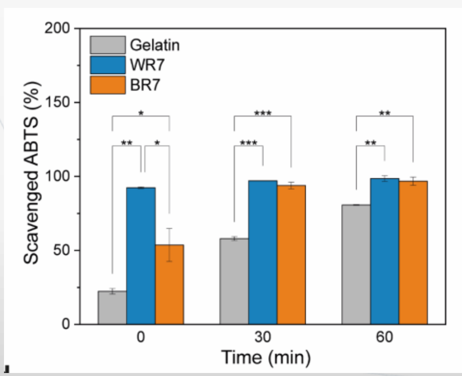


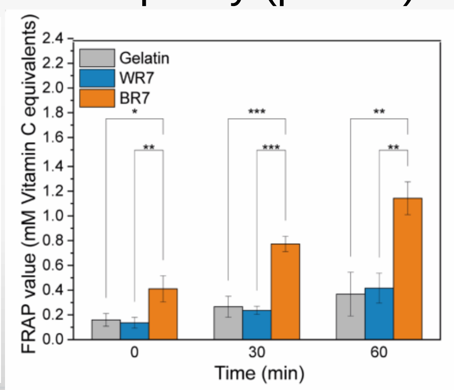


Fe^{3+}-TPTZ

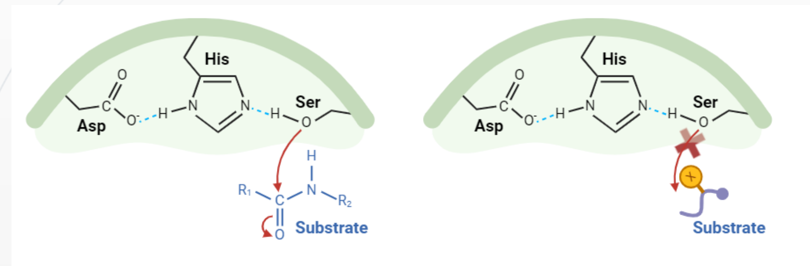
Inhibition of ABTS radicals (pH 7.4)

Fe^{3+} reducing capacity (pH 3.6)





Proteolytic stability

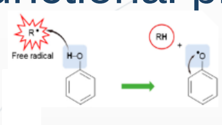


Substituting **natural amino acids** with **halogenated derivatives** can alter interactions with enzymes, potentially enhancing their stability against protease degradation.

***Adv. Funct. Mater.* 2025, in press.**

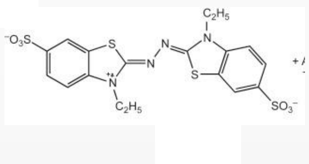
Halogenation of Elastomeric Peptides

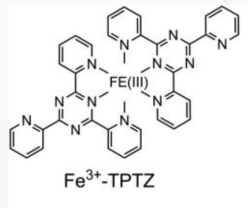
Functional properties



SDSYGAP

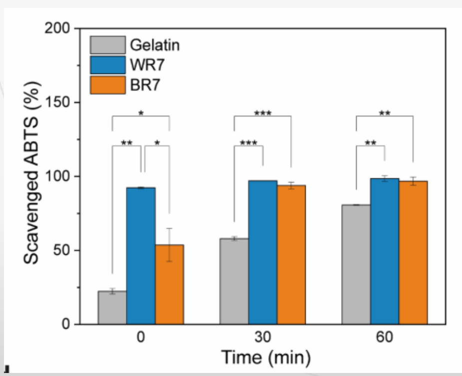
Antioxidant properties

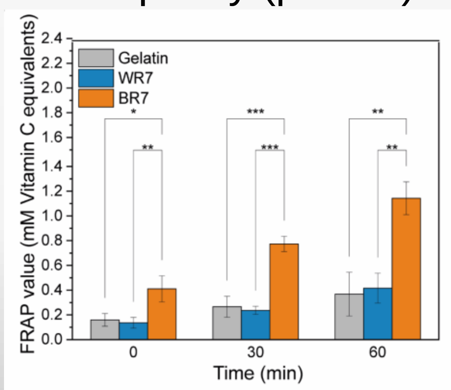


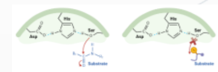


Inhibition of ABTS radicals (pH 7.4)

Fe^{3+} reducing capacity (pH 3.6)

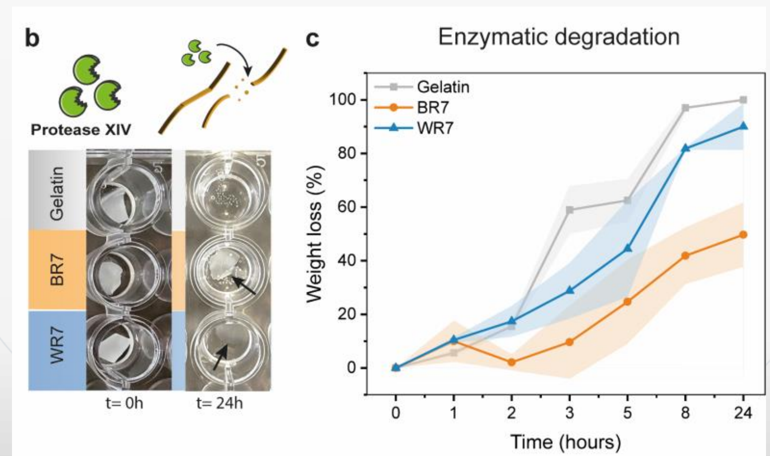






Proteolytic stability

Proteolytic stability was evaluated incubating the samples in a 0.01 U/mL protease XIV solution (pH 7.4), resulting in SDSY(3,5Br)GAP slowing down the degradation.



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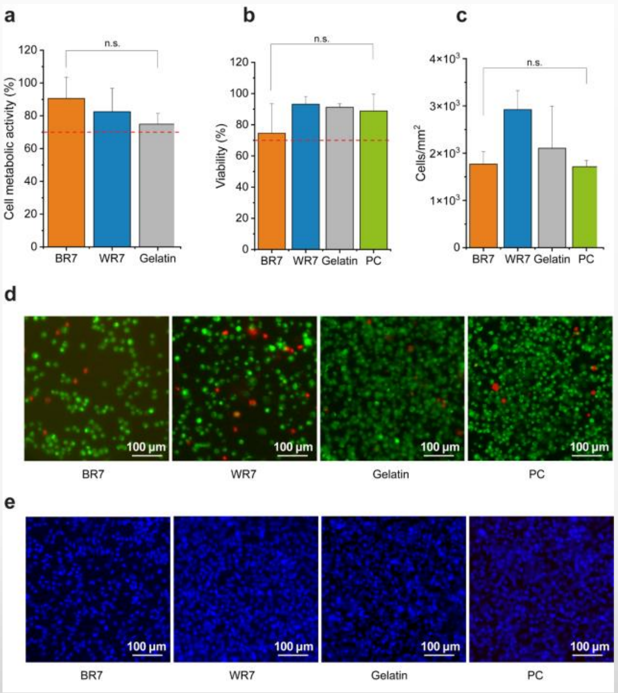
Halogenation of Elastomeric Peptides

Functional properties

Biocompatibility



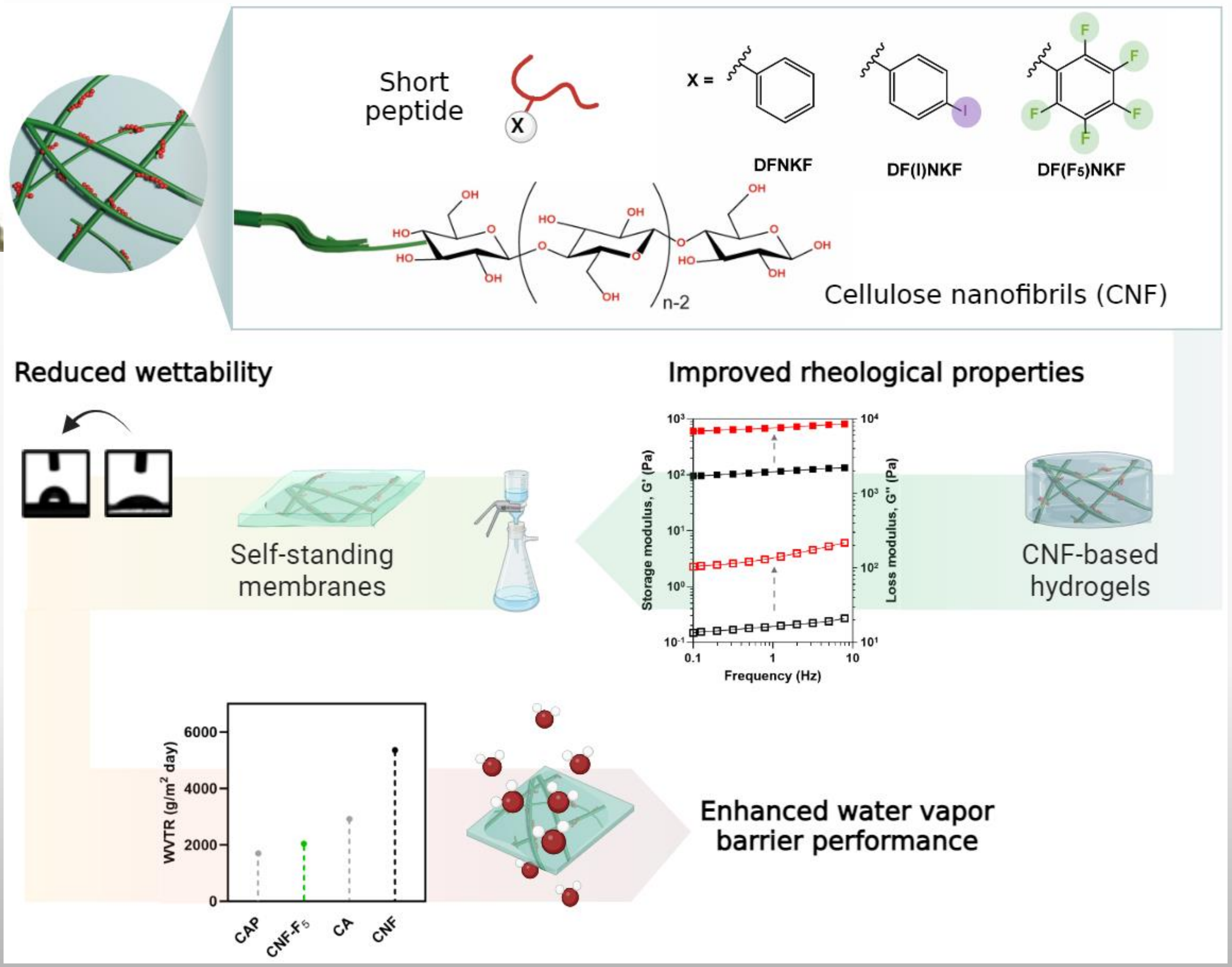
WR7 and BR7 scaffolds ensure a biocompatible environment suitable for endothelial cell culture and growing

Biological studies performed by Chiara Russo, DEIB, Politecnico di Milano

Adv. Funct. Mater.* 2025, *in press.

Nanocellulose-short peptide self-assembly for improved mechanical strength and barrier performance

E. Marelli



A. Marchetti, E. Marelli, *et. al.*,
***J. Mater. Chem. B* 2024, *12*, 9229.**

Acknowledgements

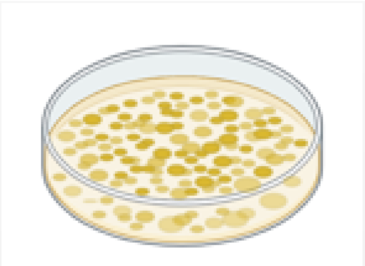
E. Marelli

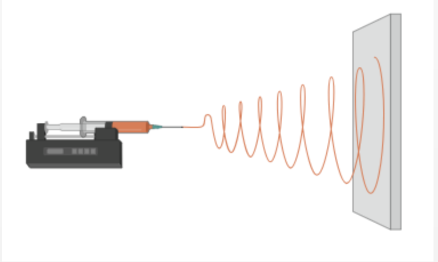
A. Marchetti

A. Marchetti, E. Marelli, *et. al.*,
***J. Mater. Chem. B* 2024, *12*, 9229.**

Keratin smARt mATerials from feather wastE

https://www.karateproject.eu/





Feather waste

4 to 10 days incubation with keratolytic bacteria strand

Keratin-materials: Electrospinning

Fondazione Cariplo is gratefully acknowledged for funding the KARATE project (2022-0437).

The global production of poultry meat is estimated to be ~130 million tons and, only in Europe, 13.4 million tons of poultry meat are produced annually, yielding 3.1 million tons of feather waste.

Conclusions

In the last two years, there has been an upsurge of examples demonstrating that using halogen bonding in supramolecular materials design is a vibrant new field of research.

- **Each of the unique characteristics inherent to halogen bonding – directionality, tunable interaction strength, hydrophobicity, and large donor atom size – is shown to bring about or enhance functionalities that are absent without these characteristic features.**
- **Iodination of amyloidogenic peptides strongly promotes their fibril formation ability and affects the structure of formed fibrils.**
- **The strong effect of iodination on peptide self-assembly is demonstrated to be general and not limited to the core sequence reported here.**
- **Iodination of aromatic amino acids may thus develop as a general strategy for the design of new hydrogels starting from unprotected peptides and without the use of organic solvents.**
- **Single atom mutations of amyloidogenic core sequences by replacing hydrogen with iodine atoms greatly promote fibril formation and self-assembly. Methods promoting the formation of mature fibrils are advantageous for use, among others, as scaffolds for tissue engineering, controlled drug release, surgical reconstruction applications, microfluidic devices, biosensors, and bioswitches.**

Acknowledgements

www.suprabionano.eu

@SupraBioNanoLab @PierMetra

Fondazione Regionale per la Ricerca Biomedica

Suomen Akatemia
Finlands Akademi • Academy of Finland

European Commission

RegioneLombardia

miur

The SupraBioNanoLab

erc
European Research Council

The project KARATE (grant no. 2022-0437) funded by Cariplo Foundation is gratefully acknowledged.

Fondazione CentroEuropeo Nanomedicina

Fondazione Cariplo

Unione Europea
Fondi strutturali e di investimento europei

Regione Lombardia

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