DESIGN, SYNTHESIS, AND CHARACTERIZATION OF A STIMULI-RESPONSIVE PEPTIDE-BASED HYDROGEL

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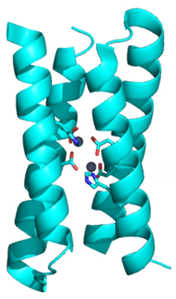
INTRODUCTION

Extreme weather events are driving the search for biosafe strategies for plant protection. **Peptide-based hydrogels** provide a sustainable solution, combining water retention, controlled bioactive release, and stimulus responsiveness[1-3]. **Metal coordination** has been widely used to guide peptide and protein **self-assembly** into well-defined nanostructures[4-5]. Here, we show that peptides inspired by ***D**ue **F**erri* metalloproteins (**hDF**, **hDF2**) can form fibrillar hydrogel networks via metal coordination and hydrophobic interactions, tunable by pH, temperature, peptide ratio, and metal ions, with potential biomedical and environmental applications.

Design of hDF

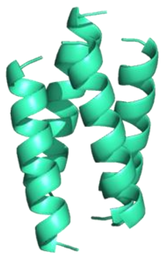


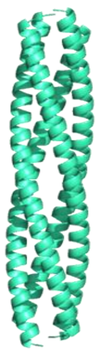
DF1 (PDBid: 1ec5)

Antiparallel four-helix bundle with two metal binding sites
[a]**Glu-x-x-**[d]**His** motif involved in the metal binding
Starting backbone coordinates

Extracted backbone

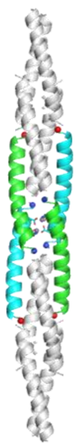
Application of the Crick equation and frequency fitting
26-residue helix as building unit





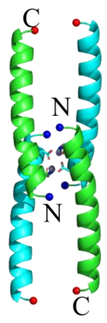
Regenerated backbone

The CCCP 'structure generator' creates a **68-residue tetrameric coiled-coil scaffold**



hDF fiber

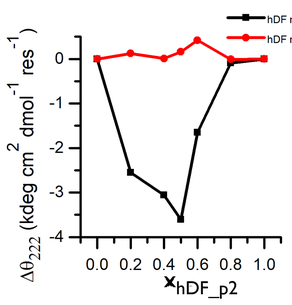
Fibrillar poly-Gly cut into **antiparallel staggered heterotetramers** with N-terminal binding site and C-terminal hydrophobic core to allow **self-assembly**



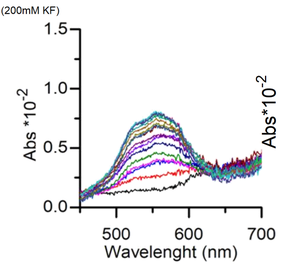
hDF heterotetramer

1st and **2nd** coordination shell residues match DF1
'**a**' and '**d**': Leu/Ile to promote an antiparallel topology
'**f**': Ala to enhance helicity and support hydrogelation
b/e,c/g: +/- Promote desired and prevent undesired topologies

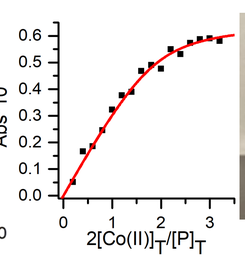
	fg	**abcdefg**	**abcdefg**	**abcdefg**	**abcdefg**	**a**
DF1-helix A	DY	LRELLKL	ELQLIKQ	YREALEY	V	
hDF-p1		LEAL	EEQLKAE	YKSALAW	LKAIKAK	L
DF1-helix B	PV	LAKILED	EEKHIEW	LETILG		
hDF-p2		IEAD	EEKHKAE	LKSILAW	LEAIEAK	L

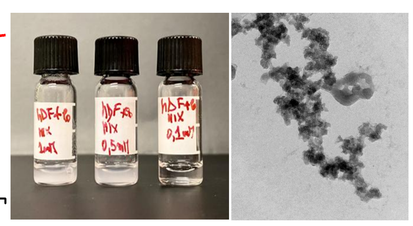


hDF-p1 and **hDF-p2** recognize each other **(1:1)**, **KF** prevents salt bridges



UV-Vis tritation
abs at 550 nm increased linearly up to **2:1 Co(II):tetramer**





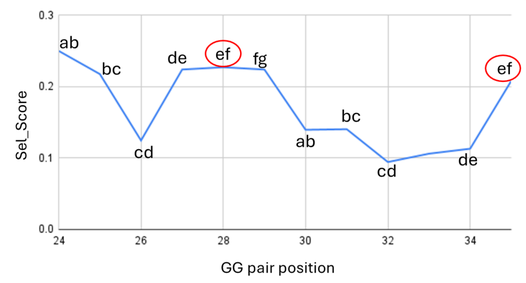
Amorphus precipitate
Too **fast** statistical assembly of fibers

Re-design of hDF2

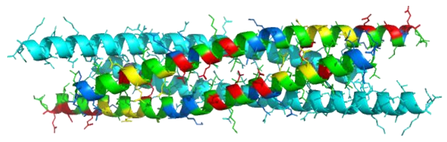
hDF → hDF2

'**c-g**' and '**b-e**' pairs were mutated

GG in '**e**' and '**f**' positions mimics the chain break and favors a staggered antiparallel heterotetramer

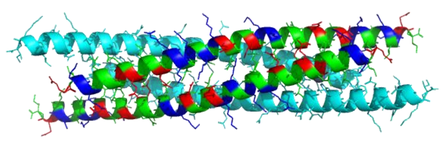


	g	**abcdefg**	**abcdefg**	**abcdefg**	**abcdefg**	**abcd**
DFtet-A	K	LKELKSK	LKELLKL	ELQAIKQ	YKELKAE	LKEL
hDF2-p1		K	LKKLLAL	EQQLLAQ	YREIKAE	LKEI**GG**
hDF2-p1.2		E	LKKLLAL	EQELLAE	YRKIKAK	LKEI
DFtet- B	E	LEELESE	LEKILED	EERHIEW	LEKLEAK	LKEL
hDF2-p2		K	LEEIEAD	EERHIAW	LQKIEAE	LEKI**GG**



hDF2-p1 + **hDF2-p2**

improved **salt bridge** interactions →



hDF2-p1.2 + **hDF2-p2**

Gelation tests

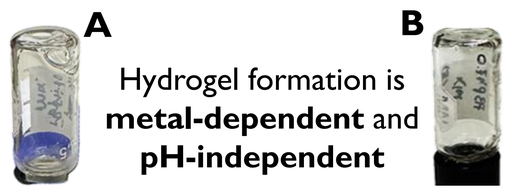
Macroscopic hydrogel formation assessed by **inverted vial tests** with different pH, metals and buffers

SOL-GEL TRANSITIONS

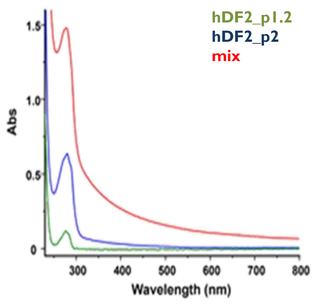
hDF2-p2
1 mM peptide in **MOPS** **pH 7.0** + **Zn(II)** on ice

Highly **pH-depended** system

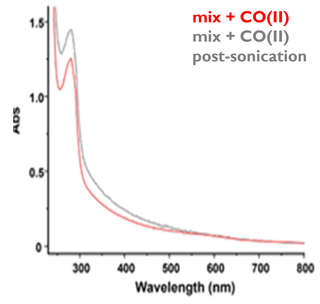
hDF2-p1.2 + **hDF2-p2**
1 mM **mix** in **PB** **pH 5.6** (**A**), **pH 7.0** (**B**) + **Co(II)** on ice



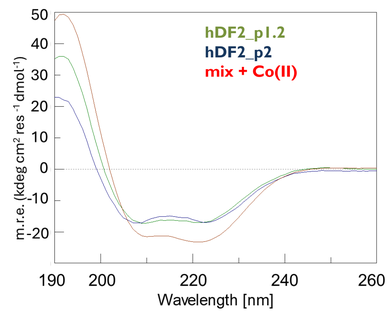
Hydrogel formation is **metal-dependent** and **pH-independent**



Aggregation of **hDF2-p1.2** and **hDF2-p2 (1:1)**
Scattering >800 nm



Spectrum unchanged after **Co(II)** addition



α-helical structures;
Red-shift: tighter helices, **hydrogel** network

CONCLUSIONS AND PERSPECTIVES

Metal-directed self-assembly of peptides inspired by ***D**ue **F**erri* metalloproteins enables the formation of tunable hydrogel networks. **hDF2-p2** forms hydrogels under specific pH-dependent conditions, while redesign of **hDF2-p1** enhances its interaction with hDF2-p2, creating a system where hydrogelation is both pH- and metal-dependent. UV-vis and CD analyses confirm peptide aggregation while preserving α-helical structure, consistent with robust hydrogel network formation, highlighting the potential of these materials for environmental applications[6–8].

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