Dehydropeptide-based self-assembled hydrogels with incorporated Gd^{3+} chelates: potential Contrast Agents for MRI?

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Introduction

- Self-assembled peptide-based hydrogels (SAPH) are the new paradigm biomaterials: soft biocompatible materials with an entangled nanofibrillar structure reminiscent of the extracellular matrix.
- Dehydropeptides *N*-capped with Naproxen (Npx, a NSAID drug) are non-toxic to cells, show enhanced stability towards proteolysis and originate self-assembled hydrogels displaying rheological properties suitable for biomedical applications.
- Dehydropeptide-based hydrogels revealed suitable nanocarriers for drug delivery applications.
- Incorporation of Superparamagnetic Iron Oxide Nanoparticles (SPION) endows dehydropeptide-based hydrogels with hyperthermia and T_{2w} MRI reporting properties – Magnetogels.
- Preliminary results from our research group suggest that Gd^{3+} complexes incorporated into dehydrodipeptide-based hydrogels retain T_{1w}MRI reporting properties.
- In this work we report novel dehydrodipeptide hydrogelators *N*-capped with succinic acid: Suc-Phe-Δ-PheOMe and Suc-Phe-Δ-PheOH.
- The dehydropeptide hydrogels with an incorporated Gd^{3+} complex are characterised as potential Contrast Agents for MRI.

Dehydropeptide Hydrogelators

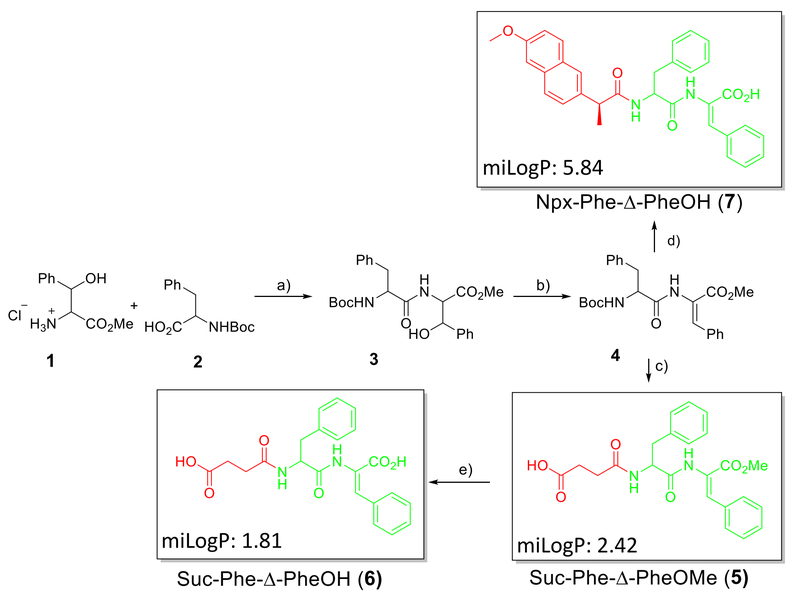

Figure 1: General methodology for the synthesis of dehydrodipeptides: a) HBTU, TEA, MeCN; b) i. Boc_2O/DMAP, MeCN, ii. TMG; c) i. TFA, ii. anhydride succinic, pyirdine; d) i. TFA, ii. Naproxen chloride, TEA; e) NaOH 1M; dioxane.

STEM

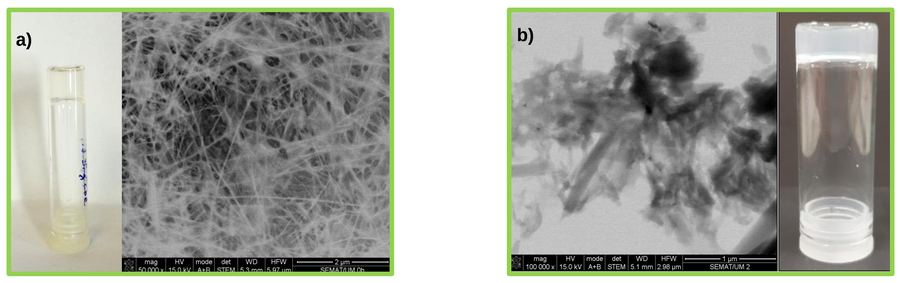

Figure 3. STEM images of hydrogels: a) Npx-Phe-Δ-PheOH (**7**) (0.4 wt%); b) Suc-Phe-Δ-PheOMe (**5**) (0.4 wt%).

Conclusions

- New dehydropeptides *N*-capped with succinic acid were synthetized following synthetic pathways developed by the research group.
- A new DOTA-type Gd^{3+} complex (Gd(Npx) functionalized with a naproxen (Npx) moiety was synthesized for incorporation into the dehydropeptide-based self-assembled hydrogels.
- The dehydrodipeptides Suc-Phe-Δ-PheOMe and Npx-Phe-Δ-PheOH afford elastic hydrogels with a fibrillar nanostructure at 0.4 wt% (4mg/ml) concentration. The Suc-Phe-Δ-PheOH hydrogelator fails to gelate at 0.4 wt% , presumably due to low hydrophobicity (LogP: 1.81).
- The CD spectra of the hydrogels suggest that the peptide backbone adopts predominantly a random coil secondary structure in the self-assembled nanofibers.
- The higher elasticity of the Npx-Phe-Δ-PheOH hydrogel in comparison with the Suc-Phe-Δ-PheOMe hydrogel can be ascribed to π-π stacking interactions of the bulky aromatic naproxen group.
- The Gd(NPx) complex displays a relaxivity value (8.42 $mM^{-1}s^{-1}$) in accordance with its intermediate molecular weight. The complex does not undergo self-association in the concentration range studied.
- The co-assembled Gd(Npx)@dehydrodipeptide hydrogels show concentration-dependent T_{1w} MRI contrast enhancement.
- The hydrogel Gd(Npx)@Npx-Phe-Δ-Phe-OH is significantly more efficacious as T_1 MRI CA than the Gd(Npx)@Suc-Phe-Δ-PheOMe and the Gd(Npx)@Suc-Phe-Δ-PheOH hydrogels, presumably due to association between the Gd(Npx)-complex and the Npx-Phe-Δ-PheOH hydrogel fibers - tuning of the rotation correlation time t_R.
- The efficacy of the co-assembled Gd(Npx)@hydrogel Contrast Agents seem to be determined by the structure of the hydrogelator.
- High relaxivity Gd@hydrogel MRI Contrast Agents can be attained by structural design of hydrogelators and Gd-complexes.
- Injectable Gd@hydrogel Contrast Agents loaded with drugs are potential cancer theragnostic.

Gd^{3+} complex

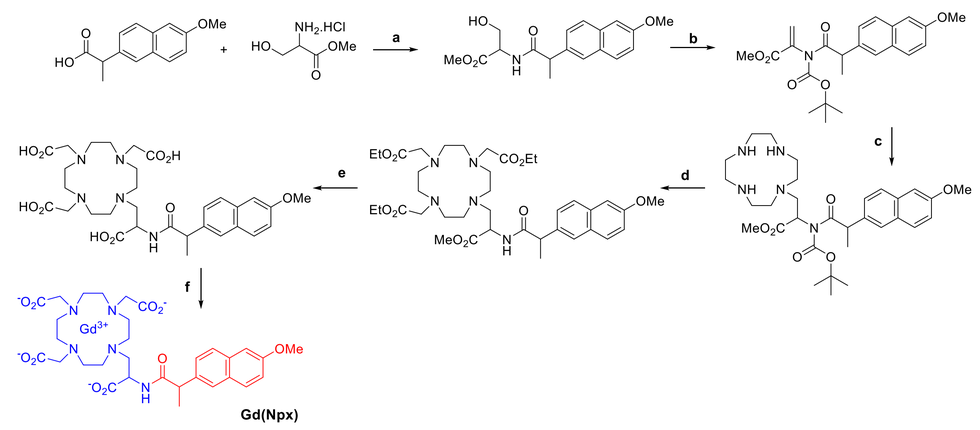

Figure 2. Synthetic pathway for the synthesis of the Gd(Npx) complex: a) DCC/HOBT, TEA, DCM; b) Boc_2O/DMAP, MeCN; c) Cyclen, K_2CO_3/MeCN; d) i. TFA/DCM; ii. K_2CO_3, MeCN; iii. ethyl bromoacetate; e) i. Dowex-1X2-OH^- H_2O/EtOH; ii. elution with HCl (0,1M); f) $GdCl_3$.

CD

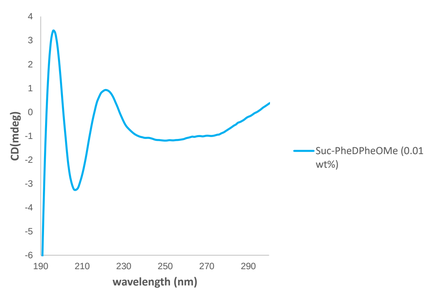

Figure 4: CD spectrum for hydrogelator Suc-Phe-Δ-PheOMe (**5**)

Rheology

Table 1. Rheological parameters: G' (elastic shear modulus) and G'' (viscosity shear modulus) for hydrogels (0.4 wt%).

	G' (kPa)	G'' (kPa)
Suc-Phe-Δ-PheOMe (5)	15.8	8.57
Suc-Phe-Δ-PheOH*(6)	0.039	0.023
Npx-Phe-Δ-PheOH (7)	39.3	4.07

* Does not gelate

MRI

Table 2. T_1 and R_1 values for co-assembled hydrogels Gd(Npx)@hydrogel.

[Gd] (mM)	0.1	0.2	0.3	0.5	0.8
Gd(Npx)					
T_1 (s)	0.70	0.64	0.45	0.27	0.10
R_1 (s^{-1})	0.002	0.13	0.78	2.35	8.89
T_1map					
Gd(Npx)@Suc-Phe-Δ-PheOMe (**5**)					
T_1 (s)	1.56	1.02	0.66	0.29	0.16
R_1 (s^{-1})	0.14	0.48	1.02	2.94	5.87
T_1map					
Gd(Npx)@Suc-Phe-Δ-PheOH (**6**)					
T_1 (s)	1.53	0.94	0.76	0.46	0.22
R_1 (s^{-1})	0.15	0.57	0.82	1.68	4.07
T_1map					
Gd(Npx)@Npx-Phe-ΔPheOH (**7**)					
T_1 (s)	3.67	2.41	1.81	1.04	1.19
R_1 (s^{-1})	0.27	0.42	0.55	0.96	0.84
T_1map					

Table 3. Relaxivity (r_1, $mM^{-1}s^{-1}$; 120 MHz; 37 °C) values for co-assembled hydrogels Gd(Npx)@hydrogel.

Gd(Npx)@hydrogel	$r_{1\ (mM^{-1}s^{-1})}$
Gd(Npx)	8.42
Suc-Phe-Δ-PheOMe (**5**)	7.71
Suc-Phe-Δ-PheOH (**6**)	5.55
Npx-Phe-Δ-PheOH (**7**)	10.29

References:
[1] M. F. Ferreira *et al.*, *Dalton Trans.*, **2014**, 43, 3162.
[2] H. Vilaça *et al.*, *Biomacromolecules*, **2015,** 16, 3562.
[3] Carvalho *et al.*, *Nanomaterials*, **2019**, 9, 541.

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