Supporting Information

Mediating Oxidation of Thioethers with Iodine—A Mild and Versatile Pathway to Trigger the Formation of Peptide Hydrogels

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1 Materials and Methods

All commercially available chemicals were used as received and were purchased from Acros Organics (Waltham, Massachusetts, USA), Aldrich (St. Louis, Missouri, USA), Alfa Aesar (Massachusetts, USA), Iris Biotech (Marktredwitz, Germany), Fluorochem (Hadfield, UK), Fluka (St. Louis, Missouri, USA), Merck (Darmstadt, Germany) and TCI (Tokyo, Japan).

1.1 Automated Column Chromatography

Automated preparative chromatography was performed on Reveleris X2 flash chromatography system (Büchi Labortechnik, Flawil, Switzerland) equipped with a Flash Pure Ecoflex 12 g C 18 (Büchi Labortechnik, Flawil, Switzerland) reverse phase column. Signals were detected on a UV detector and an evaporative light scattering (ELSD) detector.

1.2 Circular Dichroism Spectroscopy

CD spectra were recorded on a Jasco J-815 (Jasco Deutschland GmbH, Pfungstadt, Germany) using the software Spectra Manager 2.12.00 (Jasco Deutschland GmbH, Pfungstadt, Germany). Data processing was realized using OriginPro 2018 b b9.5.5.409 (ORIGINLAB Corporation, Northampton, USA). Each spectrum was measured at 20 °C and over 5 accumulations. 0.5 wt% Nap-FMDM was dissolved in MiliQ upon addition of 2 μl/ml 12 M NaOH and sonication for 10 min at 20 °C. The stock solution was diluted to 0.01 wt% gelator concentration and 0.05 vol% 1 M I2/3 M NaI solution was added. The sample was subsequently transferred into a 1 mm High Precision SUPRASIL quartz glass cuvette (Hellma Analytics GmbH, Müllheim, Germany) and rested overnight prior to the measurement. For reference, the sample was treated analogously but no triiodide was added to the alkaline precursor solution.

1.3 pH Measurements

pH Measurements were performed on a SevenCompact Duo S213 (Mettler Toledo, Columbus, USA) equipped with a InLab Expert Pro-ISM electrode (Mettler Toledo, Columbus, USA).

For triiodide concentration dependent measurements, 0.5 wt% of Nap-FF or Nap-FMDM were suspended in the respective amount of MiliQ and 2 μl/ml 12 M NaOH were added to yield a homogenous solution after sonication for 10 min. In case of the Nap-FF sample, 1 vol% of thiodiglycol was added and the sample was sonicated again for 10 min at 20 °C. The solution was subsequently transferred onto the pH meter and stirred there using a magnetic stirring bar and plate. When a constant pH was achieved, the value was noted and 1 M I2/3 M NaI solution was added to yield the stated concentration of triiodide. To ensure completion of the oxidation reaction, the sample was stirred for 24 h after each addition of triiodide solution before measurement of the pH value.
For the time dependent measurement of the pH, samples were prepared in an analogous way by sonicating 0.5 wt% of Nap-FF or Nap-FMDM in the respective amount of MiliQ upon addition of 2 μl/ml 12 M NaOH for 10 min at 20 °C. In case of the Nap-FF sample 1 vol% of thiodiglycol was added and the sample was sonicated again. The solution was subsequently transferred onto the pH meter and stirred there using a magnetic stirring bar and plate. For the Nap-FF sample 2.5 vol% and for the Nap-FMDM sample 10 vol% of 1 M I2/3 M NaI solution were added in one portion and the pH measurement was started. pH values were noted after the stated time intervals. After 6 h the sample was removed from the pH meter and stirring was continued in a closed vial to avoid solvent evaporation. Samples were only transferred back to the pH meter for the respective measurement after the stated time had passed.

1.4 HPLC-MS

The HPLC setup was based on the Agilent 1260 Infinity II series (Agilent, Santa Clara, USA) and consisted of a G7129C vial sampler, a G7112B binary pump, a G7116A column oven and a G7114A variable wavelength detector. The setup was coupled to a G6125B single quadrupole mass analyser (Agilent, Santa Clara, USA) and controlled by OpenLab CDS ChemStation Edition Rev. C.01.08 (Agilent, Santa Clara, USA). For analysis a C18 Hypersil Gold column (Thermo Scientific, Waltham, USA) was used. Measurement data were analyzed using MestReNova v14.2.3-29241 (Mestrelab Research S.L., Santiago de Compostela, Spain).

MiliQ water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B) were used for elution following the listed gradient:

| Time [min] | 0  | 20 | 30 | 40 | 41 | 45 |
|------------|----|----|----|----|----|----|
| % A        | 80 | 80 | 50 | 50 | 100| 100|
| % B        | 20 | 20 | 50 | 50 | 100| 100|

1 ml hydrogel samples were prepared as described in 1.5 [0.5 wt% Nap-FMDM (±7 mM) with 5 vol% 1 M triiodide solution (±50 mM) or 0.5 wt% Nap-FF (±10 mM) with 0.1 vol% thiodiglycol (±9.6 mM) and 2.5 vol%1 M triiodide solution (±25 mM)] and gelated overnight. 1 ml of acetonitrile was added to the gel and the sample was dissolved via shaking and sonication. The dissolved system was diluted to 100 μM gelator concentration using 1:1 MiliQ/acetonitrile and analyzed via HPLC-MS.

1.5 Hydrogel Preparation

Nap-FF, Nap-GFYE, Nap-GFFYS, Nap-FGDS, Nap-FS and Nap-GFYE

Gelator solutions were prepared in MiliQ by the addition of 2 μl/ml 12 M NaOH to a suspension of the respective amount of the gelator in MiliQ. The dispersion was sonicated for 10 min at 20 °C and the stated volume of thiodiglycol was added. After again sonicating for 10 min the stated volume of 1 M I2/3 M NaI solution was added in one portion and the sample was shaken by hand to yield a homogenous sample. Afterwards the system was left untouched overnight to ensure complete gelation.
Gelation success was monitored by standard inverted vial tests. The pH of the samples was assessed after gelation using MColorHast pH indicator strips (Merck KGaA, Darmstadt, Germany).

**Nap-FMDM**

Nap-FMDM solutions were prepared in MiliQ by the addition of 2 μl/ml 12 M NaOH to a suspension of the respective amount of Nap-FMDM in MiliQ. The dispersion was sonicated for 10 min at 20 °C and stated volume of 1 M I2/3 M NaI solution was added in one portion. Subsequently the sample was gently shaken by hand to yield a homogenous sample. Afterwards the system was left untouched overnight to ensure complete gelation. Gelation success was monitored by standard inverted vial tests. The pH of the samples was assessed after gelation using MColorHast pH indicator strips (Merck KGaA, Darmstadt, Germany).

**1.6 Rheology**

Rheological measurements were carried out on an Anton Paar Modular Compact Rheometer MCR 102 (Anton Paar GmbH, Graz, Austria) with Anton Paar RheoCompass V1.20.40.496 (Anton Paar GmbH, Graz, Austria) analysis software. Data processing was realized using OriginPro 2018 b9.5.5.409 (ORIGINLAB Corporation, Northampton, USA). The rheometer was equipped with a P PTD200 (Anton Paar GmbH, Graz, Austria) measuring cell and a CP25 2 (Anton Paar GmbH, Graz, Austria) spindle (25 mm plate diameter) with the measurement gap set to 0.106 mm. Tests were performed at 20 °C.

For amplitude sweep measurements a constant frequency of 1 rad/s was applied and data points were taken from 0.1% to 100% shear strain.

When performing frequency sweep measurements the shear strain was kept constant at 0.5% and the frequency was monitored from 1 rad/s to 100 rad/s.

Time sweep tests were performed at constant dynamic mechanical conditions with 0.5% shear strain and 5 rad/s frequency. To prevent solvent evaporation over the course of the measurement, a water soaked paper towel was placed in the measuring chamber to guarantee a saturated atmosphere.

Hydrogels were prepared as reported in 0.5 ml EPPENDORF-Cups and transferred to the rheometer after gelation overnight. To avoid sample destruction, the measurement gap was approached using a normal force controlled protocol. Excess hydrogel was carefully removed from the sides of the measurement cone. After successful approach of the measurement gap, the sample was rested for 15 min prior to the start of the measurement to release potentially built up strains from the loading procedure. For the time sweep measurements hydrogels were prepared following the same procedure but the sample was immediately transferred on the rheometer after addition of the 1 M I2/3 M NaI solution to monitor the gelation via rheology.
1.7 Scanning Electron Microscopy

SEM samples were obtained by spreading the sample on silicon wafers and drying over night under ambient conditions. Measurements were performed on a *Raith Velion FIB-SEM* (Raith Nanofabrication, Dortmund, Germany). The acceleration voltage was set to 10 kV and the secondary electrons were detected at a working distance of 9.6 mm.
2 Additional Experimental Data

Figure S 1: HPLC-MS chromatogram obtained in negative ion mode of a 0.5 wt% Nap-FF and 0.1 vol% thiodiglycol sample a) before oxidation and b) after oxidation via addition of 2.5 vol% 1 M triiodide solution. Annotated peak corresponding to Nap-FF.

Figure S 2: ESI mass spectra of a dried 5 vol% thiodiglycol solution (≅ 386 mM) after oxidation with 25 vol% 1 M triiodide solution (≅ 200 mM) with annotation of the relevant signals.
Figure S 3: Critical thiodiglycol concentration of a 0.5 wt% Nap-FF (≈10 mM) hydrogel triggered with 2.5 vol% 1 M triiodide solution (≈25 mM). Thiodiglycol concentrations: a) 0.07 vol% (≈7 mM), b) 0.14 vol% (≈13 mM), c) 0.28 vol% (≈27 mM), d) 0.57 vol% (≈55 mM) and e) 1.14 vol% (≈109 mM).

Figure S 4: Critical gelation concentration of a Nap-FF hydrogel triggered with 1 vol% thiodiglycol (≈96 mM) and 2.5 vol% 1 M triiodide solution (≈25 mM). Gelator concentrations: a) 0.1 wt% (≈2 mM), b) 0.2 wt% (≈4 mM), c) 0.3 wt% (≈6 mM) and d) 0.4 wt% (≈8 mM).
Figure S 5: Amplitude sweep curves measured at 1 rad/s frequency and 20 °C. Black data points: 0.5 wt% Nap-FF (\(\leq 10\) mM) hydrogel with 1 vol% thiodiglycol (\(\leq 96\) mM) triggered with 2.5 vol% 1 M triiodide solution (\(\leq 25\) mM). Red data points: reference sample without thiodiglycol. Blue data points: reference sample without triiodide.

Figure S 6: ESI mass spectra of a dried 0.5 wt% Nap-FMDM (\(\leq 7\) mM) hydrogel after being triggered with 5 vol% 1 M triiodide solution (\(\leq 50\) mM) with annotation of the relevant signals.
Figure S 7: HPLC-MS chromatogram of 0.5 wt% Nap-FMDM (\(\cong 7 \text{ mM}\)) triggered with 5 vol% 1 M triiodide solution (\(\cong 50 \text{ mM}\)). a) Total ion current in negative ion mode with annotated retention times and integral proportion. b) Extracted ion chromatogram for m/z = 757 Da \(\pm 0.5 \text{ Da}\) corresponding to the disulfoxide and monosulfone. c) Extracted ion chromatogram for m/z = 741 Da \(\pm 0.5 \text{ Da}\) corresponding to the monosulfoxide. d) Extracted ion chromatogram for m/z = 726 Da \(\pm 0.5 \text{ Da}\) corresponding to the unoxidized Nap-FMDM.

Figure S 8: Critical gelation concentration of a Nap-FMDM hydrogel triggered with 2.5 vol% 1 M triiodide solution (\(\cong 25 \text{ mM}\)). Gelator concentrations: a) 0.1 wt% (\(\cong 1.3 \text{ mM}\)), b) 0.2 wt% (\(\cong 3 \text{ mM}\)), c) 0.3 wt% (\(\cong 4 \text{ mM}\)) and d) 0.4 wt% (\(\cong 5 \text{ mM}\)).
Figure S 9: Amplitude sweep curve measured at 1 rad/s frequency and 20 °C of a 0.5 wt% Nap-FMDM (\( \equiv 7 \) mM) hydrogel triggered with 20 vol% 1 M triiodide solution (\( \equiv 166 \) mM).

Figure S 10: Frequency sweep curve measured at 0.5% strain and 20 °C of a 0.5 wt% Nap-FMDM (\( \equiv 7 \) mM) hydrogel triggered with 20 vol% 1 M triiodide solution (\( \equiv 166 \) mM).
Figure S 11: Time sweep of a 0.5 wt% Nap-FMDM (≈7 mM) hydrogel triggered with 10 vol% 1 M triiodide solution (≈91 mM) measured at constant dynamic mechanical conditions of 0.5% shear strain, 5 rad/s frequency and 20 °C.

Figure S 12: Time dependent analysis of the pH value of a stirred 0.5 wt% Nap-FMDM (≈7 mM) system 10 vol% 1 M triiodide solution (≈91 mM).
3 Synthesis

3.1 Solid Phase Peptide Synthesis

![Scheme 1: Schematic representation of the solid-phase peptide synthesis with Nap-FF as an example.]

**Standard Operating Procedure (SOP) 1 (Loading of the Resin)**

The first Fmoc-protected amino acid (1.5 eq. relative to the number of active functionalities on the resin) was dissolved in dry DCM (20 ml). The solution was added to the 2-chlorotrityl-resin (1.6 mmol/g) under argon atmosphere. DIPEA (2 eq. relative to the number of active functionalities on the resin) was added and the mixture was agitated for 5 min by the argon stream. A second portion of DIPEA (3 eq. relative to the number of active functionalities on the resin) was added. After agitating for 2 h by the argon stream methanol (1 ml/g resin) was added and the resulting mixture was agitated for further 15 min to quench the remaining resin functionalities. After filtration of the reaction mixture the resin was washed with DCM p.a. (3 x 20 ml), DMF p.a. (3 x 20 ml), DCM p.a. (3 x 20 ml) and methanol (3 x 20 ml). The resin was dried under vacuum to determine the loading ratio by the weight increase.

**SOP 2 (Stepwise Elongation)**

The dry resin was pre-swollen by shaking in DMF (20 ml) for 5 min. The pre-swollen resin was washed with DMF (2 x 20 ml) and the Fmoc-group was cleaved by shaking in 20% piperidine solution in DMF (20 ml). After sucking off the solution another portion
of 20% piperidine solution in DMF (20 ml) was added and shaken for 20 min to ensure complete deprotection. The resin was washed with DMF (7 x 20 ml) and the second Fmoc-protected amino acid (3 eq. relative to resin loading, 0.5 M solution in DMF) was added. HOBt (4 eq. relative to resin loading, 0.4 M solution in DMF) and DIPCDI (4 eq. relative to resin loading, 0.4 M solution in DMF) were added and the mixture was shaken for 2.5 h. After washing with DMF (3 x 20 ml) the procedure was repeated for the following (amino)acid derivatives (3 eq. to resin loading each, 0.5 M solution in DMF).

**SOP 3 (Cleavage of the Peptide from the Resin)**

The resin was suspended in a solution of TFA:H₂O:Triisopropylsilane (95:2.5:2.5, 20 ml) and stirred for 4 h. The reaction mixture was sucked off and the resin was washed with TFA (3 x 5 ml). In the following the peptide was precipitated by the addition of cold Et₂O:pentane solution (3:1). The precipitate was collected via centrifugation and the remaining water was removed via lyophilization. The dried powder was re-dissolved in a minimal amount of DMSO and precipitated by the addition of MilliQ. The precipitate was collected via centrifugation and the remaining water was removed via lyophilization. Alternatively, the lyophilized solid was dissolved in a minimal amount MilliQ/THF and purified via reverse phase automated column chromatography using a FlashPure EcoFlex C18 column (Büchi Labortechnik, Flawil, Switzerland).

### 3.2 Nap-FF

![Nap-FF](image)

For the synthesis of Nap-FF Fmoc-Phenylalanine was loaded on the resin and stepwise elongation was performed using Fmoc-Phenylalanine and naphtoxyacetic acid. After cleavage from the resin the crude peptide was purified by precipitation with cold Et₂O:pentane (3:1) and the title product was obtained as a colourless powder.
1H NMR (400 MHz, DMSO): δ [ppm] = 8.45 (d, J = 7.9 Hz, 1H, Amide-H), 8.13 (d, J = 8.6 Hz, 1H, Amide-H), 7.88 – 7.80 (m, 2H, Aryl-H), 7.73 (d, J = 8.2 Hz, 1H, Aryl-H), 7.46 (t, J = 7.6 Hz, 1H, Aryl-H), 7.36 (t, J = 7.5 Hz, 1H, Aryl-H), 7.26 – 7.13 (m, 12H, Aryl-H), 4.71 – 4.62 (m, 1H), 4.58 – 4.44 (m, 3H), 3.13 – 2.99 (m, 2H), 2.97 – 2.81 (m, 2H).

MALDI-MS: [m/z]: found: 519.25, calculated: 519.19 [M+Na]+.

3.3 Nap-FMDM

For the synthesis of Nap-FMDM Fmoc-Methionine was loaded on the resin and stepwise elongation was performed using Fmoc-Aspartic Acid(OtBu), Fmoc-Methionine, Fmoc-Phenylalanine and naphthoxyacetic acid. The dried peptide was purified via automated preparative chromatography with water/THF by ramping up the THF content from 50 vol% to 100 vol% over 16 min (retention time product: 1.7 min). The title product was isolated as a colourless powder.

1H NMR (400 MHz, DMSO): δ [ppm] = 12.59 (s, 2H, 2 x Carboxy-H), 8.34 (d, J = 8.0 Hz, 1H, Amide-H), 8.28 (d, J = 7.6 Hz, 1H, Amide-H), 8.19 (d, J = 8.1 Hz, 1H, Amide-H), 8.04 (d, J = 8.0 Hz, 1H, Amide-H), 7.87 – 7.81 (m, 2H, Aryl-H), 7.74 (d, J = 8.1 Hz, 1H, Aryl-H), 7.51 – 7.43 (m, 1H, Aryl-H), 7.40 – 7.33 (m, 1H, Aryl-H), 7.26 – 7.11 (m, 7H), Aryl-H, 4.70 – 4.48 (m, 4H), 4.40 – 4.26 (m, 2H), 3.10 – 3.01 (m, 1H), 2.95 – 2.83 (m, 1H), 2.74 – 2.63 (m, 1H), 2.58 – 2.53 (m, 1H), 2.44 – 2.37 (m, 2H), 2.04 – 1.59 (m, 10H).

MALDI-MS: [m/z]: found: 749.26, calculated: 749.23 [M+Na]+.
3.4 NMR Spectra

Figure S 11: $^1$H-NMR spectrum of Nap-FF (400 MHz in DMSO-$d_6$).

Figure S 12: $^1$H-NMR spectrum of Nap-FMDM (400 MHz in DMSO-$d_6$).