Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supplementary Information

Magneto-responsive hydrogels by self-assembly of low molecular weight peptides and crosslinking with magnetite nanoparticles

Benedikt P. Nowak, Maximilian Niehues and Bart Jan Ravoo*

Center for Soft Nanoscience and Organic Chemistry Institute, Westfälische Wilhelms Universität Münster, Busso Peus Straße 10, 48149 Münster, Germany.

E-mail: b.j.ravoo@uni-muenster.de

Materials

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).

Automated column chromatography

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

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 Corperation, Northampton, USA). If not indicated otherwise, all samples were measured at 20 °C and 10 accumulations were executed. The gelator was dissolved in MiliQ upon stepwise addition of 12 M NaOH. The sample was diluted to the reported concentration and if required Fe_3O_4 particles were added from a freshly sonicated 4 wt% stock dispersion. After sonication for 10 min while strictly maintaining the temperature at 20 °C 20 mg/ml GdL was added and dissolved *via* gentle shaking of the vial. The sample was subsequently transferred into a 1 mm *High Precision SUPRASIL quartz glass cuvettes* (Hellma Analytics GmbH, Müllheim, Germany) and gelated overnight prior to measurement.

Dye release experiments

Hydrogels for the dye release were prepared using a standard acid triggered technique as reported by Adams *et al.*[1]. Nap-GFYE stock solutions at 1 wt% were prepared in MiliQ water by stepwise addition of 12 M NaOH. To 100 µl of this solution 500 µl of a freshly sonicated 4 wt% stock dispersion of MNPs were added and diluted with 360 µl of MiliQ water. 40 µl of the FITC-Dextran stock solution (10 mg/ml in case of M_w = 4000 g/mol and M_w = 10000 g/mol; 40 mg/ml in case of M_w = 250000 g/mol) were and added and the resulting dispersion was again sonicated for 10 min while strictly controlling the temperature at 20 °C. The dispersion was immediately transferred into a vial containing 20 mg GdL, gently mixed by hand and subsequently split up into 4 disposable semi-micro PMMA cuvettes (VWR, Radnor, USA). Gelation was carried out at ≈ 5 °C overnight to reduce fluorophore decomposition and gelation success was tested after 18 h *via* inverted vial tests.

Reference hydrogels without MNPs were prepared accordingly by substitution of the MNP dispersion with water.

After gelation 1 ml of MiliQ water was added gently onto the top of the hydrogel and fluorescene intensity was measured in the supernatant using a Jasco FP 8500 spectrofluorometer (Jasco Deutschland GmbH, Pfungstadt, Germany) at 25 °C. Data were collected on on Spectra Manager 2, Spectra Manager Version 2.14.05 (Jasco Deutschland GmbH, Pfungstadt, Germany). Samples were exitated at λ = 450 nm and spectra were recorded from λ = 475 nm to λ = 700 nm.

The maximum reference was analysed by diluting 10 µl of the FITC-Dextran stock solution (10 mg/ml in case of M_w = 4000 g/mol and M_w = 10000 g/mol; 40 mg/ml in case of M_w = 250000 g/mol) in 1240 µl of MiliQ water and likewise recording of the fluorescence spectra.

The magnetized samples were placed on a 50.8 mm x 50.8 mm x 25.4 mm neodym N40 magnet and just transferred to the fluorimeter at the reported measurement intervall times. To exclude photobleaching effects all samples, including the reference systems were storred in the dark inbetween the distinct measurements. The dye release is analysed as the increase in fluorescene intensity of FITC-Dextran relative to the maximum possible fluorescence intensity in the supernatant.

Dynamic light scattering

Dynamic light scattering (DLS) was performed on a Nano-ZS Zetasizer (Malvern Instruments, Malvern, UK). Aqueous samples were measured in disposable 1 mL semi-micro PMMA cuvettes (Brand GmbH & Co. KG, Wertheim, Germany). Data analysis was realized with Malvern Zetasizer software 7.12 (Malvern Instruments, Malvern, UK).

Fourier transformed infrared spectroscopy

IR spectra were recorded on a Varian Inc. 2005 (SPECAC, Kent, UK) spectrometer by attenuated total reflection. From every spectrum, the background measured against MiliQ was subtracted. Data analysis was performed using the software Resolution Pro (SPECAC, Kent, UK). For the gel samples Nap-GFYE stock solutions at 1 wt% were prepared in MiliQ water by stepwise addition of 12 M NaOH. The samples were diluted to the reported concentration using MiliQ. If required MNPs were added from a freshly sonicated 4 wt% MNP stock dispersion. The diluted samples were again sonicated for 10 min while strictly maintaining the temperature at 20 °C. To trigger the gelation 1 M HCl (0.5 ml/ml) was added and the samples were left untouched overnight prior to measurement. As a non-assembled reference a 0.4 wt% Nap-GFYE solution at pH ≈ 12 was used.

Rheology

Rheological measurements were carried out on an Anton Paar Modular Compact Rheometer MCR 102 (Anton Paar GmbH, Graz, Austria) with Anton Paar RhepCompass V1.20.40.496 (Anton Paar GmbH, Graz, Austria) analysis software. Data processing was realized using OriginPro 2018 b b9.5.5.409 (ORIGINLAB Corperation, Northampton, USA). For frequency sweep measurements 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). The measuring gap was set to 0.106 mm and a shear strain of 0.1% was applied. The measurement was carried out at 20 °C and the frequency was monitored from 1 rad/s up to 100 rad/s.

Hydrogels were likewise prepared as reported by Adams *et al.*[1]. Nap-GFYE stock solutions at 1 wt% in MiliQ water were obtained by stepwise addition of 12 M NaOH. To 50 µl of this solution 250 µl of a freshly sonicated 4 wt% stock dispersion of MNPs were added and diluted with 200 µl of MiliQ water. The resulting dispersion was again sonicated for 10 min while strictly controlling the temperature at 20 °C. The dispersion was immediately transferred into a 0.5 ml EPPENDORF Cups containing 5 mg GdL, gently mixed by repeated pipetting. The samples were allowed to gelate overnight before transferring to the rheometer.

Reference samples containing SiO_2-NPs were prepared utilizing the same protocol while exchanging the Fe_3O_4-MNP stock solution with a freshly sonicated, 4 wt% solution of commercially available SiO_2-NPs (d = 387 nm $\pm$ 10 nm, microParticles GmBH, Berlin, Germany) in water.

Magnetized samples were obtained by placing the 0.5 ml EPPENDORF Cups containing the hydrogel on a 50.8 mm x 50.8 mm x 25.4 mm neodym N40 magnet for 24 h prior to the transfer on the rheometer.

Scanning electron microscopy

SEM samples were obtained by spreading 0.1 wt% Nap-GFYE (and 0.1 wt% MNPs) hydrogels triggered with 20 mg/ml GdL on silicon wafers and drying over night under ambient conditions. Measurements were performed on a Zeiss Crossbeam XB1540 (Carl Zeiss AG, Oberkochen, Germany) equipped with a field emission cathode Gemini 2 (Carl Zeiss AG, Oberkochen, Germany). The extractor voltage was set to 3 kV. The secondary electrons were detected at a working distance of 5 mm.

Transmission electron microscopy

Transmission electron microscopy was performed on a Thermo Scientific FEI Titan Themis G3 60-300 TEM equipped with a high brightness field emission gun (X-FEG) a monochromator, an image Cs-corrector and a fast CMOS camera (Ceta 2 speed upgrade 4k x 4k). For all measurements, the microscope was operated at an acceleration voltage of 300 kV with an extraction voltage of 3.45 kV for the X-FEG. Samples were measured on carbon coated copper grids (Plano EM, S162). Images were analyzed using TIA version 4.5 (FEI) and ImageJ version 1.52h (National Institutes of Health, USA, Java 1.8.0_66).

Additonal experimental data

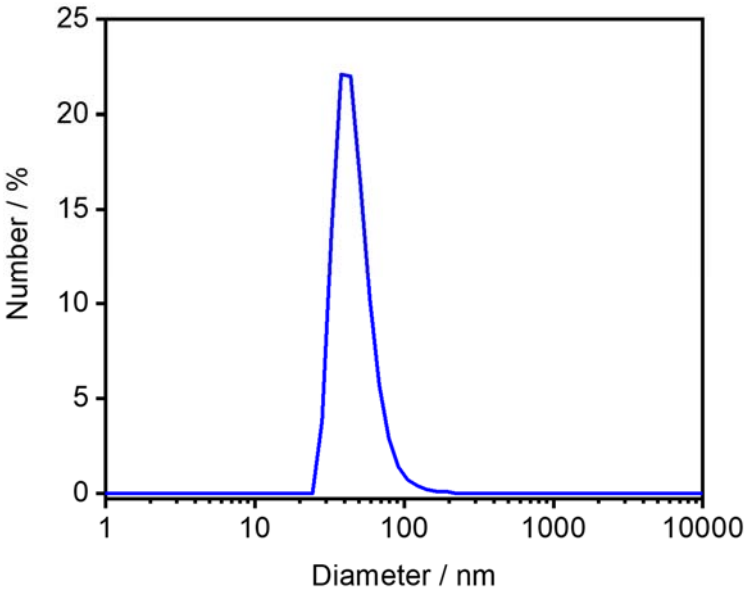


Figure S 1: Hydrodynamic diameter of Fe_3O_4 MNPs in water.

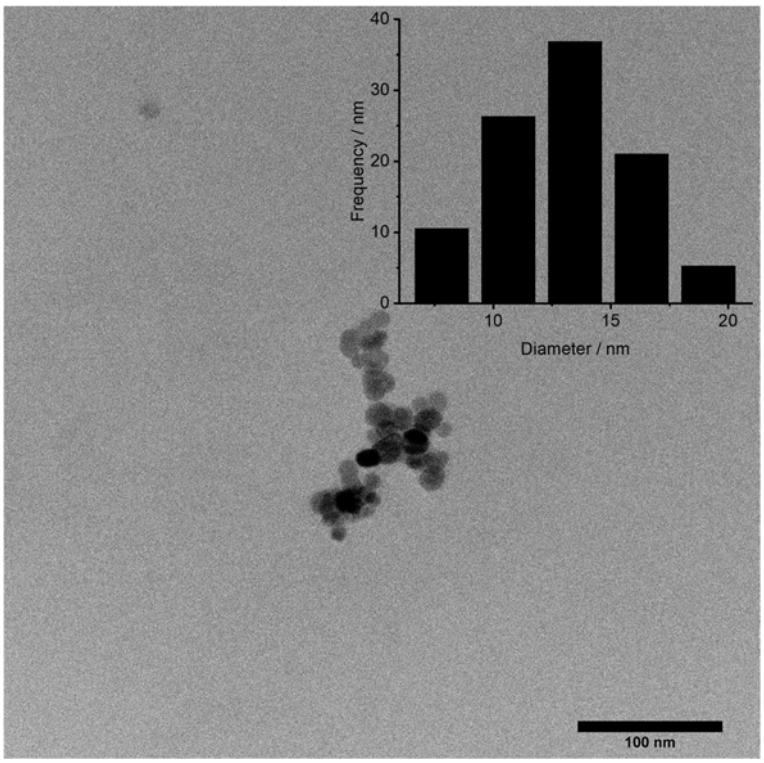


Figure S 2: Transmission electron microscopy of Fe_3O_4 MNPs with related histogram. Scale bar: 100 nm.

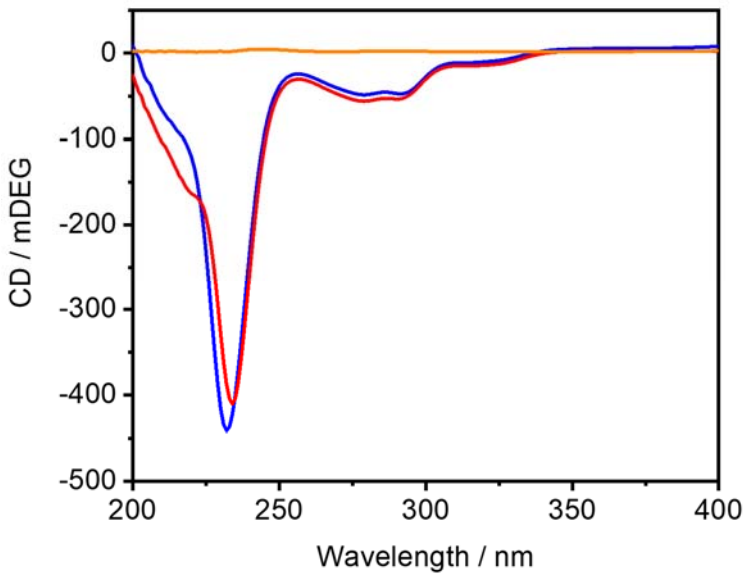


Figure S 3: CD spectra of 0.1 wt% Nap-GFYE hydrogel samples triggered with 20 mg/ml GdL. Blue data points: Without MNPs. Red data points: With 0.1 wt% MNPs. Orange data points: gelator pre-solution at pH = 12.

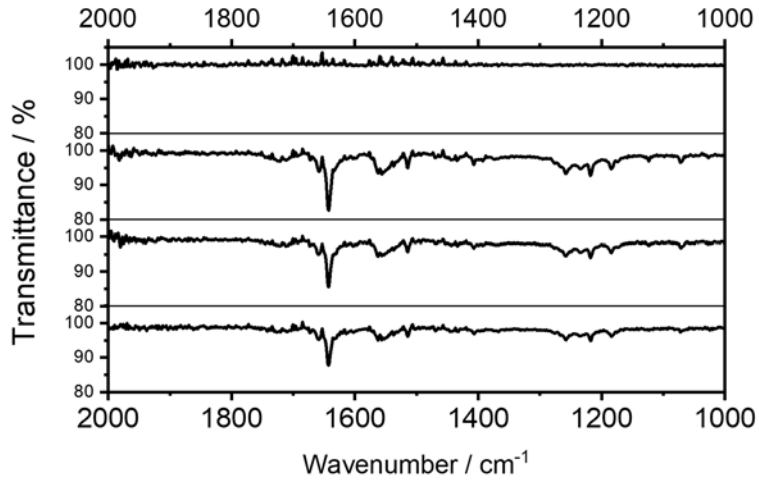


Figure S 4: IR spectra of 0.4 wt% Nap-GFYE hydrogel triggered with HCl. a) Reference sample without HCl at pH ≈ 12, b) without MNPs, c) with 0.1 wt% MNPs and d) with 0.4 wt% MNPs.

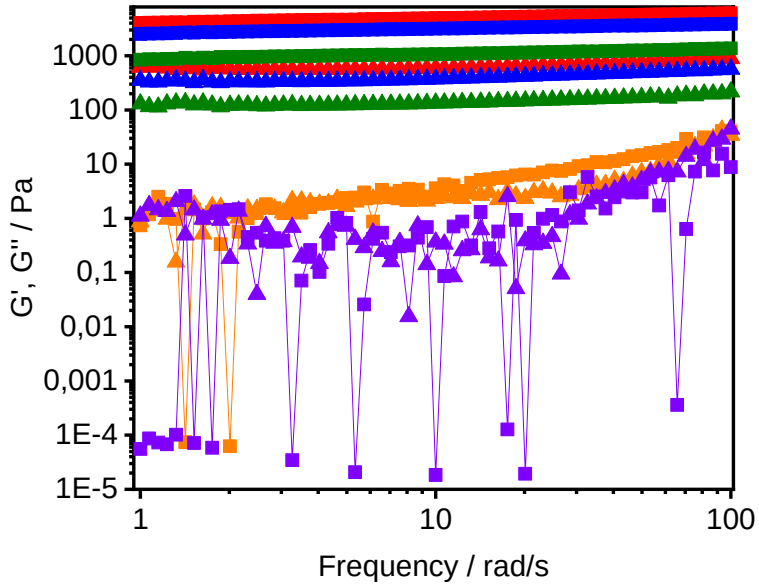


Figure S 5: Flow curves of 0.1 wt% Nap-GFYE materials triggered with 2 wt% MNPs measured at 0.1% strain and 20 °C with differing amount. Red data points: 20 mg/ml GdL (pH = 3), blue data points: 15 mg/ml (pH = 3.5), green data points: 10 mg/ml GdL (pH = 4), orange data points: 5 mg/ml GdL (pH = 4.5) and purple data points: 3 mg/ml GdL (pH = 5.5). Squares: storage modulus and triangles: loss modulus.

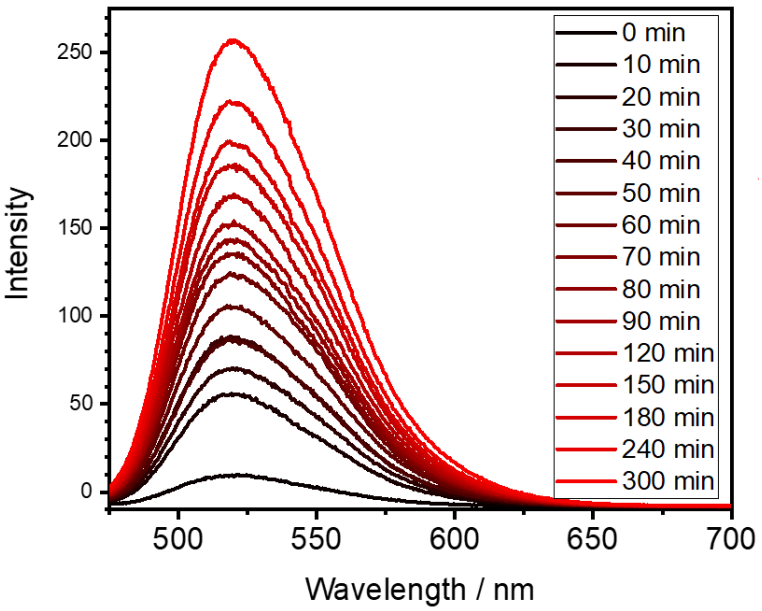


Figure S 6: Time dependent emission spectra of FITC-Dextran (M_w = 10000 g/mol) from a 0.1 wt% Nap-GFYE hydrogel triggered with 20 mg/ml GdL as a reference for the measurement of the dye release properties.

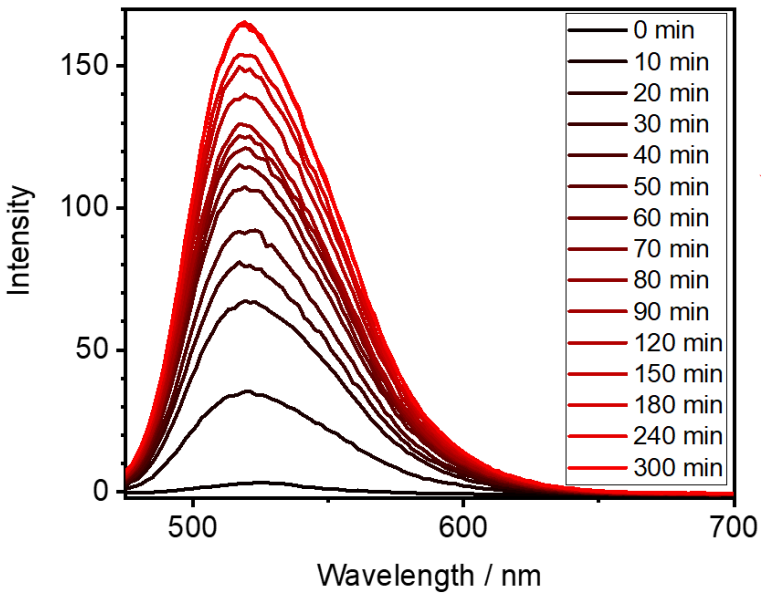


Figure S 7: Time dependent emission spectra of FITC-Dextran (M_w = 10000 g/mol) from a 0.1 wt% Nap-GFYE, 2 wt% MNP hydrogel triggered with 20 mg/ml GdL magnetized during measurement on a weak permanent magnet.

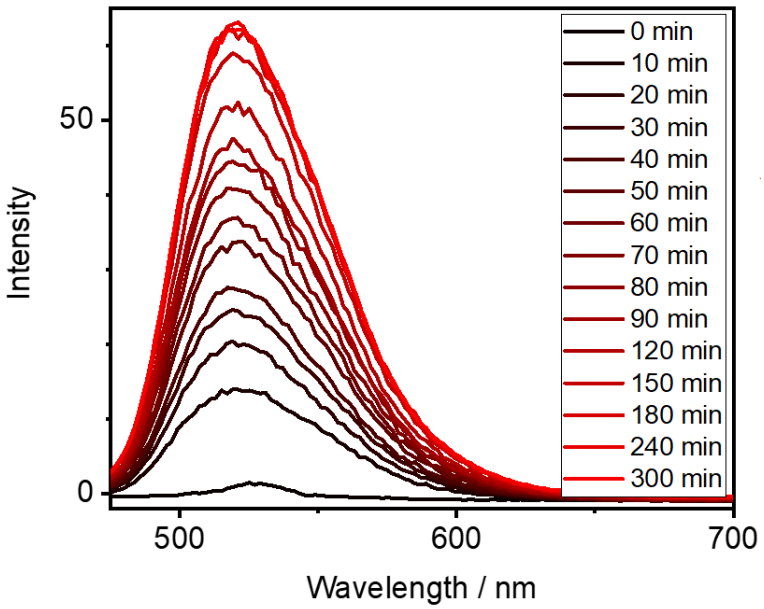


Figure S 8: Time dependent emission spectra of FITC-Dextran (M_w = 10000 g/mol) from a 0.1 wt% Nap-GFYE, 2 wt% MNP hydrogel triggered with 20 mg/ml GdL not magnetized during the measurement as a reference for the measurement of the dye release properties.

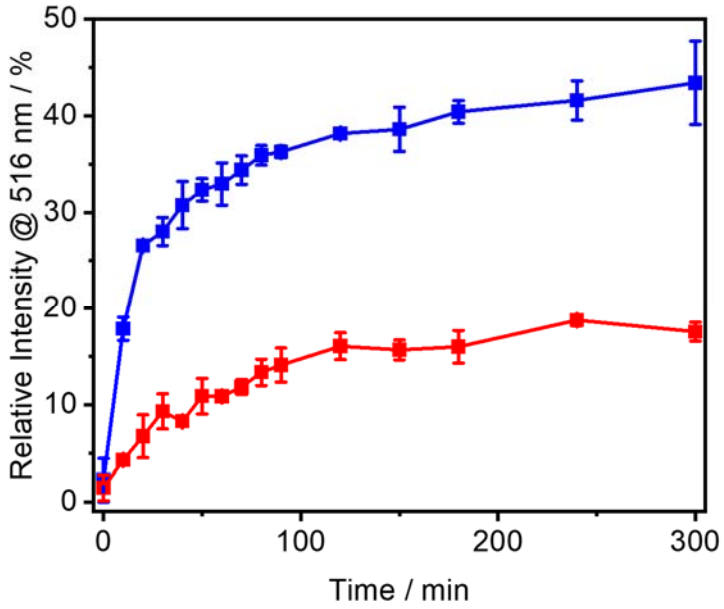


Figure S 9: Dye release of curves of FITC-Dextran (M_w = 4000 g/mol) from a 0.1 wt% Nap-GFYE hydrogel with 2 wt% MNPs triggered with 20 mg/ml GdL. Fluorescence intensity was referenced to the highest emission. Blue data points: magnetized sample, red data points: unmagnetized reference sample. The error bars reflect the standard deviation derived from a dual measurement.

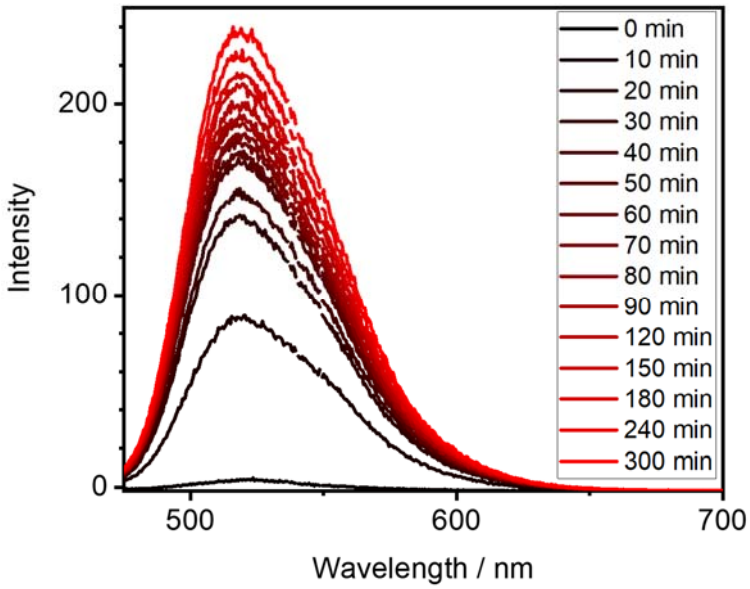


Figure S 10: Time dependent emission spectra of FITC-Dextran (M_w = 4000 g/mol) from a 0.1 wt% Nap-GFYE, 2 wt% MNP hydrogel triggered with 20 mg/ml GdL magnetized during the measurement as a reference for the measurement of the dye release properties.

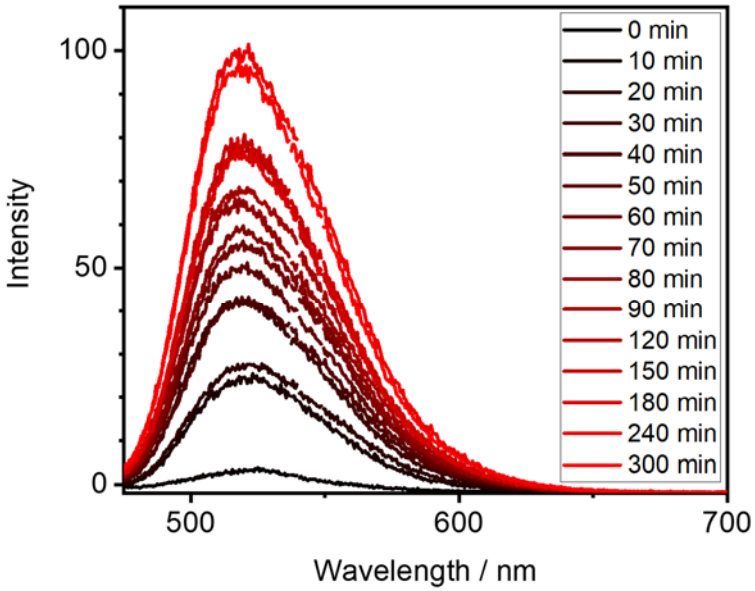


Figure S 11: Time dependent emission spectra of FITC-Dextran (M_w = 4000 g/mol) from a 0.1 wt% Nap-GFYE, 2 wt% MNP hydrogel triggered with 20 mg/ml GdL not magnetized during the measurement as a reference for the measurement of the dye release properties.

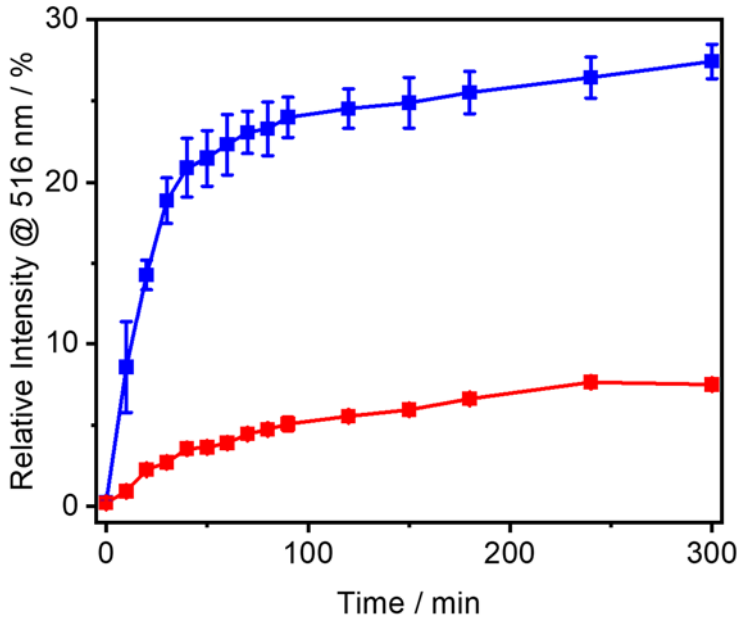


Figure S 12: Dye release of curves of FITC-Dextran (M_w = 250000 g/mol) from a 0.1 wt% Nap-GFYE hydrogel with 2 wt% MNPs triggered with 20 mg/ml GdL. Fluorescence intensity was referenced to the highest emission. Blue data points: magnetized sample, red data points: unmagnetized reference sample. The error bars reflect the standard deviation derived from a dual measurement.

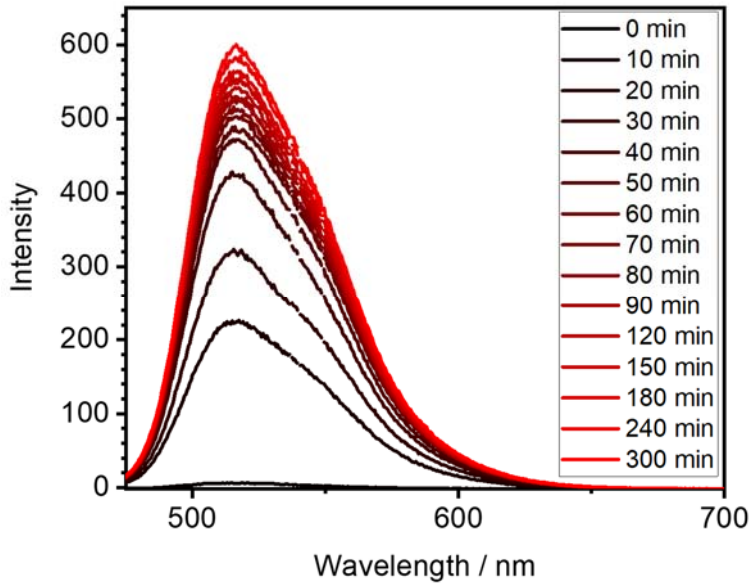


Figure S 13: Time dependent emission spectra of FITC-Dextran (M_w = 250000 g/mol) from a 0.1 wt% Nap-GFYE, 2 wt% MNP hydrogel triggered with 20 mg/ml GdL magnetized during the measurement as a reference for the measurement of the dye release properties.

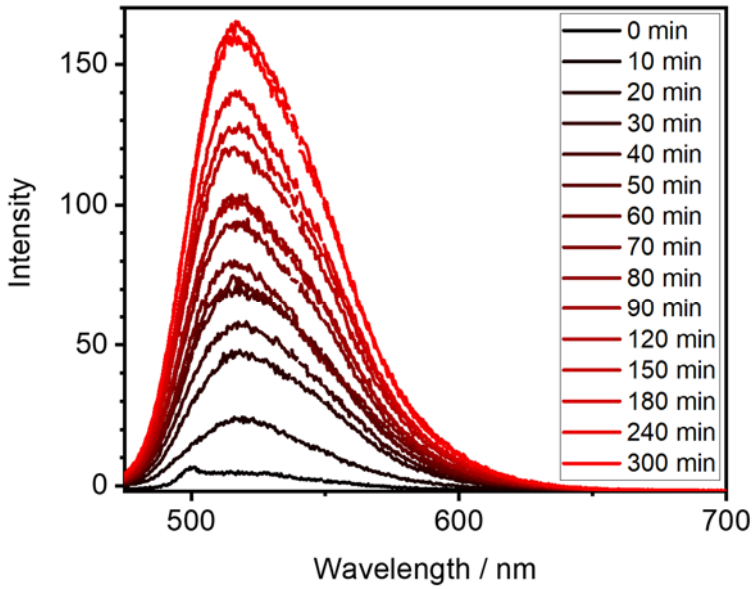


Figure S 14: Time dependent emission spectra of FITC-Dextran (M_w = 250000 g/mol) from a 0.1 wt% Nap-GFYE, 2 wt% MNP hydrogel triggered with 20 mg/ml GdL not magnetized during the measurement as a reference for the measurement of the dye release properties.

Synthesis

Magnetite nanoparticles

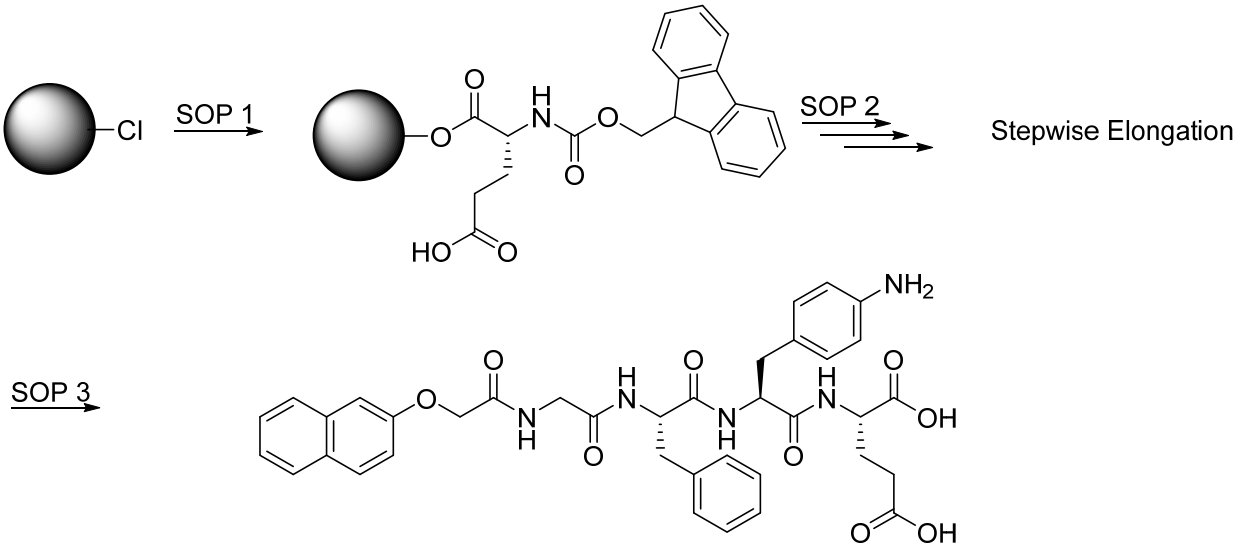
Magnetite (Fe_3O_4) nanoparticles were prepared using a simple coprecipitation technique. $Fe(II)Cl_2$ x 4 H_2O (497 mg, 2.5 mmol, 1 eq.) and $Fe(III)Cl_3$ x 6 H_2O (811 mg, 5 mmol, 2 eq.) were dissolved in MiliQ water (60 ml) under argon and degassed for 10 min. Ammonia (25%, ca. 3 ml) was added stepwise until a final pH of 11 was reached. The reaction was stirred at room temperature for 30 min and subsequently heated up to 60 °C in a sealed flask. After stirring for 1 h the reaction mixture was allowed to cool to room temperature and the resulting black MNPs were collected magnetically[2]. The particles were washed with water (2 x 50 ml) and 1 ml of the dispersion was separated from the bulk and dried in order to determine the particle loading. Finally, the MNP concentration was adjusted to 4 wt%.

Hydrodynamic diameter – DLS: 52.2 nm $\pm$ 2.7 nm

ζ-Potential:- 13.6 mV $\pm$ 0.9 mV

TEM:13.1 nm $\pm$ 2.9 nm

Solid Phase Peptide Synthesis of Nap-GFYE



Scheme 1: Schematic representation of the solid-phase peptide synthesis of Nap-GFYE.

Standard Operating Procedure (SOP) 1 (Loading of the resin)

The first Fmoc protected amino acid (Fmoc-glutamic acid(OtBu) 1.5 eq. relative to the amount 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 amount 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 amount 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 aminoacid (Fmoc-Tyrosine(tBu), 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: Fmoc-Phenylalanine, Fmoc-Glycine and napthoxyacectic acid (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:H_2O: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 Et_2O:pentane solution (3:1). The precipitate was collected *via* centrifugation and the remaining water was removed *via* lyophilisation. The dried peptide was purified *via* automated preparative chromatography with water/THF by ramping up the THF content from 20 vol% to 100 vol% over 16 min. The following chromatogram (see figure S 15) was obtained. The purified product elutes as a sharp peak around 5:30 min, whereas the peaks at higher CAN volume fraction are believed to be still protected peptide impurities.

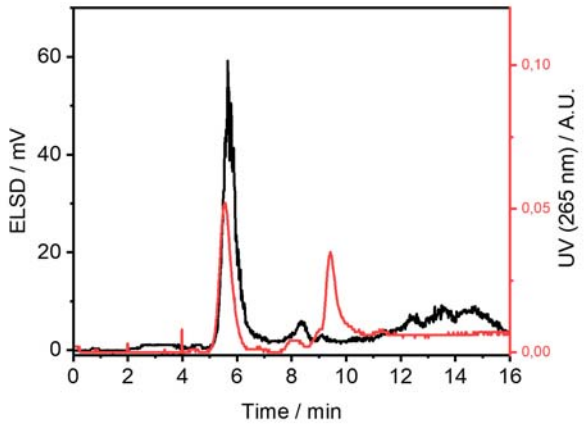


Figure S 15: Chromatogram of Nap-GFYE raw product on reverse phase column using water/THF as an eluent by ramping up the THF content form 20 vol% to 100 vol% over 16 min. Product peak at 5:30 minBlack data line: ELSD signal and red data line: UV-detector signal at λ = 265 nm.

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 8.27 (t, *J* = 5.8 Hz, 1H, Amide-H), 8.18 (dd, *J* = 9.8, 8.0 Hz, 2H, 2 x Amide-H), 8.08 (d, *J* = 8.3 Hz, 1H, Amide-H), 7.87 – 7.76 (m, 3H, Aryl-H), 7.50 – 7.43 (m, 1H, Aryl-H), 7.39 – 7.30 (m, 2H, Aryl-H), 7.27 – 7.10 (m, 6H, Aryl-H), 7.08 – 7.02 (m, 2H, 19-H, 23-H), 6.68 – 6.60 (m, 2H, 20-H, 22-H), 4.62 (s, 2H, 39-H), 4.55 – 4.40 (m, 2H, 13-H, 2-H), 4.30 – 4.19 (m, 1H, 25-H), 3.84 – 3.64 (m, 2H, 35-H), 3.04 – 2.86 (m, 2H, 17-H), 2.78 – 2.64 (m, 2H, 6-H), 2.33 – 2.24 (m, 2H, 30-H), 2.07 – 1.92 (m, 1H, 29'-H), 1.86 – 1.70 (m, 1H, 29''-H).

MALDI-MS: [*m*/*z*]: found: 721.30, calculated: 721.25 $[M+Na]^+$.

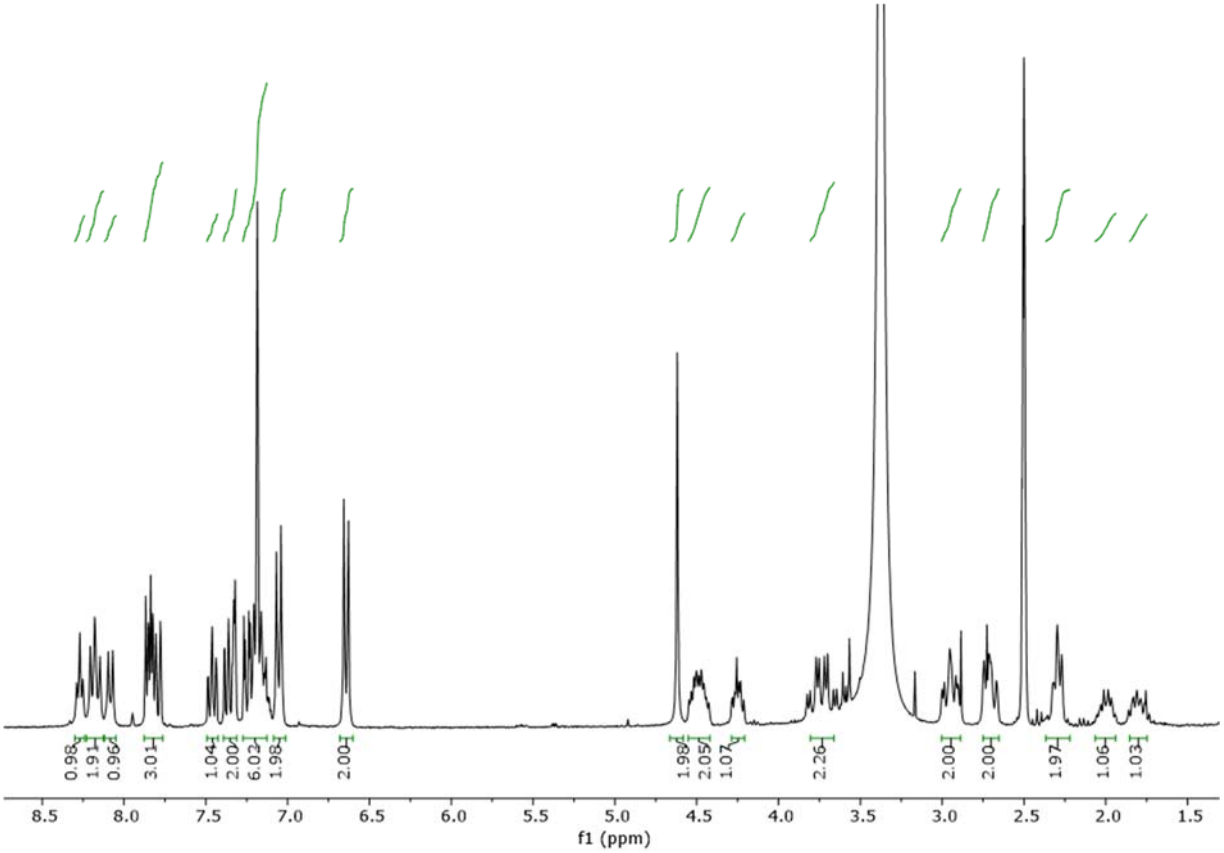


Figure S 16: ^{1}H-NMR spectra of Nap-GFYE (300 MHz in DMSO-d_6).

References

1. D. J. Adams, M. F. Butler, W. J. Frith, M. Kirkland, L. Mullen and P. Sanderson, *Soft Matter*, 2009, **5**, 1856.

2. S. Sagebiel, L. Stricker, S. Engel and B. J. Ravoo, *Chem. Commun.*, 2017, **53**, 9296.