ELECTRONIC SUPPLEMENTARY INFORMATION

Multiresponsive hydrogels and organogels based on photocaged cysteine

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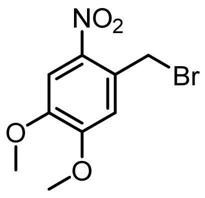
Materials and methods

All chemicals and reagents were purchased from Alfa Aesar, Sigma-Aldrich or Fluorochem and were used without further purification unless otherwise stated. NMR spectra were measured at 299 K on an Agilent DD2 500 or Agilent DD2 600 spectrometer. The chemical shifts (*δ*) were reported in ppm relative to deuterated solvents as internal standard (chloroform-d = 7.26 ppm, DMSO-d_6 = 2.50 ppm). Multiplicities are indicated as s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplet) t (triplet), q (quartet) and m (multiplet). High-resolution mass spectra were recorded on a Thermo Scientific Orbitrap LTQ XL.

Synthesis of 4,5-dimethoxy-2-nitrobenzyl bromide



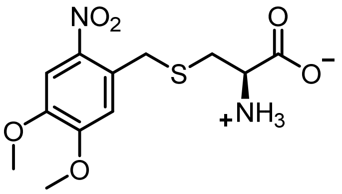
Under an argon atmosphere 4,5-dimethoxy-2-nitro-benzylalcohol (2.13 g, 10 mmol, 1.0 eq.) was suspended in 50 mL DCM and cooled to 0 °C. In a dropping funnel PBr_3 (1 mL, 11 mmol 1.1 eq.) was dissolved in 50 mL DCM and added dropwise over 30 min. The reaction mixture was stirred for 1.5 h at room temperature and neutralized with 5 M NaOH. The aqueous phase was extracted with 2x 100 mL DCM and the combined organic phases were washed with 100 mL ddH_2O and 100 mL brine subsequently. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure to yield the product as red solid. (1.875 g, 6.79 mmol, 68 %)

^{1}H-NMR (chloroform-d): δ (in ppm) = 7.65 (s, 1H), 6.93 (s, 1H), 4.85 (s, 2H), 3.96 (d, J = 15.3 Hz, 6H).

ESI-MS (TOF):

$[C_9H_{10}BrNO_4Na]^+$ calculated for (m/z) = 299.96659 found 299.96648.

Synthesis of 4,5-dimethoxy-2-nitrobenzyl-L-cysteine (DNC)



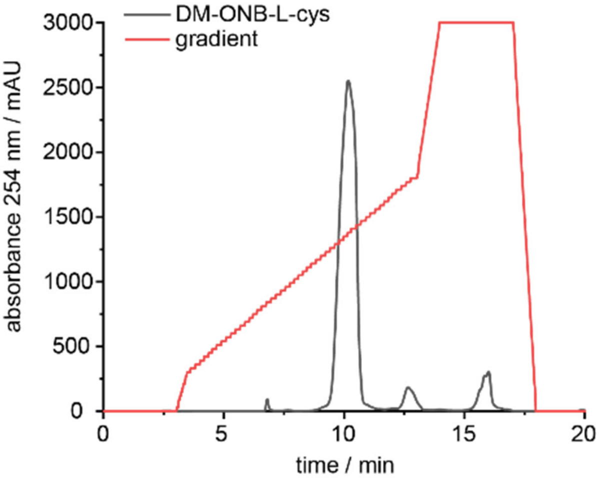
Under argon L-cysteine (789 mg, 6.51 mmol, 1.0 eq.) was dissolved in 26.0 mL freshly prepared NaOH (ddH_2O, degassed, 0.25 M, 1 eq.) and slowly added a suspension of 4,5-dimethoxy-2-nitrobenzyl bromide (1797 mg, 6.51 mmol, 1.0 eq) in 25.0 mL degassed 1,4-dioxane. The reaction was vigorously stirred at room temperature over 2.5 h. The crude mixture was acidified to pH = 6 using 1 M HCl the residual solvent was removed under reduced pressure. The crude product was dissolved in DMSO and separated via flash chromatography as stated below. The product was obtained as yellow solid (1634.8 mg, 5.17 mmol, 79 %).

^{1}H-NMR (dimethyl sulfoxide-d_6): δ (in ppm) = 7.69 (s, 1H), 7.18 (s, 1H), 4.21–4.16 (m, 1H), 4.15–4.02 (m, 2H), 3.92–3.84 (d, 6H), 2.97–2.84 (m, 2H).

ESI-MS (TOF):

$[C_{12}H_{15}N_2O_6S]^-$ calculated for (m/z) = 315.06563 found 315.06544.

Purification of 4,5-dimethoxy-2-nitrobenzyl-L-cysteine (DNC)



time / min	A: 50 mM NH_4Ac pH = 6.0	B: Acetonitrile
1-3	100 %	0 %
3	90 %	10 %
3-13	40 %	60 %

Fig. S1: Chromatogram of the preparative HPLC purification of DNC was performed on a Büchi PrepChrom C-700 equipped with Teledyne ISCO HighPerformance Gold C18-Aq. 150 g column. Desired product elutes around 10 min.

NMR and mass spectra

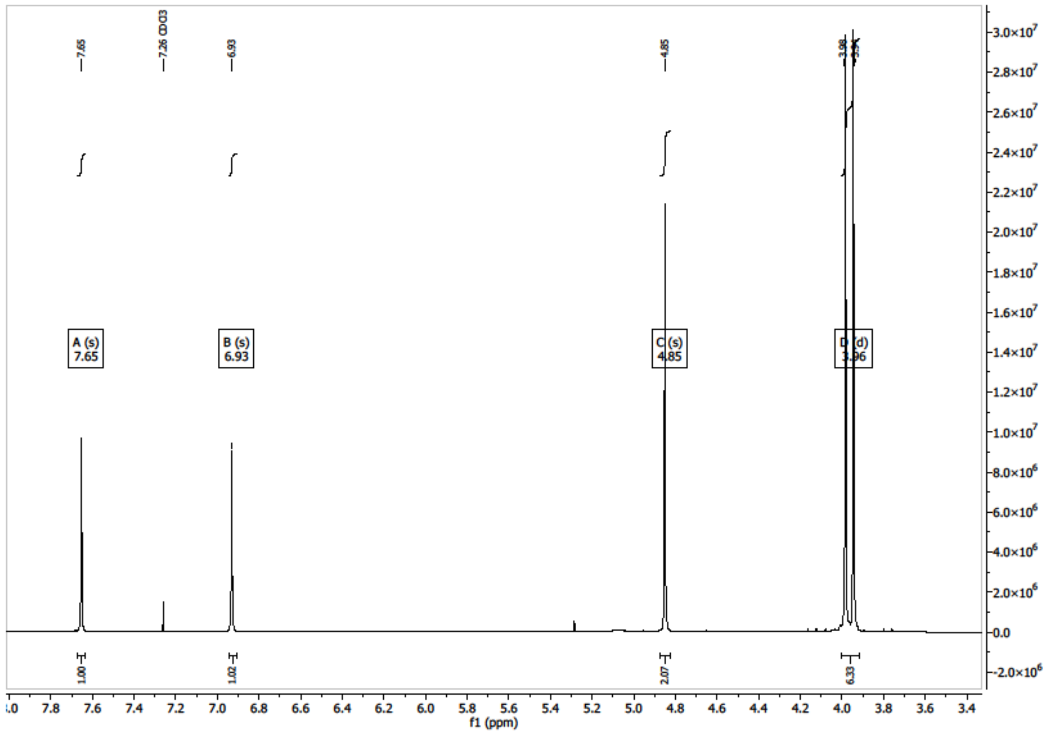


Fig. S2: ^{1}H-NMR spectrum of 4,5-dimethoxy-2-nitrobenzyl bromide in chloroform-d.

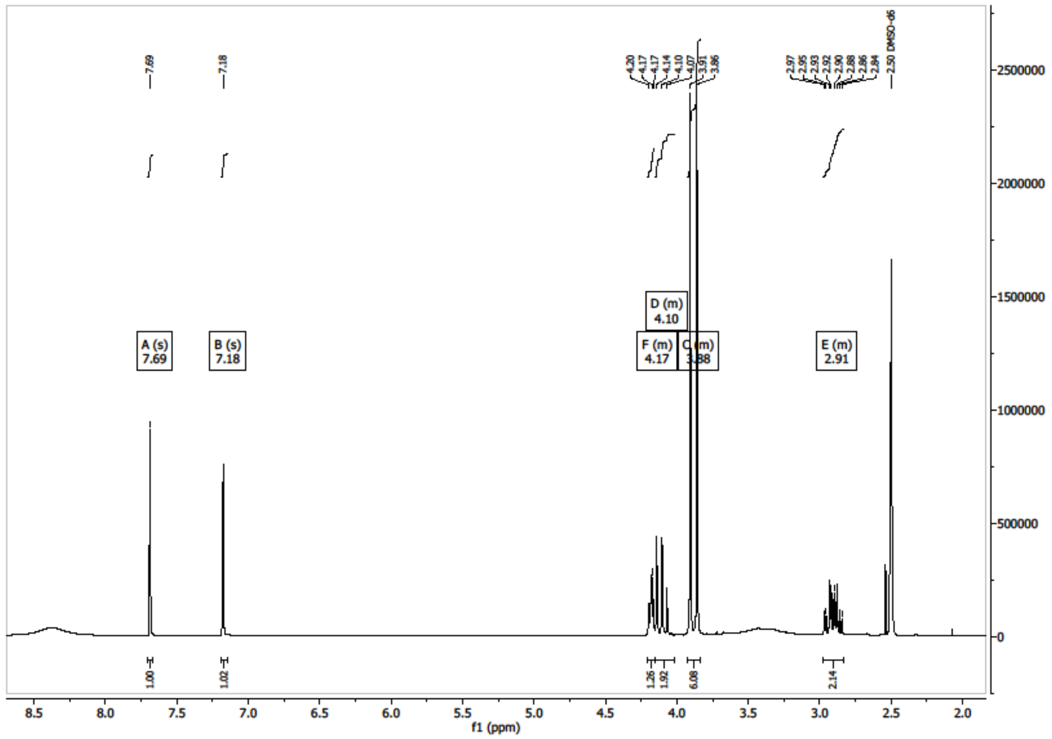


Fig. S3: ^{1}H-NMR spectrum of (**1**) in DMSO-d$_6$.

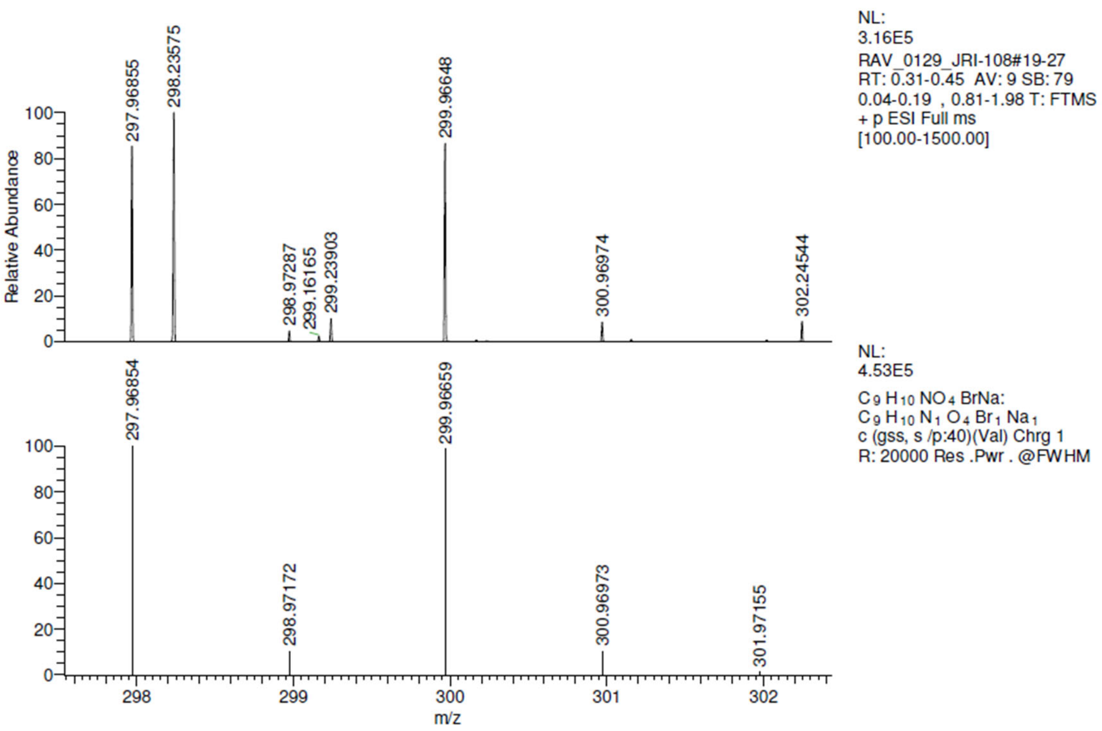


Fig. S4: HR-MS of 4,5-dimethoxy-2-nitrobenzyl bromide, top: measured; bottom: calculated.

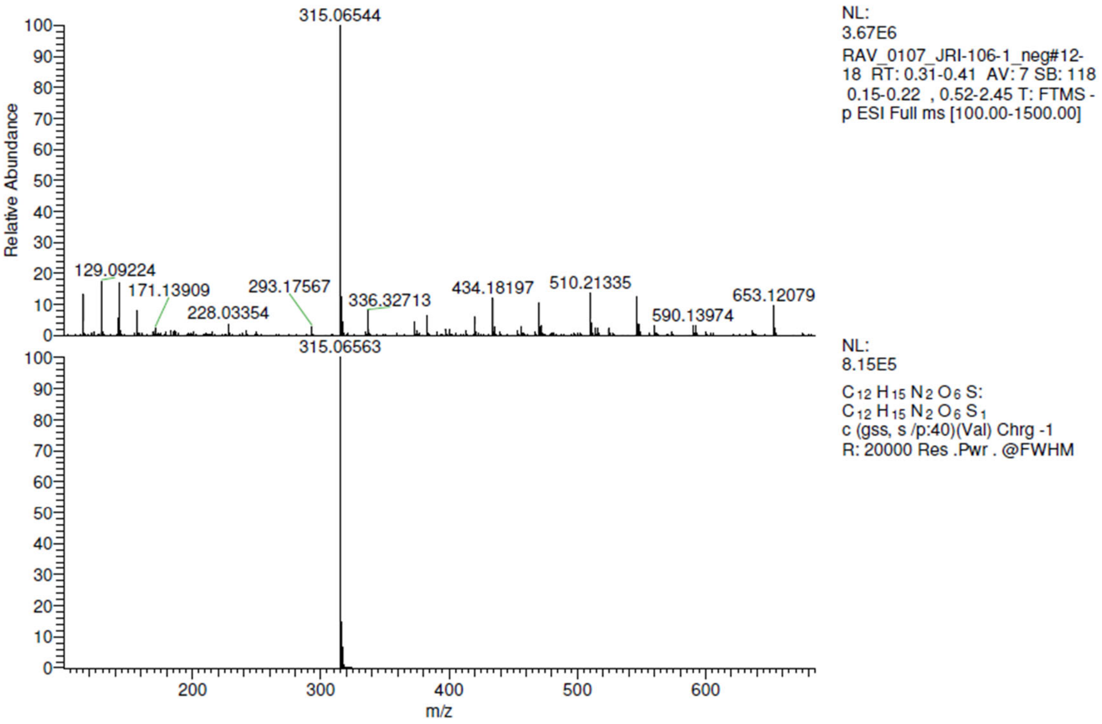


Fig. S5: HR-MS of (**1**), top: measured; bottom: calculated.

Preparation of sonication induced hydrogel

For a 3 % (w/v) gel 15 mg of DNC were transferred to a vial and 0.5 mL of aqueous NaOH (64 mM) were added. Complete dissolution was achieved by mixing with a pipette. The vial was subsequently sonicated in an Elmasonic P 70 H ultrasound bath by Elma (Elma Schmidbauer GmbH, Singen, Germany) at 220 W with 37 kHz for 1 min.

Preparation pH-induced hydrogel

For a 3 % (w/v) gel 15 mg of DNC were transferred to a vial and 0.5 mL of aqueous NaOH (64 mM) were added. Complete dissolution was achieved by mixing with a pipette. Then, the solution was transferred to a vial containing the appropriate amount of GdL. The vial was left in the dark for 1 h for gelation.

Preparation of organogels

The amount of DNC corresponding to 0.3-4.8 % (w/v) was added to a vial and 0.5 mL of the respective solvent was added. Any remaining precipitate was dispersed as homogeniously as possible and the vial was subsequently sonicated in an Elmasonic P 70 H ultrasound bath by Elma at 220 W with 37 kHz for 1 min to yield the organogels.

Rheology

Rheological measurements were performed 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. The data was processed using OriginPro 2021 9.8.0.200 (ORIGINLAB Corperation, Northampton, USA). All measurements were conducted with a CP25-2 cone plate spindle (25 mm diameter) and a P PTD200 measuring cell. Gel samples were prepared by following the above-mentioned procedure before being transferred onto the rheometer. For all rheological experiments, the measuring gap was set to 0.106 mm. Amplitude sweep measurements were performed from 0.1 Pa to 10 Pa.

Scanning electron microscopy (SEM)

Samples were prepared by spreading a thin layer of gel onto a freshly cleaned silicon wafer (10 mm x 10 mm) with a spatula and freeze-drying the sample over 3 days. The resulting xerogels were used as prepared. To increase sample conductivity, each sample was sputter-coated with gold for 3 s at 45 mA using a Quorum Technology Q150T coater (Quorum Technologies, Laughton, United Kingdom).

Scanning electron microscopy (SEM) measurements were conducted on an AURIGA Crossbeam workstation from Zeiss (Carl Zeiss AG, Oberkochen, Germany) with a field emission gun (Schottky-type). An acceleration voltage of 3.00 kV was used at a working distance between 2.9 mm and 4.1 mm.

Cysteine release from hydrogel

Release experiments were investigated via UV-vis spectroscopy using a Jasco V750 double-beam spectrometer. Gel samples were prepared by dissolving DNC in 0.2 mL NaOH (64 mM) solution at 3 wt% and immediately transferring the solution into quartz cuvettes (10 mm pathlength) for UV measurement. For a sonication-induced gel, the cuvette was then irradiated with ultrasound for 1 min. pH-triggered gels were obtained by transferring the gelator solution to a cuvette containing 1 % (w/v) GdL and moving the resulting solution through the pipette multiple times to quickly dissolve GdL before leaving it for 2h for gelation.

1 mL of Ellman´s reagent solution (35 µM in PBS buffer) was added to the gel containing cuvettes. Repeatedly, the gel blocks were irradiated with UV light before UV-vis measurements were conducted. Reference experiments were performed were the cuvettes were charged with gel and Ellman´s reagent as described above and left in the dark during the entire experiment.

Additional data: Photoresponse of hydrogel

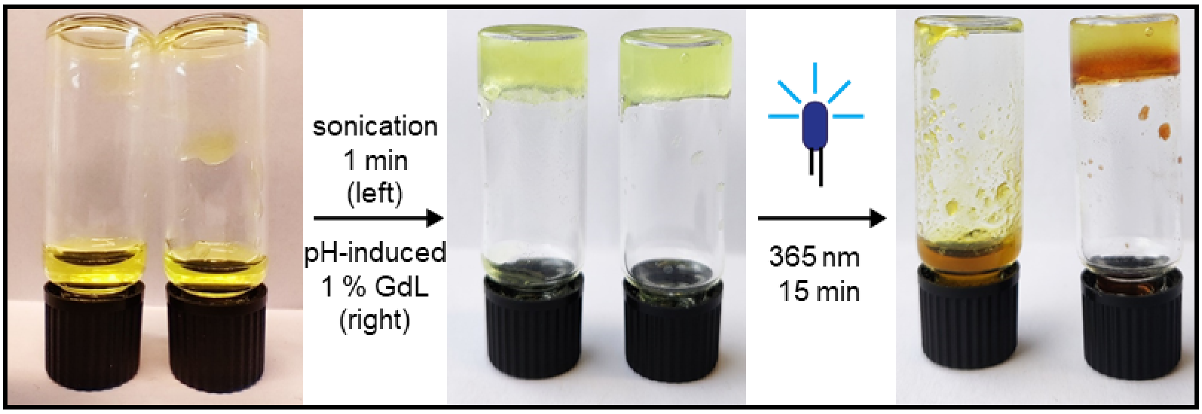


Fig S6: Sonication-induced (left vial) and pH-induced (right vial) gels were prepared as stated above. Irradiation was performed at 365 nm with a 3 W LED for 15 min.

Additional data: Cysteine release from hydrogel

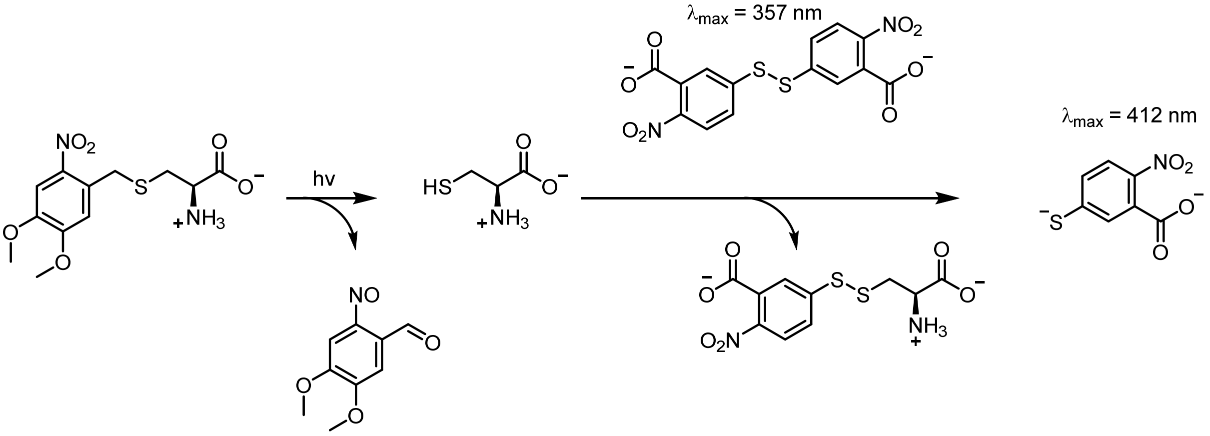


Fig. S7: Cysteine release and reaction with Ellman´s reagent.

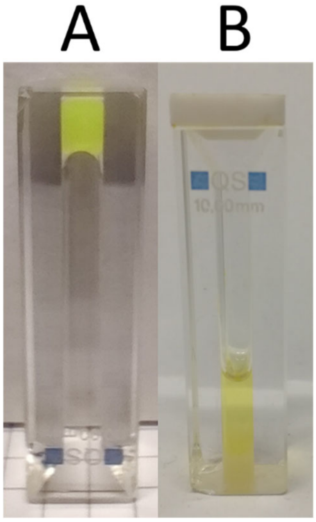


Fig. S8: Photographs of DNC hydrogel before cysteine release experiments (A) and solution after cysteine release (B).

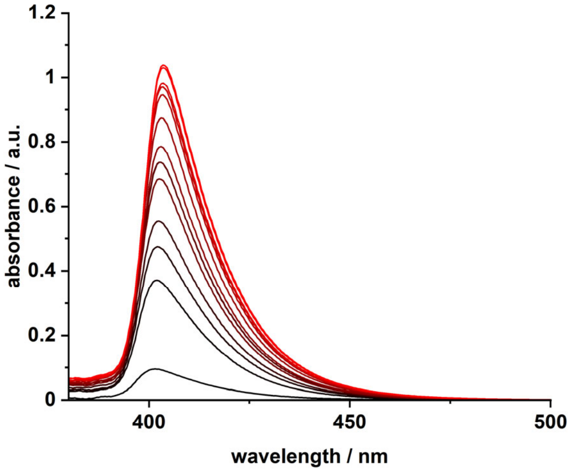


Fig. S9: UV-vis spectrum of photoresponsive cysteine release from pH-induced hydrogel, spectra were recorded every 60 s over a total period of 13 minutes.

Additional data: Multiresponse of hydrogel (acid, base, oxidation)

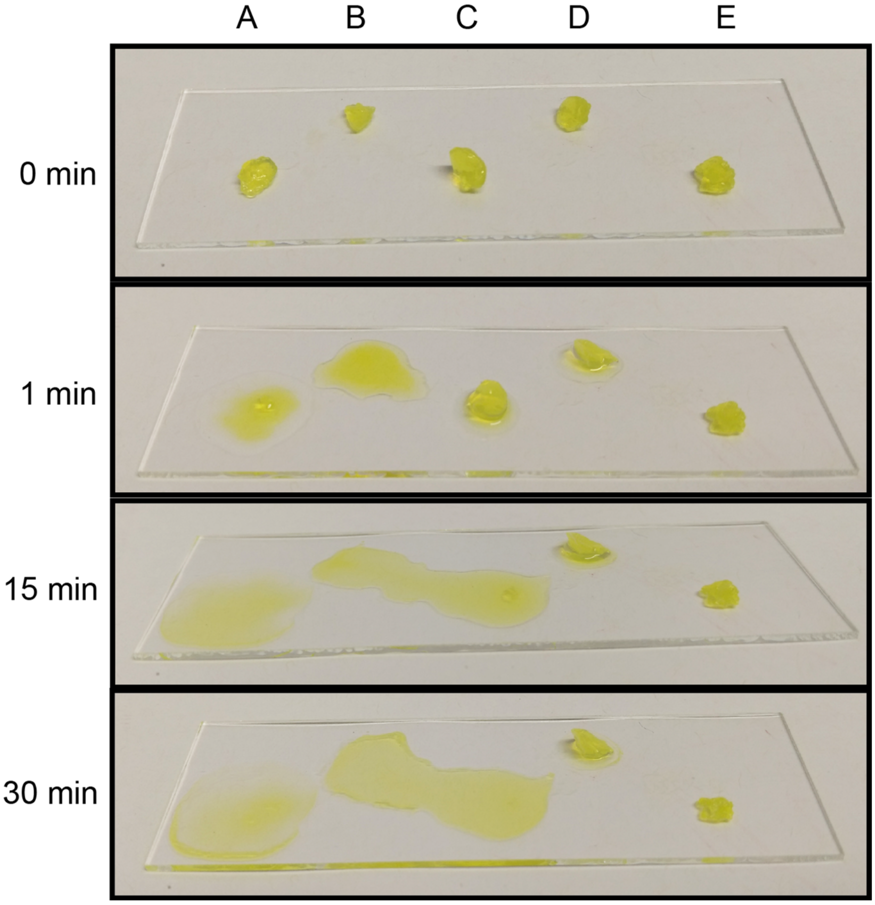


Fig S10: 20 mg pieces of sonication-induced 3 % (w/v) DNC hydrogel were transferred to a microscope slide and incubated with 20 µL of (A) 1 M HCl, (B) 1 M NaOH, (C) 30 % H_2O_2, (D) ddH_2O and (E) control.

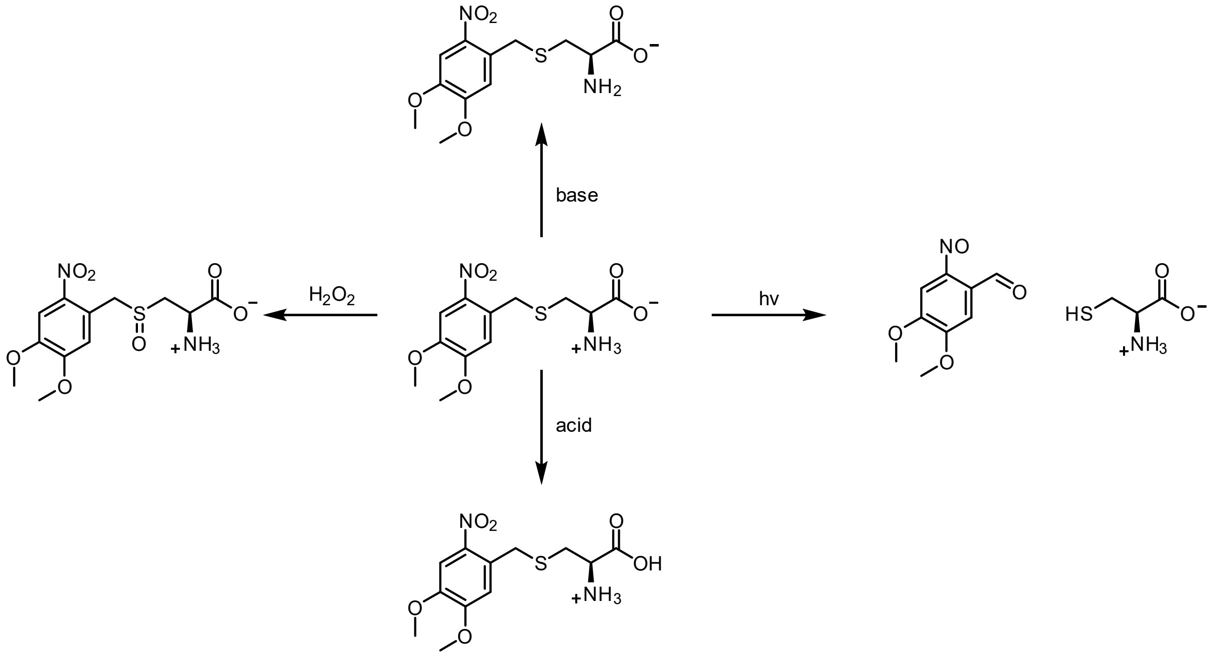


Fig. S11: Incubation with acid/base leads to protonation/deprotonation or the gelator. Oxidation with H_2O_2 is known to occur on the thioether, resulting in the corresponding sulfoxide.[1] Irradiation with 365 nm leads to photolysis by Norrish-type-II mechanism resulting in 4,5-dimethoxy-2-nitrosobenzaldehyde and L-cysteine.[2]

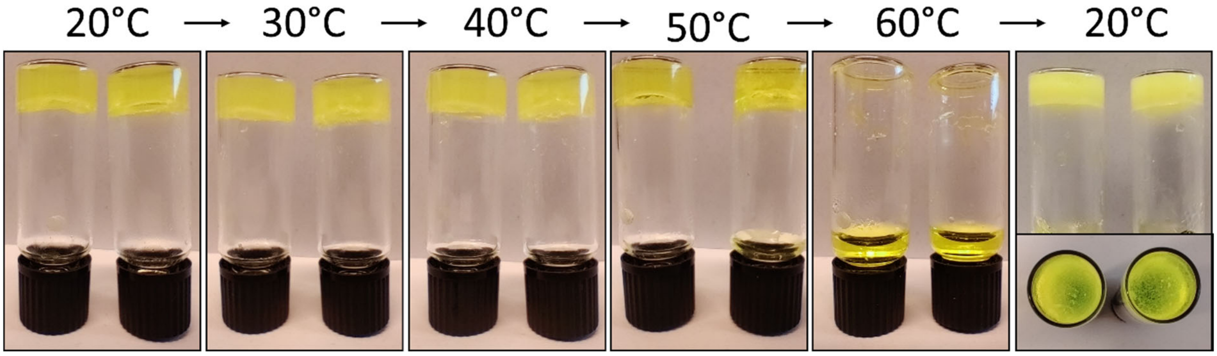


Fig. S12: Behaviour of sonication- (left vials) and pH-induced (right vials) DNC-hydrogels. Irreversible precipitation is visible after heating to 60 °C. Additional data: Photoresponse of organogel

Additional data: Rheology measurements of hydrogels and organogel

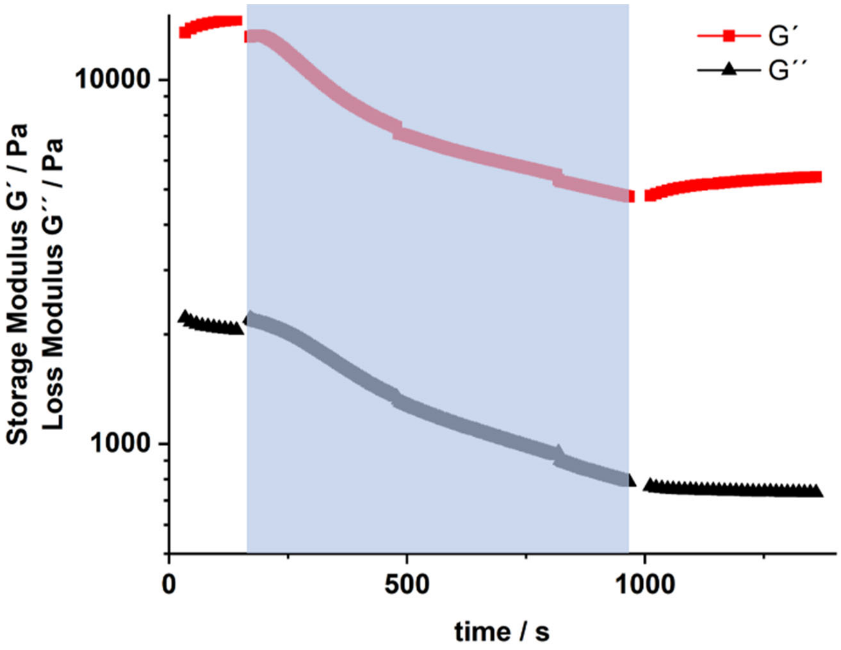


Fig. S13: Rheological behaviour of a 0.3 % (w/v) DNC organogel in acetonitrile under UV-irradiation (365 nm).

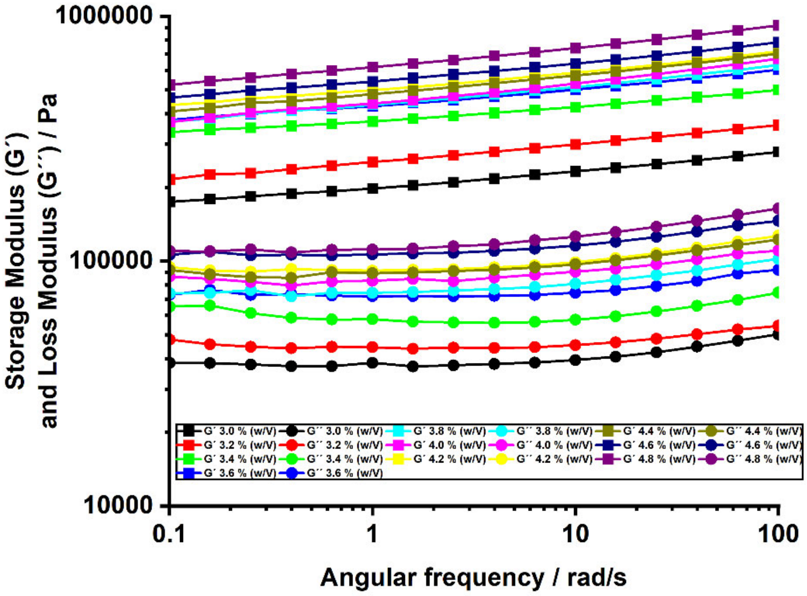


Fig. S14: Frequency sweep of sonication-induced hydrogels at given DNC concentrations, performed at a constant shear strain of 0.1 %.

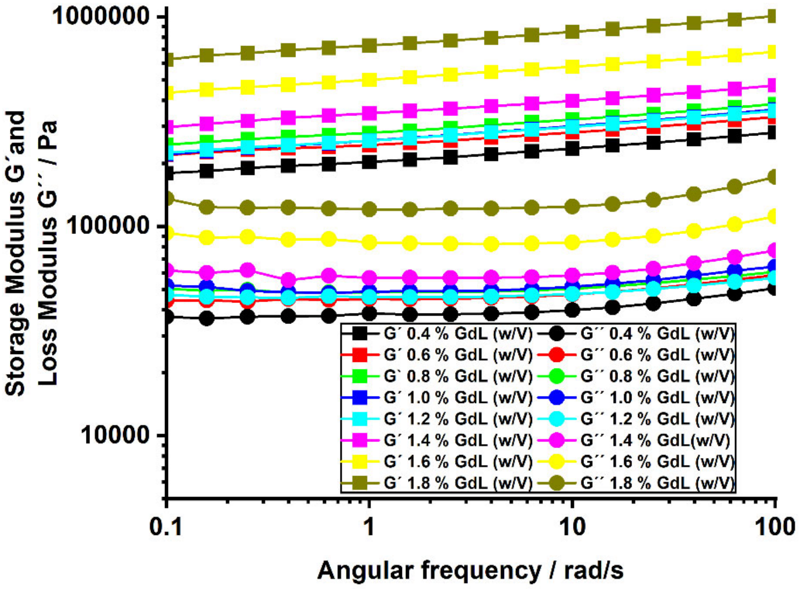


Fig. S15: Frequency sweep of pH-induced hydrogels at given GdL concentrations and at 3 % (w/V) DNC, performed at a constant shear strain of 0.1 %.

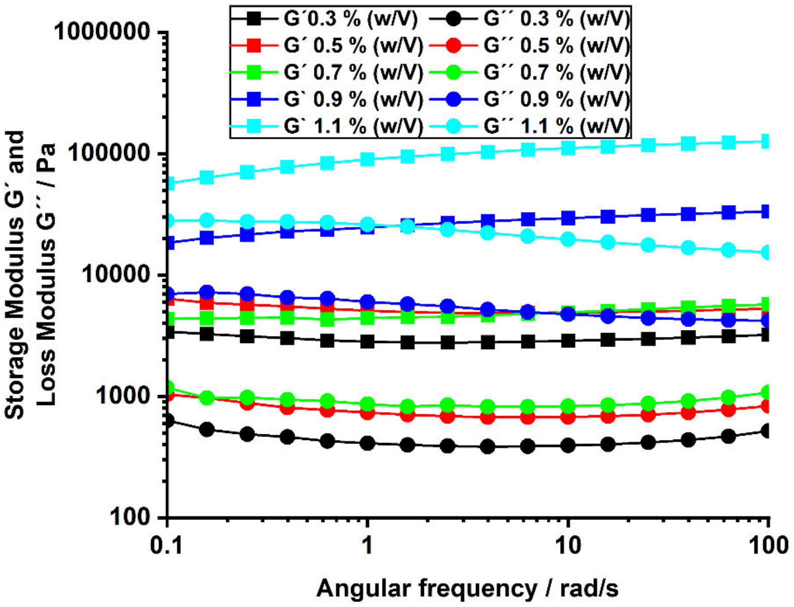


Fig. S16: Frequency sweep of sonication-induced organogels in acetonitrile at given DNC concentrations, performed at a constant shear strain of 0.1 %.

References

1. J. Pan and K. S. Carroll, *Org Lett*, 2015, **17**, 6014-6017.
2. C. G. Bochet, *J. Chem. Soc., Perkin Trans. 1*, 2002, **2**, 125-142.