Electronic Supplementary Information

Photoresponsive hybrid hydrogel with a dual network of agarose and a self-assembling peptide

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<u>Materials</u>

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).

UV/vis spectroscopy

UV/vis spectra were measured on a Jasco V-770 spectrophotometer (Jasco Deutschland GmbH, Pfungstadt, Germany) using High Precision SUPRASIL quartz glass cuvettes (Hellma Analytics GmbH, Müllheim, Germany). The spectra were recorded with Spectra Manager 2, Spectra Manager Version 2.14.06 (Jasco Deutschland GmbH, Pfungstadt, Germany). The samples were dissolved in 20 mM HEPES buffer (pH = 8.01) and the baseline was measured against the same solvent. Data analysis was realized using OriginPro 2018 b b9.5.5.409 (ORIGINLAB Corperation, Northampton, USA).

<u>Rheology</u>

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).

 $250 \ \mu$ l of every hydrogel sample were prepared individually and gelated overnight in 0.5 ml EPPENDORF-Cups. Prior to the measurement the material inside one EPPENDORF-Cup was transferred onto the rheometer. After moving to the measuring position the sample was equilibrated for 10 min before starting the first measurement.

Photo rheological measurements were performed by equipping the rheometer with a P PTD200 + H PTD200 (Anton Paar GmbH, Graz, Austria) photo cell and a CP25-2 (Anton Paar GmbH, Graz, Austria) spindle (25 mm plate diameter, 2 degree angle). The sample was continuously measured at 20 °C, 0.1% shear strain and a frequency of 1 Hz during irradiation with UV (λ = 365 nm) or vis light (λ = 520 nm) for 2 min each. For irradiation at λ = 365 nm a UV LED Gen2 Emitter (radiant flux 1.2 W, LED Engin Inc., San Jose, USA) and at λ = 520 nm a LSC-G HighPower-LED (radiant flux 87 Im, Cree Inc., Durham, USA) were used.

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 0.5% was applied. The measurement was carried out at 20 °C and the frequency was monitored from 0.1 Hz up to 100 Hz.

Shape memory experiments

Hydrogel rods were prepared using a simple heating-cooling cycle. The desired amount of AAP-FGDS, agarose and KOH (1 eq.) were weighed in a 1 ml vial and MiliQ was added. The obtained yellow suspension was heated to 90 °C until all components were completely dissolved. The hot solution was transferred into a pre-heated glas pipette. After sealing of the pipette with parafilm the system was left unmoved overnight to ensure complete gelation.

A gel rod of approximately 1.5 cm length was gently pressed out of the pipette and placed in front of an angle scale to enable graphical angle analysis. The rod was subsequently irradiated for 3 min using UV light (λ = 365 nm) giving rise to a form reported as the permanent shape. After bending the rod upwards with gentle mechanical force, the gel was irradiated for 3 min with visible light (λ = 520 nm). The obtained silhouette is reported as the programmed shape. Removal of the force usually led to a relaxation of the bent material towards the ground. To ensure that no further unintended relaxation took place the sample was rested for 15 min. The shape kept after this time is referred to as the temporary shape. In order to restore the original bend, the material was irradiated for 3 min using UV light (λ = 365 nm). The final shape is reported as the recovered shape. Three cycles of irradiation, bending, resting and irradiation were executed to analyze fatigue phenomena. Quantification was achieved by measurement of the angles of the bent rod as visible on the angle scale. The vertical axis was set as 0° bend.

Constant light intensity was ensured by placing the light source 2 cm above the glass-rod and utilizing the same LEDs for every cycle. For irradiation at λ = 365 nm a UV LED Gen2 Emitter (radiant flux 1.2 W, LED Engin Inc., San Jose, USA) and at λ = 520 nm a LSC-G HighPower-LED (radiant flux 87 lm, Cree Inc., Durham, USA) were used.

Solvent loss was estimated by transferring a short piece of the hydrogel rod onto a pre-weighed glass slide. The rod was subsequently irradiated with UV light (λ = 365 nm) for 3 min, visible light (λ = 520 nm), rested for 15 minutes under ambient conditions and finally irradiated for 3 min using UV light (λ = 365 nm) in order to replicate the conditions of the shape memory experiment. Three cycles of irradiation, resting and irradiation were executed. The solvent loss is referred as the weight reduction of the sample after each cycle.

Additional experimental data



Figure S1: Determination of the critical gelation concentration of AAP-FGDS with equivalent of KOH *via* inverted vial tests (pH = 11). Gelation was achieved through a heating-cooling cycle. Gelator concentration: a) 5 wt%, b) 4 wt%, c) 3 wt%, d) 2 wt% and e) 1 wt%.



Figure S2: Determination of the critical gelation concentration of AAP-FGDS at acidic pH *via* inverted vial tests. Gelation was achieved through slow acidification of a basic solution using glucono- δ -lactone. Gelator concentration: a) 1 wt%, b) 0.5 wt%, c) 0.3 wt% and e) 0.1 wt%.



Figure S3: Photoresponse of a 2 wt% AAP-FGDS/0.7 wt% agarose hydrogel with 1 eq. of KOH (pH = 11). Photographs were taken after 10 min of irradiation for each wavelength.



Figure S4: Frequency sweep rheology measured at 0.5% deformation and 20 °C. Squares: Storage modulus, triangles: Loss modulus. Blue data points: 5 wt% AAP-FGDS hydrogel with 1 eq. of KOH (pH = 11). Red data points: 1.7 wt% agarose hydrogel with 1 eq. KOH (equivalents of KOH related to 5 wt% AAP-FGDS, pH = 13). Green data points: 5 wt% AAP-FGDS/1.7 wt% agarose hydrogel with 1 eq. of KOH (pH = 11).



Figure S5: Amplitude sweep rheology measured at 1 Hz frequency and 20 °C. Squares: Storage modulus, triangles: Loss modulus. Blue data points: 5 wt% AAP-FGDS hydrogel with 1 eq. of KOH (pH = 11). Red data points: 1.7 wt% agarose hydrogel with 1 eq. KOH (equivalents of KOH related to 5 wt% AAP-FGDS, pH = 13). Green data points: 5 wt% AAP-FGDS/1.7 wt% agarose hydrogel with 1 eq. of KOH (pH = 11).



Figure S6: a) Schematic representation of the shape memory experiment: The programmed shape is achieved after gentle mechanic deformation of the hydrogel rod in combination with 3 min of green light ($\lambda = 520$ nm) irradiation. The small photograph was taken directly after removal of the mechanical force. The temporary shape photography was recorded after 15 min of waiting. Recovered shape pictures were obtained after 3 min of irradiation with UV light ($\lambda = 365$ nm). b) Shape memory cycle 2 of a 15 wt% AAP-FGDS, 5 wt% agarose and 1 eq. KOH hydrogel (pH = 11).

	Cycle 1	Cycle 2	Cycle 3
Solvent loss [wt%]	18,9	34,2	46,1
Standard deviation [±wt%]	1,1	1,4	1,4

Table S1: Solvent loss of a 15 wt% AAP-FGDS, 5 wt% agarose and 1 eq. KOH hydrogel (pH = 11) during the shape memory cycles.

<u>Synthesis</u>

Synthesis of 3-(2-phenylhydrazono)pentane-2,4-dione



Aniline (1,86 g, 20 mmol, 1.82 ml, 1 eq.) and HCl (12 M, 4,6 ml) were dissolved in AcOH (30 ml) at 0 °C. NaNO₂ (1,66 g, 24 mmol, 1.2 eq.) dissolved in a minimum amount of water (ca. 5 ml) was added dropwise and stirred for 1 h. The resulting mixture was dropwise added into a suspension of NaOAc (2.46 mg, 30 mmol, 3 eq.) and pentane-2,4-dione (2.60 g, 26 mmol, 2.65 ml, 1.3 eq.) in EtOH/water 10:6 (32 ml). The mixture was stirred for 1 h and the yellow precipitate was collected *via* filtration. The solid was washed with EtOH (50 ml) and water/EtOH 1:1 (50 ml) and dried under vacuum. The product was isolated as a yellow solid in a yield of 2.52 g (12.3 mmol, 62%)¹.

¹**H NMR**: (400 MHz, CDCl₃) δ [ppm]= 14.74 (s, 1H, -NH-), 7.49 – 7.35 (m, 4H, *o,m*-Ph **H**), 7.24 – 7.15 (m, 1H, *p*-Ph **H**), 2.61 (s, 3H, -C**H**₃), 2.50 (s, 3H, -C**H**₃).

¹³**C NMR**: (101 MHz, CDCl₃) δ [ppm]= 197.97, 197.16, 141.53, 133.22, 129.68, 125.92, 116.28, 31.71, 26.67.

ESI-MS: [*m*/*z*]: found: 227.0798 [M+Na]⁺, calculated: 227.0791

Synthesis of 3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazole (AAP)



3-(2-Phenylhydrazono)pentane-2,4-dione (2.52 g, 12.3 mmol, 1 eq.) and hydrazine x hydrate (396 mg, 12.3 mmol, 0.48 ml, 1 eq.) were dissolved in EtOH (125 ml) and the solution was refluxed for 3 h. The orange solution was concentrated under reduced pressure and the 3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazole was isolated as a yellow solid without further purification in a yield of 2.392 g (11.95 mmol, 97%)¹.

¹**H NMR**: (400 MHz, CDCl₃) δ [ppm]= 10.20 (s, 1H, -NH-), 7.87 – 7.74 (m, 2H, *o*-Ph **H**), 7.52 – 7.44 (m, 2H, *m*-Ph **H**), 7.43 – 7.36 (m, 1H, *p*-Ph **H**), 2.64 (s, 6H, -C**H**₃).

¹³**C NMR**: (101 MHz, CDCl₃) δ [ppm]= 153.55, 141.49, 134.74, 129.51, 128.94, 121.86, 12.20.

ESI-MS: [*m*/*z*]: found: 201.1146 [M+H]⁺, calculated: 201.1135

¹ L. Stricker, E. C. Fritz, M. Peterlechner, N. L. Doltsinis, B. J. Ravoo, J. Am. Chem. Soc., 2016, **138**, 4547–4554.

Synthesis of methyl (E)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetate



AAP (13.57 g, 67.8 mmol, 1 eq.), K_2CO_3 (28.11 g, 203.4 mmol, 3 eq.) and methyl 2bromoacetate (13.39 g, 88.14 mmol, 1.3 eq.) were dissolved in ACN (150 ml) and refluxed for 2 h. The reaction was allowed to cool and afterwards stirred over night at room temperature. The orange suspension was concentrated under vacuum and the residue was re-dissolved in EtOAc/water (300 ml, 1:1). After separation of the phases the aqueous layer was extracted with EtOAc (2 x 70 ml). The combined organic layers were dried over MgSO₄ and the solvent was removed under vacuum. The resulting residue was purified *via* column chromatography (DCM/MeOH 98:2; $R_f = 0.8$) and the title product was isolated as an orange oil in a yield of 7.5 g (27.56 mmol, 41%)^{Fehler! Textmarke nicht definiert.}

¹**H NMR**: (400 MHz, CDCl₃) δ [ppm]= 7.83 – 7.76 (m, 2H), 7.46 (t, *J* = 8.2, 7.7, 1.8 Hz, 2H), 7.41 – 7.35 (m, 1H), 4.85 (s, 2H), 3.79 (s, 3H), 2.57 (s, 3H), 2.51 (s, 3H).

¹³**C NMR**: (101 MHz, CDCl₃) δ [ppm]= 167.86, 153.54, 143.39, 139.97, 135.47, 129.54, 128.92, 121.85, 52.82, 50.43, 14.03, 9.85.

ESI-MS: [m/z]: found: 295.1189 [M+Na]⁺, calculated: 295.1165

Synthesis of (E)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetic acid (AAP-carboxylic acid)



Methyl (*E*)-2-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)acetate (7.5 g, 27.6 mmol, 1 eq.) was dissolved in THF/water (250 ml, 4:1) and LiOH was added (0.99 g, 41.3 mmol, 1.5 eq.) in one portion. After stirring overnight at room temperature the solvent was removed under vaccum and the residue was re-dissolved in water (400 ml). The mixture was acidified to pH \approx 2 using concentrated HCl followed by extraction with DCM (3 x 150 ml). The title product was obtained after drying the combined organic layers over MgSO₄ and evaporation of the solvent under vacuum as a yellow solid in a yield of 3.71 g (14.4 mmol, 52%)¹.

¹**H NMR**: (400 MHz, DMSO-*d*₆) δ [ppm]= 13.27 (s, 1H), 7.77 – 7.71 (m, 2H), 7.55 – 7.49 (m, 2H), 7.47 – 7.41 (m, 1H), 4.96 (s, 2H), 2.52 (s, 3H), 2.38 (s, 3H).

¹³**C NMR**: (101 MHz, DMSO-*d*₆) δ [ppm]= 169.64, 153.43, 141.36, 141.23, 135.05, 130.08, 129.66, 121.90, 50.95, 14.31, 9.84.

ESI-MS: [*m*/*z*]: found: 257.1045 [M-H]⁻, calculated: 257.1044

Solid phase peptide synthesis of AAP-FGDS



Scheme S1: Schematic representation of the solid-phase peptide synthesis of AAP-FGDS.

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

The first Fmoc-protected aminoacid (Fmoc-Serine(tBu), Fmoc-Ser*-AAP or Fmoc-Ser*-Ad, 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 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-Aspartic acid(OtBu)), 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-Glycine, Fmoc-Phenylalanine and AAP-carboxylic 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: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* lyophilisation. 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* lyophilisation. 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* lyophilisation. The title product was obtained as a yellow powder.

¹**H NMR**: (400 MHz, DMSO-*d*₆) δ [ppm]= 8.55 – 8.44 (m, 2H), 8.23 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 7.6 Hz, 2H), 7.51 (t, J = 7.6 Hz, 2H), 7.42 (t, J = 7.3 Hz, 1H), 7.32 – 7.24 (m, 4H), 7.23 – 7.17 (m, 1H), 4.86 – 4.53 (m, 4H), 4.29 – 4.20 (m, 1H), 3.85 – 3.69 (m, 3H), 3.68 – 3.60 (m, 1H), 3.14 – 3.04 (m, 1H), 2.91 – 2.63 (m, 3H), 2.35 (s, 3H), 2.30 (s, 3H).

¹³C NMR: (101 MHz, DMSO-*d*₆) δ [ppm]= 172.16, 171.77, 171.07, 169.05, 166.50, 153.45, 141.44, 141.06, 138.18, 134.97, 129.99, 129.69, 129.65, 128.56, 126.76, 121.86, 61.70, 55.28, 54.43, 51.89, 49.62, 42.41, 38.26, 36.75, 18.30, 14.32, 12.53, 9.76.

MALDI-MS: [*m*/*z*]: found: 665.36 [M+H]⁺, calculated: 665.27



Figure S7: ¹H-NMR spectra of AAP-FGDS (400 MHz in DMSO- d_6).