Electronic Supplementary Material (ESI) for ChemComm.


Electronic Supporting Information for:

# Photoresponsive Self-healing Supramolecular Hydrogels for Light-induced Release of DNA and Doxorubicin

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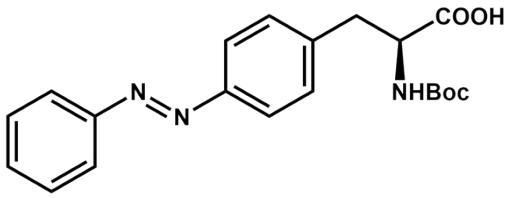
## *1. General Information:*

All reagents and starting materials are commercially available and were used as supplied unless otherwise indicated. In particular, htDNA (Deoxyribonucleic acid sodium salt from herring testes, Sigma-Aldrich, cat.#: D6898, containing polydisperse double-stranded DNA with the approximate length of c.a. 1300 bp) was used in all experiments that involved oligonucleotides. All experiments were conducted in air unless otherwise noted. All experiments were performed in deionized water (Millipore) unless otherwise noted. Column chromatography was performed on Silica gel 60 (0.063×0.200 mm, 70–230 mesh ASTM) (Merck) or Geduran® Silica gel 60 (0.040×0.063 mm, 230–400 mesh ASTM) (Merck). Deuterated solvents were purchased from Eurisotop and used as received. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a 300 MHz NMR spectrometer (Brucker 300), with working frequencies of 300.17 MHz for $^{1}$H nuclei, and 75.48 MHz for $^{13}$C nuclei. Chemical shifts are quoted in ppm relative to tetramethylsilane (TMS), using the residual solvent peak as the reference standard. UV-Vis spectra were recorded on a Lambda 750 (PerkinElmer) or Nanodrop-1000 UV-Vis spectrophotometer. IR spectra were recorded on a Bruker IFS 88 using DRIFT (Diffused Reflectance Infrared Fourier Transform Spectroscopy) or ATR (Attenuated Total Reflection) for solids. The intensities of the peaks are given as follows: vs=very strong 0-10% T, s=strong 10-40% T, m=medium 40-70% T, w=weak 70-90% T, vw=very weak 90-100% T. Abbreviations: ν=stretching vibration, δ=deformation vibration. All spectroscopy samples were taken at room temperature. Analytical thin layer chromatography was carried out using silica coated aluminium plates (silica 60, F254, layer thickness: 0.25 mm) with fluorescence indicator by Merck. Detection proceeded under UV light at λ=254 nm. SEM images were obtained using a QUANTA 650-FEG scanning electron microscope from FEI Company, with an accelerating voltage of 10-20 kV. TEM measurements were performed using Zeiss 912 Omega microscope on 400 Mesh carbon-coated copper grids, with the sample sprayed on the surface using a built-in ultrasound device. Rheological measurements were performed using ARES-G2 Rheometer (TA Instruments). Photoisomerization of samples was performed using 10 W LED diodes from LED Engin (UV: 365 nm, blue: 460 nm). For the time of irradiation, samples were placed in a water-cooled aluminium block and the temperature of 20 $^{0}$C (+/- 2 $^{0}$C) was maintained.

## *2. Synthesis:*

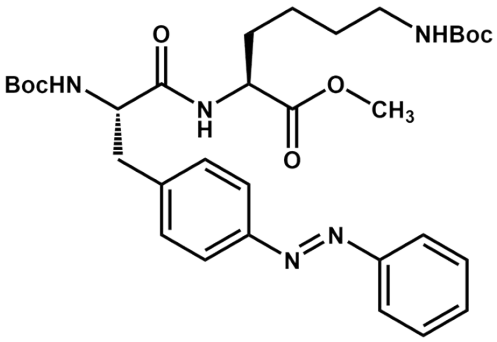



**(*S*,*E*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(phenyldiazenyl)phenyl)propanoic acid**

**(2)** *Boc-PAP-OH*:

Commercially available reagents: nitrosobenzene (2.9 g, 27.1 mmol, 1.7 eq.) and (S,E)-2-((tert-butoxycarbonyl)amino)-3-(4-aminophenyl)propanoic acid [saled as: *(S)-N*-Boc-4-aminophenylalanine] (4.4 g, 15.7 mmol, 1.0 eq.) were dissolved in glacial acetic acid (100% AcOH, 65 mL) in a round-bottom flask and stirred for 3 days at room temperature. The solvent was then evaporated under reduced pressure (rotavap) and the residual oil crude was co-evaporated three times with 100 mL toluene. This way, most of the unreacted nitrosobenzene was removed from the crude. The product was purified with flash chromatography on silicagel. The crude was dissolved in DCM and applied on the column that was pre-equilibrated with DCM. The column was eluted with pure DCM until the initial yellow fractions (containing mainly unreacted nitrosobenzene) were collected and the flow-through liquid became colorless. The pure product was then eluted with 2% MeOH in DCM. After evaporation of the solvents and drying *in vacuo*, 4.8 g (13 mmol, 83% yield) of the product **2** was obtained as orange solid.

**TLC :** $R_f$ = 0.4 (developed in DCM:MeOH 10:1); **$^1$H NMR (DMSO-d$^6$) δ(ppm):** 12.69 (1H, br s), 7.80-7.88 (4H, m), 7.58 (3H, m), 7.46 (2H, m), 7.21 (1H, d, *J* = 9 Hz), 4.18 (1H, br m), 2.80-3.20 (2H, br m), 1.31 (9H, s); **$^{13}$C NMR (DMSO-d$^6$) δ(ppm):** 173.37, 155.42, 151.91, 150.58, 142.09, 131.15, 130.15, 129.39, 128.82, 122.41, 78.06, 54.85, 36.29, 28.09; **HRMS (ESI)**: calcd. for $C_{20}H_{24}O_4N_3$ $[M+H]^+$: 370.1767 Da, found: 370.1770 Da (Δ = 0.8 ppm).

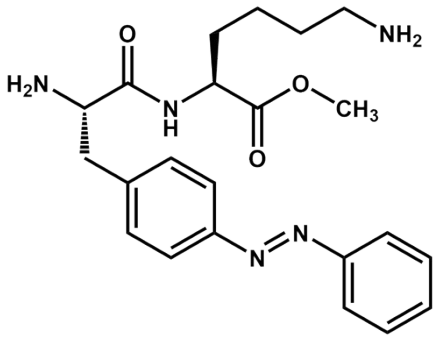



**Methyl $N^6$-(tert-butoxycarbonyl)-$N^2$-{(*S*)-2-[(tert-butoxycarbonyl)amino]-3-[4-((*E*)-phenyldiazenyl)phenyl]propanoyl}-*L*-lysinate**

**(3)** *Boc-PAP-Lys(Boc)-OMe*:

compound **2** (Boc-PAP-OH, 1.30 g, 3.54 mmol, 1.0 eq.), HBTU (1.34 g, 3.54 mmol, 1.0 eq.) and DIPEA (1.47 mL, 8.9 mmol, 2.5 eq.) were dissolved in anhydrous DMF (10 mL) and stirred for 15 min. at room temperature under argon. The same amount of DIPEA (1.47 mL, 8.9 mmol, 2.5 eq.) was added together with solid *ω*-*N*-Boc-lysine methyl ester hydrochloride (H-Lys(Boc)-OMe*HCl, 1.05 g, 3.54 mmol, 1.0 eq.). The reaction mixture was stirred further at room temperature under argon and the reaction progress was followed by TLC. After 3 hours, full conversion of the starting material **2** (Boc-PAP-OH) was observed. The reaction mixture was quenched with sat. aq. $NH_4Cl$ solution (200 mL) and extracted once with EtOAc (200 mL). The organic layer was washed with sat. aq. $NH_4Cl$ solution (3 x 200 mL), brine (1 x 200 mL), dried with anhydrous $Na_2SO_4$ and the solvent was evaporated (rotavap) to dryness. The crude was purified with flash chromatography. Initial elution with cH:EtOAc 3:1 washed out colorless non-polar impurities. Then the column was eluted with cH:EtOAc 2:1. Evaporation of combined orange fractions and drying under reduced pressure (oil pump) resulted in 1.64 g (76% yield) of the product **3** as orange solid. During evaporation we observed formation of a gel in this solvent system before the product was completely dried.

**TLC :** $R_f$ = 0.45 (developed in cH:EtOAc 1:1); **$^1$H NMR ($CDCl_3$) δ(ppm):** 7.90-7.82 (4H, m), 7.56-7.42 (3H, m), 7.36 (2H, m), 6.68 (1H, m), 5.23 (1H, m), 4.81 (1H, br s), 4.60-4.40 (2H, m), 3.61 (3H, s), 3.25-2.98 (4H, m), 1.88-1.56 (2H, br m), 1.42 (9H, s), 1.40 (9H, s), 1.52-1.31 (2H, m), 1.24 (2H, br s); **$^{13}$C NMR ($CDCl_3$) δ(ppm):** 172.30, 171.14, 156.13, 155.61, 152.67, 151.70, 140.10, 131.16, 130.37, 129.30, 123.45, 122.87, 80.45, 79.16, 55.62, 52.77, 52.09, 45.92, 39.76, 32.04, 28.65, 28.44, 22.36; **HRMS (ESI)**: calcd. for $C_{32}H_{45}O_7N_5Na$ $[M+Na]^+$: 634.3217 Da, found: 634.3225 Da (Δ = 1.26 ppm).

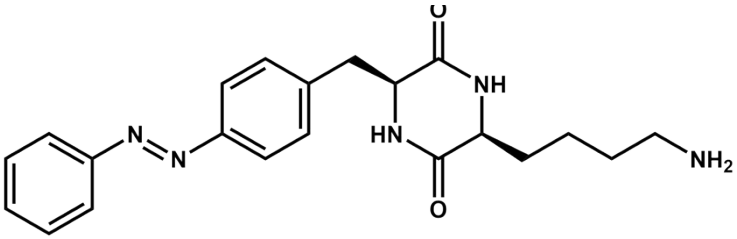


**Methyl $N^2$-{(*S*)-2-amino-3-[4-((*E*)-phenyldiazenyl)phenyl]propanoyl}-*L*-lysinate**

**(4)** *H-PAP-Lys-OMe*:

compound **3** (Boc-PAP-Lys(Boc)-OMe, 1.45 g, 2.37 mmol, 1.0 eq.) was dissolved in DCM (20 mL) yielding orange solution. Trifluoroacetic acid (TFA, 20 mL) was added at room temperature and color of the mixture changed to deep red. The mixture was stirred for 1 h at room temperature. Then 100 mL of toluene was added and the mixture was co-evaporated to dryness. The resulting crude (obtained quantitatively as a TFA salt) was used without further workup for the next step – cyclization to diketopiperazine. If necessary, it can be purified with flash chromatography: the crude dissolved in DCM is applied on a silicagel column pre-equilibrated with DCM. The column is washed with pure DCM, followed by DCM containing 5%, 10% and 25% of methanol. Small amounts of impurities are washed away at the eluent with 5% methanol and the product is eluted with 25% methanol (DCM:MeOH 3:1). After evaporation to dryness, the pure product is obtained as TFA salt.

**TLC :** $R_f$ = 0.3 (developed in DCM/MeOH 10:1); **$^1$H NMR ($CD_3OD$) δ(ppm):** 7.90-7.79 (4H, m), 7.52-7.40 (5H, m), 4.42 (1H, m), 3.95 (1H, br s), 3.62 (3H, s), 3.23-2.95 (2H, br m), 2.87 (2H, t, *J* = 7.8 Hz), 1.91-1.51 (4H, m), 1.46-1.30 (2H, m); **$^{13}$C NMR ($CDCl_3$) δ(ppm):** 171.72, 171.49, 152.33, 151.50, 139.04, 130.73, 129.93, 128.69, 122.56, 122.18, 54.67, 51.89, 51.28, 41.12, 38.81, 30.48, 26.38, 22.03; **HRMS (ESI)**: calcd. for $C_{22}H_{30}O_3N_5$ $[M+H]^+$: 412.2349 Da, found: 412.2343 Da (Δ = 1.46 ppm).




**(3*S*,6*S*)-3-(4-aminobutyl)-6-{4-[(*E*)-phenyldiazenyl]benzyl}piperazine-2,5-dione**

**(1)** *PAP-DKP-Lys*:

the crude compound **4** from the previous experiment (H-PAP-Lys-OMe, 2.37 mmol, 1.0 eq.) was dissolved in 2-butanol (*sec*-BuOH, 100 mL). It was mixed with 100 % acetic acid (500 µL), *N*-methylmorpholine (333 µL) and *N,N*-diisopropyl-*N*-ethylamine (Hünig's base, DIPEA, 600 µL). The resulting mixture was refluxed for 2 h (oil bath, external heating 120 °C), then cooled down. Next, approximately 60% of the solvent (c.a. 60 mL) was removed on the rotavap. Cooling down to room temperature resulted in orange precipitation. The precipitate was filtered off, washed with small amount of cold 2-butanol (2 x 5 mL) on the filter, dried on the filter and *in vacuo* using oil pump overnight resulting in 810 mg of the product (2.13 mmol, 90% yield) as orange solid.

**$^1$H NMR (DMSO-d$^6$) δ(ppm):** 8.26 (1H, s), 8.12 (1H, s), 7.92-7.75 (4H, m), 7.58 (3H, m), 7.39 (2H, d, *J* = 8.4 Hz), 4.28 (1H, s), 3.68 (1H, s), 3.28-2.92 (2H, br m), 2.54 (2H, t, *J* = 7.5 Hz), 1.22 (2H, m), 1.05-0.76 (4H, br m); **$^{13}$C NMR (DMSO-d$^6$) δ(ppm):** 167.01, 166.26, 151.97, 150.85, 140.31, 131.31, 129.40, 122.54, 122.20, 119.70, 54.99, 53.41, 38.43, 37.85, 32.43, 26.51, 20.43; **HRMS (ESI)**: calcd. for $C_{21}H_{26}O_2N_5$ $[M+H]^+$: 380.2087 Da, found: 380.2090 Da (Δ = 0.8 ppm). **IR (KBr):** ν = 2957 (w), 1662 (s), 1460 (m), 1334 (w), 1182 (s), 1122 (s), 1009 (w), 924 (w), 833 (m), 799 (m), 769 (m), 720 (m), 687 (m), 572 (m), 525 (w), 433 (m) $cm^{-1}$.

### *3. Photophysical properties*

Photoisomerization of the compound **1** (PAP-DKP-Lys) in solution was examined by irradiating a sample (2 mL of 60 µM solution in deionized water) with 10 W UV-LED (365 nm) diode. The photostationary state $PSS_{365}$ with predominant *cis*-isomer was already achieved upon short (1 s) irradiation. Longer exposure on the UV light did not result in any further changes of the UV-Vis spectrum. Short (1 s) irradiation of the second sample with blue light (10 W LED diode, 460 nm) did not cause significant *trans*-to-*cis* photoisomerization. Its spectrum was almost identical to the spectrum from a sample which was not irradiated at all ("the dark state").

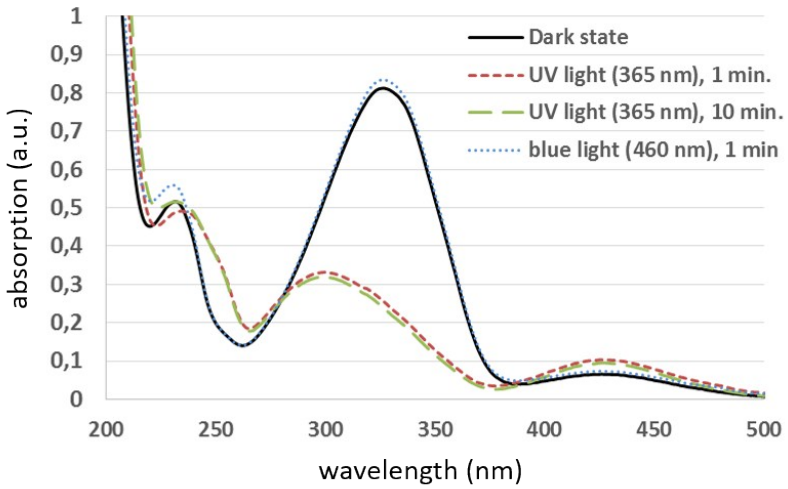



**Figure S1.** UV-Vis absorbance of the PAP-DKP-Lys **1** in absence of light or upon irradiation with UV (365 nm) or blue (460 nm) light (60 µM solutions in di$H_2O$).

Although the isomer distribution in different photostationary states in solutions of the unmodified azobenzene was described in numerous publications [1], we wanted to verify the isomer ratio in our particular experimental system consisted of the molecule **1** (15 g/L) and htDNA (2 g/L) dissolved in 50 mM NaCl solution in $D_2O$. The *cis/trans* (*E/Z*) isomer ratio in darkness and upon irradiation with 365 nm UV light was determined by $^1$H NMR measurements and integration of peaks at the aromatic spectral region. The following peaks were identified as contributions from the respective isomer:

*trans*-PAP-DKP-Lys, *E*-**1**: $^1$H NMR (300 MHz, $D_2O$): δ=7.87 (d, 4 H, J=7.6 Hz), 7.64 (m, 3 H), 7.42 (d, 2 H, J=6.8 Hz), 4.57 (br s, 1 H, CH); *cis*-PAP-DKP-Lys: *Z*-**1**: $^1$H NMR (300 MHz, $D_2O$): δ=7.33 (d, 4 H, J=7.8 Hz), 7.13 (m, 3 H, J=8.2 Hz), 6.94 (d, 2 H, J=8.0 Hz), 4.57 (br s, 1 H, CH)

The peak at 7.87 ppm for the *trans*-isomer of **1** was well separated from the other ones. By comparing the integration of that peak to the summary integration of all peaks in the aromatic region we were able to estimate the isomer ratio of our compound in darkness and upon 30 min. irradiation with 365 nm (**Table S1**). It remains in good agreement with literature data for the unsubstituted azobenzene.

**Table S1.** E/Z ratio of 15 g/L PAP-DKP-Lys, 2 g/L H-DNA in 50 mM NaCl in $D_2O$

| Procedure | E/Z ratio | Peaks for calculation |
|---|---|---|
| Dark state | Approx. 100% | 7.87 / all $A_r$ |
| 30 min 365 nm | 23%/77% | 7.87 / all $A_r$ |
| Ref. [1] $PSS_{365}$ | 20%/80% | . - |

## *4. Gelation properties of PAP-DKP-Lys (1).*

*Solubility of **1** in water and organic solvents*

20 mg of **1** as finely crushed powder was mixed in a 10 mL glass vessel with 1 mL of solvent. **1** was readily dissolved in DMF, DMSO and methanol. Samples in water, acetonitrile and toluene were shortly boiled and slowly cooled down to room temperature. **1** was not soluble in MeCN and toluene. **1** was fully soluble in boiling water and this solution formed a gel upon cooling.The following table (**Table S2**) summarizes solubility of the compound **1** in different solvents**.**

**Table S2.** Solubility of the compound **1** in various solvents (conc. of 2% in each case)

| ***Solvent*** | water | MeOH | DMF | DMSO | MeCN | toluene |
|---|---|---|---|---|---|---|
| ***Solubility*** | *Gel* | *soluble (gel)** | *Soluble* | *soluble* | *Insoluble* | *insoluble* |

*) the 5 % solution of **1** in MeOH forms a gel with Tm of c.a. -22 $^{0}$C

*Procedure for preparation of the hydrogels*

In a 10 mL glass vessel was added the finely crushed powder of the PAP-DKP-Lys **1** (40.0 mg, 0.105 mmol for 2% gels or proportionally less for gels of lower concentration) and deionized water (between 1.5 mL and 2.0 mL). The closed glass vessel was instantly treated with ultrasonic in a water bath for 1 min with gentle stirring. To this opaque suspension was added, if needed, stock solution (400 mM) of NaCl and/or htDNA stock solution (6.0 g/L) in deionized water to reach the desired concentration of the components in the total volume of 2.0 mL. The closed glass vessel was warmed up vertical in a water bath at 80 °C for 5 minutes. The yellow suspension was dissolved in the closed glass vessel after heating it up to the boiling point with a heat gun. The hot fluid turned to an orange solution and this fluid was gelated after 5 minutes at room temperature. In the case of incomplete gelation the fluid was incubated overnight at room temp. in darkness

*Measuring the melting temperature of the hydrogels.*

2.0 mL samples of the hydrogels in a 10 mL cylindrical glass vials are swimming horizontal on the surface of water in water bath stirred with 60 rpm at 25 °C, which is heated up with a heating rate between 1 °C/min and 2 °C/min. The hydrogel will start melting slowly before the hydrogel will abrupt flow down at a certain moment. The measurement was repeated 5 times and the average melting point was reported.

Gels prepared from **1** in deionized water without any additives were stable and homogenous in the range between 20 g/L and 30 g/L (2-3 wt% of **1**). Below that concentration gelation was slow (overnight) or did not occur at all. Moreover, the gels containing less than 2 wt% of **1** were very sensitive on mechanical deformation and almost immediately turned into liquid upon slight shaking of the vial.

Addition of trifluoroacetic acid (1 wt%, 10 g/L) or sodium chloride (to the final concentration of 50 mM) to the gel **A** resulted in a significant increase of the melting point as well as significantly improved the mechanical properties of the resulting materials, that could be examined both by optical observation of samples and verified by rheometry (*vide infra*). It can be intuitively understood by protonation of the lysine side chain in case of acids or increasing ionic strength of the medium – both can increase the strength of electrostatic and polar interactions that in turn stabilize fibers and the entire hydrogel structure.

Addition of long dsDNA (htDNA 2g/L) alone (gel **C**) did not significantly improve the mechanical properties, but increased melting temperature of resulting gels, although not to the same extent as in the case of TFA (DNA is much less acidic) or NaCl. That could be explained as a combination of the ionic interactions between the amines from lysine side chains and phosphates of DNA together the additional templating effect of the covalent DNA backbone.

As expected, combination of NaCl and htDNA led to formation of gels that were mechanically most stable and had the highest melting temperatures in comparison with all other combinations at the same concentration of **1** (PAP-DKP-Lys).

**Table S3.** Melting temperatures of hydrogels containing **1** and additives (TFA, NaCl, htDNA).

| *Composition of the gel* | $T_m$ ($^0$C) | Remarks |
|---|---|---|
| 15 g/L **1** (1.5 wt%) | 38 ±4 | |
| 16 g/L **1** | 41 ±4 | |
| 17 g/L **1** | 43 ±4 | |
| 18 g/L **1** | 46 ±8 | |
| 19 g/L **1** | 48 ±6 | |
| 20 g/L **1** (2 wt%) | 51 ±4 | gel **A** |
| 30 g/L **1** (3 wt%) | 69 ±2 | |
| | | |
| 20 g/L **1**, 2 g/L htDNA | 64 ±8 | gel **C** |
| 20 g/L **1**, 10 g/L TFA | 68 ±1 | |
| 20 g/L **1**, 50 mM NaCl | 70 ±1 | gel **B** |
| 20 g/L **1**, 2 g/L htDNA, 50 mM NaCl | 71 ±2 | |
| | | |
| 15 g/L **1**, 2 g/L htDNA | 44 ±3 | |
| 15 g/L **1**, 50 mM NaCl | 51 ±4 | |
| 15 g/L **1**, 2 g/L htDNA, 50 mM NaCl | 60 ±3 | gel **D** |

## *5. Light-induced gel-sol transitions*

Gel samples were irradiated at maximal power for 30 min at 20°C± 2 °C with two 10 W 365 nm LED diodes (LED Engin) in a water-cooled aluminium chamber. Immediately after irradiation the glass was turned upside down. The state of the sample was assigned as fluid, unstable gel (that liquefied after slewing), or stable gel (resistant on moderate shaking). The following table (**Table S4**) summarizes the results of gel irradiation with UV light.

**Table S4.** Mechanical properties of various **1**-based gels in darkness and upon UV irradiation.

| Gel composition | dark | 365 nm, 30’ | | Gel composition | dark | 365 nm, 30’ |
|---|---|---|---|---|---|---|
| 3% **1** | stable gel | stable gel | | 1.5% **1** | unstable gel | fluid |
| 2% **1** | stable gel | unstable gel | | | | |
| 2% **1**, 1% TFA | stable gel | stable gel | | | | |
| 2% **1**, 50 mM NaCl | stable gel | stable gel | | 1.5% **1**, 50 mM NaCl | stable gel | fluid |
| 2% **1**, 0.2% htDNA | stable gel | unstable gel | | 1.5% **1**, 0.2% htDNA | unstable gel | fluid |
| 2% **1**, 0.2% htDNA 50 mM NaCl | stable gel | stable gel | | 1.5% **1**, 0.2% htDNA 50 mM NaCl | stable gel | fluid |

The fluid samples containing 1.5% of the DKP **1** after UV light irradiation were irradiated for 30 min at 20 °C± 2 °C with a single 10 W 460 nm (blue) LED (LED Engin) and left overnight in darkness. Upon that time they formed gels with mechanical properties similar to the gels formed with the same composition and left without any irradiation. In comparison, UV-irradiated fluid samples incubated in darkness without previous blue light irradiation remained liquids for at least one week.

That behavior can be explained, as follows: the existence and rigidity of hydrogels depends on the presence of fibers that constitute its inner structure. Upon UV light irradiation and photoisomerization around three quarter of molecules (as judged by the NMR signals) loose the flat unpolar aromatic system capable of strong π-π stacking interactions. That causes fibers to break and dismantle. In case of 2% PAP-DKP-Lys gels, the remaining fraction of *trans* molecules can still sufficiently support the inner structure. For 1.5% PAP-DKP-Lys samples the remaining *trans* fraction is too low and the whole gel structure tends to collapse. Thermal back-isomerization (*cis*-to-*trans*) rate for azobenzene is in the range of 5-10 days and this corresponds well to the rate of gel regeneration in darkness. Irradiation of such samples with blue light speeds up this process because it increases the population of the *trans*-isomer. However, the process of fiber reconstitution is slow and therefore the gel structure is reconstituted after a few hours.

## *6. Rheology*

The rheological characterization of hydrogels formed by the compound **1** in water or 50 mM aqueous NaCl was performed in presence or in absence of htDNA. The following results are in accordance with our macroscopic observations that indicate that both the addition of NaCl and DNA to hydrogels formed by **1** increase their mechanical stiffness and resistance on sample shaking.

Hydrogel samples for rheological measurements were generated by cooling their solutions directly on the rheometer plate from the boiling point to room temperature. Strain sweep experiments were performed at 10 rad/s to determine the linear viscoelastic regime and the mechanical strength of the hydrogel. Frequency experiments were performed at low strain within the linear viscoelastic region (LVR) of the sample. For regeneration experiments, the samples were exposed to a deformation of 100% for thirty seconds to destroy the supramolecular network, afterwards the regeneration of G' was measured at low strain within the LVR.

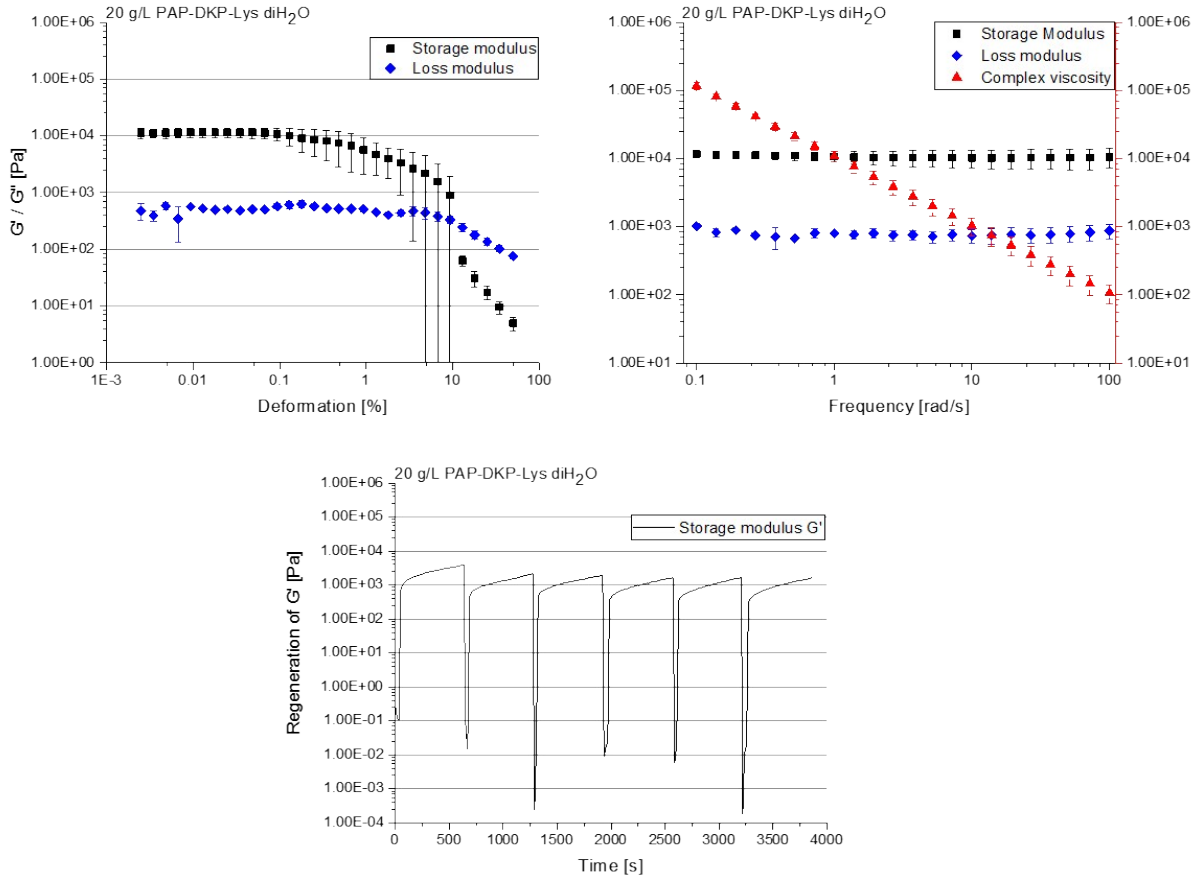



**Fig S2** Gel A: 20 g/L (2%) of **1** in di$H_2O$. Strain sweep experiment (top left); frequency sweep experiment (top right); regeneration of G' after shearing the gel for 30 seconds at 100% deformation (bottom);

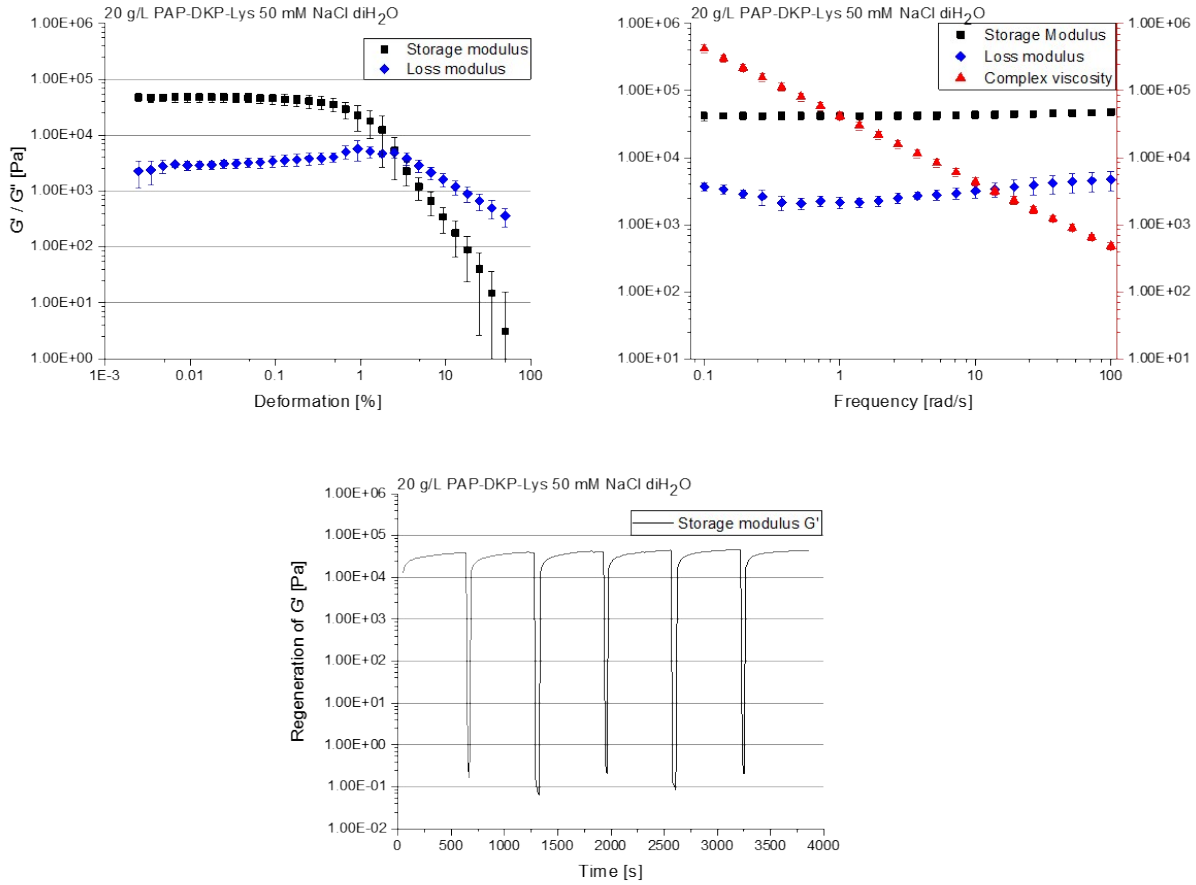



**Fig S3.** Gel **B**: 20 g/L (2%) of **1** in 50 mM aqueous NaCl. Strain sweep experiment (top left); frequency sweep experiment (top right); regeneration of G' after shearing the gel for 30 seconds at 100% deformation (bottom).

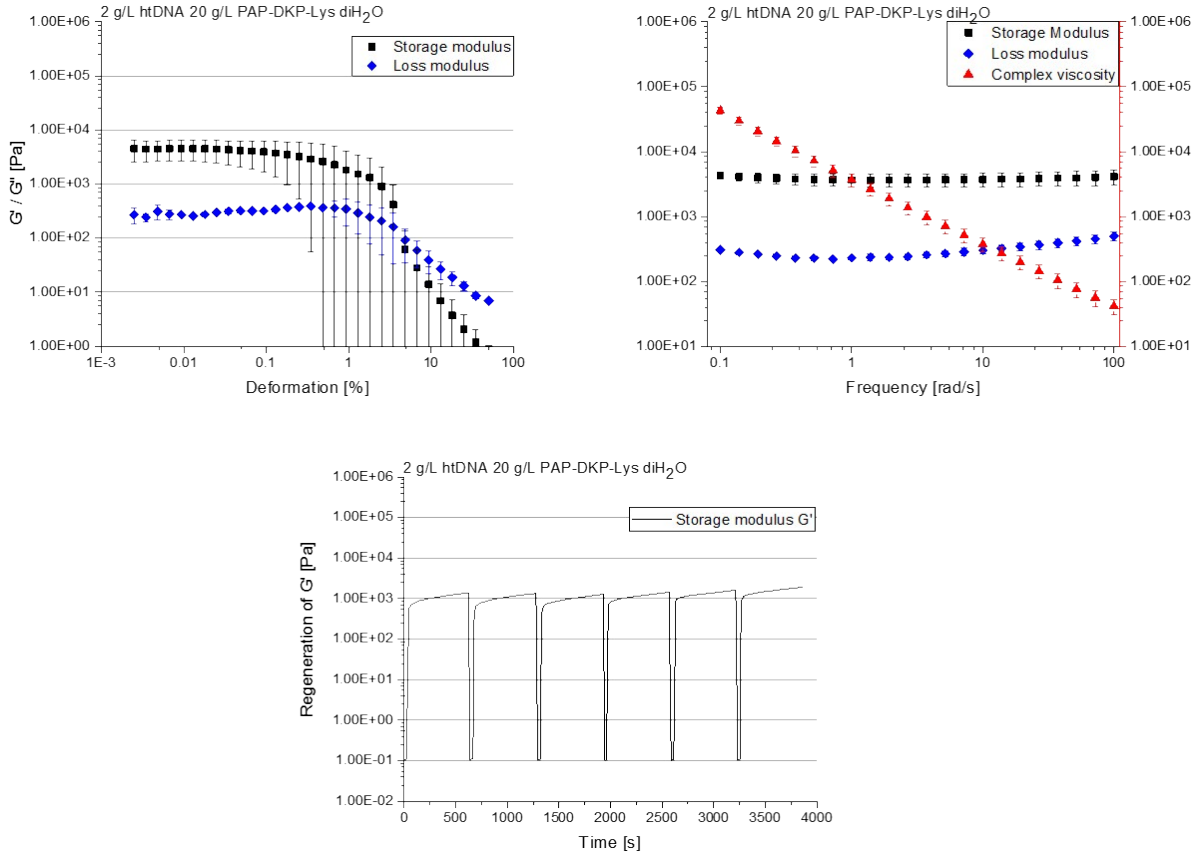



**Fig S4.** Gel **C**: 20 g/L (2 %) of **1** and 2 g/L of htDNA in di$H_2O$. *S*train sweep experiment (top left); frequency sweep experiment (top right); regeneration of G' after shearing the gel for 30 seconds at 100% deformation (bottom).

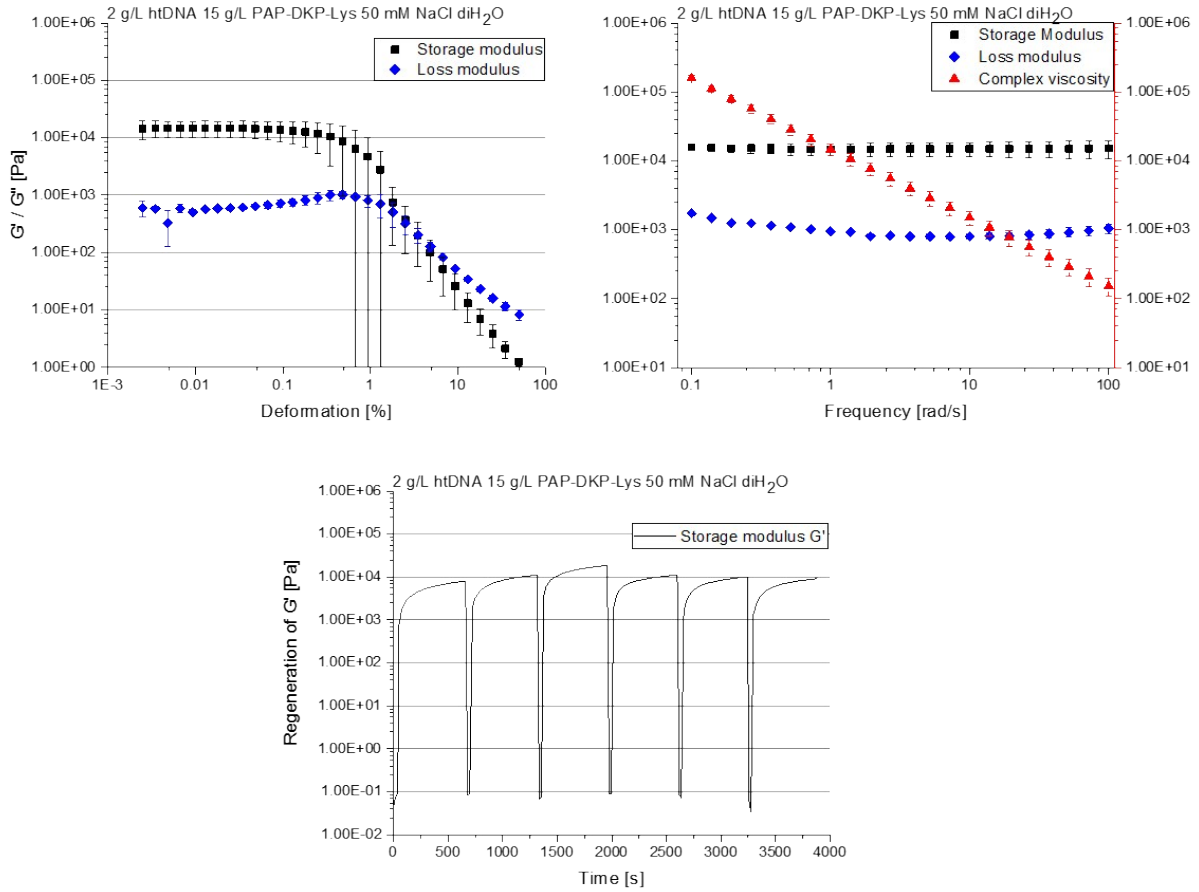



**Fig S5.** Gel **D**: 15 g/L (1.5%) of **1** and 2 g/L of htDNA in 50 mM aqueous NaCl. *S*train sweep experiment (top left); frequency sweep experiment (top right); regeneration of G' after shearing the gel for 30 seconds at 100% deformation (bottom).

### *7. SEM and TEM characterization*

We characterized with scanning electron microscopy the samples previously characterized by rheology. Samples for high vacuum mode SEM were prepared by lyophilization of gels that were rapidly frozen with liquid nitrogen. The dried SEM images of the samples were obtained with a QUANTA 650-FEG scanning electron microscope from FEI Company (accelerating voltage: 10 kV, spot size: 3.0). Before imaging, all the samples were sputter-coated with platinum. For environmental SEM (ESEM) mode, a small piece of the hydrogel was placed on the sample holder without sputter-coating and the ESEM images were directly recorded using the same microscope (accelerating voltage: 20 kV, spot size: 3.0).

***High-vacuum SEM***

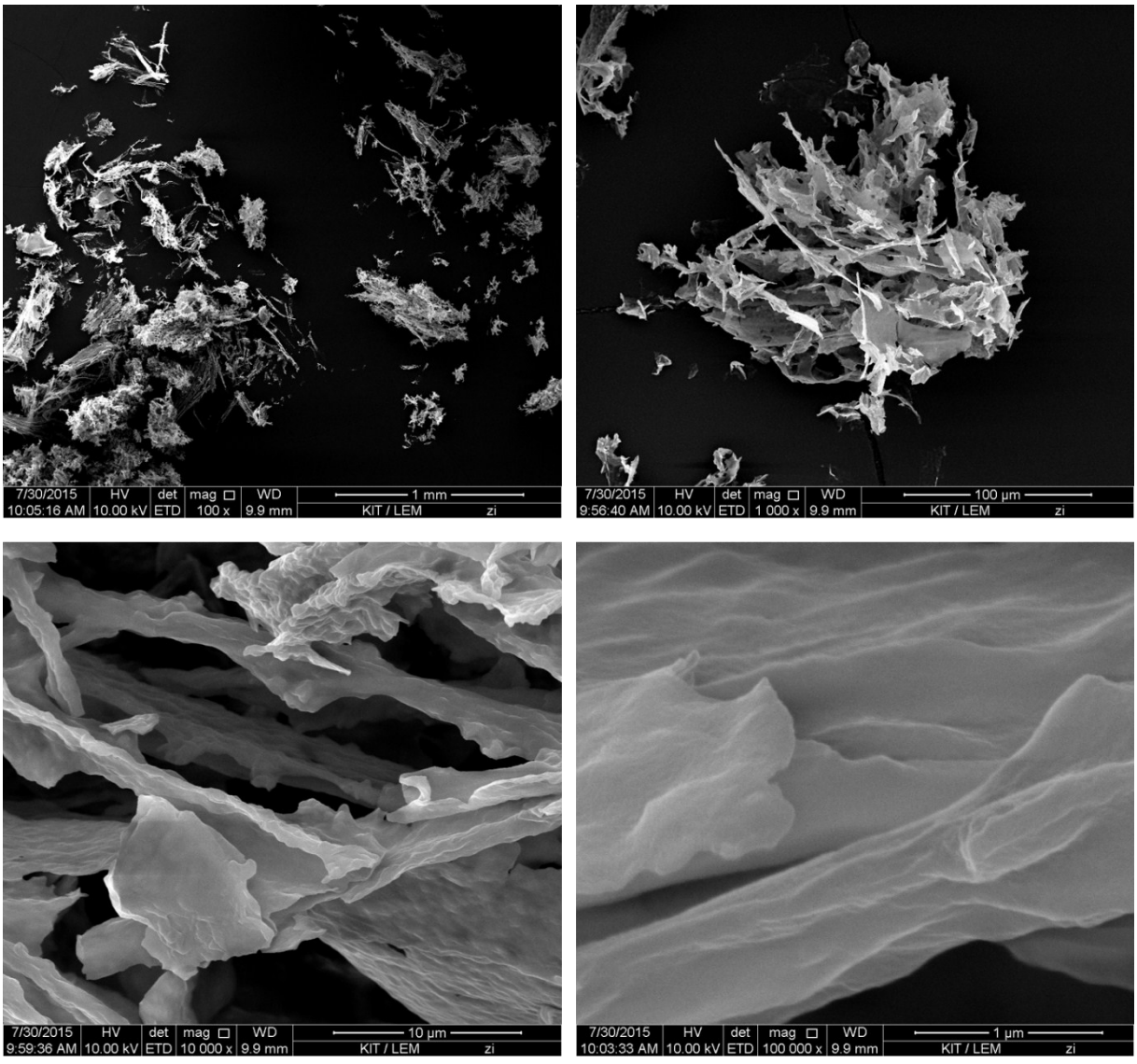



**Figure S6.** 2% of **1** in di$H_2O$.

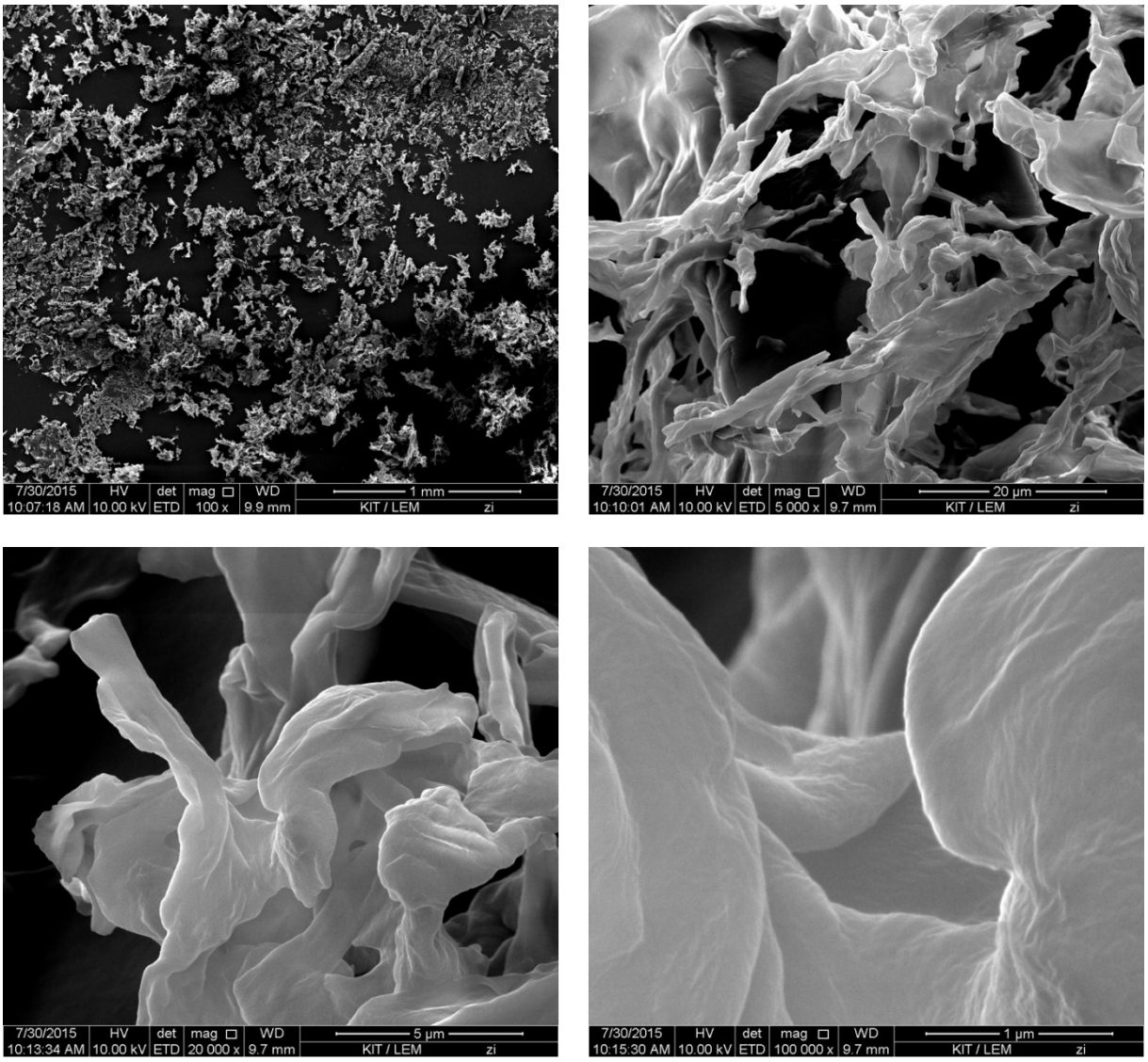



**Figure S7.** 2% of **1** and 0.2% of htDNA in di$H_2O$.

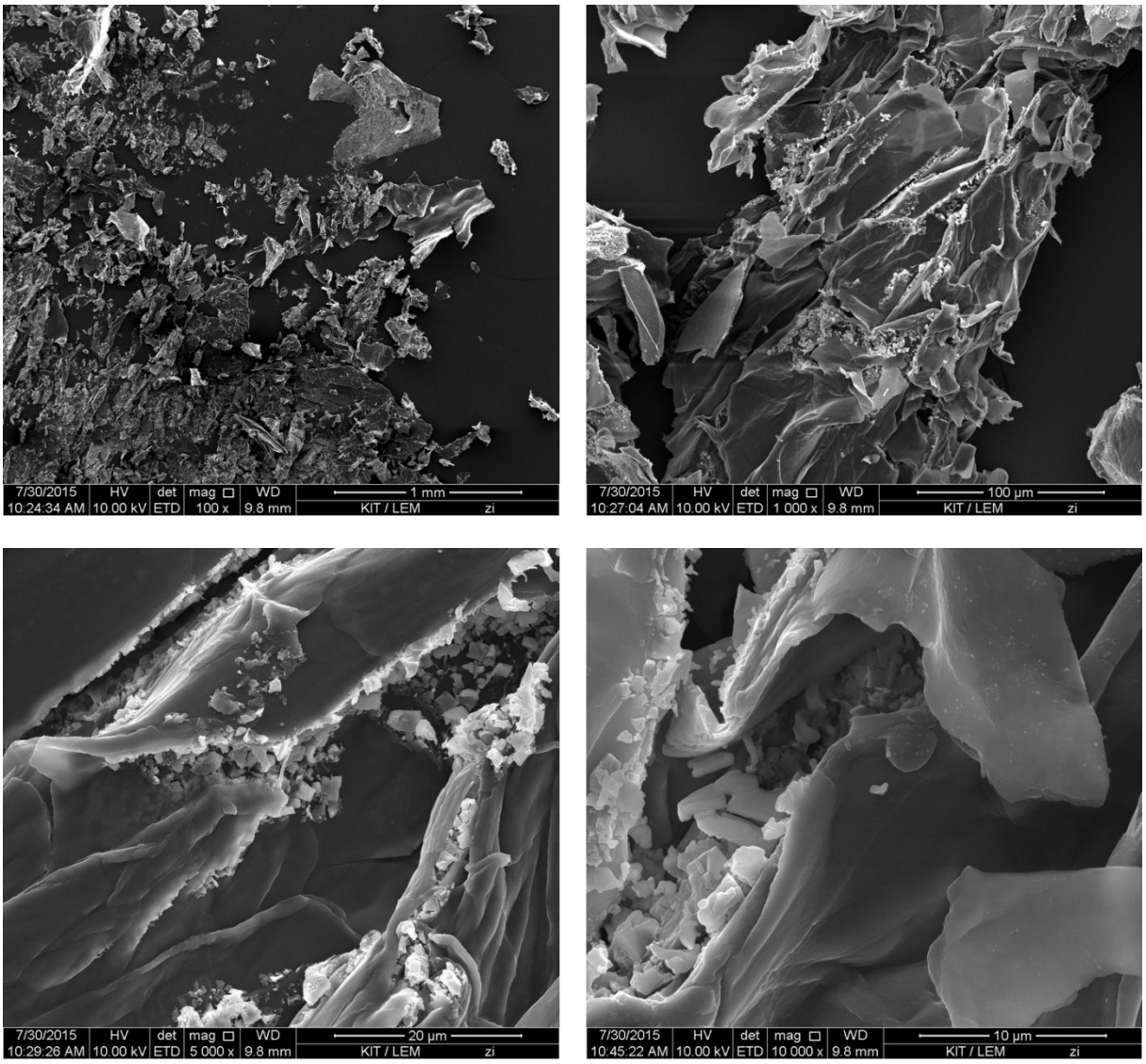



**Figure S8.** 2% of **1** in 50 mM aqueous NaCl.

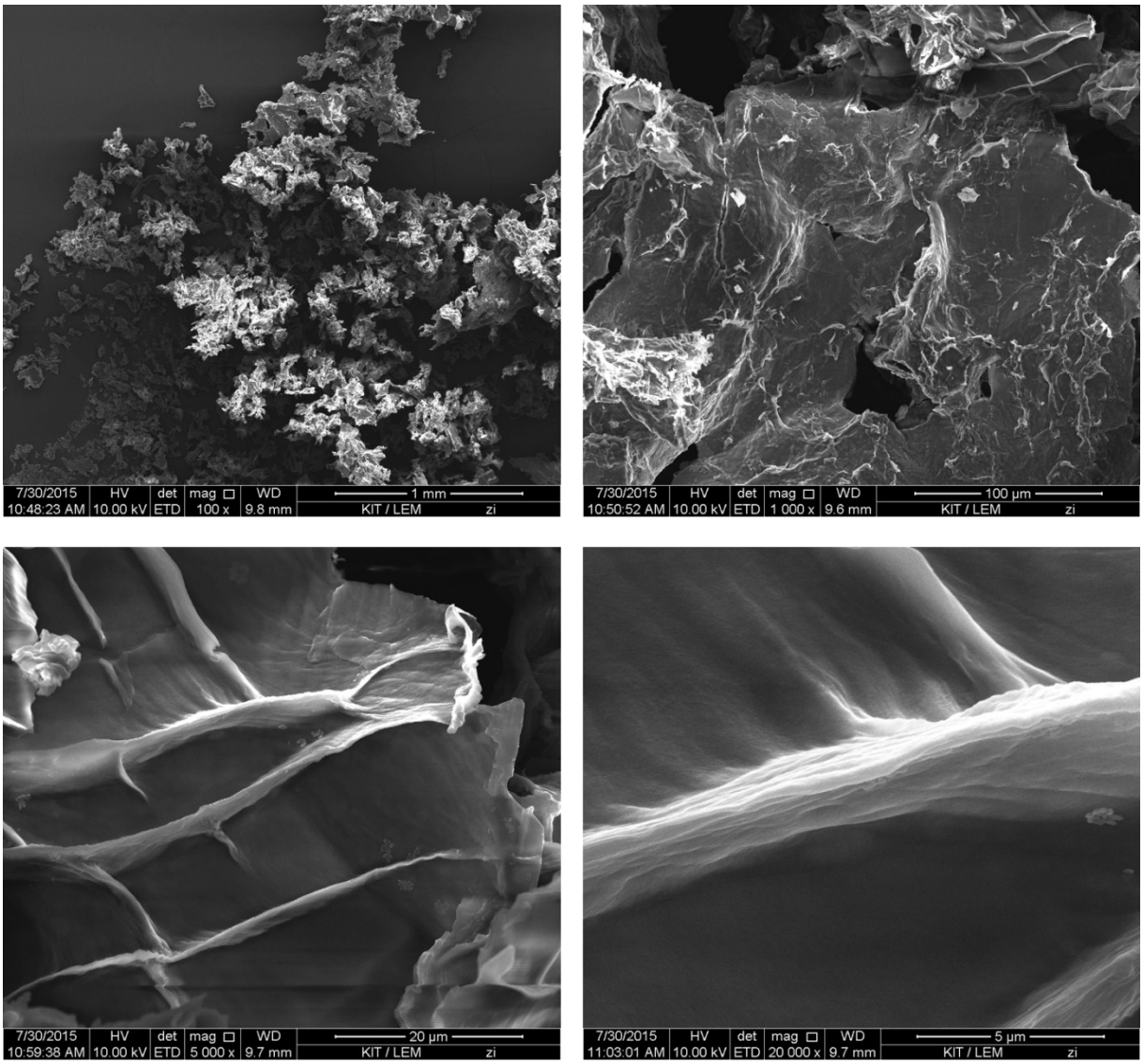



**Figure S9.** 1.5% of **1** and 0.2% of htDNA in 50 mM aqueous NaCl.

***Environmental SEM (ESEM)***

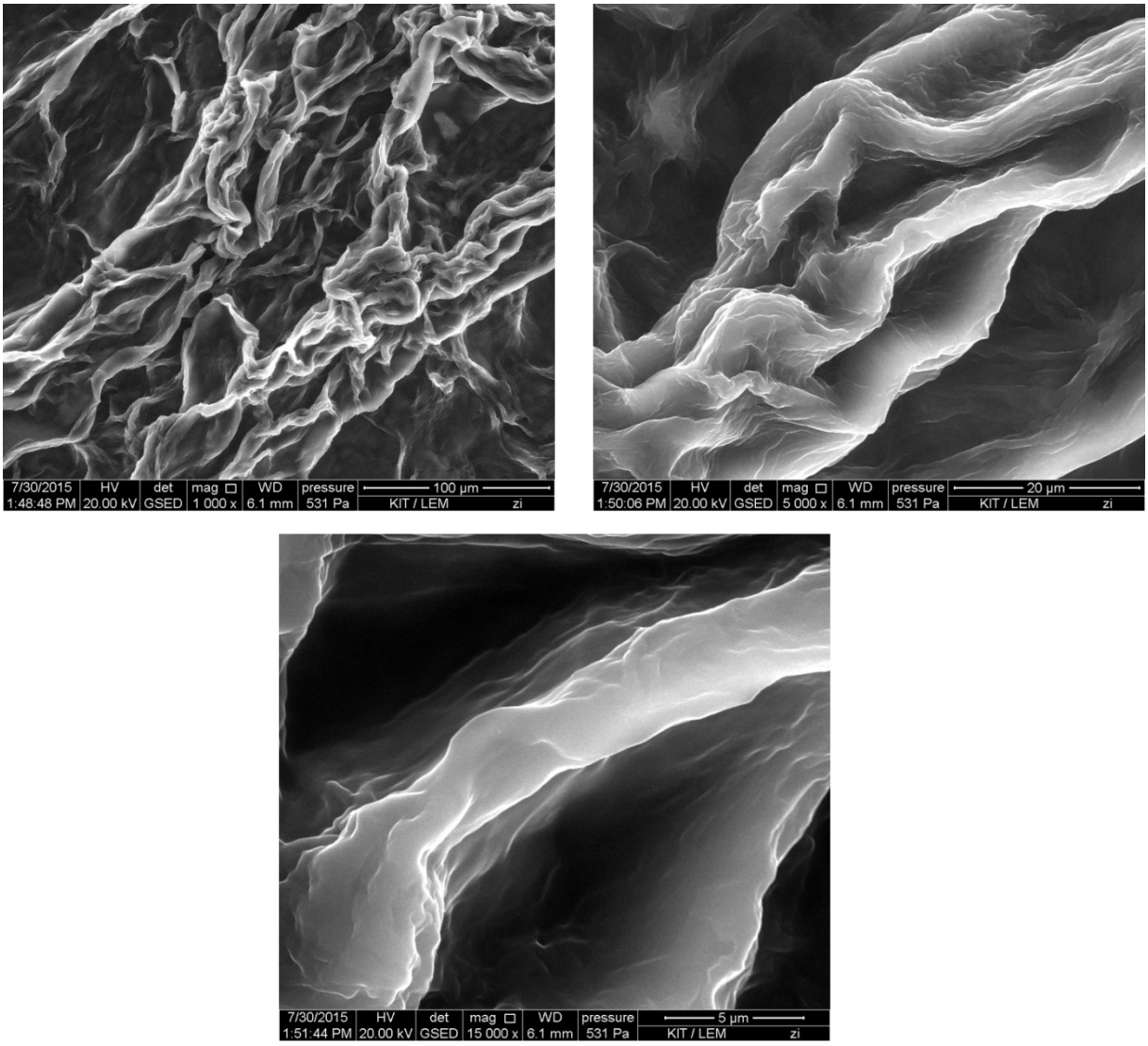



**Figure S10.** 1.5% of **1** and 0.2% of htDNA in 50 mM aqueous NaCl (no UV light irradiation).

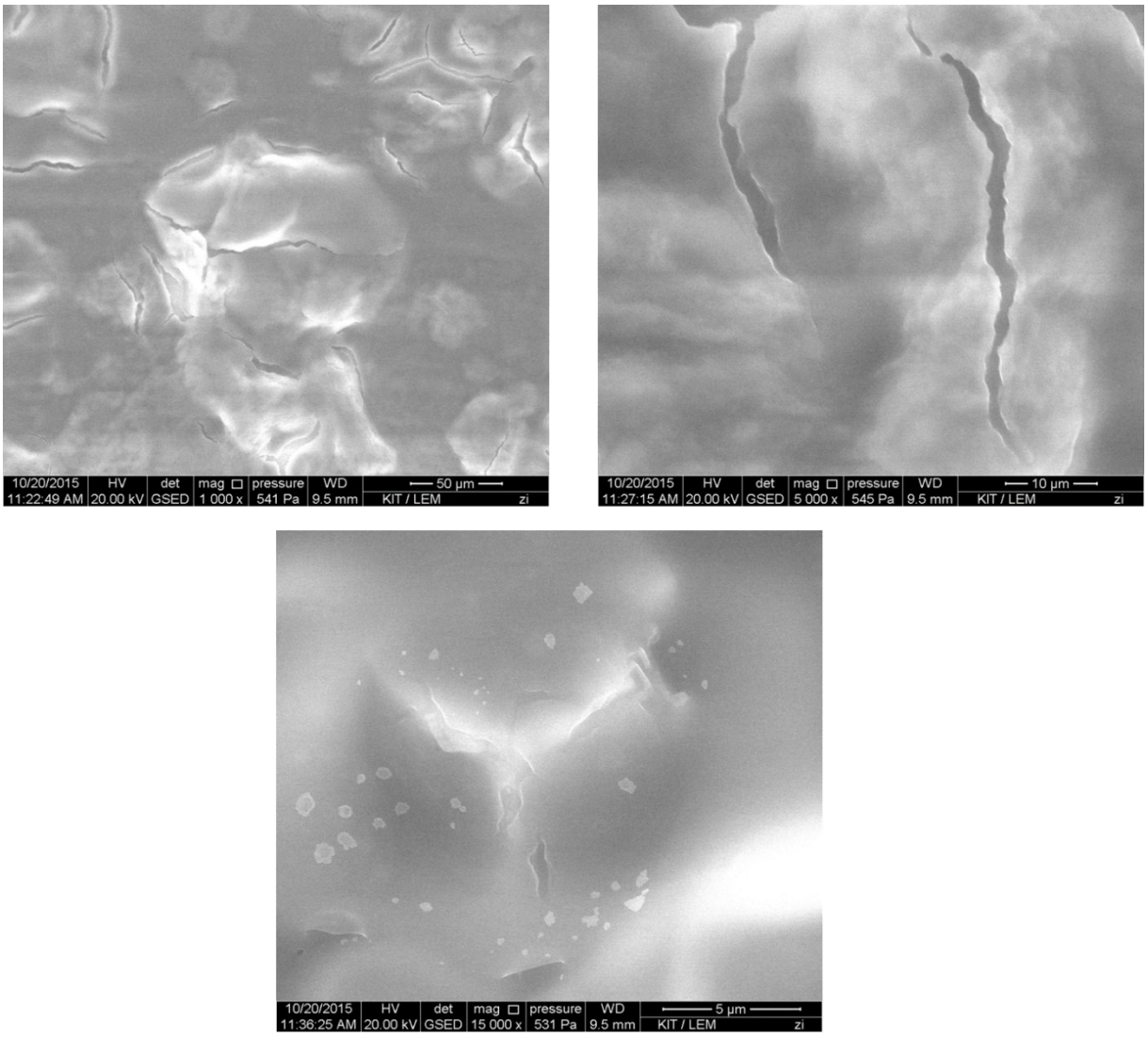



**Figure S11.** 1.5% of **1** and 0.2% of htDNA in 50 mM aqueous NaCl [after UV light irradiation (365nm, 20 W, 30 min.].

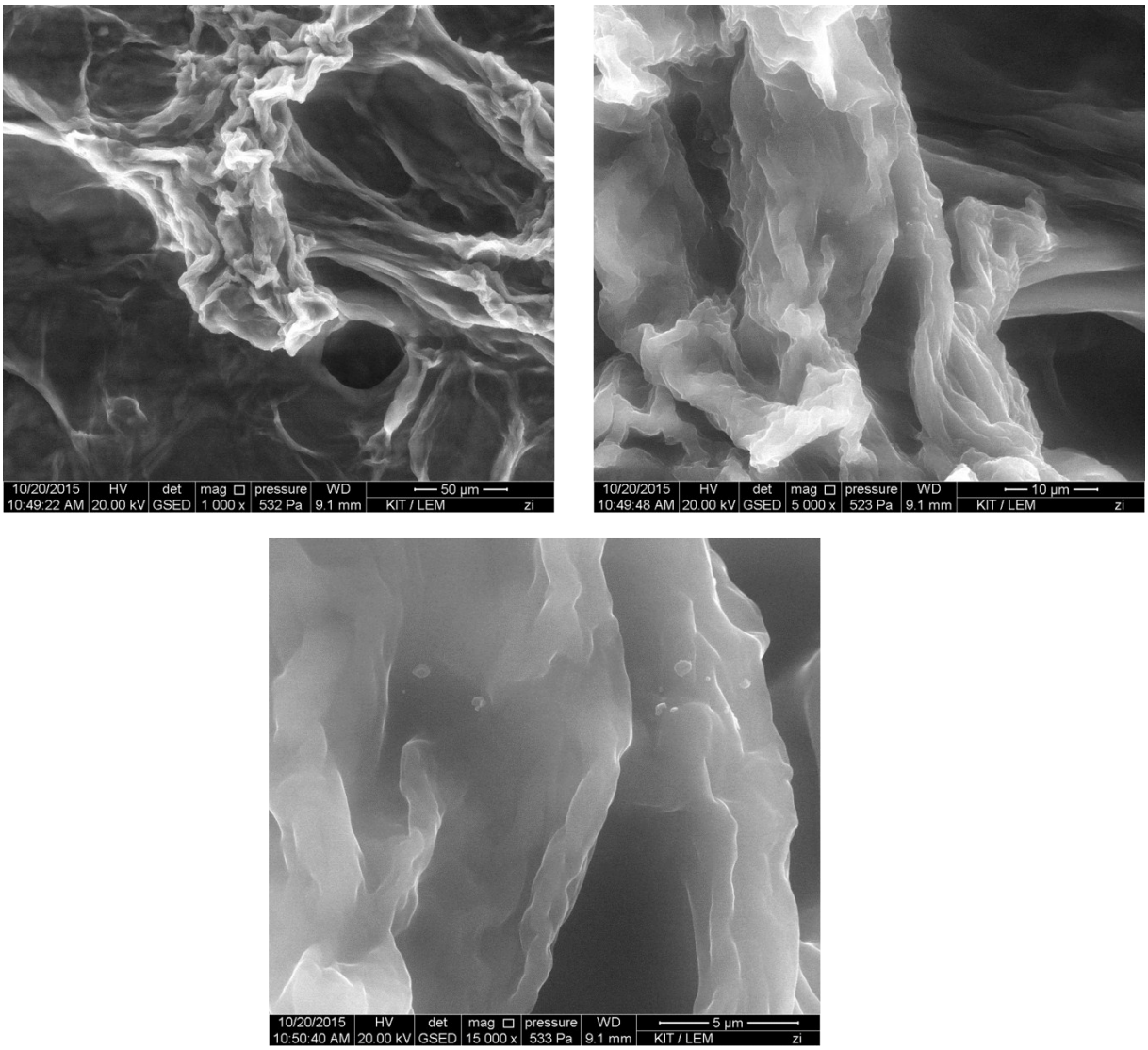



**Figure S12.** 1.5% of **1** and 0.2% of htDNA in 50 mM aqueous NaCl [after UV light irradiation (365nm, 20 W, 30 min.) followed by blue light irradiation (460 nm, 10 W, 30 min.)].

***TEM characterization***

Carbon-coated copper grids (400 Mesh) were covered with diluted solution of the compound **1** in aqueous NaI or NaCl by short exposure on spray generated from the samples with ultrasounds. The grids dried under atmospheric pressure and were examined using "Zeiss 912 Omega" transmission electron microscope. Under these conditions, fine fibrous structure of our material was revealed.

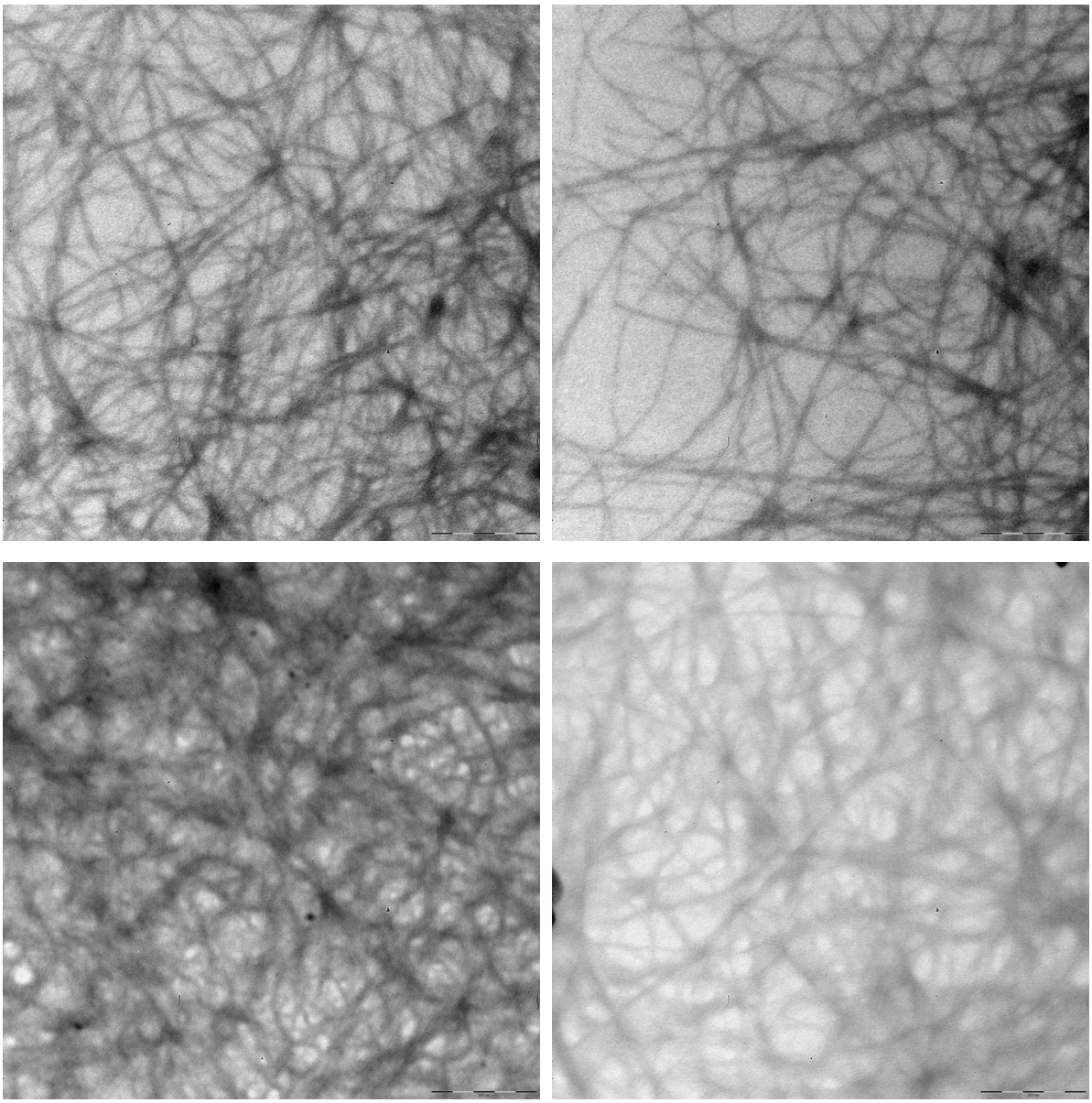


**Figure S13.** TEM of the sample 5 g/L PAP-DKP-Lys **1**. Staining with 50 mM NaI (top), 50 mM NaCl (bottom left) and 500 mM NaCl (bottom right). Scalebar = 200 nm.

## 8. Stability of the PAP-DKP-Lys – photodegradation, multiple switching cycles

Photostability of the compound **1** (PAP-DKP-Lys) was investigated by irradiation of its 60 µM solution in di$H_2O$ for 24 h with UV light (10 W, 365 nm) at 20 °C (± 2 °C) in a sealed cuvette and measurement of its UV-Vis spectra. No significant changes were observed in the spectrum after 24 h of irradiation with UV light (**Fig. S14**, empty triangles) in comparison to the same sample photoisomerized shortly (10 sec. irradiation) with UV light (**Fig. S14**, empty squares, $PSS_{365}$). The sample after 24 h of UV light irradiation was shortly (10 sec.) irradiated with blue light (460 nm, **Fig. S14**, black triangles). Again, no significant changes in comparison to the $PSS_{460}$ (**Fig. S14**, black dots) achieved after 10 sec. irradiation of the fresh sample with blue light was observed. From these results we conclude that the compound **1** does not undergo significant photodegradation on the timescale of at least 24 hours.

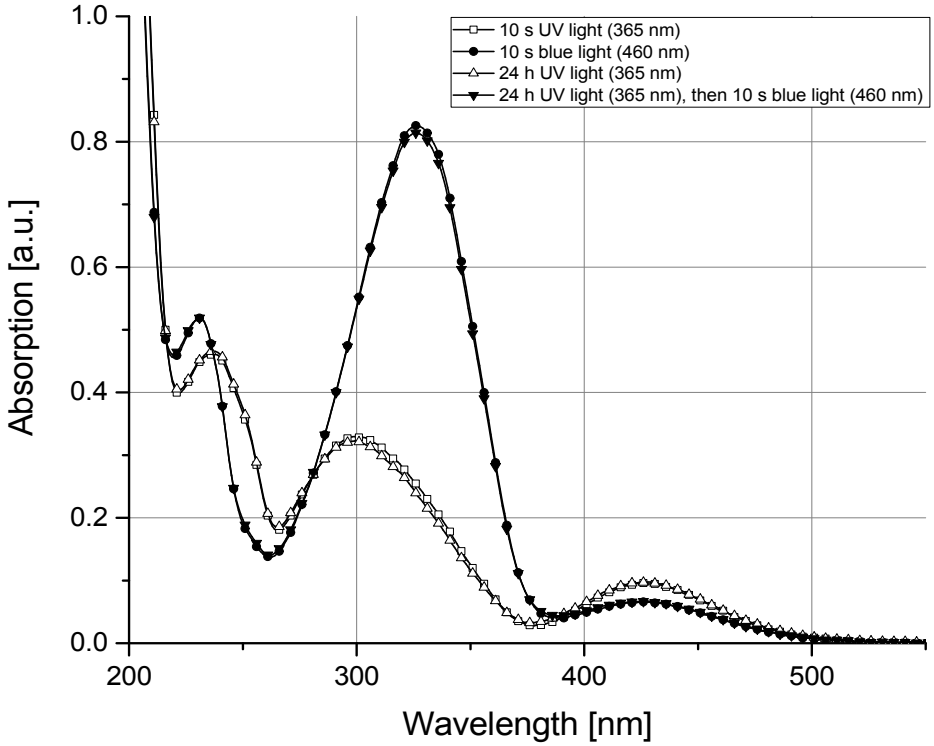



**Figure S14.** UV-Vis spectra: 60 µM PAP-DKP-Lys **1** in di$H_2O$. Photostationary states (10 sec. irradiation) at 365 nm (10 W, empty squares) – and 460 nm (10 W, black dots). The same sample after 24 h irradiation with UV light (365 nm, 10 W, empty triangles), and additionally 10 sec. irradiation with blue light (460 nm, 10 W, black triangles).

The resistance of the material to multiple photoisomerization cycles was also investigated by UV-Vis spectrophotometry. A 60 µM solution of the compound **1** (PAP-DKP-Lys) was alternatively irradiated with UV (365 nm, 10 W) and blue (460 nm, 10 W) light 100 times each. At the end, the respective spectra were compared to the photostationary state achieved after single irradiation with the given wavelength. We have found no meaningful differences in the behavior of the freshly prepared material and the same material after 100 full photoisomerization cycles.

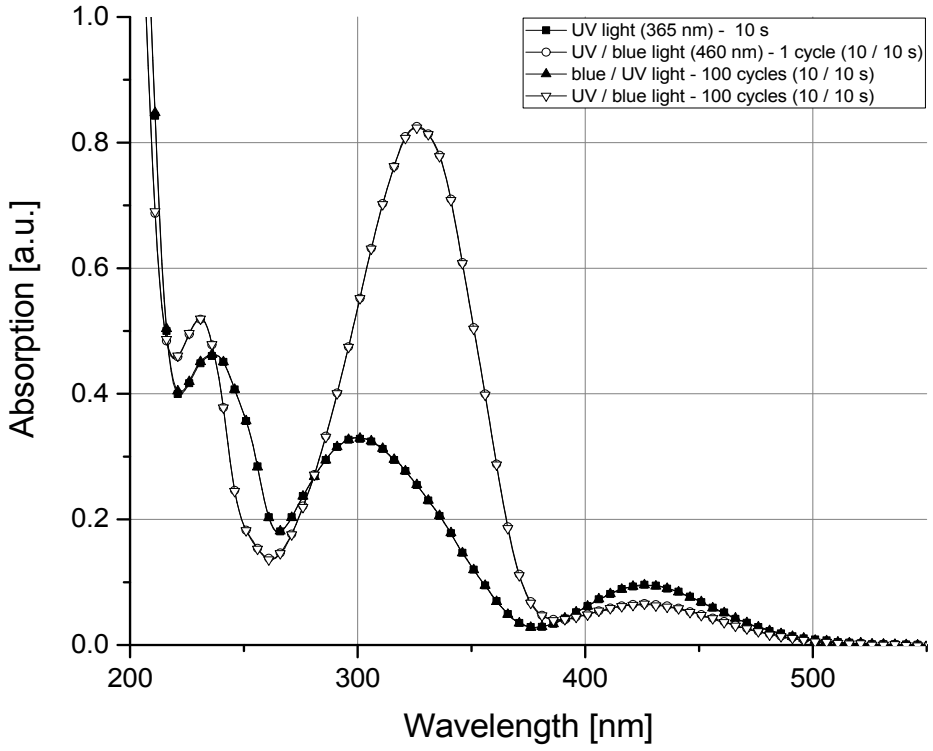



**Figure S15.** UV-Vis spectra: 60 µM PAP-DKP-Lys **1** in di$H_2O$. Photostationary states (10 sec. irradiation) at 365 nm (10 W, black squares) – and 460 nm (10 W, empty dots). The same sample after 100 full switching cycles (10 sec. each) ended with UV light irradiation (black triangles), or blue light irradiation (empty triangles).

### *9. Guest release from the gel matrix*

Here we wanted to investigate how efficient are hydrogels based on PAP-DKP-Lys **1** in releasing various types of encapsulated guest molecules by means of diffusion (in darkness) or decomposition of the inner gel structure (upon UV irradiation). As the gel matrix we have chosen the following composition: 1.5% (15 g/L) of the DKP **1** in 50 mM NaCl. As described in the section 5 of this supporting information, it forms a stable gel in absence of light that is completely converted to fluid upon 30 min. of UV light irradiation.

For the release experiments we prepared (in the same way as described above – section 4) six samples of 1 mL volume containing 2 g/L htDNA and 15 g/L DKP **1** in 50 mM NaCl and 6 samples of 1 mL containing 2 g/L doxorubicin (DOX) and 15 g/L DKP **1** in 50 mM NaCl. The samples were warmed up until the stage of homogenous liquid and left in darkness overnight to produce uniformed gels upon slow cooling.

All the release measurements (in darkness and upon UV light irradiation) were performed in triplicates and the average values were taken for the final conclusions and result plots.

1 mL of 50 mM aq. NaCl was slowly added on top of a gel sample (on the wall of the vial) and immediately removed with micropipette, to wash away unbound or loosely bound guest molecules from the surface. Addition of fresh 1 mL of 50 mM aq. NaCl followed. The gel was incubated together with the liquid on the top in darkness for 5 min. and 1 mL of the liquid was collected after 5 minutes by gently turning the vial sideways and pipetting off the liquid from the side wall of the vial. Then, fresh 1 mL of 50 mM aq. NaCl was added on the side wall of the vial, removed after 5 min. the same way as described above, and that process was repeated for the total duration of 30 minutes (by collecting 6 subsequent 1 mL-volume aliquots). After that time, both gel types (with DNA and DOX) remained visually unaffected. By measuring the remaining liquid volume after removal of the last 1 mL aliquot from the top of the gel we estimate that the total decay of the gel volume was lower that 15%.

To measure the release process upon UV light irradiation, we exactly repeated the procedure described above, but after initial washing of the gel surface the sample was placed in an irradiation chamber and illuminated with two 10 W LED diodes. Short breaks in irradiation (<30 sec.) were taken for the replacement of 1 mL aliquot with fresh 1 mL of 50 mM NaCl every 5 minutes, but the summary irradiation time was 30 min. That period was sufficient to fully convert all the gel samples into liquid. In the case of htDNA, slight pale-yellowish precipitate was observed on the bottom of the sample and aliquots. That might correspond to partial precipitation or aggregation of some DNA material, as that precipitation was not observed for the gel samples containing DOX or the DKP **1** alone in 50 mM NaCl solutions.

*Release of doxorubicin*

The resulting 1 mL aliquots of solutions collected above the gels in the time course of experiments were analyzed using UV-Vis absorption spectrophotometry (**Fig. S16** top left).
The concentration of doxorubicin was measured by quantifying the sample absorbance at λ = 485 nm. At that wavelength there is no spectral overlap with the PAP-DKP-Lys **1** molecule. Calibration was performed using solutions of doxorubicin at the concentration range of 10 mg/L to 1000 mg/L (the maximal possible DOX concentration in case if 100% of the material was instantly released from the gel) and a mixture of DOX and DKP **1** in the ratio 1:7.5 (identical as the ratio in the gel) at the same concentration range of doxorubicin.

*Release of htDNA*

Quantification of the released DNA was more complicated, as the DKP **1** molecule strongly absorbs in the UV light range that is normally used to quantify DNA. After comparing various techniques (HPLC alone or in combination with chemical reduction of azobenzene or acetylation of the lysine side chain) to separate the two molecules from our samples we obtained the best results using size-exclusion filtration (SEC). The 1 mL sample (yellow solution, the color comes from **1**) directly from the release experiment was applied on the DextraSEC PRO10 size-exclusion cartridge (AppliChem, cat.#: A8822,0050), previously equilibrated with 50 mM aq. NaCl, and eluted according to the attached protocol to obtain 1.5 mL of the filtrate as colorless liquid. The yellow band containing DKP **1** remained on the column and could be fully removed with further 15 mL of the eluent. The amount of DNA in the filtrate was quantified using Nanodrop-1000 with the standard procedure for dsDNA quantification. Every fraction was additionally controlled with standard UV-Vis spectrum. The DKP **1** has a strong absorption maximum at λ = 327 nm, but no peak at that wavelength was detected in the filtrates from the size-exclusion column, which confirms good separation of both components.
As the size-exclusion filtration leads to certain material losses due to various factors (e.g. incomplete material separation, aggregation, incomplete collection of the material in the fractions collected according to standard protocols), to measure the material recovery after size-exclusion filtration we prepared (in triplicate) a 1 mL sample of 100 mg/L htDNA and 750 mg/L DKP **1** in 50 mM NaCl. Those amounts exactly corresponded to 10% of the total material that could be released after full degradation of our gel sample. The sample was applied on the SEC column and eluted in the identical way as described above to the final volume of 1.5 mL. The average value of DNA concentration measured for three output samples was multiplied by the factor of 10 and the resulting absorbance value was used as the reference: “100% material release”.
The 1 mL aliquots from the htDNA release experiments performed in absence of light or under UV irradiation were purified on the SEC columns. The DNA concentration was measured and compared with the reference absorbance “100% material release” described above. In the samples incubated in darkness, the DNA release was well within the

experimental error, so the total 1.42% material release reported on the plot below is the maximal value, but it rather reflects the sum of baseline fluctuations during the absorbance measurements. Even that result is, however, in the striking contrast with the amount of htDNA recovered after UV irradiation of gel samples. Within 30 min. all examined gels fully degrade to fluid phase. We would therefore expect total recovery of DNA at the same range as for irradiated gel samples with doxorubicin (above 90%, as the material losses during size exclusion chromatography are already taken into account). The value of 42% was initially a little puzzling. However, after careful inspection of the used aliquots and the remaining glass vials we have noticed subtle flakes of precipitation that were most likely some form of DNA aggregation (no such material was found in analogous DOX/DKP gels). This insoluble material was obviously retained on the top of SEC columns during filtration so it could not be properly re-solubilized.

In summary, we have recovered around half of the total oligonucleotide material in soluble form after size-exclusion purification (**Fig. S16** top right).

The outcome of these release experiments was also summarized on the **Fig. 5** of the manuscript.

The total material recovery in a soluble form is compared on the bottom of the **Figure S16** for both types of the guest molecules in darkness and upon irradiation.

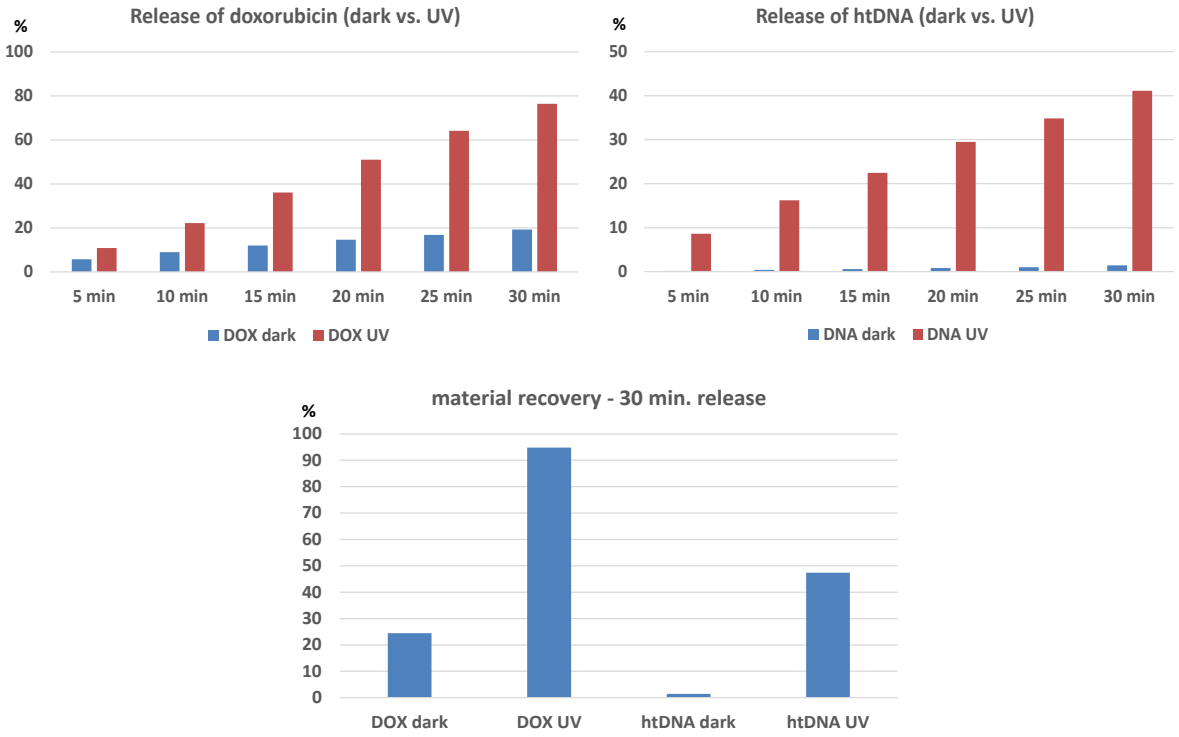
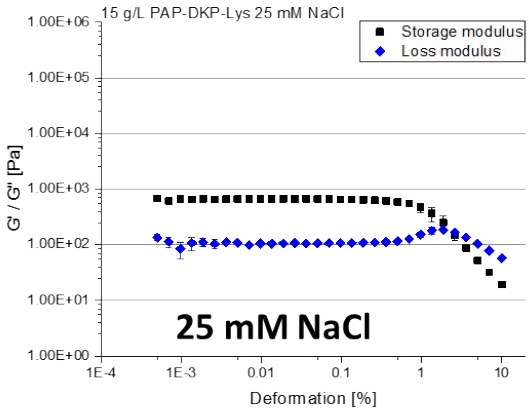
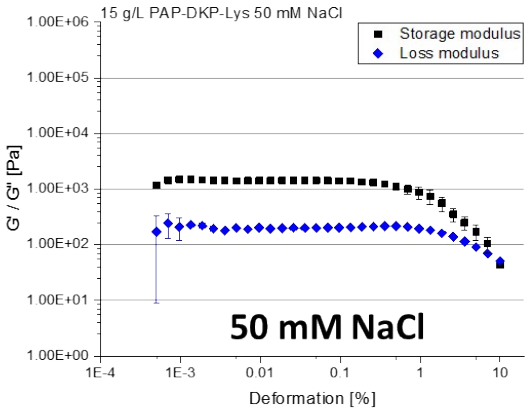


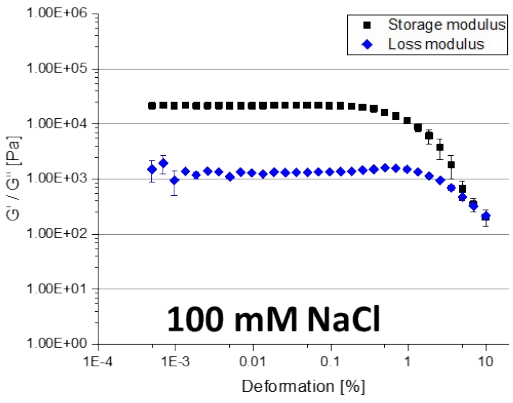

**Figure S16.** Guest release experiments. Release of doxorubicin (**top left**) upon UV irradiation (red) or in darkness by diffusion (blue); release of htDNA (**top right**) upon UV irradiation (red) or in darkness by diffusion (blue); total recovery of encapsulated material (**bottom**) after 30 min. of the release experiments – in darkness ("dark") or under UV light irradiation ("UV") - from left to right: doxorubicin recovered in absence of light, doxorubicin recovered from UV-irradiated gels, soluble htDNA recovered in absence of light, soluble htDNA recovered from UV-irradiated samples.

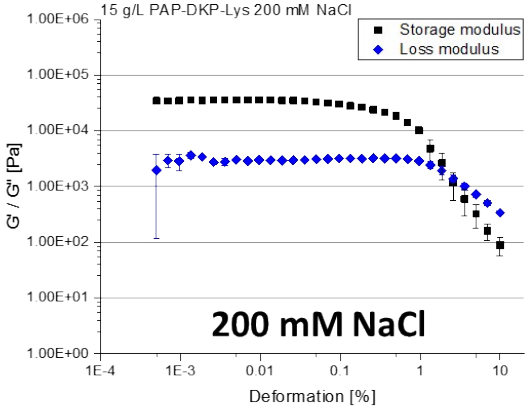
### *10. Influence of NaCl concentration on rigidity of the hydrogels*

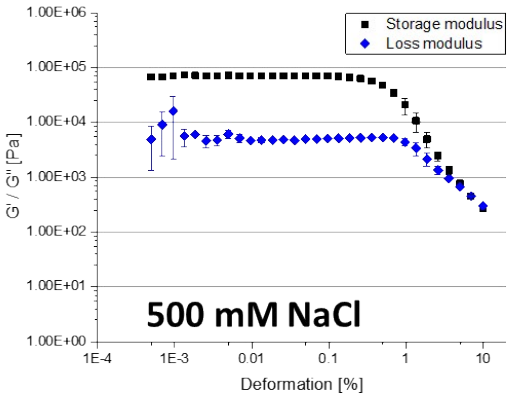
Three gels with constant concentration of **1** (15 g/L) and variable concentrations of NaCl in water were characterized by rheology. Additionally, the results for a gel sample composed of 30 g/L of **1** in 2M aqueous NaCl solution are demonstrated. Based on these results, we can see that the rigidity of gels consistently increases with the NaCl content, and the increase is the fastest in the range between 0 mM and 200 mM NaCl.

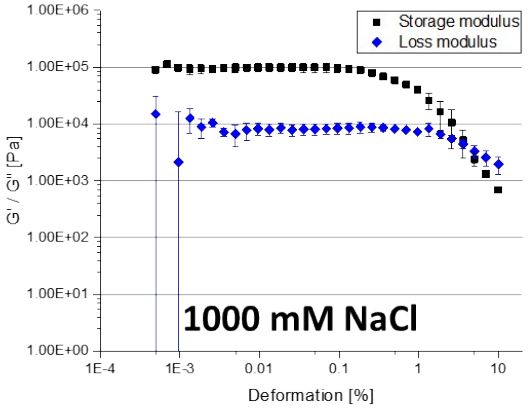


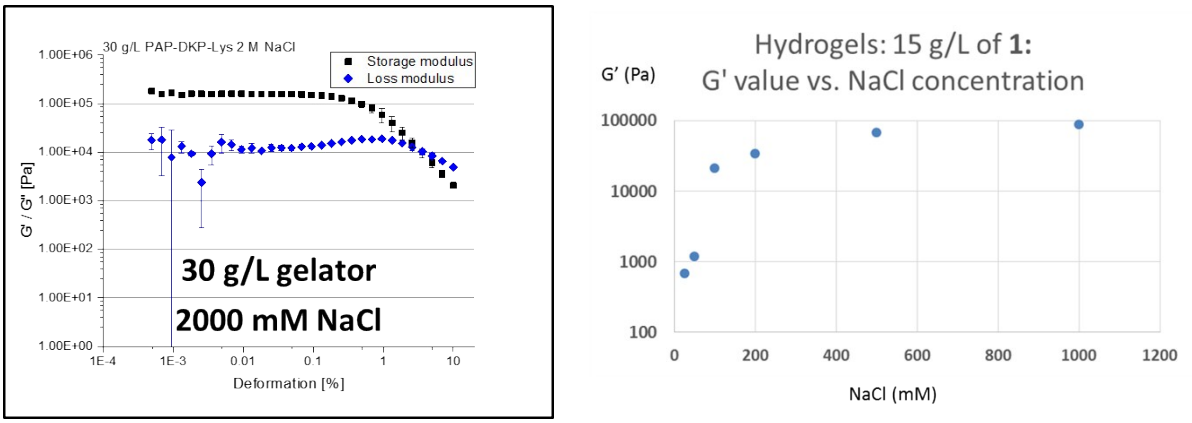


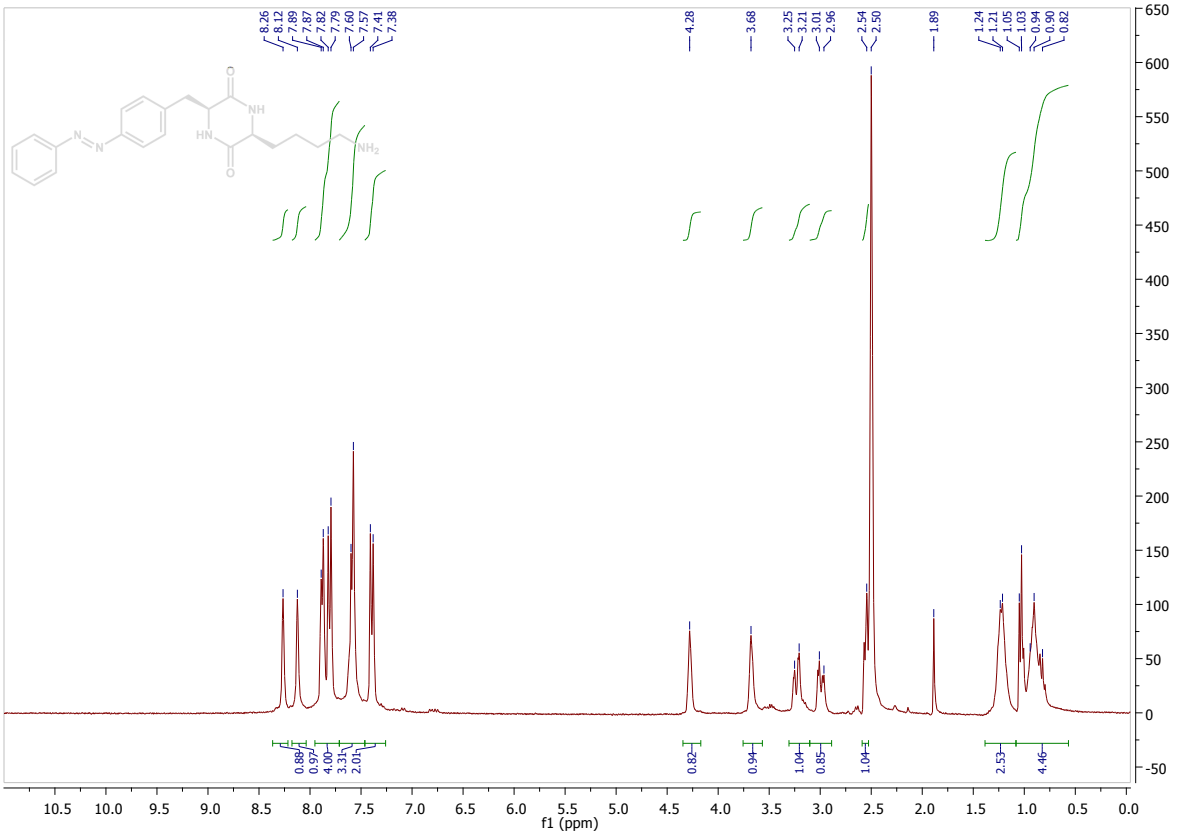

**Fig S17.** *S*train sweep experiments: hydrogels composed of 15 g/L **1** and various amounts of NaCl (25 mM – 1000 mM) in water. Inside a black frame - the analogous experiment for 30 g/L of **1** and 2 M NaCl. Bottom right: a logarithmic plot of the G' values vs. NaCl concentration (for the 15 g/L of **1**).

## *11. References*

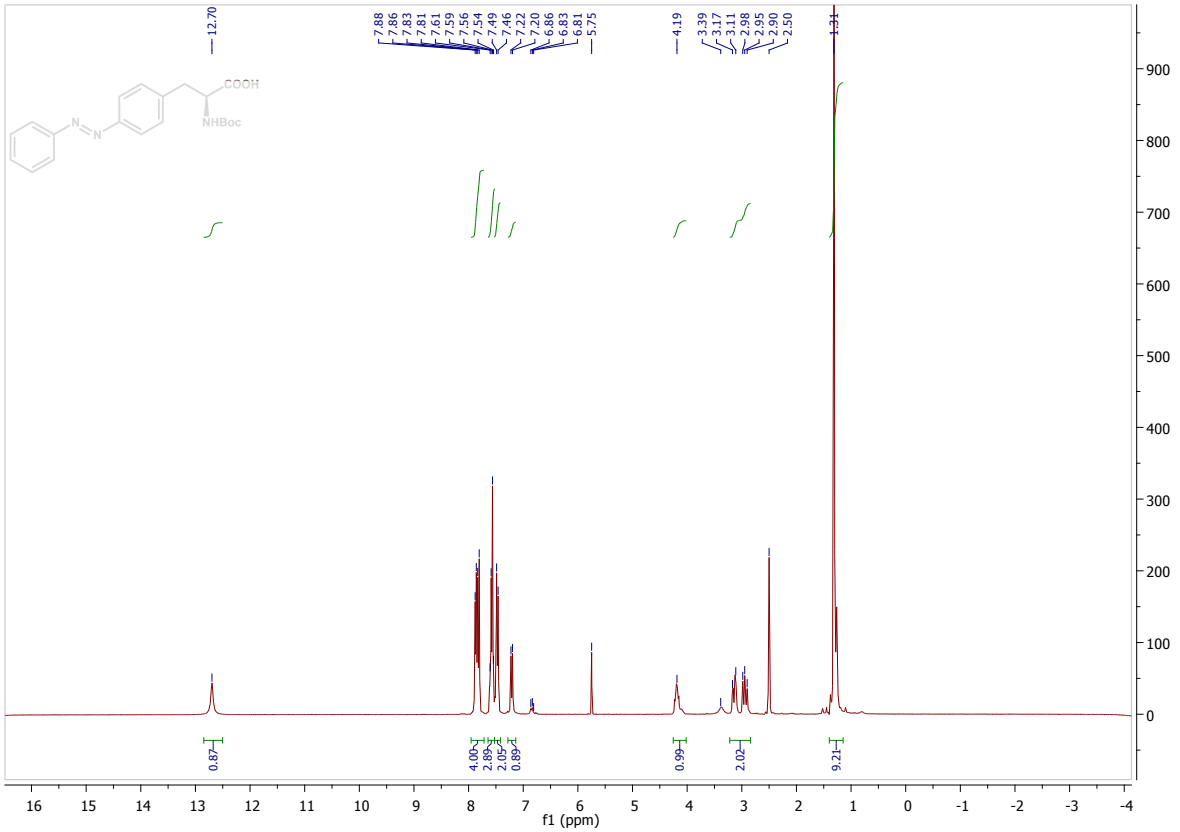
[1] Woolley, A. G.; Beharry, A. A. *Chem. Soc. Rev.* **2011***, 40,* 4422-4437.

## 12. $^{1}$H NMR Spectra of the synthesized compounds

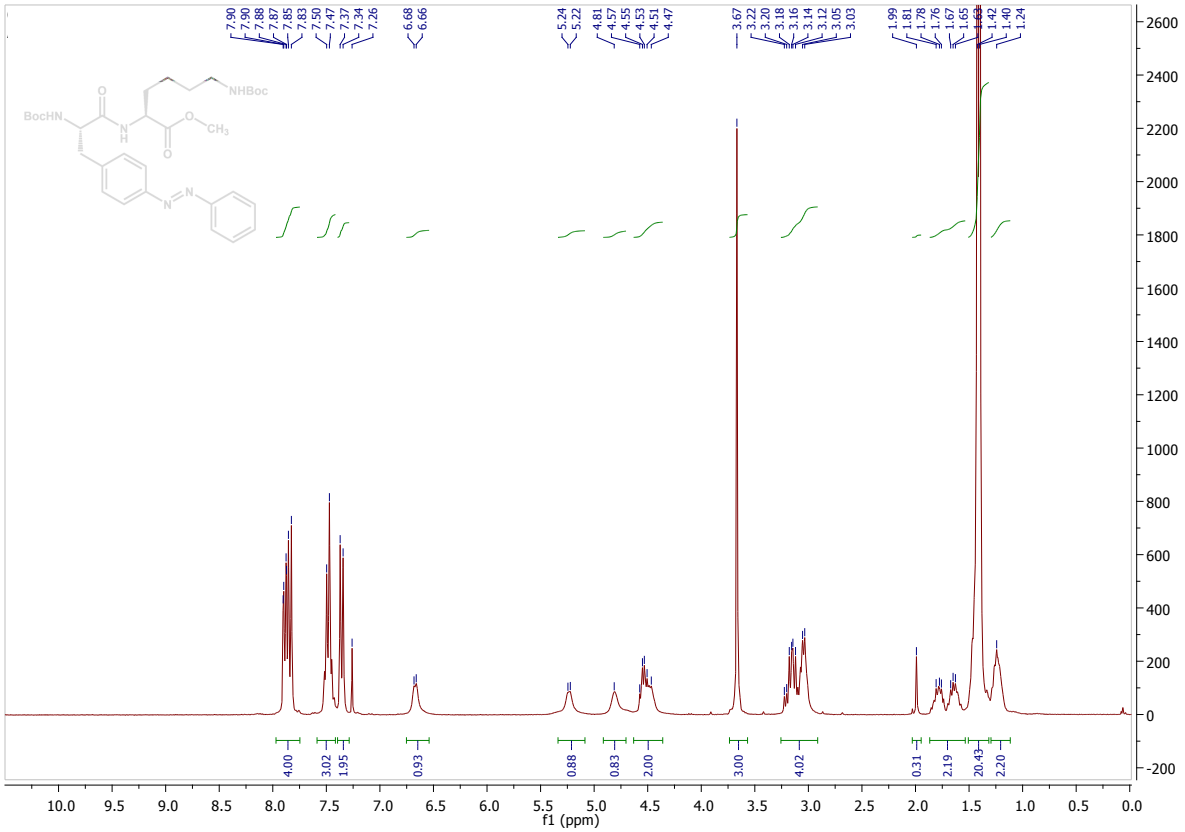
PAP-DKP-Lys **1** (DMSO-*d*$^{6}$):




Boc-PAP-OH **2** (DMSO-*d*$^{6}$):



Boc-PAP-Lys(Boc)-OMe **3** ($CDCl_3$):




H-PAP-Lys-OMe **4** ($CD_3OD$):