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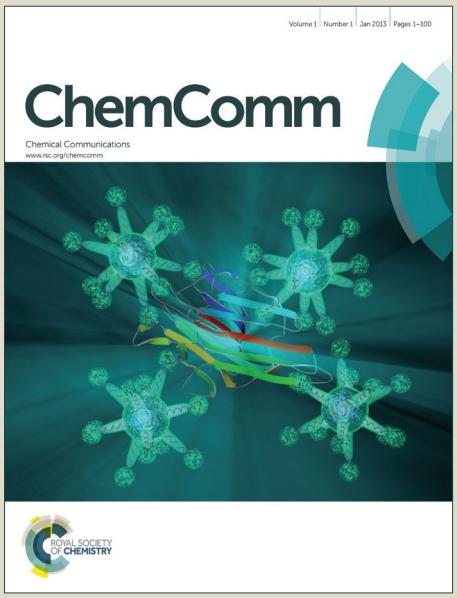

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Photoresponsive self-healing supramolecular hydrogels for light-induced release of DNA and doxorubicin

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An azobenzene-containing cyclic dipeptide PAP-DKP-Lys is a photoresponsive low-MW hydrogelator. The gelation process can be triggered with temperature, pH, light, and ionic strength. The resulting self-healing gels can encapsulate dsDNA or an anticancer drug doxorubicin, and release them in a light-dependent manner.

Hydrogels are materials used for tissue engineering,[1] drug delivery systems,[2] construction of self-healing materials[3] and environmental sensors.[4] They contain over 90% of water absorbed into covalent or supramolecular fibril networks, and possess the degree of flexibility similar to natural tissues. Low-molecular weight gelators (LMWGs) are small molecules that form supramolecular hydrogels stabilised by π-π stacking, hydrogen bonding, ionic and/or van der Waals interactions. Particularly privileged scaffolds for LMWG are peptides, ureas and nucleobases, due to their extensive hydrogen-bonding networks that promote self-assembly.[5] Such gels can react on triggers like changes of pH, temperature, ion concentrations,[5,6] redox potential[7] or light.[8] Numerous light-responsive hydrogel systems were designed based on photocaged compounds,[9] photoswitches[8] and light-activated molecular motors.[10] Particularly, the perspective of using light to release bioactive or therapeutically relevant compounds (e.g. drugs or oligonucleotides) from hydrogels is very attractive and could find numerous therapeutic applications.[11]

Azobenzenes, diarylethylenes or spiropyrans are common examples of molecular photoswitches which can reversibly transform light into molecular motion or polarity changes. They were used to photomodulate activities and structures of bioactive compounds,[12] biopolymers[13,14] and artificial nanoscale systems.[15] They can be added as dopants to hydrogels,[16] covalently attached to, or partially replacing the structure of known gelators.[8, 17]

Controlled delivery of therapeutic oligonucleotides, and their protection from premature degradation, are crucial issues for successful gene therapy methods. Encapsulation of DNA for gene delivery has been studied using polymeric hydrogels, like collagen, alginate, PEG/PLA systems or engineered silk elastin. To slow down rapid DNA diffusion typical for such systems, DNA can be also organized into nanoparticles (by condensing with cationic peptides, lipids or polymers) encapsulated inside fibrin or PEG hydrogels.[18]

In this communication we describe the first, to our knowledge, supramolecular hydrogel based on LMWG that preferentially encapsulates oligonucleotides inside its fibrous network and can efficiently release them upon irradiation. We designed and synthesized a LMWG **1** containing a molecular photoswitch and a basic residue that can specifically interact with nucleic acids. We investigated its gelating properties under variety of conditions, including the presence of DNA (**Fig. 1**).

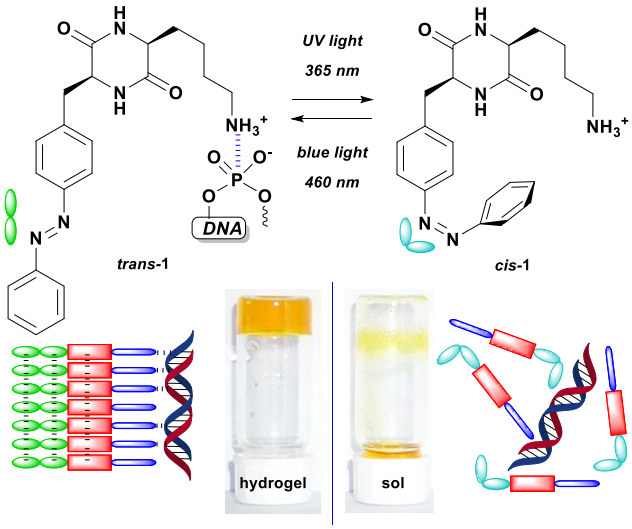

Figure 1 The supramolecular hydrogel formed from PAP-DKP-Lys **1** in water (left) becomes liquid upon UV light irradiation (right). The gel is reconstituted after irradiation with blue light. Polyacids (e.g. DNA) may additionally stabilize the fibres.

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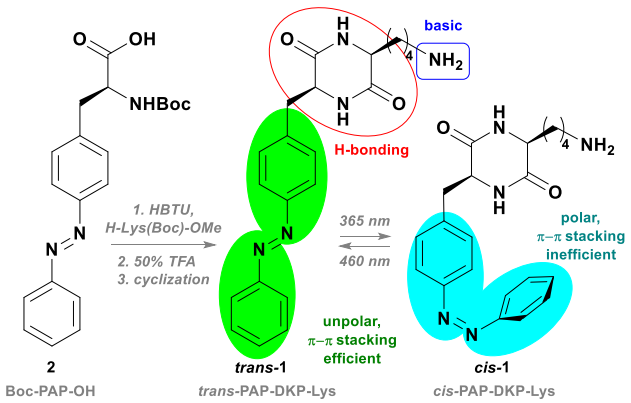

Figure 2 Synthesis and photoisomerization of the supramolecular hydrogelator **1** (PAP-DKP-Lys)

The compound **1** can form supramolecular hydrogels alone or with a variety of dopants like NaCl, acids, or DNA. Some of the gels exhibit excellent self-healing parameters (thixotropy). DNA oligomers and an anticancer drug doxorubicin can be released from hydrogels based on **1** in the light-dependent manner.

Our goal was to create a light-responsive hydrogel with specific affinity towards DNA. We hypothesised that the significant change in molecular geometry and polarity, which is characteristic for azobenzene photoisomerization, will be sufficient to strongly destabilise and eventually destroy the gel structure and cause its transition into sol. The design was based on known 2,5-diketopiperazine-based gelators[19] stabilised by hydrogen bonding provided by the heterocycle and the π-π stacking of non-polar phenyl rings. Replacing the phenyl with non-polar and planar *trans*-azobenzenes retains this pattern of stabilization. But photoswitching them to polar and non-planar *cis*-azobenzenes (**Fig. 2**, right) would disturb tight packing of the molecules in fibres and possibly destroy the long-distant self-assembly.

We synthesized the desired molecule **1** starting from an unnatural photochromic aminoacid **2** Boc-PAP-OH.[14] It was coupled with protected *L*-lysine and cyclised to the corresponding 2,5-diketopiperazine (**Fig. 2**, left). The compound **1** is insoluble in acetonitrile or toluene, and soluble in methanol, DMF and DMSO. It is also soluble (upon short boiling) in hot water to the maximal concentration of 3%. After cooling down, such aqueous solutions (above 1.5% of **1**) form hydrogels which are however fragile and become liquid after shaking. Replacing water with 50 mM aqueous NaCl solution results in striking improvement of mechanical properties and increased melting temperatures of gels. Similar behaviour is observed after lowering the pH with trifluoroacetic acid (TFA). In that case, the minimal concentration of **1** necessary for gel formation is 1.0%. Generally, both the presence of salt or acids improves rigidity of the gels.

In comparison to monoprotic acids, polyacids like DNA should stabilise structures of gels formed by **1** to even higher extent by cooperative effect of multiple salt bridges. To verify that hypothesis, we used commercially available double-stranded

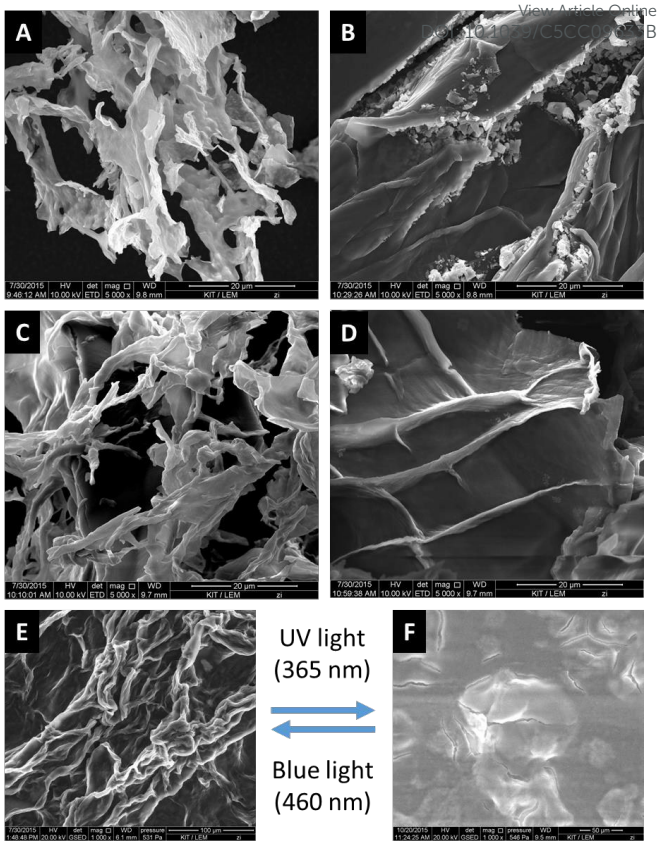

Figure 3 Scanning electron microscopy (SEM). **A-D**: SEM images of xerogels. Gels **A-C**: 20 g/L of **1**, gel **D**: 15 g/L of **1**; gels **A**, **C** - in diH_2O; gels **B**, **D** - in 50 mM aq. NaCl; gels **C** and **D**: additionally 2 g/L htDNA; **E-F**: environmental SEM of the hydrogel **D** in darkness (**E**) and upon UV light irradiation (**F**)

DNA oligomers (htDNA, c.a. 1300 bp long). Hydrogels formed with htDNA (2 g/L) and 2% (20 g/L) of the gelator **1** in water did not exhibit significantly improved mechanical properties, however their melting temperatures increased by more than 10 °C (see **Table S2**) in comparison to the undopped 2% gel (20 g/L of **1** in diH_2O). Addition of htDNA to hydrogels formed by **1** in 50 mM aqueous NaCl resulted in both elevated melting temperatures and improved mechanical stability comparing to hydrogels formed by **1** in water without additives.

After examining various proportions of the components, we continued detailed characterization with the gel composed of 2 g/L htDNA and 15 g/L (1.5%) of **1** in 50 mM aqueous NaCl (gel **D**) due to the most appealing mechanical properties.

The morphology of the gel samples described above was determined using scanning electron microscopy (SEM) (**Fig. 3**, also **Fig. S6-S9**, Supp. Info). We examined xerogels formed by lyophilization of the hydrogel samples containing only **1** (gel **A**) or **1** and htDNA (gel **C**) in water, or gels containing the same ingredients in 50 mM aq. NaCl (gels **B** and **D**, respectively). Additionally, a diluted sample of **1** was characterized using transmission electron microscopy (TEM) to visualize in details its fibrous structure (**Fig. S13**).

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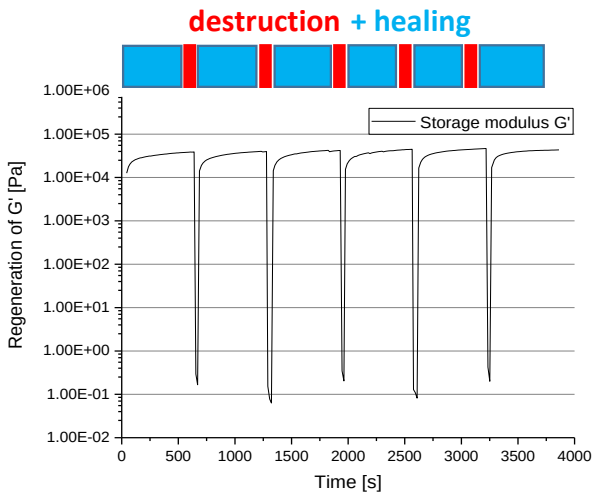

Figure 4 Self-healing properties of the gel **B** (20 g/L of **1** in 50 mM NaCl). The storage modulus is rapidly (< 1 min.) recovered after 100% deformation.

The samples of gel **D** irradiated with UV light for 30 min. (365 nm, 20 W) became mechanically very fragile and turned into liquid upon inversion of the vial or slight shaking. That observation is in agreement with our hypothesis that the *trans*–to-*cis* photoisomerization should break the long-distant aromatic stacking between the molecules, then cause the fibres to dissipate, and ultimately lead to gel dissolution. The liquid could be stored in darkness at room temperature for several days without significant changes, which is in agreement with the reported thermal half-life of the unsubstituted *cis*-azobenzene (on the scale of days at room temperature[20]). If the same liquid, however, is irradiated for 30 min. with blue light (460 nm, 10 W), the samples gelate fully in the timescale of a few hours in darkness yielding clear, homogenous and mechanically stable hydrogels. This time is apparently needed to initiate fibre formation that ultimately reconstitutes the original gel structure. The sample **D** was also imaged (**Fig. 3 E-F**, also **Fig. S10-S12**, Supp. Info) under low-vacuum ("environmental") SEM conditions (eSEM). The observed structures (**Fig. 3E, S10**) almost entirely disappeared (**Fig. 3F, S11**) after 30 min. of irradiation with UV light, along with the gel-to-sol transition of the sample. Similar structures were observed again (**Fig. S12**) in the samples where the photoisomerization cycle was completed with blue light and resulted in regeneration of the rigid gel.

Mechanical properties of the gels were characterized by rheological strain sweep and frequency sweep experiments (Supp. Info, **Fig. S2-S5**). The storage modulus (G') and the loss modulus (G") differed by one row of magnitude, indicating the viscoelastic behaviour. The mechanical properties improve with increasing NaCl concentration (Page **S17**, Supp. Info).

All the investigated samples revealed self-healing properties (thixotropy). Samples **B** and **D** recovered their initial mechanical properties (plotted as regeneration of the G' value) in the time range below one minute from deformation. Samples **A** and **C** regenerated with slightly slower rates. Repeated stress did not lead to the significant material fatigue (**Fig. 4** and **Fig. S2-S5** of the Supp. Info).

The compound **1** is resistant on photodegradation with UV light for at least 24 hours (see Supp. Info **Fig. S14**) and can undergo over 100 full cycles of photoisomerization. (**Fig. S15**) without degradation.

Finally, to investigate the potential of hydrogels formed by **1** for oligonucleotide storage and their light-controlled release we measured diffusion of htDNA from the gel **D** [2 g/L htDNA and 15 g/L (1.5%) **1** in 50 mM NaCl] in darkness and upon UV light irradiation. To have a comparison with guest molecules of small MW, we also prepared a hydrogel composed of 2 mg/L doxorubicin (DOX, a common anticancer drug) and 15 mg/L (1.5%) of **1** in 50 mM NaCl. Both gel samples (1 mL each) were irradiated with UV light for 30 min. and incubated with 1 mL of 50 mM aq. NaCl changed every 5 minutes. Concentration of the guest molecule in every fraction was measured afterwards. The results were compared with guest concentrations in fractions collected over gel samples without UV irradiation.

As demonstrated on the **Fig. 5**, diffusion of DOX from the gel in darkness is at least 3-fold slower than its release under UV light irradiation. Much more striking difference was observed in the case of htDNA: in absence of UV light the DNA was retained inside the gel within the detection limit of our experimental setup, whereas UV light released most of the oligonucleotides within 30 minutes (See also **Fig. S16**).

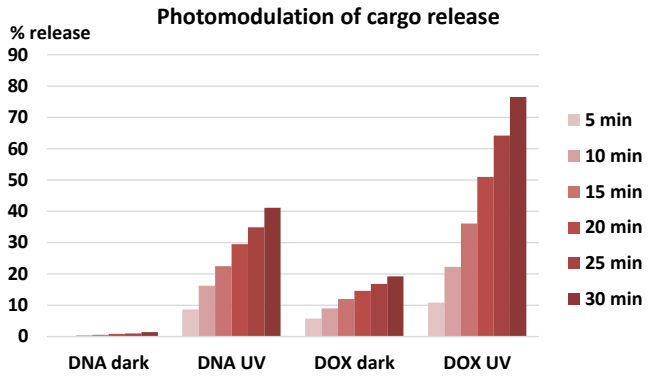

Figure 5 Photomodulation of the cargo release from hydrogels. Gel composition: "DNA" 2 g/L htDNA + 15 g/L **1** in 50 mM NaCl, "DOX" 2 g/L doxorubicin (DOX) + 15 g/L **1** in 50 mM NaCl; release of the guest molecules upon UV irradiation (DNA UV, DOX UV) or in darkness (DNA dark, DOX dark), data points in 5 min. intervals.

To conclude, prior research on supramolecular hydrogels formed by LMWG molecules demonstrated that such materials can quickly respond to numerous physical and chemical stimuli. In contrary to polymeric materials, light-stimulated oligonucleotide release from LMWG-based hydrogels was, however, not reported. By the rational design of cationic LMWG **1** we were able to prepare photoresponsive gels that tightly bind long dsDNA oligomers inside their fibrous structures and efficiently release them upon light irradiation. Doxorubicin can be also encapsulated in gels formed by **1** and released in a light-dependent manner. These results indicate

that light can be an effective and selective trigger for releasing oligonucleotides and other types of bioactive molecules from LMWG-based hydrogels. Mechanical and physical properties of hydrogels formed by **1** can be extensively modified by changing concentration of the gelator, as well as the pH and concentrations of inorganic ions. The latter features indicate prospective applicability of the material in the area of environmental sensing. Together with the observed self-healing behaviour it indicates considerable potential of compositions based on **1** as smart materials for therapeutic purposes, particularly for photodynamic therapy based on release of cytotoxic drugs or therapeutic oligonucleotides. To consider applications in living organism, further studies will be performed with the focus on issues like general toxicity of the gelator, photoswitching with visible light for better tissue penetration, or tuning the aminoacid residue to the particular cargo types.

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