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Photoswitchable fluorescent crystals obtained by the photoreversible co-assembly of a nucleobase and an azobenzene intercalator

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Abstract

Self-assembled nucleobases, such as G-quartets or quadruplexes, have numerous applications but light-responsive structures are limited to small, non-crystalline motifs. In addition, the assembly of the widely exploited azobenzene photochromic compounds can produce fluorescent crystals of extended dimensions but at the prize of sacrificing their photoswitchability. Here we overcome inherent limitations of self-assembly with a new concept of supramolecular co-assembly leading *in fine* to materials with unprecedented properties. We show that the co-assembly of guanosine monophosphate (GMP) with an azobenzene-containing DNA intercalator produce supramolecular crystals arranged through a combination of π - π , electrostatic and hydrogen bond interactions. The resulting crystals are 100 μm long, pH-sensitive, fluorescent and can be photoreversibly disassembled/reassembled upon UV/blue irradiation. This allows us to perform operations such as dynamic photocontrol of a single crystal growth, light-gated permeability in membrane-like materials and photoswitchable fluorescence. We believe this concept critically expands the breadth of multifunctional materials attainable by self-assembly.

Introduction

Systems with light-switchable functions are appealing for fields ranging from biology to physics and material science.¹⁻³ Azobenzene is one of the most attractive photochromes used in such systems because of its robust, rapid and reversible E/Z isomerization that leads to large changes in molecular geometry and polarity.⁴ Applications of azobenzene derivatives in holographic memory storage, optoelectronic devices, optofluidics, molecular machines, responsive materials, energy conversion and drug design have been widely explored by taking advantage of their robust photoisomerization characteristics.⁵⁻¹¹ However, this remarkable photoisomerization ability tends to hinder photochromic azobenzene derivatives from exhibiting fluorescence properties. By imparting azobenzene with steric rigidity, bright fluorescence can be achieved but this is usually done at the price of sacrificing the photoisomerization capability.^{12,13} Realizing a system displaying both azobenzene fluorescence and photoisomerization capability, an interesting combination for instance to optically modulate fluorescence, is thus a desirable challenge that has been achieved in a few rare cases. The typical strategy has consisted in controlling with light the aggregation behavior of azobenzene derivatives which, by exploiting the fluorescence enhancement effect upon aggregation, led to a variety of light-dependent aggregating systems with optically tunable emission characteristics.¹⁴⁻¹⁹ These materials were however usually morphologically ill-defined and poorly structured. Conversely, well-defined azobenzene crystals with bright fluorescence properties were obtained but did not display any photoswitching characteristics²⁰⁻²². Finally light-responsive organized assembly of azobenzenes were reported but the fluorescence properties were either not documented²³ or modestly affected by light.²⁴ Note that most of the above-mentioned examples were based on the assembly of hydrophobic or amphiphilic compounds in organic solvents. To our knowledge, highly-organized azobenzene-based supramolecular structures, such as crystals, with both light-responsive organization and on/off photoswitchable fluorescence has never been realized. Here we report the first light-responsive azobenzene crystals with photoswitchable fluorescence in water. These crystals were obtained following a new strategy consisting in the co-assembly of azobenzene-containing DNA intercalators and nucleobases as non-covalently interacting building bricks. By exploiting the natural self-arrangement characteristics of nucleobases, we found that this new way of organizing azobenzenes allowed us to obtain crystals with aggregation-induced fluorescence emission while maintaining a strong and reversible response to light stimulation.

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3 Among nucleobases, guanine represents a particularly interesting component for
4 scaffolding supramolecular organizations. Due to the presence of both self-complementary
5 donor and acceptor sites for hydrogen bonds and aromatic rings favouring π - π stacking, it can
6 self-assemble into multiple hydrogen-bonding structure like G-quartets or quadruplexes,²⁵
7 showing potential in biosensors, material science and organic electronics.²⁶⁻²⁸ Efforts have
8 also been made to expand the dynamics of the structure, which resulted in a variety of
9 stimuli-responsive systems, the majority of them being controlled by solution composition
10 (pH, ionic composition). Conversely, external control by a light stimulus has been achieved
11 in much fewer cases and only with G-quartet structures by covalent modification or
12 noncovalent π - π stacking strategies to incorporate a photosensitive moiety.²⁹⁻³⁸ However, all
13 of these light-sensitive structures have consisted of a few repeating units, and neither μ -
14 sized nor crystalline materials have been constructed. Here we describe for the first time
15 light-responsive, highly organized, supramolecular architectures containing guanosine
16 monophosphate (GMP) with a needle-like geometry and a length of several hundreds of
17 micrometers. Both structure and light-responsiveness resulted from the co-assembly of GMP
18 with a hydrophilic, azobenzene-containing DNA intercalator, called AzoDiGua.³⁹ This
19 process relied on a network of hydrogen bonds and π - π stacking involving both components,
20 resulting in a structure that is different from the G-quartet-based architecture that was
21 reported for most GMP-based systems. In this paper, we describe the protocol for co-
22 assembling GMP and AzoDiGua, determine the structure of the resulting crystals,
23 characterize their reversible assembling character in response to pH or light stimulation, and
24 analyze their fluorescence properties. We finally exploit the light-responsiveness of these
25 new assemblies to demonstrate a combination of unique properties, such as photoreversible
26 assembly, optical growth control, light-gated permeability and photoswitchable fluorescence.
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46 **Preparation of the supramolecular hybrid crystals**

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48 Figure 1 shows the concept of our co-assembly method to generate hybrid crystals
49 with both fluorescence and light-responsiveness. It consisted in the co-assembly of the GMP
50 nucleobase with a photochrome, here an azobenzene-containing symmetric divalent
51 guanidinium compound (AzoDiGua) having photosensitive DNA intercalating properties³⁹
52 (Fig. 1A). GMP (5 mM) and AzoDiGua (0.2 mM) were mixed in acetic acid buffer (10 mM
53 in water, pH = 3.8) at room temperature, heated to 90 °C prior to slowly cooling down to
54 4 °C at a speed of 1 °C/min (Fig. 1B). After assembly, the suspension was brought back at
55 room temperature (RT) and all experiments were then performed at RT. This process resulted
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3 in phase separation occurring only in the presence of both GMP and AzoDiGua (Fig. 1C).
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5 Optical, electron and atomic force microscopy observations of the dense phase revealed the
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7 presence of well-organized needle-like architectures with an average length and width of 101
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9 ± 46 μm and 1.9 ± 0.7 μm respectively (Figs. 1D, 1E, S1). Elementary analysis showed that
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11 this solid material was hexahydrated with a stoichiometry AzoDiGua:GMP of 1:2 (Table S1),
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13 corresponding to electrostatic neutralization of AzoDiGua by 2 GMP moieties in their
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15 monoanionic form $\text{H}(\text{GMP})^-$ (Fig. S2). Increasing pH of the assembling buffer resulted in a
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17 progressive deprotonation of $\text{H}(\text{GMP})^-$ (Fig. S2) and led to assembled materials with reduced
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19 sizes and irregular shapes (Fig. S3). Meanwhile, sample prepared at pH 3.8 but with inosine
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21 monophosphate (IMP) or adenosine monophosphate (AMP) instead of GMP remained
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23 homogenous (Fig. S4). Compared with GMP, IMP lacks the 2-amino group that is typically
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25 engaged in one of the three H-bonds of the GC DNA base pair. Similarly, AMP provides one
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27 less hydrogen bond formation site compared with GMP. All these observations suggest that
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29 both multiple noncovalent hydrogen bonds and electrostatic interactions were involved in the
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31 successful co-assembly of GMP and AzoDiGua into extended and highly-ordered
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33 supramolecular structures.

32 X-ray structural analysis

34 We acquired X-ray diffraction data on a single crystal to gain better insight into the
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36 assembled structure and its stabilizing interactions (Fig. 2, Table S2). Interestingly, although
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38 GMP bases were not covalently bound, the relative organization of AzoDiGua and GMP was
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40 found to be reminiscent to a DNA intercalation motif (Figs. 2A, 2B), emphasizing the interest
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42 of using a photosensitive intercalator as one of the two building bricks of the supramolecular
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44 structures. The analysis showed that the azobenzene moieties in the crystal were in a twisted
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46 configuration with a C-C-N=N dihedral angle of 17.9° between the benzene rings and the
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48 diazo group, with the two GMP molecules arranged in a nearly parallel manner with respect
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50 to the closest benzene plane (Fig. 2B). The distance between the centroids of the AzoDiGua
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52 benzene rings and the guanine planes was ~ 3.5 \AA , which was within the π - π interaction
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54 distance.⁴⁰ The arrangement of the molecules also allowed the formation of a network of
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56 hydrogen bonds with neighbouring units to stabilize the structure (Figs. 2C, 2D, Table S3).
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58 The analysis revealed that a pair of guanine bases formed two $\text{NH}_2 \cdots \text{N}7$ hydrogen bonds
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60 (2.09 \AA , 174.5°), which explains the observed crucial role of the 2-amino group for the
ordered structure formation. P-O \cdots H-O hydrogen bonds (1.76 \AA , 159.5°) were also found
between the phosphate groups of adjacent GMPs (Figure S5), which explains the importance of

pH as the monoprotection of the phosphate group was necessary for assembly to occur. Moreover, the guanidinium group of AzoDiGua not only helped to reduce the repulsion of charged phosphate groups, but also contributed to the assembly through the formation of a network of hydrogen bonds with lengths measured to be within the range 1.98–2.22 Å (Figure 2D). All these results show the crucial roles of the two components in the self-assembly process leading to the observed crystalline structure: both AzoDiGua and GMP synergistically participated through the cooperative action of electrostatics, intercalation mechanisms, π - π interactions and hydrogen bond network formation.

pH-responsiveness of the crystals

We analysed how the produced self-assembled crystals could be reversibly affected by the application of different stimuli targeting the non-covalent interactions involved in their structure. First, increasing the pH of a solution containing crystals to pH = 8.5 resulted in their rapid dissolution (Fig. 3A), due to the deprotonation of GMP and subsequent ruptures of the related hydrogen bonds. Interestingly, when the same solution was brought back to pH = 4, the sample underwent phase separation again. The re-assembled material displayed needle-like geometries of similar size and shape than the initial crystals (Fig. 3B), demonstrating that the co-assembled AzoDiGua-GMP supramolecular structure was reversibly addressable through a straightforward pH stimulus.

Photoreversible crystal formation/dissolution

We fixed the pH of the sample suspension (pH = 3.8) and studied the effect of light on the co-assembled crystals (Fig. 4). Irradiation by UV light (365 nm) at RT resulted in the total dissolution of the crystals in a few minutes (Fig. 4A, Movie S1), while subsequent blue irradiation (440 nm) of the same solution resulted in the reformation of needle-like crystals (Fig. 4A, Movie S2). A close up analysis of the UV-induced dissolution process showed that it started by a bending of the structure prior to breaking and disappearance (Fig. 4B). A representative plot of the length of a single crystal vs time showed a two-step dissolution kinetics composed of a first slow process followed by a much faster one (Figure 4C, Movie S3). When green irradiation (535 nm) where AzoDiGua did not absorb, was used instead of UV, neither the dissolution process nor any morphology transformation of the microstructure was detected. We thus conclude that the disassembling process comes from the photoisomerization of AzoDiGua from *trans* to *cis* isomer upon UV irradiation.³⁹ The UV-induced bending of the crystal at the early stage of irradiation was attributed to anisotropic

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3 exposure to the UV stimulus leading to uneven *trans-cis* AzoDiGua photoisomerization
4 features at the surface of the crystals. This speculation was supported by observing the
5 recovery of a straight needle by applying blue irradiation (30 s) after a very short UV pulse
6 (0.5 s), *i.e.*, as soon as the crystal started to bend (Fig. S6). After repeating the process twice,
7 a defect was observed on the crystal surface, indicating a preferential location on the crystal
8 surface for the dissolution to start. To know whether the formation of *cis*-AzoDiGua upon
9 UV irradiation was occurring only at the crystal surface or also in its core, we irradiated a
10 crystal suspension by a moderate UV irradiation (4 min) so that the photosensitive crystals
11 were only partially dissolved. The suspension was centrifuged immediately after UV
12 irradiation and we compared by absorption spectroscopy the composition of the supernatant
13 to that of the pellet re-dissolved in water (Fig. S7). Clearly, the pellet, and thus the remaining
14 crystal structures after UV were composed of *trans*-AzoDiGua while *cis*-AzoDiGua was
15 detected in the supernatant only, *i.e.*, in the surrounding medium of the irradiated crystals.
16 We can thus propose the following UV-induced crystal dissolution process. UV irradiation
17 induced the *trans-cis* isomerization of AzoDiGua molecules present on the surface of the
18 crystals, which disrupted the local π - π and hydrogen bond interaction network. The *cis*-
19 AzoDiGua molecules disengaged from the surface and diffused into the bulk as a result of
20 their hydrophilicity. This led to the exposure of a fresh crystal surface to the light and the
21 disassembly process continued in a cascade-like manner until the full dissolution of the
22 crystal. This suggested diffusion-limited mechanism explains why the full crystal dissolution
23 required significantly more time than that for AzoDiGua to reach the photostationary state in
24 solution under UV irradiation.³⁹ Blue irradiation after such UV-induced dissolution was
25 shown to trigger a re-crystallization process (Fig. 4A, Movie S2). The re-assembled material
26 displayed needle-like structures similar to the ones before UV irradiation, but with a length
27 typically 30% longer (Fig. 4E). Figure 4B bottom depicts the track of a representative single
28 structure, which elongated from both ends with increased time, with an estimated initial
29 growth speed of around $0.4 \mu\text{m}\cdot\text{s}^{-1}$ (Fig. 4D, Movie S3). Since AzoDiGua was shown to be
30 rapidly converted into a *trans*-rich photostationary state upon blue irradiation,³⁹ we propose
31 the following mechanism of blue-induced recrystallization. After UV-irradiation, the
32 photostationary *cis*-rich state contained a small fraction of *trans*-AzoDiGua molecules which
33 were probably engaged in *trans*-AzoDiGua-GMP clusters that were too small to be detected
34 by optical microscopy. Blue irradiation strongly enriched the solution into *trans*-AzoDiGua
35 molecules that progressively either co-assembled on preformed structures or nucleated new
36 ones. The presence of *trans*-AzoDiGua-GMP clusters after UV irradiation might thus act as
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3 nuclei and explain the formation, after blue irradiation, of crystals that were larger and in a
4 smaller number than before the UV irradiation step (Fig. S8). All these results show that the
5 photoisomerization properties of AzoDiGua were successfully preserved in the self-
6 assembled structures to generate 100 μm -long azobenzene-GMP crystals with
7 photoreversible assembly capability.
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13 **Dynamic photocontrol of a single crystal growth**

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15 In materials science, controlling the kinetics of material growth until desired
16 dimensions, once the parameters like temperature and concentration are set, is a desirable
17 challenge. We thus explored how the dimension of a single needle-shaped crystal could be
18 controlled in a remote and dynamic way by light (Figs 4F, 4G, Movie S4). To this end, we
19 locally irradiated a crystal suspension using a focused UV beam to fully dissolve the solid
20 material in the irradiated area. Then a much smaller focused laser spot of blue light was
21 applied in front of a selected needle and switched on and off in a dynamic manner. Figs. 4F
22 and 4G show that the growth of the needle was strictly correlated with the application of the
23 light stimulus. The needle grew at almost constant speed when the light was on while the
24 growth was momentarily stopped as long as the light was kept off. This unique feature
25 allowed us to dynamically control the growth of the targeted single crystal by light with
26 precise spatiotemporal resolution.
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38 **Light-gated permeable material**

39 We then explored how the photoreversible assembly/disassembly properties
40 demonstrated on individual crystals could be exploited to control the permeability of
41 extended materials composed of a large number of photoresponsive crystals. We first
42 prepared a thin layer of crystals and found that the successive application of focused UV and
43 blue light allowed us to dynamically open and close a single pore at the lit area (Figs. 5A, 5B,
44 Movie S5). The UV-induced pore formation was also successful with a thicker layer (~ 100
45 μm) but required prolonged irradiation time. Interestingly, the size and shape of the light-
46 induced pore reproduced well the spot dimensions, allowing us to create a hole in the material
47 with an arbitrary, user-defined shape (Figs. 5C, 5D, Movie S6). We then embedded the
48 photoresponsive crystals inside a thin layer of agarose gel to form a 0.9 mm thick membrane
49 that we placed, in a sandwich configuration, between two aqueous buffer compartments (Fig.
50 5E). The upper compartment was initially loaded with Rhodamine B dye (RhB) and we
51 measured the absorbance of the lower compartment solution 20 min after loading RhD in the
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3 upper chamber. The absorbance spectrum displayed a peak around the absorption maximum
4 of RhB (554 nm) showing that the dye diffused through the membrane. Interestingly, the
5 absorbance was approximately 6 times higher when the membrane was exposed to UV,
6 demonstrating an enhanced permeability of the membrane material upon UV exposure. These
7 simple proof-of-concept demonstrations show that the photoreversible assembly character of
8 the crystals can be easily implemented to design and build materials with user-controlled and
9 light-gated permeability.
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17 **Photoswitchable fluorescence**

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19 We also found that the supramolecular AzoDiGua-GMP crystals were fluorescent
20 upon blue excitation (Ex/Em 488/493-575 nm) allowing us to observe them by fluorescence
21 microscopy (Fig. 6A). The crystal material displayed a maximal emission at around 600 nm
22 and a wide excitation range from 350 nm to 500 nm (Fig. 6B). Notably, under the same
23 excitation conditions, the fluorescence emission of the hybrid crystal solution was 58 times
24 stronger than AzoDiGua alone (Fig. S9). Apart from a much lower intensity, the emission
25 spectrum of AzoDiGua alone displayed similar features as that of the crystal. This shows that
26 the fluorescence properties mainly emerged from the specific supramolecular organization of
27 AzoDiGua within the co-assembled crystal. Compared to AzoDiGua alone, the absorption of
28 AzoDiGua in the crystal displayed a red-shift attributed to the π - π and hydrogen bond
29 interaction (Figure S10). The absorption band that associated with the π - π^* ($S_2 \leftarrow S_0$) transition
30 of the azobenzene unit shifted from 357 nm to 370 nm. Meanwhile, a new strong shoulder
31 band appeared at around 470 nm, where the forbidden n - π^* ($S_1 \leftarrow S_0$) transition of the *trans*
32 isomer is located. In principle, since the fluorescence from the S_1 excited state in solution is
33 extremely weak due to its short lifetime and its optically forbidden nature, it is hardly
34 observed. We thus attribute the observed fluorescence in needle crystals to a relaxation
35 mechanism of the S_1 excited state in the co-crystalline state that is different from that of
36 AzoDiGua alone in solution. Crystallography data showed a restricted conformation of
37 AzoDiGua due to its arrangement with neighbouring GMPs making rotation or inversion
38 relaxation processes upon photoexcitation not favourable. We thus propose that the
39 suppression of the nonradiative relaxation was responsible for the greatly enhanced
40 fluorescence emission. Similar effects have been regularly reported in azobenzene crystals
41 but all fluorescent crystalline systems reported so far lost most of their photoisomerization
42 ability upon crystallization. Here, the unique supramolecular nature of the co-assembled
43 AzoDiGua-GMP crystals allowed us to maintain their photoswitching characteristics (Figs. 4-
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3 5). We thus exploited the combination of the photoreversible assembly with the
4 crystallization-induced fluorescence to analyse how fluorescence could be modulated by light
5 in our system. Interestingly, applying UV on a crystal suspension allowed us to switch off the
6 fluorescence while subsequent blue irradiation on the same solution induced a remarkable
7 recovery of the fluorescence (Fig. 6C).
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13 **Conclusion**

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15 We have proposed a new concept of supramolecular assembly associating a
16 nucleobase, here GMP, with an azobenzene-containing photosensitive DNA intercalator
17 called AzoDiGua. This strategy allowed us to obtain properties that could not be achieved by
18 conventional assembly of the same family of compounds taken separately. For instance, we
19 obtained the first 100 μm long GMP-based crystals, while conventional photoresponsive
20 guanine assemblies involving G-quartets or quadruplexes have been mainly limited to small
21 non-crystalline structures of a few repeating units. Similarly, this co-assembly principle
22 allowed us to build a new family azobenzene crystals combining for the first time
23 fluorescence and photoswitchability. Held by a synergic network of $\pi - \pi$, electrostatic and
24 hydrogen bond interactions, the resulting crystals presented a combination of unique
25 properties such as photoreversible assembly, optical shapability and photoswitchable
26 fluorescence, and could be integrated in membrane-like materials to achieve interesting
27 functionality such as light-gated permeability. We believe that this concept can be easily
28 extended to other molecular bricks carrying additional functionality, thus constituting a
29 promising framework for the facile preparation of a variety of user-defined, stimuli-
30 responsive, and multifunctional self-assembled materials.
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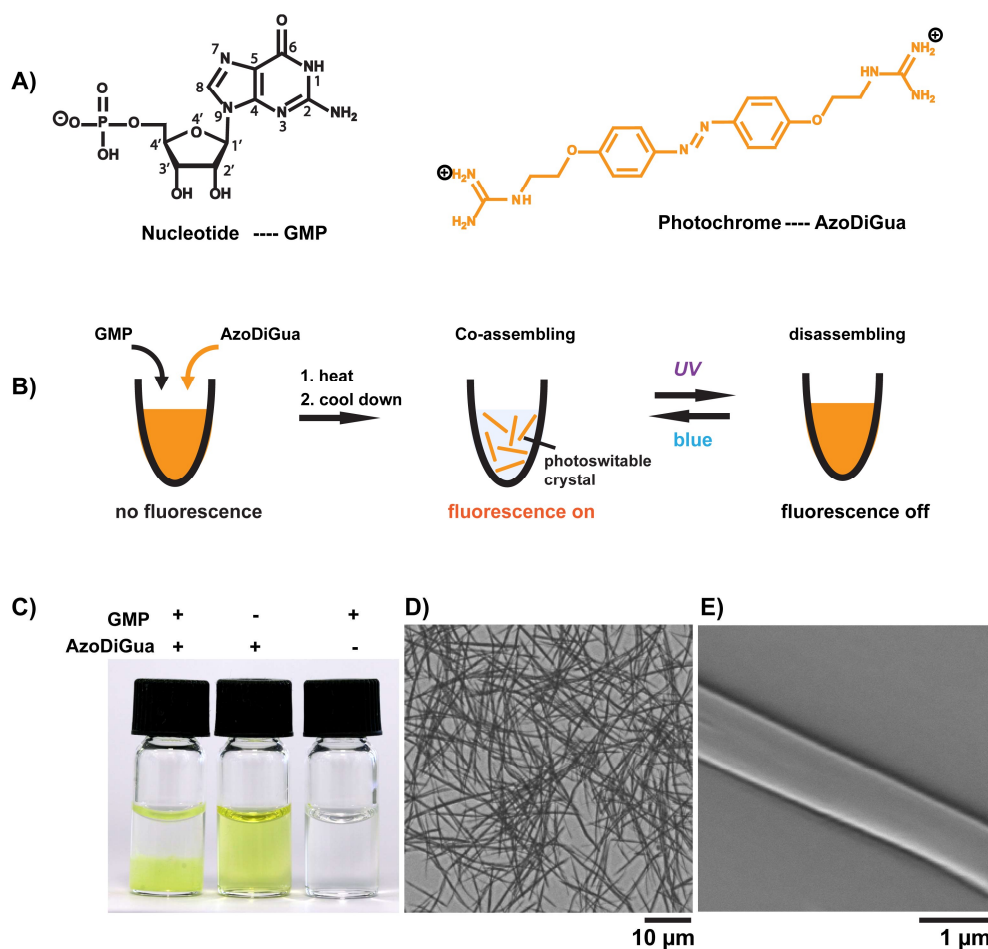


Figure 1. Building a fluorescent, photo-switchable crystal by co-assembly of a nucleobase and a photochromic intercalator. **A)** Molecular structures of the nucleobase, guanosine monophosphate (GMP), and the photochrome, a symmetric azobenzene diguanidinium compound (AzoDiGua), used in this study. **B)** GMP (5mM) and AzoDiGua (0.2 mM) were assembled in acetic acid buffer (10 mM in water, pH = 3.8), heated to 90 °C and slowly cooled down to 4 °C to form photoswitchable fluorescent crystals. After assembly, crystals were characterized and manipulated at room temperature (RT). **C)** Photographs of solutions prepared by different combinations of GMP and AzoDiGua, 20 min at RT after the assembly process. **D-E)** Transmission optical (D) and scanning electron (E) microscopy images of the resulting crystals deposited on glass after drop casting and water evaporation.

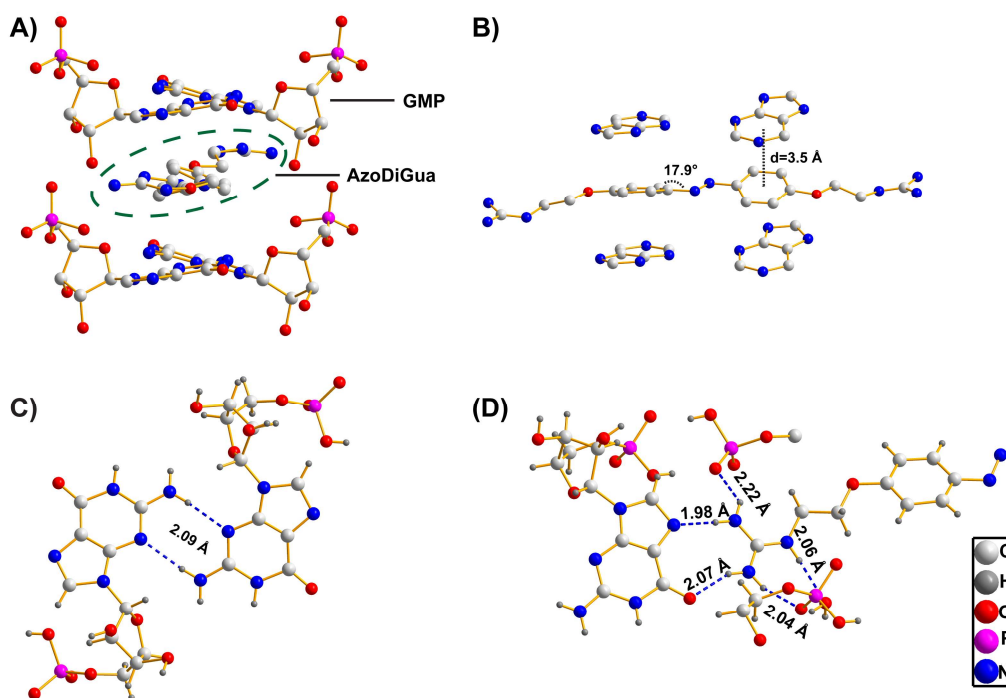


Figure 2. Single crystal X-ray crystallography reveals the structure of the GMP/AzoDiGua assemblies. A) Packing diagram of GMP/AzoDiGua crystal. B) π - π interaction between AzoDiGua and GMP in the crystal. C) Intermolecular hydrogen bonds between two adjacent GMPs. D) The guanidium group of AzoDiGua forms a network of hydrogen bonds with neighbouring units. In (A) and (B), hydrogen atoms and functional groups are omitted for clarity.

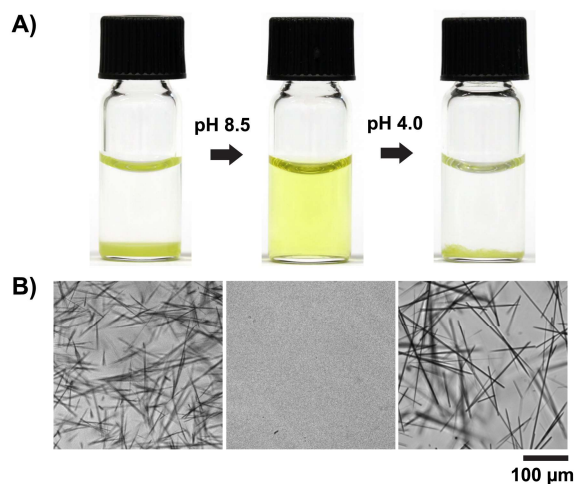


Figure 3. Hybrid crystals are pH-responsive. Photograph of the suspension (A) and *in situ* transmission optical microscopy images (B) before (left) and after successive mixing of NaOH (middle) and HCl (right) at RT. The pH of the solution was 3.8, 8.5 and 4.0, respectively.

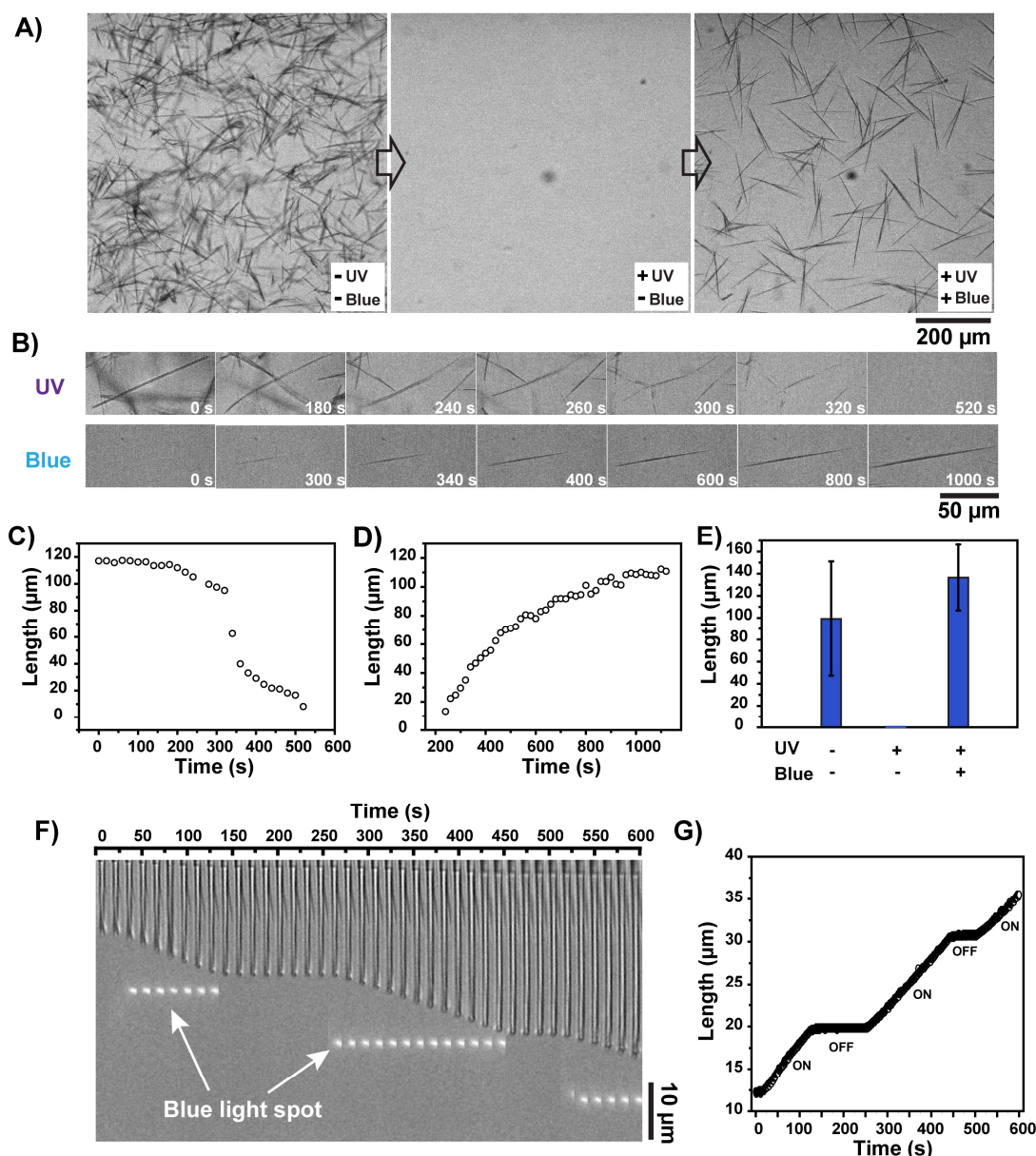


Figure 4. Reversible photocontrol of crystallization. A-E) *In situ*, real-time transmission optical microscopy characterization of a suspension of crystals before (-UV -Blue), and after successive irradiation by UV (365 nm, 2.3 mW·cm⁻²) for 10 min (+UV -Blue,) and by blue (440 nm, 16 mW·cm⁻²) for 5 min (+UV +Blue). Wide-field representative images in a given irradiated area (A). Time lapse observation and corresponding crystal length of the UV-induced dissolution (B top, C) and of the blue-induced recrystallization (B bottom, D) of a single crystal. Average crystal length (mean ± sd on 100 crystals) for the successive irradiation conditions (E). F-G) High-magnification transmission optical microscopy observation of the recrystallization of a single crystal guided by a blue light spot (488 nm, spot size 1.6 μm) in a solution previously irradiated by a larger UV light spot (365 nm, spot size 412 μm) to locally dissolve crystals in the irradiated area. The blue spot irradiation zone

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3 was made visible by the fluorescence emission of fluorescein added to the buffer (1 nM).
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5 Kymograph of the crystal growth (F). Length of the crystal versus time (G).
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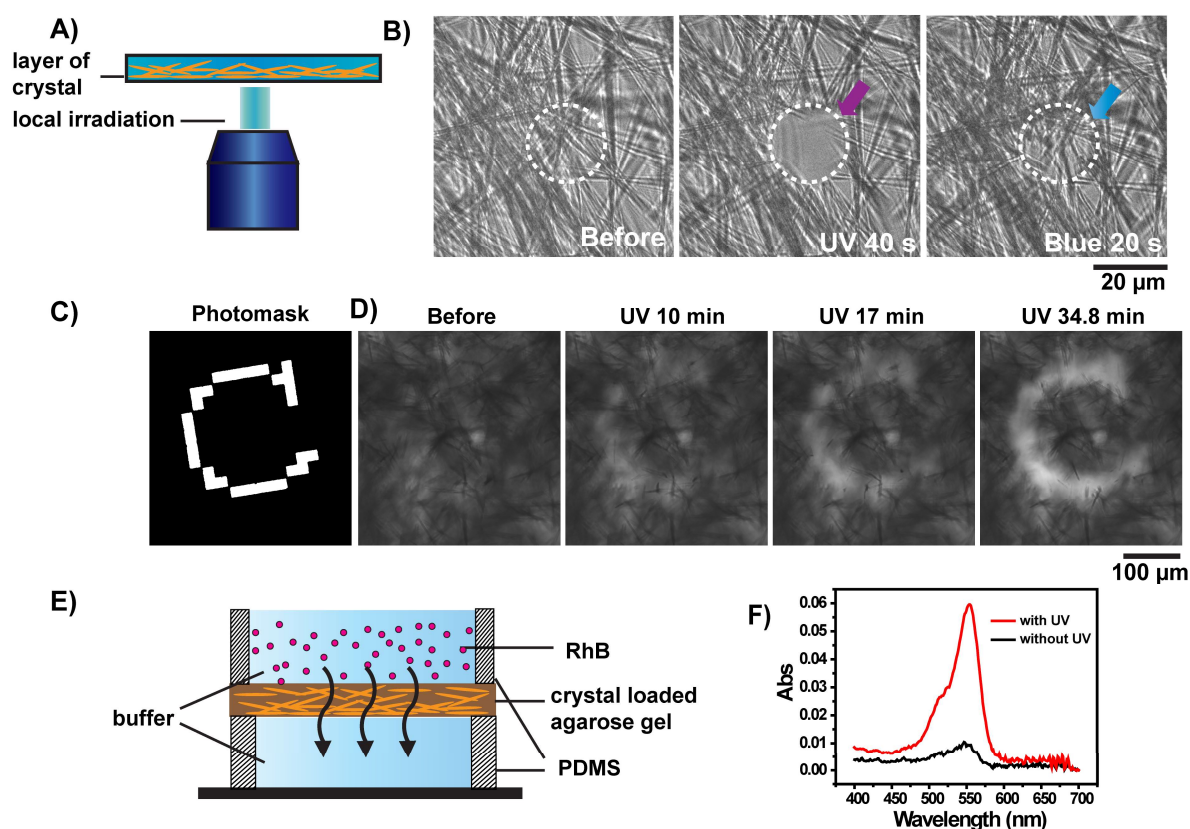


Figure 5. Light-gated permeability in a crystal layer. A-B) A thin layer of crystals is locally irradiated with a circular spot ($921 \mu\text{m}^2$) inside the suspension buffer (A). *In situ* transmission microscopy images (B) before (left) and after successive irradiation by UV (365 nm , $4 \text{ mW}\cdot\text{cm}^{-2}$) for 40 s (middle) and blue (440 nm , $28 \text{ mW}\cdot\text{cm}^{-2}$) for 20 s (right). The dashed circle indicates the irradiated area. C-D) A thick layer (approx. $100 \mu\text{m}$) of crystals is locally irradiated by UV through a photomask displaying the letter “C” (C). *In situ* transmission of the crystal layer a function of irradiation time (D). E-F) A 0.9 mm thick hybrid membrane composed of agarose and crystals is placed between two polydimethylsiloxane (PDMS) compartments. The upper (8 mm diameter) and the lower (3 mm diameter) compartments contain GMP (4.6 mM) in acetic acid buffer (10 mM in water, $\text{pH} = 3.8$) while Rhodamine B (RhB, 0.1 mM) is supplemented only in the upper one (E). Absorption of the solution in the bottom compartment 20 min after the addition of RhB, with (red curve) or without (black curve) continuous irradiation by UV (365 nm , $11 \text{ mW}\cdot\text{cm}^{-2}$) (F).

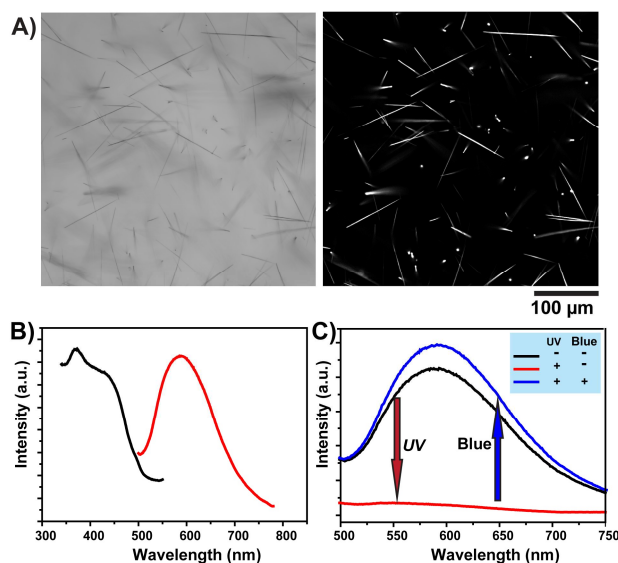


Figure 6. Photoswitchable fluorescent crystals. **A)** Transmission (left) and fluorescence (right, 488/493-575 nm) images of crystals in the suspension buffer. **B)** Fluorescence excitation (at 597 nm emission, black curve) and emission (at 400 nm excitation, red curve) spectrum of the crystal suspension. **C)** Fluorescence emission spectrum (excitation 410 nm) of a crystal suspension (200 μL , 0.6 $\text{g}\cdot\text{L}^{-1}$) before (black curve) and after successive irradiation by UV (365 nm, 11 $\text{mW}\cdot\text{cm}^{-2}$) for 25 min (red curve) and blue (440 nm, 16 $\text{mW}\cdot\text{cm}^{-2}$) for 10 min (blue curve).