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Fast Fluorescence Switching within Hydrophilic Supramolecular Assemblies

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Abstract: We designed a supramolecular strategy to modulate fluorescence in water under optical control. It is based on the entrapment of fluorophore–photochrome dyads within the hydrophobic interior of an amphiphilic polymer. The polymeric envelope around the dyads protects them from the aqueous environment, while imposing hydrophilic character on the overall supramolecular construct. In the resulting assemblies, the photochromic component can be operated reversibly on a microsecond timescale under the influence of ultraviolet stimulations. In turn, the reversible transformations control the emission intensity of the adjacent fluorophore. As a result, the fluorescence of such nanostructured constructs can be photomodulated for hundreds of cycles in water with microsecond switching speeds. Thus, our protocol for fast fluorescence switching in aqueous solutions can eventually lead to the realization of functional probes for the investigation of biological samples.

Keywords: fluorescence · heterocycles · micelles · photochromism · polymers

Introduction

The ability to switch fluorescence under optical control can offer the opportunity to monitor the translocation of labeled targets within a biological sample and provide valuable information on the fundamental factors governing the diffusion of biorelevant species.[1–3] Similarly, fluorescence photoswitching can permit the separation in time of spatially indistinguishable probes and allow the reconstruction of images with subdiffraction resolution.[2c,e,4–13] As a result, photoswitchable fluorophores are becoming invaluable analytical tools in the biomedical laboratory for the investigation of cellular dynamics and the visualization of cellular substructures. Indeed, the significant implications that such molecular switches can have in biomedical research, together with the need to advance our basic understanding on the excitation dynamics of organic chromophores, are encouraging the identification of practical mechanisms to activate fluorescence under the influence of optical stimulations.[14–19] In this context, photochromic compounds[20–25] can be valuable components for the assembly of photoswitchable fluorescent constructs. In fact, their photoinduced and reversible transformations can be engineered to regulate the emission of fluorescent partners.[25–28] Specifically, the integration of fluorescent and photochromic components within the same molecular or supramolecular assembly can be exploited to modulate the emission of the former by operating the latter with optical inputs. Generally, electron or energy transfer processes are invoked to establish communication between the functional components in the excited state and manipulate the excitation dynamics of the emissive species.[29–37] In particular, the excited fluorophore can be designed to exchange an electron with or transfer energy to only one of the two interconvertible states of the photochromic component. Under these conditions, the photochromic transformation activates or suppresses fluorescence reversibly. Indeed, numerous examples of fluorophore–photochrome constructs have already been developed successfully on the basis of these mechanisms.[38]

Photochromic spiropyrans undergo ring-opening reactions, followed by *cis*→*trans* isomerizations, upon ultraviolet irradiation to produce merocyanine chromophores.[39–50] The photogenerated isomers revert back to the original species either thermally, over the course of hundreds of seconds, or photochemically, upon visible illumination. Such reversible structural transformations are accompanied by pronounced absorbance changes in the visible region as well as by significant shifts in redox potentials. In the presence of compatible fluorescent partners, the photoinduced modification of these spectroscopic and electrochemical parameters can activate energy- and electron-transfer pathways, respectively, and quench fluorescence.[38] On the basis of these operating principles, we have been able to modulate the emission of a diversity of fluorophores in liquid solutions[51] and within

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rigid matrices.[52] In search of strategies to impose biocompatibility on these photoswitchable systems, we then devised a supramolecular protocol to transport mixtures of borondipyrromethene (BODIPY) fluorophores and spiropyran photochromes within the intracellular environment and operate them successfully within live cells.[53] Our method is based on the encapsulation of separate fluorescent and photochromic components within the hydrophobic interior of appropriate polymer micelles. Nonetheless, the slow switching speeds and modest fatigue resistances of the photochromic components limit significantly the performance of these photoswitchable supramolecular assemblies. Specifically, a single switching cycle requires more than 10^4 s and significant photodegradation occurs within a few cycles.

In parallel to our investigations on fluorescence photoswitching with spiropyrans,[51–53] we developed a new family of photochromic compounds with fast switching speeds and excellent fatigue resistance.[54] They are based on the photoinduced opening and thermal closing of an oxazine ring. These photochromic switches can complete a single cycle on a submicrosecond timescale and tolerate hundreds of cycles with no sign of degradation even in air. In fact, we have been able to modulate the emission of BODIPY and coumarin fluorophores with microsecond switching speeds and for hundreds of cycles on the basis of such photochromic transformations.[54k–m,r] As a result, we envisaged the possibility of introducing appropriate members of this family of photochromic compounds within our biocompatible supramolecular constructs, in place of their spiropyran components, with the ultimate goal of improving their performance. In this article, we report the design and synthesis of two hydrophobic photochromic oxazines, their introduction within biocompatible polymer micelles as well as the photochemical and photophysical properties of the resulting supramolecular assemblies and appropriate model compounds.

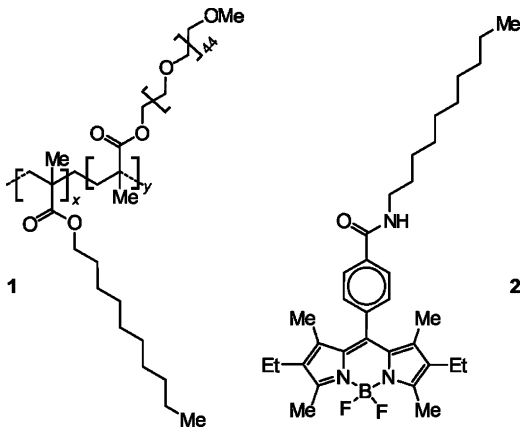

Figure 1. Amphiphilic co-polymer **1** and hydrophobic fluorophore **2**.

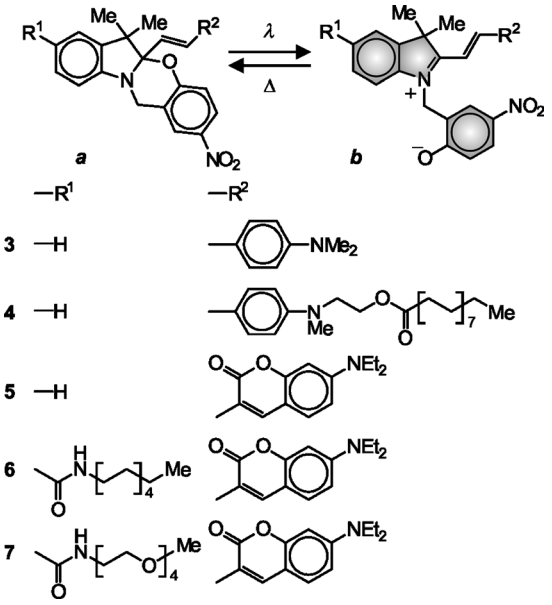

Figure 2. Photoinduced and reversible transformation of the oxazines **3a**–**7a** into the corresponding zwitterionic isomers **3b**–**7b**.

Results and Discussion

Design and synthesis: The co-polymer **1** (Figure 1) has multiple hydrophobic and hydrophilic side chains along a common poly(methacrylate) backbone. In aqueous environments, this particular amphiphilic macromolecule forms micellar assemblies with a hydrodynamic diameter of approximately 40 nm that are capable of trapping organic dyes in their hydrophobic interior.[53] Specifically, the BODIPY fluorophore **2** can be embedded within these supramolecular constructs together with a spiropyran photochrome. The photoinduced transformation of the spiropyran into the corresponding merocyanine activates electron- and energy-transfer pathways from the excited fluorophore to the photochrome that encourage the nonradiative deactivation of the former. In principle, the spiropyran component can be replaced with the photochromic oxazine **3a** (Figure 2) to enhance the switching speeds and fatigue resistance of the overall fluorescent construct. Indeed, the oxazine ring of this particular compound opens to produce the zwitterionic isomer **3b** in less than 6 ns upon ultraviolet illumination in acetonitrile.[54j] The photogenerated isomer reverts spontaneously to the original one in less than 10 μs, under these experimental conditions, and the photochromic system can be switched back and forth between its two states for hundreds of cycles with no sign of degradation. The photoinduced transformation of **3a** into **3b** brings the 4-dimethylaminostyryl appendage in conjugation with the resulting 3*H*-indolium cation. This extended chromophoric fragment absorbs[54j] in the same range of wavelengths at which **2** emits[53] and, therefore, can accept the excitation energy of this fluorophore. Furthermore, the redox potentials of similar compounds[54k] indicate that the transfer of an electron from the excited fluorophore to the 3*H*-indolium chromophore is exergonic with a free energy change of −0.5 eV. Thus, both electron- and energy-transfer pathways can be

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activated with the photoinduced transformation of **3a** into **3b** to quench the emission of **2**, if fluorescent and photochromic components are both trapped within the same supramolecular assembly. On the basis of these considerations, we envisaged the possibility of appending an oligo(methylene) tail to this photochromic oxazine, in the form of compound **4a** (Figure 2), to encourage the entrapment of the resulting molecule within micelles of the amphiphilic co-polymer **1**. In particular, we prepared **4a** in two synthetic steps (Figure S1 in the Supporting Information), starting from commercial and known precursors.

The integration of **2** and **4a** within the same micellar construct can offer the opportunity to suppress the emission of the former with the photoinduced ring opening of the latter. A similar photochemical transformation, however, can also be exploited to activate, rather than deactivate, fluorescence. Specifically, the photoinduced opening of the oxazine ring of **5a** (Figure 2) brings its coumarin appendage in conjugation with the 3*H*-indolium cation of **5b** and shifts the main absorption band of this fluorescent appendage from 410 to 570 nm in acetonitrile.[54m,r] As a consequence, illumination at 532 nm results in significant fluorescence only after the photoinduced opening of the oxazine ring. Thus, the introduction of such a fluorophore–photochrome dyad within micellar assemblies of the amphiphilic co-polymer **1** can translate into the realization of photoactivatable and biocompatible fluorescent constructs. Therefore, we designed an analogue of **5a** with a pendant oligo(methylene) tail, in the form of compound **6a** (Figure 2), to facilitate the encapsulation of the resulting dyad within micelles of **1**. In particular, we prepared **6a** in four synthetic steps (Figure S2 in the Supporting Information), starting from commercial and known precursors. Following a similar synthetic procedure (Figure S3 in the Supporting Information), we also prepared the hydrophilic analogue **7a** (Figure 2). The pendant oligo(ethylene glycol) tail of **7a** is designed to impose aqueous solubility on the fluorophore–photochrome dyad and, therefore, permit the investigation of its photochemical and photophysical properties in aqueous solutions lacking any micellar host.

Steady-state spectroscopy: The absorption spectra (a and b in Figure 3) of acetonitrile solutions of **3a** and **4a** are very similar, indicating that the hydrophobic tail of the latter has a negligible influence on the photophysical properties of the heterocyclic core. In both instances, the spectrum shows bands at 305 and 555 nm that correspond to the 4-nitrophenoxy fragment of the ring-closed isomer and the 3*H*-indolium cation of the ring-open species respectively.[54j] Indeed, the absorption in the visible region resembles that of the model compound **8** (c in Figure 3 and Figure 4), which has essentially the same chromophoric fragment of the ring-open isomers **3b** and **4b**. Thus, the two isomers of each system coexist under these experimental conditions with a ratio of 90:10 in favor of the ring-closed species.[55]

The two oxazines **3a** and **4a** are relatively hydrophobic and, as a result, they are sparingly soluble in aqueous solutions. However, they readily dissolve in neutral phosphate buffer saline (PBS) in the presence of the amphiphilic co-polymer **1**. The absorption spectra of the resulting dispersions (d and e in Figure 3) predominantly show bands at 390 and 560 nm for the 4-nitrophenolate anion[56] and 3*H*-indol-

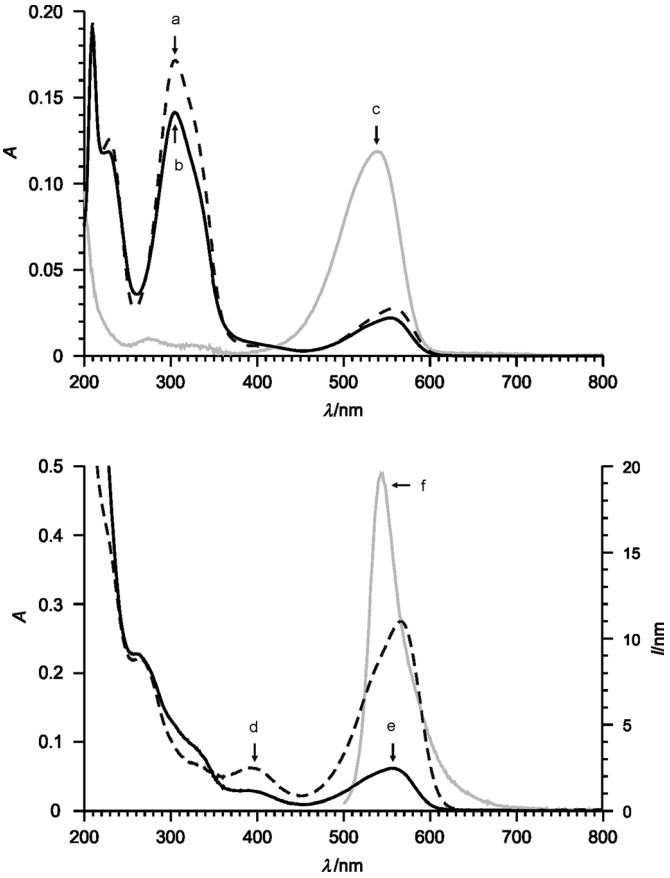

Figure 3. Absorption spectra of solutions (MeCN, 25 °C) of **3a** (a, 5 μM), **4a** (b, 5 μM), and the hexafluorophosphate salt of **8** (c, 1 μM). Absorption spectra of dispersions (PBS, pH 7.0, 25 °C) of **1** (50 μg mL^{-1}) and either **3a** (d, 5 μg mL^{-1}) or **4a** (e, 5 μg mL^{-1}). Emission spectrum (f) of a dispersion (PBS, pH 7.0, 25 °C, λ_{Ex} = 490 nm) of **1** (50 μg mL^{-1}) and **2** (5 μg mL^{-1}).

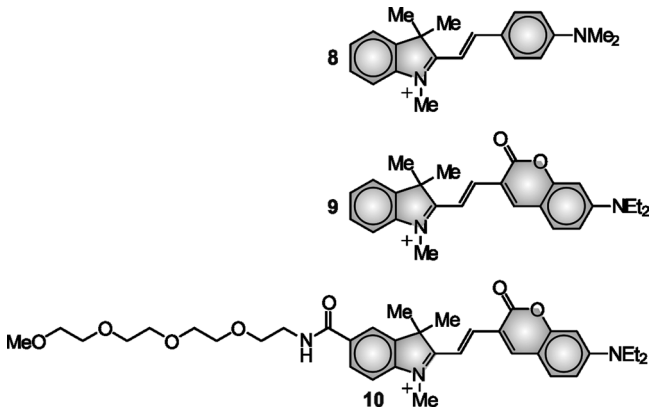

Figure 4. Model 3*H*-indolium chromophores **8**–**10**.

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ium cation, respectively, of the zwitterionic species **3b** and **4b**. These observations suggest that both molecular switches are partially exposed to the aqueous medium, despite their interactions with the amphiphilic co-polymer **1**. Indeed, the ring-open isomers are significantly more polar than their ring-closed counterparts and, as a result, the transition from organic to aqueous environments tends to encourage their population. Specifically, the absorbance of the 3*H*-indolium chromophore in the visible region indicates that the ratio between **3a** and **3b** is 30:70, whereas that between **4a** and **4b** is 85:15.[55,57] The different ratios suggest that the two molecular switches reside in different domains within their polymeric host. Presumably, the hydrophobic tail of one system limits the exposure of the heterocyclic core to the polar aqueous environment and, therefore, decreases the fractional concentration of ring-open isomer relative to that associated with the other system.

In agreement with our design logic, the absorption band of the 3*H*-indolium chromophore of the ring-open isomers **3b** and **4b** is, indeed, positioned in the same range of wavelengths at which **2** emits (f in Figure 3).[53] This pronounced spectral overlap together with the redox potentials of model compounds[54k] suggests that the concomitance of energy- and electron-transfer pathways can quench the fluorescence of **2**, if this component and either one of the two molecular switches are trapped within the same micellar assembly. Nonetheless, the fraction of ring-open isomer is relatively large for both systems, under these experimental conditions. This unexpected complication prevents the implementation of our operating principles for photoinduced fluorescence suppression, which instead require a significant population of the quenching species only after ultraviolet illumination.

The absorption spectra (a and b in Figure 5) of acetonitrile solutions of **5a** and **6a** show a band at 410 nm for the coumarin appendage of the ring-closed isomer,[54m,r] but do not reveal any significant absorbance for the 3*H*-indolium cation of the ring-open species. Indeed, the spectrum (c in Figure 5) of the model compound **9** indicates that this particular cationic chromophore absorbs at 570 nm. Analogous to **3a** and **4a**, compounds **5a** and **6a** are also relatively hydrophobic and sparingly soluble in water, but can readily be dispersed in aqueous solution together with the amphiphilic co-polymer **1**. In contrast to **3a** and **5a**, however, the spectra (d and e in Figure 5) of the resulting aqueous dispersions are very similar to those of the acetonitrile solutions. Once again, only the band for the coumarin appendage of the ring-closed isomers **5a** and **6a** can be observed at 400 nm. Similarly, the absorption spectrum (a in Figure S5 in the Supporting Information) of an acetonitrile solution of their hydrophilic analogue shows exclusively a band at 405 nm for the ring-closed isomer **7a**. However, an additional band is clearly present at 610 nm in the spectrum (b in Figure S5 in the Supporting Information) of the very same species dissolved in PBS in the absence of **1**. This band resembles that (c in Figure S5 in the Supporting Information) of the model 3*H*-indolium **10** (Figure 4) and indicates the co-existence of **7a** and **7b** in a ratio of 90:10 under these conditions.[57,58]

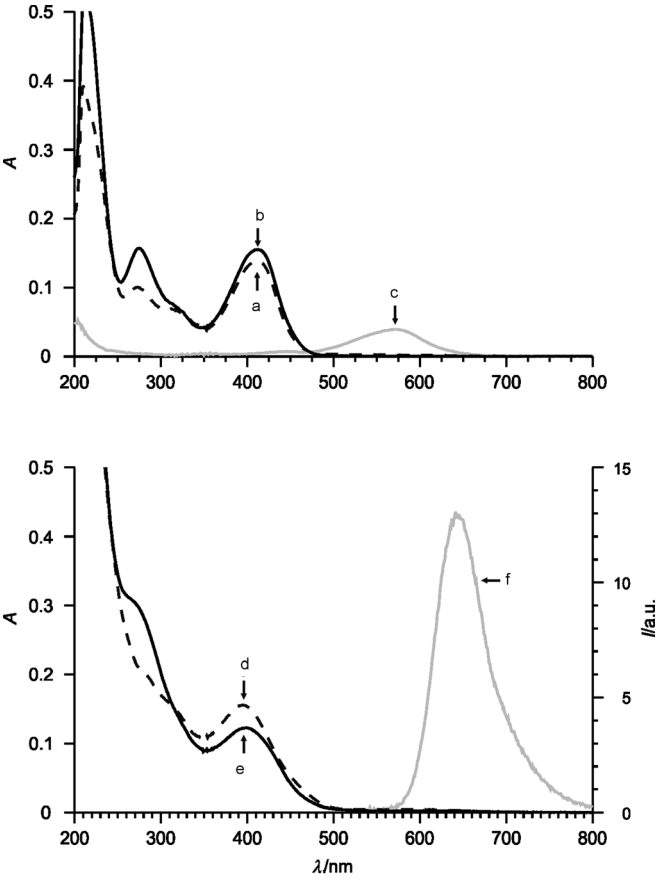

Figure 5. Absorption spectra of solutions (MeCN, 25 °C) of **5a** (a, 5 μM), **6a** (b, 5 μM), and the hexafluorophosphate salt of **9** (c, 1 μM). Absorption spectra of dispersions (PBS, pH 7.0, 25 °C) of **1** (50 μg mL^{-1}) and either **5a** (d, 5 μg mL^{-1}) or **6a** (e, 5 μg mL^{-1}). Emission spectrum (f) of a solution (MeCN, 25 °C, λ_{Ex} = 530 nm) of the hexafluorophosphate salt of **9** (10 μM).

These observations demonstrate that the micellar host effectively protects **5a** and **6a** from the aqueous environment and discourages the population of their ring-open isomers **5b** and **6b**. Thus, the supramolecular encapsulation of these fluorophore–photochrome dyads within the hydrophobic interior of the amphiphilic co-polymer offers the opportunity to implement our operating principles for fluorescence photoactivation, which demand the population of the emissive isomer only after ultraviolet stimulations. Indeed, the photoinduced opening of the oxazine ring of **5a** and **6a** within the micellar assemblies can extend the conjugation of the coumarin fluorophore within the photogenerated isomers **5b** and **6b** to produce a 3*H*-indolium chromophore able to emit at 645 nm, according to the emission spectrum of the model compound **9** (f in Figure 5).[59]

Time-resolved spectroscopy: The illumination of an aqueous dispersion of **1** and either **5a** or **6a** at 355 nm with a pulsed laser opens the oxazine ring of the molecular switch, entrapped within the polymeric construct, to generate the zwitterionic isomer **5b** or **6b** within the laser pulse (6 ns). Consis-

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tently, the absorption spectrum (a in Figure 6 and Figure S6 in the Supporting Information) of the dispersion, recorded 0.1 μs after illumination, shows the appearance of band at 620 nm. This transient absorption resembles the steady-state band (c in Figure 5) observed for the model compound **8** and corresponds to a ground-state absorption of the 3*H*-indolium chromophore of the ring-open isomer **5b** or **6b**. Essentially the same transient absorption is observed also in the spectrum (a in Figure S7 in the Supporting Information) of an aqueous solution of the hydrophilic analogue **7a** in the absence of the polymeric host, under otherwise identical conditions. However, the dependence of the absorbance of this band on the laser intensity indicates the quantum yield for the photochromic transformation to be approximately 0.02 for **5a** and **6a** within the micellar assemblies and only 0.006 for **7a** in water. These values suggest that the polymeric envelope around the photochromic components limits their exposure to the aqueous environment, in agreement with the indications that emerged from the analysis of the steady-state spectra. Furthermore, the quantum yield for the photochromic transformation of **5a** and **6a** in the micellar assemblies is identical to that measured for **5a** in acetonitrile,[54m,r] suggesting that the polymeric host provides an environment similar to this particular organic solvent.

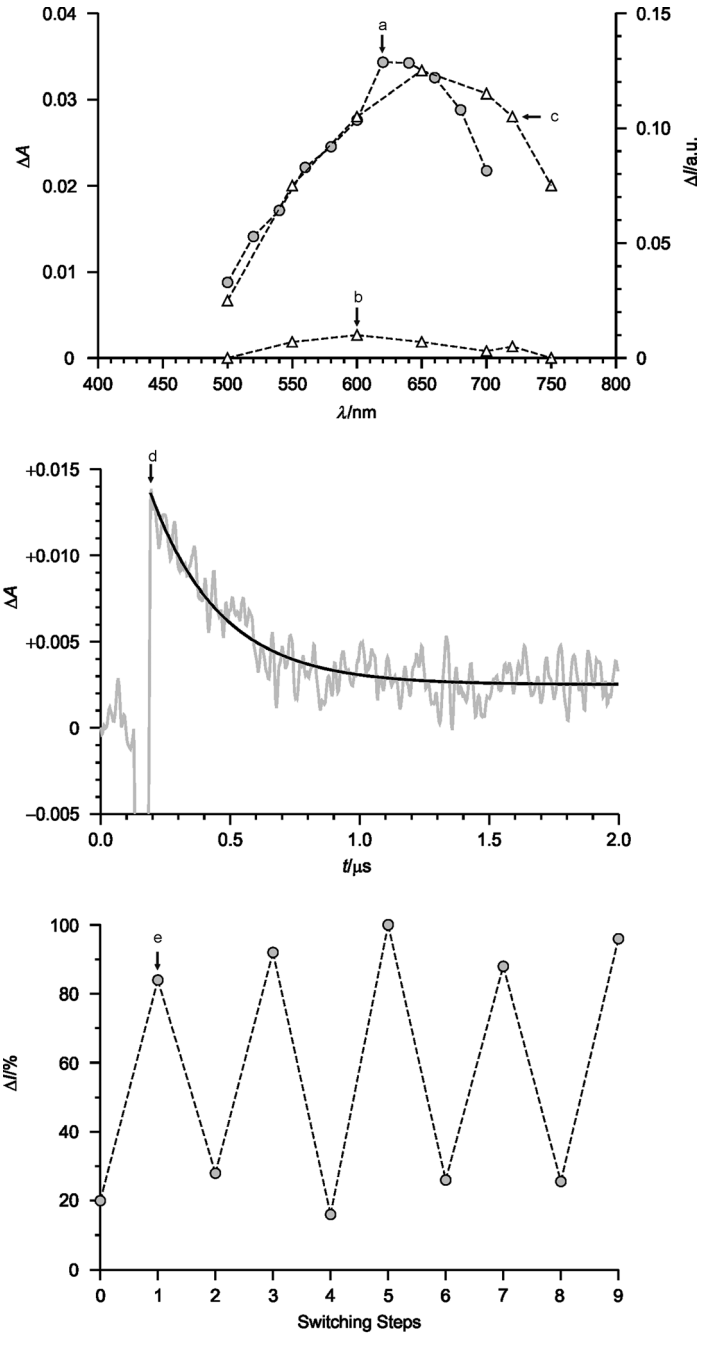

Figure 6. Absorption spectrum (a) of a dispersion (H_2O, 25 °C) of **1** (2 mg mL^{-1}) and **6a** (9 μg mL^{-1}), recorded 0.1 μs after pulsed illumination (355 nm, 6 ns, 15 mJ). Emission spectra of the same dispersion, recorded upon pulsed irradiation at 532 nm (6 ns, 30 mJ) without (b) and with (c) simultaneous pulsed illumination at 355 nm. Absorbance evolution at 620 nm of the same dispersion upon pulsed irradiation at 355 nm with the corresponding monoexponential fitting (d). Relative emission intensity at 650 nm of the same dispersion, recorded upon pulsed irradiation at 532 nm without and with simultaneous pulsed illumination at 355 nm (e).

The isomers **5b** and **6b**, photogenerated within the polymer micelles, as well as the ring-open species **7b**, produced in the absence of the polymeric host, all revert spontaneously back to the ring-closed species with first-order kinetics. As a result, the absorbance for their 3*H*-indolium chromophore decays monoexponentially (d in Figure 6 and Figures S6 and S7 in the Supporting Information). Curve fitting of the temporal absorbance profiles indicates the lifetime of these species to be approximately 0.2 μs in all instances. This value is essentially identical to that of **5b** measured in acetonitrile.[54m,r] Furthermore, all three photochromic systems can be switched back and forth between their two states for hundreds of cycles with no sign of degradation. Thus, the environment around the photochromic components has a negligible influence on their reisomerization kinetics and fatigue resistances.

The illumination of **5a** and **6a** within the polymer micelles and of **7a** in the absence of the polymeric host at 532 nm does not result in any significant fluorescence (b in Figure 6 and Figures S6 and S7 in the Supporting Information). Indeed, the coumarin fluorophore of **5a**–**7a** does not absorb at this particular wavelength and, therefore, it cannot emit. After the photoinduced ring-opening process, however, the coumarin absorption shifts from 400 to 620 nm (a in Figure 6 and Figures S6 and S7 in the Supporting Information). Therefore, the fluorescent appendage within the ring-open isomers **5b**–**7b** can absorb the exciting radiations at 532 nm and emit as a result. In fact, the simultaneous illumination of the sample at 355 nm, to open the oxazine ring of **5a**–**7a**, and at 532 nm, to excite the coumarin fluorophore of **5b**–**7b**, is accompanied by the appearance of an intense band at 650 nm in the corresponding emission spectrum (c in Figure 6 and Figures S6 and S7 in the Supporting Information). This band disappears after the spontaneous reisomerization of **5b**–**7b** back to **5a**–**7a**. As a consequence, the emission intensity at 650 nm can be switched on and off for multiple cycles (e in Figure 6) simply by turning on and

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off an excitation source at 355 nm, while illuminating the sample at 532 nm.

Conclusion

Hydrophobic fluorophore–photochrome dyads can be dissolved in aqueous environments with the assistance of an amphiphilic polymer. In the resulting supramolecular assemblies, the photochromic component retains its photochemical properties essentially unaltered. Specifically, its oxazine ring opens upon ultraviolet illumination to bring the adjacent coumarin fluorophore in conjugation with a 3*H*-indolium cation. This structural transformation shifts the main absorption of the fluorophore to the visible region. As a result, the visible illumination of aqueous dispersions of such nanostructured constructs results in significant fluorescence only under concomitant ultraviolet irradiation. Furthermore, the photochromic component reverts to the original state on a microsecond timescale after the spontaneous closing of its oxazine ring. In fact, the fluorescence of these supramolecular assemblies can be modulated for hundreds of cycles with microsecond switching speeds simply by turning on and off an ultraviolet source under visible illumination. Thus, our operating principles for fluorescence modulation under optical control can translate into the realization of switchable probes compatible with aqueous environments and, ultimately, lead to valuable analytical tools for the investigation of biological samples.

Experimental Section

Materials and methods: Chemicals were purchased from commercial sources and used as received with the exception of MeCN, which was distilled over CaH_2. Compounds **1**, **2**, **3a**, **5a**, **8**, **9**, **12a**, **13**, and **16** were prepared according to literature procedures.[53,54a,j,m,60–62] All reactions were monitored by thin-layer chromatography, using aluminum sheets coated with silica (60, F_{254}). Electrospray ionization mass spectra (ESIMS) were recorded with a Bruker micrOTO-Q II spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 400 spectrometer. Steady-state absorption spectra were recorded with a Varian Cary 100 Bio spectrometer, using quartz cells with a path length of 1.0 cm. Steady-state emission spectra were recorded with a Varian Cary Eclipse spectrometer in aerated solutions. Time-resolved absorption and emission spectra were recorded with a Luzchem Research mLFP-111 spectrometer in aerated solutions by illuminating orthogonally the sample with a Continuum Surelite II-10 Nd:YAG pulsed laser. For absorption measurements, the laser was operated at 355 nm (10 mJ) and the transmittance was measured in the 350–700 nm spectral range. For fluorescence measurements, the laser was operated simultaneously at 355 (10 mJ) and 532 nm (30 mJ) and the emission intensity was measured in the 400–800 nm spectral range. The quantum yields for the photochromic transformation were determined with a benzophenone standard, following a literature protocol.[54m]

Synthesis of 4a: A solution of **11** (417 mg, 1.0 mmol), **12a** (300 mg, 1.0 mmol), and trifluoroacetic acid (TFA, 72 μL, 0.967 mmol) in EtOH (10 mL) was heated under reflux for 24 h. After cooling down to ambient temperature, the solvent was distilled off under reduced pressure and the residue was dissolved in CH_2Cl_2 (15 mL) and washed with H_2O (20 mL). The organic phase was dried over Na_2SO_4 and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (SiO_2: Hexane/EtOAc (2:1, *v/v*)) to give **4a** (350 mg, 50%) as a red oil. ^{1}H NMR ($CDCl_3$): $\delta = 0.91$ (t, $J = 7$ Hz, 3H), 1.28 (s, 26H), 1.59–1.61 (m, 2H), 2.28 (t, $J = 7$ Hz, 2H), 3.02 (s, 3H), 3.62 (t, $J = 6$ Hz, 2H), 4.25 (t, $J = 6$ Hz, 2H), 7.51 (d, $J = 8$ Hz, 1H), 4.61 (s, 2H), 6.15 (d, $J = 16$ Hz, 1H), 6.64–6.76 (m, 4H), 6.89 (t, $J = 8$ Hz, 2H), 7.11–7.17 (m, 2H), 7.32 (d, $J = 9$ Hz, 2H), 7.96–8.01 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): $\delta = 13.9$, 22.8, 24.2, 25.1, 29.3, 29.6, 29.9, 32.1, 34.1, 38.2, 44.3, 50.7, 61.3, 109.3, 111.2, 111.6, 112.3, 116.4, 117.9, 122.7, 123.1, 123.2, 124.3, 124.6, 125.3, 128.2, 131.7, 133.5, 136.1, 140.7, 141.9, 162.5, 173.2 ppm; HRMS (ESI): *m/z* calcd for $C_{45}H_{62}N_3O_5$: 724.4689 $[M+H]^+$; found: 724.4711.

Synthesis of 6a: A solution of **15a** (71 mg, 0.3 mmol), **16** (120 mg, 0.2 mmol), and TFA (0.5 mL, 6.5 mmol) in EtOH (10 mL) was heated for 4 h under reflux. After cooling down to ambient temperature, the solvent was distilled off under reduced pressure and the residue was dissolved in CH_2Cl_2 (20 mL). The organic phase was dried over Na_2SO_4 and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (SiO_2: Hexane/EtOAc (3:2, *v/v*)) to give **6a** (24 mg, 14%) as a green solid. ^{1}H NMR ($CDCl_3$): $\delta = 0.88$ (t, $J = 7$ Hz, 3H), 1.20–1.40 (m, 26H), 1.55–1.60 (m, 2H), 3.42 (q, $J = 7$ Hz, 6H), 4.63 (d, $J = 9$ Hz, 3H), 6.03 (t, $J = 5$ Hz, 1H), 6.47 (d, $J = 3$ Hz, 1H), 6.56–6.63 (m, 3H), 6.87–6.95 (m, 2H), 7.23 (d, $J = 2$ Hz, 1H), 7.51–7.56 (m, 2H), 7.61 (d, $J = 2$ Hz, 1H), 7.99–8.05 ppm (m, 2H); HRMS (ESI): *m/z* calcd for $C_{43}H_{53}N_4O_6$: 721.3965 $[M+H]^+$; found: 721.3959.

Synthesis of 7a: A solution of **18a** (48 mg, 0.1 mmol), **16** (21 mg, 0.1 mmol), and trifluoroacetic acid (20 mg, 0.2 mmol) in EtOH (20 mL) was heated under reflux for 18 h. After cooling down to ambient temperature, the solvent was distilled off under reduced pressure and the residue was dissolved in CH_2Cl_2 (20 mL). The resulting solution was washed with H_2O (2×20 mL), dried over Na_2SO_4 and then the solvent was distilled off under reduced pressure to yield **7a** (55 mg, 80%) as a blue solid. ^{1}H NMR ($CDCl_3$): $\delta = 1.14$ (t, $J = 6$ Hz, 6H), 1.25 (br s, 3H), 1.37 (br s, 3H), 3.23 (br s, 3H), 3.39–3.57 (m, 22H), 4.60 (d, $J = 6$ Hz, 1H), 4.70 (d, $J = 6$ Hz, 1H), 6.44 (s, 1H), 6.62 (d, $J = 9$ Hz, 1H), 6.64–6.77 (m, 2H), 6.90 (d, $J = 9$ Hz, 1H), 7.10–7.20 (m, 1H), 7.28 (d, $J = 9$ Hz, 1H), 7.60 (d, $J = 6$ Hz, 1H), 7.76 (s, 1H), 7.94 (dd, $J = 3$, 9 Hz, 1H), 8.06 ppm (d, $J = 3$ Hz, 1H); HRMS (ESI): *m/z* calcd for $C_{42}H_{51}N_4O_{10}$: 771.3600 $[M+H]^+$; found: 771.3596.

Synthesis of the hexafluorophosphate salt of 10: A solution of **19** (317 mg, 0.6 mmol) and **16** (146 mg, 0.6 mmol) in EtOH (10 mL) was heated under reflux for 18 h. After cooling to ambient temperature, the solvent was distilled off under reduced pressure and the residue was dissolved in CH_2Cl_2 (3 mL). The addition of Et_2O (20 mL) caused the formation of a precipitate, which was filtered off to give the hexafluorophosphate salt of **10** (40 mg, 94%) as a blue solid. ^{1}H NMR (CD_3CN): $\delta = 1.24$ (t, $J = 8$ Hz, 6H), 1.80 (s, 6H), 3.23 (s, 3H), 3.51–3.65 (m, 20H), 3.90 (s, 3H), 6.60 (s, 1H), 6.85 (d, $J = 8$ Hz, 1H), 7.51 (d, $J = 8$ Hz, 1H), 7.65 (d, $J = 8$ Hz, 1H), 7.85 (d, $J = 16$ Hz, 1H,), 8.02 (d, $J = 8$ Hz, 1H), 8.13 (s, 1H), 8.19 (d, $J = 16$ Hz, 1H), 8.42 ppm (s, 1H); ^{13}C NMR (CD_3CN): $\delta = 12.3$, 26.2, 34.0, 40.1, 44.7, 45.7, 52.1, 58.3, 69.5, 70.4, 70.5, 70.6, 72.0, 97.1, 110.0, 110.5, 112.0, 113.0, 114.2, 122.1, 129.0, 133.1, 135.3, 143.5, 144.5, 151.2, 151.9, 155.3, 158.8, 160.0, 166.2, 183.0 ppm; HRMS (ESI): *m/z* calcd for $C_{36}H_{48}N_3O_7$: 634.3487 $[M-PF_6]^+$; found: 634.3506.

Synthesis of 11: A solution of *N*,*N*-dicyclohexylcarbodiimide (DCC, 205 mg, 1.7 mmol) in CH_2Cl_2 (5 mL) was added dropwise over the course of 10 min to a solution of *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde (300 mg, 1.7 mmol), heptadecanoic acid (453 mg, 1.7 mmol) and 4-(dimethylamino)pyridine (DMAP, 205 mg, 1.7 mmol) in CH_2Cl_2 (20 mL) maintained at 0 °C under Ar. The reaction mixture was allowed to warm to ambient temperature and stirred for 24 h under these conditions. The solvent was distilled off under reduced pressure and the residue was purified by column chromatography (SiO_2, Hexane/EtOAc (1:1, *v/v*)) to afford **11** (670 mg, 93%) as a yellow solid.; ^{1}H NMR ($CDCl_3$): $\delta = 0.89$ (t, $J = 6$ Hz, 3H), 1.27 (s, 26H), 1.56–1.59 (m, 2H), 2.25 (t, $J = 7$ Hz, 2H), 3.10 (s, 3H), 3.71 (t, $J = 6$ Hz, 2H), 4.30 (t, $J = 6$ Hz, 2H), 6.77 (d, $J = 9$ Hz, 2H), 7.75 (d, $J = 9$ Hz, 2H), 9.76 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 13.9$, 22.9, 25.0, 29.3, 29.6, 29.9, 32.2, 34.0, 38.5, 50.7, 61.3, 111.6, 126.1, 131.8, 154.0, 173.1, 189.5 ppm; HRMS (ESI): *m/z* calcd for $C_{27}H_{46}NO_3$: 432.3478 $[M+H]^+$; found: 432.3471.

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Synthesis of 14: A solution of DCC (764 mg, 3.7 mmol) in CH_2Cl_2 (5 mL) was added dropwise over the course of 10 min to a solution of **13** (500 mg, 2.5 mmol), *N*-hydroxysuccinimide (NHS, 425 mg, 3.7 mmol), and DMAP (30 mg, 0.2 mmol) in CH_2Cl_2 (80 mL) maintained at 0°C under Ar. The reaction mixture was allowed to warm to ambient temperature and was stirred for 15 h under these conditions. The resulting precipitate was filtered off and *n*-decylamine (580 mg, 3.7 mmol) was added dropwise to the filtrate over the course of 10 min. The solution was stirred for 12 h at ambient temperature. The resulting precipitate was filtered off and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (SiO_2, Hexane/EtOAc (1:2, *v*/*v*)) to afford **14** (542 mg, 43%) as a white solid. ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, J = 6 Hz, 3H), 1.28–1.36 (m, 20H), 1.62–1.73 (m, 2H), 2.32 (s, 3H), 3.47 (q, J = 6 Hz, 2H), 6.18 (br s, 1H), 7.54 (d, J = 8 Hz, 1H), 7.66 (dd, J = 2, 8 Hz, 1H), 7.81 ppm (d, J = 2 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ = 14.0, 15.5, 22.6, 22.8, 27.0, 29.2, 29.3, 29.5, 29.7, 31.8, 40.2, 53.8, 119.4, 120.8, 126.4, 131.8, 146.0, 156.2, 157.2, 167.7 ppm; HRMS (ESI): *m*/*z* calcd for $C_{22}H_{35}N_2O$: 343.2749 $[M+H]^+$; found: 343.2734.

Synthesis of 15a: A solution of **14** (342 mg, 1.2 mmol) and 4-nitro-2-chloromethyl-phenol (225 mg, 1.2 mmol) in MeCN (20 mL) was heated for 24 h under reflux and Ar. After cooling to ambient temperature, the solvent was distilled off under reduced pressure and the residue was dissolved in CH_2Cl_2 (15 mL) and washed with H_2O (20 mL). The organic phase was dried over Na_2SO_4 and the solvent was distilled off under reduced pressure and the residue was purified by column chromatography (SiO_2, Hexane/EtOAc (2:1, *v*/*v*)) to afford **15a** (180 mg, 36%) as a white solid. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, J = 7 Hz, 3H), 1.18–1.30 (m, 20H), 1.54–1.66 (m, 5H), 3.39 (q, J = 6 Hz, 2H), 4.65 (s, 2H), 6.21 (t, J = 6 Hz, 1H), 6.55 (d, J = 8 Hz, 1H), 6.72 (d, J = 9 Hz, 1H), 7.50 (dd, J = 2, 8 Hz, 1H), 7.62 (d, J = 2 Hz, 1H), 7.95 (dd, J = 3, 9 Hz, 1H), 8.07 ppm (d, J = 3 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ = 13.9, 16.2, 18.5, 22.8, 27.3, 29.6, 29.8, 30.1, 32.1, 34.0, 39.9, 48.1, 103.7, 108.3, 118.2, 121.9, 124.6, 124.7, 127.9, 128.5, 138.6, 141.1, 150.1, 159.2, 167.0 ppm; HRMS (ESI): *m*/*z* calcd for $C_{29}H_{40}N_3O_4$: 494.3019 $[M+H]^+$; found: 494.2987.

Synthesis of 17: DCC (405 mg, 2.0 mmol) was slowly added to a solution of **13** (200 mg, 1.0 mmol), NHS (227 mg, 2.0 mmol), and DMAP (25 mg, 0.2 mmol) in CH_2Cl_2 (30 mL) maintained at 0°C under Ar. The mixture was allowed to warm up to ambient temperature, stirred under these conditions for 15 h and filtered. 2-{2-[2-(2-Methoxyethoxy)ethoxy]ethoxy}-ethylamine (400 mg, 2.0 mmol) was added to the filtrate and the resulting solution was stirred for 12 h at ambient temperature. The solvent was distilled off under reduced pressure to give **17** (300 mg, 90%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 1.19 (s, 6H), 2.56 (s, 3H), 3.18 (t, J = 4 Hz, 2H), 3.23 (t, J = 4 Hz, 2H), 3.40–3.54 (m, 15H), 7.43 (d, J = 8 Hz, 1H), 7.65 (t, J = 6 Hz, 1H), 7.75 ppm (d, J = 8 Hz, 1H); ESIMS: *m*/*z* = 393 $[M+H]^+$.

***S*ynthesis of 18a**: A solution of **17** (100 mg, 0.3 mmol) and 2-chloromethyl-4-nitrophenol (48 mg, 0.3 mmol) in MeCN (10 mL) was heated for 24 h under reflux. After cooling to ambient temperature, the solvent was distilled off under reduced pressure and the residue was dissolved in CH_2Cl_2 (20 mL). The resulting solution was washed with H_2O (20 mL), dried over Na_2SO_4 and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography (SiO_2: $CHCl_3$/MeOH (99:1→95:5, *v*/*v*) to afford **18a** (80 mg, 59%) as a yellow oil. ^{1}H NMR (CD_3CN): δ = 1.54 (s, 3H), 1.59 (s, 3H), 2.40 (s, 3H), 3.28 (s, 3H), 3.34–3.52 (m, 16H), 4.59 (d, J = 16 Hz, 1H), 4.73 (d, J = 6 Hz, 1H), 6.69 (d, J = 8 Hz, 1H), 6.73 (d, J = 8 Hz, 1H), 7.12 (br s, 1H), 7.58 (d, J = 8 Hz, 1H), 7.63 (s, 1H), 7.93 (d, J = 8 Hz, 1H), 8.14 ppm (s, 1H); ^{13}C NMR (CD_3CN): δ = 16.8, 18.9, 26.1, 40.4, 40.5, 48.7, 58.8, 70.2, 70.9, 71.1, 72.5, 104.1, 108.9, 118.6, 120.5, 22.5, 124.6, 124.9, 127.8, 128.5, 139.4, 141.5, 150.8, 159.7, 167.8 ppm; ESIMS: *m*/*z* = 544 $[M+H]^+$.

Synthesis of the hexafluorophosphate salt of 19: A solution of **17** (314 mg, 0.8 mmol) and methyl iodide (0.5 mL, 8 mmol) in MeCN (20 mL) was heated for 24 h under reflux. After cooling down to ambient temperature, the solvent was distilled off under reduced pressure and the residue was dissolved in CH_2Cl_2 (3 mL). The addition of Et_2O (20 mL) caused the formation of a precipitate, which was filtered off and dissolved in Me_2CO (5 mL). After the addition of a saturated aqueous solution of NH_4PF_6 (5 mL), the mixture was concentrated under reduced pressure to half of its original volume and the resulting suspension was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and the solvent was distilled off under reduced pressure to give the hexafluorophosphate salt of **22** (317 mg, 74%) as dark red oil. ^{1}H NMR ($CDCl_3$): δ = 1.56 (s, 6H), 2.75 (s, 3H), 3.27 (s, 3H), 3.45–3.47 (m, 2H), 3.52–3.73 (m, 14H), 3.98 (s, 3H), 7.80 (d, J = 8 Hz, 1H), 8.05 (d, J = 8 Hz, 1H), 8.13 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 14.9, 22.3, 35, 40.8, 55.7, 5.89, 70.0, 70.2, 70.9, 71.1, 72.5, 72.6, 116.1, 123.2, 129.6, 137.1, 142.9, 145.1, 167.0, 167.1, 199.5 ppm; ESIMS: *m*/*z* = 407 $[M-PF_6]^+$.

Polymer micelles: A solution of **1** (2.5 mg mL^{-1}, 200 μL) in $CHCl_3$ was added to a solution of **2**, **3a**, **4a**, **5a**, or **6a** (0.1 mg mL^{-1}, 50 μL) in $CHCl_3$. Alternatively, a solution of **1** (2.5 mg mL^{-1}, 200 μL) in $CHCl_3$ was mixed with solutions of **2** (0.1 mg mL^{-1}, 20 μL) and **3a**, **4a**, **5a**, or **6a** (0.1 mg mL^{-1}, 100 μL) in $CHCl_3$. Each mixture was heated at 40°C in an open vial. After the evaporation of the solvent, the residue was purged with air and dispersed in PBS (1 mL, pH 7.0). After vigorous shaking, the dispersion was filtered and the filtrate was used for the spectroscopic and imaging experiments without further purification.

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[1] S. R. Adams, R. Y. Tsien, *Annu. Rev. Physiol.* **1993**, *55*, 755–784.
[2] a) J. Lippincott-Schwartz, N. Altan-Bonnet, G. H. Patterson, *Nat. Cell. Biol.* **2003**, S7–S14; b) J. Lippincott-Schwartz, G. H. Patterson, *Science* **2003**, *300*, 87–91; c) J. Lippincott-Schwartz, G. H. Patterson, *Trends Cell Biol.* **2009**, *19*, 555–565; d) S. Manley, J. M. Gillette, J. Lippincott-Schwartz, *Methods Enzymol.* **2010**, *475*, 109–120; e) J. Lippincott-Schwartz, *Developmental Cell* **2011**, *21*, 5–10.
[3] I. Johnson, M. T. Z. Spence, *The Molecular Probes Handbook—A Guide to Fluorescent Probes and Labeling Technologies*, 11th ed.; Life Technologies: Carlsbad, **2010**.
[4] a) S. W. Hell, *Nat. Biotechnol.* **2003**, *21*, 1347–1355; b) S. W. Hell, M. Dyba, S. Jakobs, *Curr. Op. Neurobiol.* **2004**, *14*, 599–609; c) S. W. Hell, *Phys. Lett. A* **2004**, *326*, 140–145; d) S. W. Hell, L. Kastrup, *Nachr. Chem.* **2010**, *55*, 47–50; e) S. W. Hell, *Science* **2007**, *316*, 1153–1158; f) S. W. Hell, *Nat. Methods* **2009**, *6*, 24–32; g) S. W. Hell, R. Schmidt, A. Egner, *Nat. Photonics* **2009**, *3*, 381–387.
[5] a) M. Sauer, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 9433–9434; b) M. Heilemann, P. Dedecker, J. Hofkens, M. Sauer, *Laser Photonics Rev.* **2009**, *3*, 180–202; c) S. van de Linde, S. Wolter, M. Sauer, *Aust. J. Chem.* **2011**, *64*, 503–511.
[6] M. Fernández-Suárez, A. Y. Ting, *Nat. Rev. Mol. Cell. Biol.* **2008**, *9*, 929–943.
[7] a) B. Huang, M. Bates, X. Zhuang, *Annu. Rev. Biochem.* **2009**, *78*, 993–1016; b) J. C. Vaughan, X. Zhuang, *Nat. Biotechnol.* **2011**, *29*, 880–881.
[8] a) D. Toomre, J. Bewersdorf, *Annu. Rev. Cell Dev. Biol.* **2010**, *26*, 285–314; b) Y. Xu, T. J. Melia, D. K. Toomre, *Curr. Opin. Chem. Biol.* **2011**, *15*, 822–830.
[9] J. Vogelsang, C. Steinhauer, C. Forthmann, I. H. Stein, B. Person-Skergo, T. Cordes, P. Tinnefeld, *ChemPhysChem* **2010**, *11*, 2475–2490.
[10] M. A. Thompson, J. S. Biteen, S. J. Lord, N. Conley, W. E. Moerner, *Methods Enzymol.* **2010**, *475*, 27–59.
[11] a) C. Flors, *Biopolymers* **2011**, *95*, 290–297; b) C. Flors, W. C. Earnshaw, *Curr. Opin. Chem. Biol.* **2011**, *15*, 838–844.
[12] J. Cusido, S. Impellizzeri, F. M. Raymo, *Nanoscale* **2011**, *3*, 59–70.
[13] J. W. Lichtman, W. Denk, *Science* **2011**, *334*, 618–623.

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[14] T. J. Mitchison, K. E. Sawin, J. A. Theriot, K. Gee, A. Mallavarapu, *Methods Enzymol.* **1998**, *291*, 63–78.
[15] A. P. Pelliccioli, J. Wirz, *Photochem. Photobiol. Sci.* **2002**, *1*, 441–458.
[16] *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules* (Eds.: M. Goeldner, R. Givens), Wiley-VCH, Weinheim, **2005**.
[17] G. C. Ellis-Davies, *Nat. Methods* **2007**, *4*, 619–628.
[18] L. M. Wysocki, L. D. Davis, *Curr. Opin. Chem. Biol.* **2011**, *15*, 752–759.
[19] D. Puliti, D. Warther, C. Orange, A. Specht, M. Goeldner, *Bioorg. Med. Chem.* **2011**, *19*, 1023–1029.
[20] G. H. Dorion, A. F. Wiebe, *Photochromism*, Focal Press, New York, **1970**.
[21] *Photochromism* (Ed.: G. H. Brown), Wiley, New York, **1971**.
[22] *Organic Photochromes* (Ed.: A. V. El'tsov), Consultants Bureau, New York, **1990**.
[23] *Photochromism: Molecules and Systems* (Eds.: H. Bouas-Laurent, H. Dürr), Elsevier, Amsterdam, **1990**.
[24] *Organic Photochromic and Thermochromic Compounds* (Eds.: J. C. Crano, R. Guglielmetti), Plenum Press, New York, **1999**.
[25] M. Irie, *Chem. Rev.* **2000**, *100*, 1683–1890.
[26] M. G. Kuz′min, M. V. Koz'menko in *Organic Photochromes* (Ed.: A. V. El'tsov), Consultants Bureau, New York, **1990**, pp. 245–265.
[27] a) F. M. Raymo, M. Tomasulo, *Chem. Soc. Rev.* **2005**, *34*, 327–336; b) F. M. Raymo, M. Tomasulo, *J. Phys. Chem. A* **2005**, *109*, 7343–7352; c) J. Cusido, E. Deniz, F. M. Raymo, *Eur. J. Org. Chem.* **2009**, 2031–2045; d) I. Yildiz, E. Deniz, F. M. Raymo, *Chem. Soc. Rev.* **2009**, *38*, 1859–1867; e) J. Cusido, E. Deniz, F. M. Raymo, *Curr. Phys. Chem.* **2011**, *1*, 232–241.
[28] C. Yun, J. You, J. Kim, J. Huh, E. Kim, *J. Photochem. Photobiol. C* **2009**, *10*, 111–129.
[29] T. Inada, S. Uchida, Y. Yokoyama, *Chem. Lett.* **1997**, 321–322.
[30] a) A. J. Myles, N. R. Branda, *J. Am. Chem. Soc.* **2001**, *123*, 177–178; b) A. Myles, N. R. Branda, *Adv. Funct. Mater.* **2002**, *12*, 167–173.
[31] a) J. L. Bahr, G. Kodis, L. de La Garza, S. Lin, A. L. Moore, T. A. Moore, D. Gust, *J. Am. Chem. Soc.* **2001**, *123*, 7124–7133; b) J. Andréasson, G. Kodis, Y. Terazono, P. A. Liddell, S. Bandyopadhyay, R. H. Mitchell, T. A. Moore, A. L. Moore, D. Gust, *J. Am. Chem. Soc.* **2004**, *126*, 15926–15927; c) Y. Terazono, G. Kodis, J. Andréasson, G. Jeong, A. Brune, T. Hartmann, H. Dürr, T. A. Moore, A. L. Moore, D. Gust, *J. Phys. Chem. B* **2004**, *108*, 1812–1814; d) S. D. Straight, J. Andréasson, G. Kodis, S. Bandyopadhyay, R. H. Mitchell, T. A. Moore, A. L. Moore, D. Gust, *J. Am. Chem. Soc.* **2005**, *127*, 9403–9409.
[32] a) L. Giordano, T. M. Jovin, M. Irie, E. A. Jares-Erijman, *J. Am. Chem. Soc.* **2002**, *124*, 7481–7489; b) E. A. Jares-Erijman, L. Giordano, C. Spagnuolo, J. Kawior, R. J. Verneij, T. M. Jovin, *Proc. SPIE-Int. Soc. Opt. Eng.* **2004**, *5323*, 13–26.
[33] a) M. Irie, T. Fukaminato, T. Sasaki, N. Tamai, T. Kawai, *Nature* **2002**, *420*, 759–760; b) M.-S. Kim, T. Kawai, M. Irie, *Opt. Mater.* **2002**, 275–278; c) T. Fukaminato, T. Sasaki, T. Kawai, N. Tamai, M. Irie, *J. Am. Chem. Soc.* **2004**, *126*, 14843–14849; d) Y. Odo, T. Fukaminato, M. Irie, *Chem. Lett.* **2007**, *36*, 240–241; e) T. Fukaminato, T. Umemoto, Y. Iwata, S. Yokojima, M. Yoneyama, S. Nakamura, M. Irie, *J. Am. Chem. Soc.* **2007**, *129*, 5932–5938; f) T. Fukaminato, T. Doi, M. Tanaka, M. Irie, *J. Phys. Chem. C* **2009**, *113*, 11623–11627; g) T. Fukaminato, T. Tanaka, T. Doi, N. Tamaoki, T. Katayama, A. Mallick, Y. Ishibashi, H. Miyasaka, M. Irie, *Photochem. Photobiol. Sci.* **2010**, *9*, 181–187; h) T. Fukaminato, T. Doi, N. Tamaoki, K. Okuno, Y. Ishibashi, H. Miyasaka, M. Irie, *J. Am. Chem. Soc.* **2011**, *133*, 4984–4990.
[34] R. T. Jukes, V. Adamo, F. Hartl, P. Belser, L. De Cola, *Inorg. Chem.* **2004**, *43*, 2779–2792.
[35] a) M. Bossi, V. Belov, S. Polyakova, S. W. Hell, *Angew. Chem.* **2006**, *118*, 7623–7627; *Angew. Chem. Int. Ed.* **2006**, *45*, 7462–7465; b) A. de Meijere, L. Zhao, V. N. Belov, M. Bossi, M. Noltemeyer, S. W. Hell, *Chem. Eur. J.* **2007**, *13*, 2503–2516; c) J. Fölling, S. Polyakova, V. Belov, A. von Blaaderen, M. L. Bossi, S. W. Hell, *Small* **2008**, *4*, 134–142.
[36] L.-H. Liu, K. Nakatani, R. Pansu, J.-J. Vachon, P. Tauc, E. Ishow, *Adv. Mater.* **2007**, *19*, 433–436.
[37] M. Berberich, A.-M. Krause, M. Orlandi, F. Scandola, Würthner, *Angew. Chem.* **2008**, *120*, 6718–6721; *Angew. Chem. Int. Ed.* **2008**, *47*, 6616–6619.
[38] Mechanisms for fluorescence switching with photochromic compounds are reviewed in ref. [27]. Representative examples of fluorophore–photochrome dyads operating on the basis of electron and energy transfer are reported in refs. [29–37].
[39] a) R. C. Bertelson in *Photochromism* (Ed.: G. H. Brown) Wiley, New York, **1971**, pp. 45–431; b) R. C. Bertelson in *Organic Photochromic and Thermochromic Compounds* (Eds.: J. C. Crano, R. Guglielmetti) Plenum Press, New York, **1999**, vol. 1, pp. 11–83.
[40] A. S. Kholmanskii, K. M. Dyumanev, *Russ. Chem. Rev.* **1987**, *56*, 136–151.
[41] R. Guglielmetti in *Photochromism: Molecules and Systems* (Eds.: H. Dürr, H. Bouas-Laurent), Elsevier, Amsterdam, **1990**, pp. 314–466 and 855–878.
[42] a) K. Ichimura in *Photochromism: Molecules and Systems* (Eds.: H. Dürr, H. Bouas-Laurent), Elsevier, Amsterdam, **1990**, pp. 903–918; b) K. Ichimura, T. Seki, Y. Kawamishi, Y. Suzuki, M. Sakuragi, T. Tamaki in *Photo-Refractive Materials for Ultrathin Density Optical Memory* (Ed.: M. Irie), Elsevier, Amsterdam, **1994**, pp. 55–83; c) K. Ichimura in *Photochromic Polymers* (Eds.: J. C. Crano, R. Guglielmetti), Plenum Press, New York, **1999**, vol. 2, pp. 9–63; d) K. Ichimura, *Chem. Rev.* **2000**, *100*, 1847–1873.
[43] J. C. Crano, W. S. Kwak, C. N. Welch in *Applied Photochromic Polymer Systems* (Ed.: C. B. McArdle) Blackie, Glasgow, **1992**, pp. 31–79.
[44] a) V. Krongauz in *Applied Photochromic Polymer Systems*, C. B. McArdle (Ed.) Blackie: Glasgow, **1992**, p. 121–173; b) G. Berkovic, V. Krongauz, V. Weiss, *Chem. Rev.* **2000**, *100*, 1741–1754.
[45] M. Irie in *Applied Photochromic Polymer Systems* (Ed.: C. B. McArdle) Blackie, Glasgow, **1992**, pp. 174–206.
[46] J. Hibino, T. Hashida, M. Suzuki in *Photo-Refractive Materials for Ultrathin Density Optical Memory* (Ed.: M. Irie), Elsevier, Amsterdam, **1994**, pp. 25–53.
[47] S. Kawata, Y. Kawata, *Chem. Rev.* **2000**, *100*, 1777–1788.
[48] J. A. Delaire, K. Nakatani, *Chem. Rev.* **2000**, *100*, 1817–1845.
[49] N. Tamai, H. Miyasaka, *Chem. Rev.* **2000**, *100*, 1875–1890.
[50] V. I. Minkin, *Chem. Rev.* **2004**, *104*, 2751–2776.
[51] a) F. M. Raymo, S. Giordani, *Org. Lett.* **2001**, *3*, 1833–1836; b) F. M. Raymo, S. Giordani, *J. Am. Chem. Soc.* **2002**, *124*, 2004–2007; c) M. Tomasulo, I. Yildiz, F. M. Raymo, *Aust. J. Chem.* **2006**, *59*, 175–178; d) M. Tomasulo, E. Deniz, R. J. Alvarado, F. M. Raymo, *J. Phys. Chem. C* **2008**, *112*, 8038–8045; e) S. Silvi, E. C. Constable, C. E. Housecroft, J. E. Beves, E. L. Dunphy, M. Tomasulo, F. M. Raymo, A. Credi, *Chem. Commun.* **2009**, 1484–1486.
[52] a) M. Tomasulo, S. Giordani, F. M. Raymo, *Adv. Funct. Mater.* **2005**, *15*, 787–794; b) M. Tomasulo, F. M. Raymo, *J. Mater. Chem.* **2005**, *15*, 4354–4360; c) E. Deniz, M. Tomasulo, R. A. DeFazio, B. D. Watson, F. M. Raymo, *Phys. Chem. Chem. Phys.* **2010**, *12*, 11630–11634.
[53] I. Yildiz, S. Impellizzeri, E. Deniz, B. McCaughan, J. F. Callan, F. M. Raymo, *J. Am. Chem. Soc.* **2011**, *133*, 871–879.
[54] a) M. Tomasulo, S. Sortino, A. J. P. White, F. M. Raymo, *J. Org. Chem.* **2005**, *70*, 8180–8189; b) M. Tomasulo, S. Sortino, F. M. Raymo, *Org. Lett.* **2005**, *7*, 1109–1112; c) M. Tomasulo, S. Sortino, A. J. P. White, F. M. Raymo, *J. Org. Chem.* **2006**, *71*, 744–753; d) M. Tomasulo, S. Sortino, F. M. Raymo, *Asian Chem. Lett.* **2007**, *11*, 219–222; e) M. Tomasulo, S. Sortino, F. M. Raymo, *Adv. Mater.* **2008**, *20*, 832–835; f) M. Tomasulo, S. Sortino, F. M. Raymo, *J. Org. Chem.* **2008**, *73*, 118–126; g) M. Tomasulo, S. Sortino, F. M. Raymo, *J. Photochem. Photobiol. A* **2008**, *200*, 44–49; h) M. Tomasulo, E. Deniz, T. Benelli, S. Sortino, F. M. Raymo, *Adv. Funct. Mater.* **2009**, *19*, 3956–3961; i) M. Åxman Petersen, E. Deniz, M. Brøndsted Nielsen, S. Sortino, F. M. Raymo, *Eur. J. Org. Chem.* **2009**, 4333–4339;

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j) E. Deniz, M. Tomasulo, S. Sortino, F. M. Raymo, *J. Phys. Chem. C* **2009**, *113*, 8491–8497; k) E. Deniz, S. Ray, M. Tomasulo, S. Impellizzeri, S. Sortino, F. M. Raymo, *J. Phys. Chem. A* **2010**, *114*, 11567–11575; l) E. Deniz, S. Sortino, F. M. Raymo, *J. Phys. Chem. Lett.* **2010**, *1*, 1690–1693; m) E. Deniz, S. Sortino, F. M. Raymo, *J. Phys. Chem. Lett.* **2010**, *1*, 3506–3509; n) M. Tomasulo, E. Deniz, S. Sortino, F. M. Raymo, *Photochem. Photobiol. Sci.* **2010**, *9*, 136–140; o) E. Deniz, S. Impellizzeri, S. Sortino, F. M. Raymo, *Can. J. Chem.* **2011**, *89*, 110–116; p) E. Deniz, M. Tomasulo, J. Cusido, S. Sortino, F. M. Raymo, *Langmuir* **2011**, *27*, 11773–11783; q) E. Deniz, J. Cusido, S. Swaminathan, M. Battal, S. Impellizzeri, S. Sortino, F. M. Raymo, *J. Photochem. Photobiol. A* **2012**, *229*, 20–28; r) E. Deniz, M. Tomasulo, J. Cusido, I. Yildiz, M. Petriella, M. L. Bossi, S. Sortino, F. M. Raymo, *J. Phys. Chem. C* **2012**, *116*, 6058–6068.

[55] The relative concentrations of the two isomers of each system at equilibrium were estimated from the absorbance of the 3*H*-indolium chromophore of **3b** and **4b** in the visible region, using the molar extinction coefficient of the model compound **8**.

[56] The pH dependence of the absorption spectrum (a and b in Figure S4 in the Supporting Information) of 4-nitrophenol confirms the assignment of the band at 390 nm to the anionic chromophore of the ring-open isomers **3b** and **4b**. Indeed, this band resembles the absorption observed for the 4-nitrophenolate anion at a pH of 8.0 (b in Figure S4 in the Supporting Information).

[57] The pK_a of 4-nitrophenol is 7.15 (*CRC Handbook of Chemistry and Physics* (Ed.: W. M. Haynes), CRC Press/Taylor and Francis, Boca Raton, **2012**). This value suggests that a significant fraction of the ring-open isomer has the 4-nitrophenolate anion in a protonated form.

[58] The relative concentrations of the two isomers at equilibrium were estimated from the absorbance of the 3*H*-indolium chromophore of **7b** in the visible region, using the molar extinction coefficient of the model compound **10**.

[59] The fluorescence quantum yield of the hexafluorophosphate salt of **9** is 0.09 in acetonitrile[54m,r] and 0.04 in PBS (pH 7.0). Thus, the transition from organic to aqueous solutions tends to discourage the radiative deactivation of this particular fluorophore. In the presence of the amphiphilic co-polymer **1**, however, the quantum yield in PBS increases to 0.13. These observations suggest that the polymeric host limits the exposure of the fluorescent guest to the aqueous environment.

[60] The synthesis of **12a** was originally reported in: a) A. A. Shachkus, J. Degutis, A. Jezerskaite in *Chemistry of Heterocyclic Compounds* (Eds.: J. Kovac, P. Zalupsky), Elsevier, Amsterdam, **1987**, vol. 35 pp. 518–520; b) A. A. Shachkus, J. Degutis, A. G. Urbonavichyus, *Khim. Geterotsikl. Soedin.* **1989**, *5*, 672–676; A. A. Shachkus, Yu. A. Degutis, A. G. Urbonavichyus, *Chem. Heterocycl. Compd.* **1989**, *25*, 562–565.

[61] M. Tomasulo, S. L. Kaanumal, S. Sortino, F. M. Raymo, *J. Org. Chem.* **2007**, *72*, 595–605.

[62] J. Wu, W. Liu, X. Zhuang, F. Wang, P. Wang, S. Tao, X. Zhang, S. Wu, S. T. Lee, *Org. Lett.* **2007**, *9*, 33–36.

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Supramolecular Chemistry

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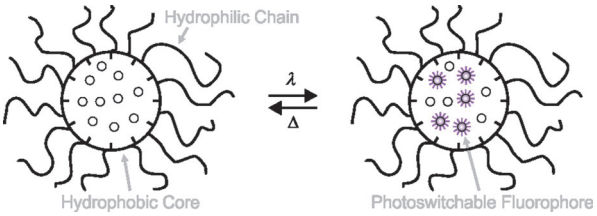
Fast Fluorescence Switching within Hydrophilic Supramolecular Assemblies



Photoswitchable fluorophores can be entrapped within the hydrophobic interior of an amphiphilic polymer. The fluorescence of the resulting supramolecular assemblies can be switched for hundreds of cycles on a microsecond timescale in aqueous environments (see scheme).

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