Postfach 10 11 61
69451 Weinheim
Germany
Courier services:
Boschstraße 12
69469 Weinheim
Germany
Tel.: (+49) 6201 606 581
Fax: (+49) 6201 606 510
E-mail: macromol@wiley-vch.de

WILEY-VCH

Dear Author,

Please correct your galley proofs carefully and return them no more than four days after the page proofs have been received.

The editors reserve the right to publish your article without your corrections if the proofs do not arrive in time.

Note that the author is liable for damages arising from incorrect statements, including misprints.

Please note any queries that require your attention. These are indicated with a Q in the PDF and a question at the end of the document.

Please limit corrections to errors already in the text; cost incurred for any further changes or additions will be charged to the author, unless such changes have been agreed upon by the editor.

Reprints may be ordered by filling out the accompanying form.

Return the reprint order form by fax or by e-mail with the corrected proofs, to Wiley-VCH : macromol@wiley-vch.de

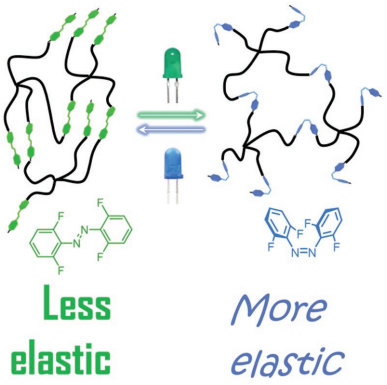
To avoid commonly occurring errors, please ensure that the following important items are correct in your proofs (please note that once your article is published online, no further corrections can be made):

- **Names** of all authors present and spelled correctly
- **Titles** of authors correct (Prof. or Dr. only: please note, Prof. Dr. is not used in the journals)
- **Addresses** and **postcodes** correct
- **E-mail address** of corresponding author correct (current email address)
- **Funding bodies** included and grant numbers accurate
- **Title** of article OK
- All **figures** included
- **Equations** correct (symbols and sub/superscripts)

Corrections should be made directly in the PDF file using the PDF annotation tools. If you have questions about this, please contact the editorial office. The corrected PDF and any accompanying files should be uploaded to the journal's Editorial Manager site.

COMMUNICATIONS

Q1 xxxx



Phototuning hydrogels' mechanical properties with visible light are achieved by incorporating all-visible azobenzene derivatives within PEGylated networks.

Macromolecular Rapid Communications
www.mrc-journal.de

Reversible Modulation of Elasticity in Fluoroazobenzene-Containing Hydrogels Using Green and Blue Light

*Fangli Zhao, Aurelio Bonasera, Ulrich Nöchel, Marc Behl, and David Bléger**

Hydrogels are soft materials that have found multiple applications in biomedicine and represent a good platform for the introduction of molecular switches and synthetic machines into macromolecular networks. Tuning their mechanical properties reversibly with light is appealing for a variety of advanced applications and has been demonstrated in the past; however, their activation typically requires the use of UV light, which displays several drawbacks related to its damaging character and limited penetration in tissues and materials. This study circumvents this limitation by introducing all-visible *ortho*-fluoroazobenzene switches into a hydrophilic network, which, as a result, can be activated with green or blue light. Photoisomerization of the photochromic moieties is accompanied by a reversible tuning of the elastic modulus. The translation of molecular isomerization within the network into macroscopic modulation of its mechanical properties is attributed to different aggregation tendencies of the *E* and *Z* isomers of the azobenzene derivatives.

1. Introduction

Hydrogels are 3D networks with high water content that have found many applications in modern medicine, e.g., biomedical implants, adhesives, contact lenses, or scaffolds for tissue engineering.[1] Their mechanical properties, in particular the elasticity, are essential and hence a key parameter is the shear elastic modulus (G'), with different applications requiring moduli across the 10^2–10^7 Pa range.[2] For instance, stem cells are known to remember past mechanical environments and differentiate depending on the elastic modulus of the substrate.[3] Hence, creating hydrogels whose elasticity can be tuned on demand is promising for inducing environmental mechanical variations that mimic the extracellular matrix dynamics and dictate cells' fate.[4]

F. Zhao, Dr. A. Bonasera, Dr. D. Bléger
Department of Chemistry and IRIS Adlershof
Humboldt-Universität zu Berlin
Brook-Taylor-Str. 2, 12489 Berlin, Germany
E-mail: david.bleger@chemie.hu-berlin.de
Dr. U. Nöchel, Dr. M. Behl
Institute of Biomaterial Science and Berlin-Brandenburg Center for Regenerative Therapies (BCRT)
Active Polymers Department
Helmholtz-Zentrum Geesthacht
Kantstraße 55, 14513 Teltow, Germany
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/marc.201700527.

DOI: 10.1002/marc.201700527

Gels are especially sensitive to their environment and can be designed to readily respond to variations in, typically, pH or temperature.[5] Light is another trigger of choice offering the advantage of precise photopatterning[4] and allows to alter hydrogels' mechanical properties either permanently by implementing photocleavable groups[6] or reversibly using photochromic molecules, such as spiropyrans[7,8] or azobenzenes.[9] Following the latter strategy, noncovalent physical gels typically lead to materials with light-induced sol–gel transitions,[10–13] whereas covalent chemical gels can exhibit more finely tuneable phenomena such as photoinduced motion,[14–18] or softening/hardening. Photoswitchable hydrogels exhibiting modulation of their mechanical properties have been described;[16,19] however, their activation requires UV light, which displays several drawbacks related to its damaging character for the materials and its surrounding, as well as its limited light penetration.[20]

Here, we present a polyethylene glycol (PEG) hydrogel incorporating *ortho*-fluoroazobenzenes[21] as cross-linkers (see ***F4-azo-PEG***; **Figure 1**) and exhibiting reversible photomodulation of elasticity using blue and green light. *Ortho*-fluoroazobenzenes (abbreviated to F-azos in the following) were selected as photochromic moieties due to their full addressability with visible light and very high thermal stabilities of the thermodynamically less stable *Z*-isomers. F-azos were functionalized in *para*-positions with amide linkers, since such electron withdrawing groups maximize the separation of *E* and *Z* isomers' n→π^* bands in the visible region[22] (see **Figure 2**a) and hence promote higher photoisomerization yields using blue (*Z*→*E*) or green (*E*→*Z*) light.

2. Results and Discussion

F4-azo-PEG gel samples were prepared via strain-promoted click cycloaddition between a tetra-armed PEG macromonomer (M_n = 10 kg mol^{-1}) terminated with azide groups (***tetra-N3-PEG***) and the F-azo derivative functionalized with aza-dibenzocyclooctynes[23,24] (***F4-azo-bis-DBCO***). This reaction typically exhibits fast kinetics at room temperature and does not necessitate the presence of a Cu-catalyst, which might be beneficial for the formation of the gel, since once the gelation starts, diffusion of the reactants is reduced. The two starting materials were dissolved in stoichiometric amount in

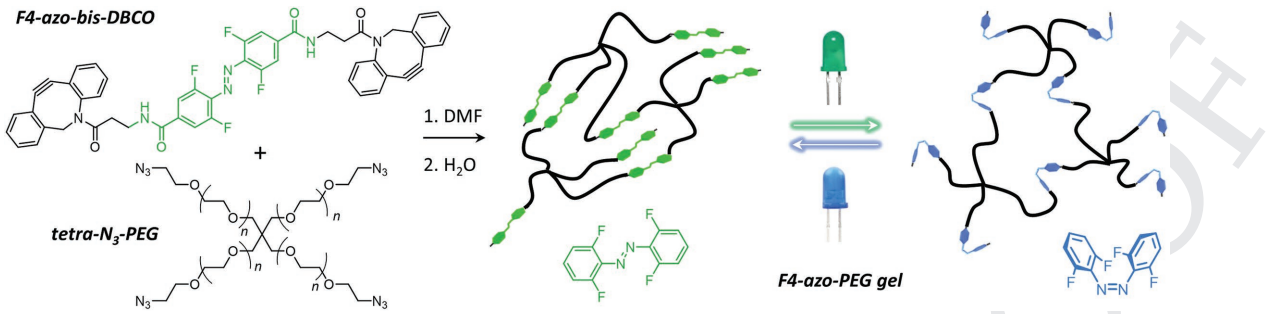


Figure 1. Design and synthesis of photochromic hydrogels responsive to green and blue (LED) irradiation.

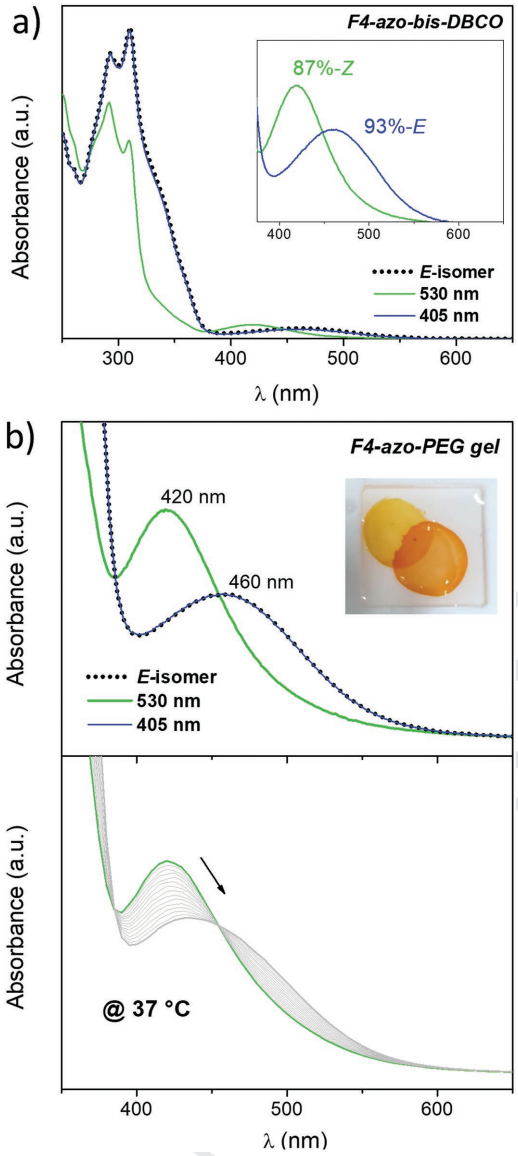


Figure 2. UV–vis absorbance spectra. a) ***F4-azo-bis-DBCO*** in acetonitrile at 25 °C (5.5×10^{-5} mol L^{-1}); the inset shows the n⟶π* bands and indicates the compositions of the PSS mixtures (as determined by liquid chromatography) upon irradiation with visible light. b) ***F4-azo-PEG*** gel in water at 25 °C (top, λ_{max} are indicated next to the curves; the inset highlights the photochromism of the hydrogels) and thermal $Z \rightarrow E$ relaxation at 37 °C (bottom, each curve was taken with 4 h interval; total measurement time: 60 h). The inset shows the redshift of the n→π* band over time.

DMF in a disc-shaped mold and stirred at room temperature for ≈3 min. When the solution became viscous, the stirring bar was removed and the gel was kept at room temperature for 1 h, followed by 4 h at 45 °C in order to obtain homogenous samples. After cooling to room temperature, solvent exchange with distilled water for 3 d yielded fully swollen hydrogel samples.

The photoisomerization of the F-azo moieties within ***F4-azo-PEG*** hydrogel samples was investigated by UV–vis spectroscopy (see Figure 2b) and compared with the starting material (***F4-azo-bis-DBCO***) in solution (see Figure 2a). As typically observed for F-azo derivatives,[21] the green-light-induced $E \rightarrow Z$ isomerization is characterized by a blueshift of the n→π* band from 460 to 420 nm, while subsequent blue-light irradiation almost completely recovers the initial *E*-isomer spectrum. This reversible Z/E isomerization can be repeated for several cycles without photobleaching (see **Figure 3**b). Based on very similar absorption spectra of the hydrogels and ***F4-azo-bis-DBCO*** in solution, it can be concluded that photoisomerization within the gel is not hindered and similar photostationary states (PSS) are obtained, i.e., 87% of *Z*-isomer and 93% of *E*-isomer upon green-light and blue-light irradiation, respectively (see Figure S1, Supporting Information). The kinetics of the thermal $Z \rightarrow E$ relaxation was then measured at 37 °C. After irradiation with green light until reaching the *Z*-rich PSS, spectral changes were monitored in the dark (see Figure 2b, bottom). After 60 h, the n→π* band experienced a redshift of 15 nm, corresponding to a decrease of ≈30% of *Z*-isomer (from 87% to 57%). Such a slow thermal relaxation, corresponding to a thermal half-live of ≈15 d at 37 °C, is characteristic of F-azos and similar to the free derivatives in solution.[22] The absence of significant acceleration of the $Z \rightarrow E$ thermal isomerization compared to the free molecules in solution indicates that no large mechanical strain is generated within the polymer chains of the networks upon $E \rightarrow Z$ photoisomerization.

Dynamic time sweep rheological experiments were conducted to monitor the response of ***F4-azo-PEG*** gel in situ upon exposure to visible light. The shear moduli were recorded after 2 h of equilibration in water with the hydrogel sample loaded into the rheometer. The moduli were measured for frequencies ranging from 0.001 to 100 Hz at a constant shear strain of 1% (see Figure S3, Supporting Information). Over this frequency range, the storage modulus (G') was larger than the loss modulus (G''), indicating a preponderance of the elastic behavior within the samples and demonstrating successful cross-linking (in a liquid or melt $G' < G''$).

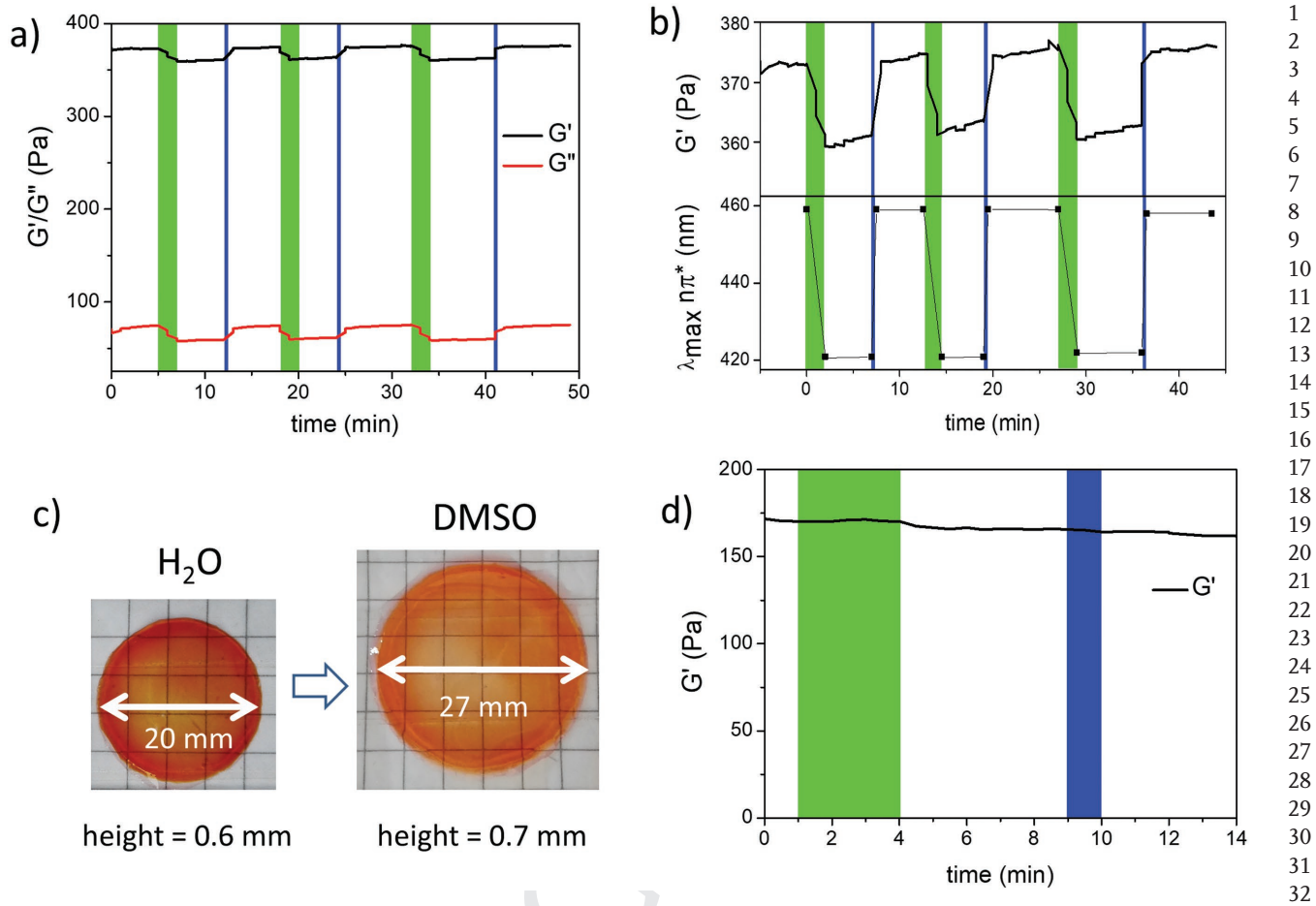
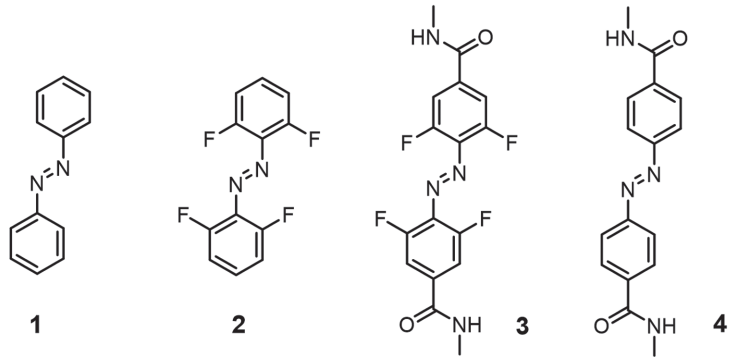


Figure 3. Rheology of ***F4-azo-PEG*** hydrogel. a) Cyclic changes in G' and G'' obtained upon alternating irradiation with green (530 nm, 2 min) and blue (405 nm, 30 s) LEDs. b) Zoom-in of the G' curve (top) and corresponding spectral changes recorded within the same time period (bottom, absorption maxima have been connected with a line to guide the eyes), highlighting the synchronicity between photomodulation of G' and kinetics of photoisomerization. The origin of the time was set at the beginning of the first irradiation cycle. c) Solvent exchange (water → DMSO) experiment and d) subsequent measurement of G', which was not affected by irradiation with the green or blue LEDs.

The evolution of the moduli over time was measured at a frequency of 10 Hz with three different samples. The initial value of G' was found to be constant for all samples, at around 375 Pa (corresponding to a cross-linking density of ≈150 mmol m^{-3}; see the Supporting Information), indicating the reproducibility of the synthesis procedure. Upon irradiation with green light, G' decreased and stabilized after 2 min, while subsequent irradiation with blue light quickly restored the initial elasticity (see Figure 3a). Both green and blue-light-irradiated states could be kept with stable G' values for a few minutes after the light was switched off. However, a slight increase of G' was observed over time for both states, possibly due to small changes in the swelling of the gel during isomerization. Several switching cycles could be performed alternating between cross-linking density without exhibiting any fatigue. Remarkably, photomodulation of the mechanical properties occurred at the same speed as photoisomerization within the gel (i.e., the value of G' stabilized as soon as the PSS was reached; see Figure 3b).

In order to understand the mechanism of the photomodulation of G', the gel's mechanical properties were also investigated in an organic solvent. Exchanging the swelling agent (water) with DMSO causes tremendous swelling of the sample, as the diameter increased from 20 to 27 mm, the thickness increased from 0.6 to 0.7 mm (see Figure 3c), and the gel became much more elastic (G' of 170 Pa in DMSO vs 375 Pa in water; see Figure 3d). In addition, no photomodulation of G' could be observed in the DMSO sample. In view of these last experiments, it is assumed that the photomodulation of G' occurring in the hydrogel arises from reversible disruption of noncovalent interactions between F-azos in water. Since *E*-azobenzenes are planar and more hydrophobic than the *Z*-isomers (see **Figure 4**), their tendency to assemble (via π-stacking and hydrophobic effects) is higher, especially in water.[16] As a result, *E*-isomers most likely act as noncovalent additional cross-linkers, leading to less elastic hydrogels, while $E \rightarrow Z$ isomerization could disrupt these physical cross-links hence softening the gel. By contrast, the organogel swollen with DMSO does not exhibit any change in G' upon irradiation because the physical cross-links induced by the hydrophobicity of the *E*-azo moiety in water readily dissociate in the organic solvent. The disruption of



Compound	μ (*E*)	μ (*Z*)	|Δμ|	Φ (*E*)	Φ (*Z*)
1	0.0	4.5	4.5	0.1	51.4
2	0.4	7.4	7.0	30.2	58.2
3	4.6	9.0	4.4	31.1	58.0
(3')	(8.4)	(8.6)	(0.2)	(31.1)	(58.0)
4	0.6	4.6	4.0	1.8	50.0
(4')	(9.4)	(7.9)	(1.5)	(1.8)	(50.0)

Figure 4. Dipole moments (μ) and CCNN dihedral angles (Φ) of compounds **1–4**, computed at the B3LYP/6-311+G(d,p) level of theory. Compounds **3** and **4** can display alternative stable conformations, where the two amido groups point toward the same direction (referred to as **3'** and **4'**; see the corresponding values in parentheses). Dipole moments are given in debye (D) and dihedral angles in degrees (°).

secondary physical cross-links can also explain the large expansion of the gel during solvent exchange (Figure 3c) in addition to a higher affinity of the network for DMSO than for water.

Although the hydrogels described here display significant (yet moderate) photomodulation of elasticities, we did not observe any concomitant macroscopic contraction–expansion motion as reported recently[16] in PEG hydrogels of similar architecture incorporating parent (nonfluorinated) azobenzenes (see compound **4**; Figure 4), which might be due to different association tendency of the photochromic moieties. Three driving forces can be involved in the association of amido-azobenzenes in water: hydrogen bonds, hydrophobic effects, and π-stacking. Although it is difficult to evaluate differences in H-bonding ability between F-azos and parent azobenzenes, it is possible to gain insight into π-stacking ability by comparing their deviation from planarity, and into hydrophobic interactions by comparing their dipole moments (μ). The minimum geometries and dipoles of a series of relevant azobenzenes were computed using density functional theory (DFT; see Figure 4). According to the calculations, plain *E*-azobenzene as well as its amido derivative (compounds **1** and **4**) is nearly planar, whereas *E*-F-azos (compounds **2** and **3**) exhibit significant deviation from planarity (CCNN twist angle of ≈30° for the minimum geometry of the *E*-isomers; see Figure 4 and Figure S4, Supporting Information).[25] Consequently, the π-stacking ability of the *E*-isomer should be weakened, supporting the moderate photomodulation observed in F-azos as well as the absence of photoinduced macroscopic motion. As a consequence of the less planar geometry, the calculated dipoles for the *E*-isomers of F-azos are higher compared to parent azobenzenes (see Figure 4), hence weakening hydrophobic effects in the hydrogels. A last reason for the different observed behaviors could be a smaller difference of dipole moments between *E* and *Z*-isomers ($\Delta\mu$) for F-azos, which would affect the amplitude of the swelling/deswelling of the hydrogels. Nevertheless, DFT calculations indicate that $\Delta\mu$ are similar for compounds **3** and **4**; hence, at this stage, we believe that the system could be optimized mainly via planarization of the visible-light responsive moiety.

3. Conclusions

Hydrogels whose elasticity can be reversibly phototuned with visible light have been presented. The use of UV light could be avoided, which is generally beneficial regarding the photostability of the materials and their surroundings as well as their future use for biological applications. The origin of the light-induced responses is attributed to the disruption of secondary physical cross-links between photochromic moieties upon isomerization, as inferred by solvent exchange in

DMSO, which in addition to suppressing any photoreponses induces a large expansion of the samples. DFT calculations have been performed to gain insight into the association tendency of F-azobenzenes, revealing significant deviation from planarity for the *E*-isomers, which is detrimental to the formation of noncovalent crosslinks. Optimizing the design of F-azobenzenes regarding their planarity, for example, employing only one *ortho*-F per phenyl ring, could increase the amplitude of *G′* phototuning and possibly induce macroscopic motion of the materials for the preparation of synthetic macromolecular machines.[26]

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Generous support from the German Research Foundation (DFG, through research grants BL1269/1-1 and BL1269/1-3) is gratefully acknowledged.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

azobenzenes, hydrogels, photodynamic materials, visible light

Received: August 2, 2017
Revised: September 29, 2017
Published online:

[1] D. Seliktar, *Science* **2012**, *336*, 1124.
[2] M. Zhong, R. Wang, K. Kawamoto, B. D. Olsen, J. A. Johnson, *Science* **2016**, *353*, 1264.
[3] A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, *Cell* **2006**, *126*, 677.
[4] A. M. Rosales, K. S. Anseth, *Nat. Rev. Mater.* **2016**, *1*, 15012.
[5] S. Chaterji, I. K. Kwon, K. Park, *Prog. Polym. Sci.* **2007**, *32*, 1083.
[6] A. M. Kloxin, A. M. Kasko, C. N. Salinas, K. S. Anseth, *Science* **2009**, *324*, 59.
[7] K. Sumaru, M. Kameda, T. Kanamori, T. Shinbo, *Macromolecules* **2004**, *37*, 4949.
[8] J. ter Schiphorst, S. Coleman, J. E. Stumpel, A. Ben Azouz, D. Diamond, A. P. H. J. Schenning, *Chem. Mater.* **2015**, *27*, 5925.
[9] M. Irie, D. Kunwatchakun, *Macromolecules* **1986**, *19*, 2476.
[10] S. Yamaguchi, S. Matsumoto, K. Ishizuka, Y. Iko, K. V. Tabata, H. F. Arata, H. Fujita, H. Noji, I. Hamachi, *Chem.—Eur. J.* **2008**, *14*, 1891.
[11] Y.-L. Zhao, J. F. Stoddart, *Langmuir* **2009**, *25*, 8442.
[12] S. Tamesue, Y. Takashima, H. Yamaguchi, S. Shinkai, A. Harada, *Angew. Chem., Int. Ed.* **2010**, *49*, 7461.
[13] D. Wang, M. Wagner, H.-J. Butt, S. Wu, *Soft Matter* **2015**, *11*, 7656.
[14] T. Ikeda, M. Nakano, Y. Yu, O. Tsutsumi, A. Kanazawa, *Adv. Mater.* **2003**, *15*, 201.
[15] N. Hosono, M. Yoshikawa, H. Furukawa, K. Totani, K. Yamada, T. Watanabe, K. Horie, *Macromolecules* **2013**, *46*, 1017.
[16] K. Iwaso, Y. Takashima, A. Harada, *Nat. Chem.* **2016**, *8*, 625.
[17] Q. Li, G. Fuks, E. Moulin, M. Maaloum, M. Rawiso, I. Kulic, J. T. Foy, N. Giuseppone, *Nat. Nanotechnol.* **2015**, *10*, 161.
[18] J. T. Foy, Q. Li, A. Goujon, J.-R. Colard-Itté, G. Fuks, E. Moulin, O. Schiffmann, D. Dattler, D. P. Funeriu, N. Giuseppone, *Nat. Nanotechnol.* **2017**, *12*, 540.
[19] A. M. Rosales, K. M. Mabry, E. M. Nehls, K. S. Anseth, *Biomacromolecules* **2015**, *16*, 798.
[20] D. Bléger, S. Hecht, *Angew. Chem., Int. Ed. Engl.* **2015**, *54*, 11338.
[21] D. Bléger, J. Schwarz, A. M. Brouwer, S. Hecht, *J. Am. Chem. Soc.* **2012**, *134*, 20597.
[22] C. Knie, M. Utecht, F. Zhao, H. Kulla, S. Kovalenko, A. M. Brouwer, P. Saalfrank, S. Hecht, D. Bléger, *Chem. Eur. J.* **2014**, *20*, 16492.
[23] M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. J. T. Rutjes, J. C. M. van Hest, F. L. van Delft, *Chem. Commun.* **2010**, *46*, 97.
[24] M. Chen, Y. Gu, A. Singh, M. Zhong, A. M. Jordan, S. Biswas, L. T. J. Korley, A. C. Balazs, J. A. Johnson, *ACS Cent. Sci.* **2017**, *3*, 124.
[25] Note that in a single crystal the F-azo moiety was found to be essentially planar, see ref. (22).
[26] *Macromol. Rapid Commun.* **2018**.

Q7

Query

Q1: Please provide TOC keyword.

Q2: Please provide the highest academic title (either Dr. or Prof.) for all authors, where applicable.

Q3: Please define 'LED' at the first occurrence in the text.

Q4: Please define 'DMF' at the first occurrence in the text.

Q5: Please define 'DMSO' at the first occurrence in the text.

Q6: Please define 'CCNN' at the first occurrence in the text.

Q7: Please provide author names, volume number and page number for ref. (26), if available now.

Macromolecular Rapid Communication

Editorial Office:
Wiley-VCH Verlag
Boschstraße 12, 69469 Weinheim
Germany

Tel.: +49 (0) 6201 – 606 – 581
Fax: +49 (0) 6201 – 606 – 510
Email: macromol@wiley-vch.de

Reprint Order Form 2017

Short DOI: marc. _____

Please send me and bill me for

☐ **Reprints** via ☐ airmail (+ 25 Euro)
☐ surface mail

Please send me and bill me for a:

☐ **Issue copies** (25 Euro)

☐ **high-resolution PDF file** (330 Euro).

My Email address:

_____

Please note: It is not permitted to present the PDF file on the internet or on company homepages.

Information regarding VAT

Please note that from German sales tax point of view, the charge for **Reprints, Issues or Posters** is considered as **"supply of goods"** and therefore, in general, such delivery is a subject to German sales tax. However, this regulation has no impact on customers located outside of the European Union. Deliveries to customers outside the Community are automatically tax-exempt. Deliveries within the Community to institutional customers outside of Germany are exempted from the German tax (VAT) only if the customer provides the supplier with his/her VAT number. The VAT number (value added tax identification number) is a tax registration number used in the countries of the European Union to identify corporate entities doing business there. It starts with a country code (e.g. FR for France, GB for Great Britain) and follows by numbers.

VAT no.: _____

(Institutes / companies in EU countries only)

Purchase Order No.: _____

Delivery address / Invoice address:
Name of recipient, University, Institute, Street name and Street number, Postal Code, Country

_____

_____

_____

_____

_____

_____

_____

Date and Signature: _____

Credit Card Payment (optional) - You will receive an invoice.

VISA, MasterCard, AMERICAN EXPRESS

Please use the Credit Card Token Generator located at the website below to create a token for secure payment. The token will be used instead of your credit card number.

Credit Card Token Generator:
https://www.wiley-vch.de/editorial_production/index.php

Please transfer your token number to the space below.

Credit Card Token Number

Price list for reprints (The prices include mailing and handling charges. All Wiley-VCH prices are exclusive of VAT)

No. of pages	Price (in Euro) for orders of					
	50 copies	100 copies	150 copies	200 copies	300 copies	500 copies
1-4	345	395	425	445	548	752
5-8	490	573	608	636	784	1077
9-12	640	739	786	824	1016	1396
13-16	780	900	958	1004	1237	1701
17-20	930	1070	1138	1196	1489	2022
for every additional 4 pages	147	169	175	188	231	315

Wiley-VCH Verlag GmbH & Co. KGaA; Location of the Company: Weinheim, Germany;
Trade Register: Mannheim, HRB 432833, Chairman of the Board: Mark Allin
General Partner: John Wiley & Sons GmbH, Location: Weinheim, Germany
Trade Register Mannheim, HRB 432296, Managing Director: Sabine Steinbach

WILEY-VCH