

Molecular design of light-responsive hydrogels, for in-situ generation of fast and reversible valves for microfluidic applications

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Materials

All reagents and chemicals were obtained from commercial sources unless stated otherwise and used without further purification. Dichloromethane was dried over an alumina oxide column prior to use in the synthesis of 6-bromohexyl acrylate. The UV-photoinitiator (phenylphosphoryl)-bis(mesitylmethanone) (Irgacure 819) was purchased from Ciba Speciality Chemicals. All other chemicals were purchased from Sigma-Aldrich.

¹H NMR and ¹³C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer using CDCl₃ as solvent and tetramethylsilane (TMS) as internal standard. The splitting patterns are designated as: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. The Matrix-Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-ToF) mass spectrometry was performed using a Biosystems Voyager-De Pro spectrometer with α -cyano-4-hydroxycinnamic acid or *trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenylidene]malononitrile as a matrix. **1** was purified on recycle GPC using a Shimadzu LC-10AD VP liquid chromatograph, a Shimadzu SPD-10AV VP UV-Vis detector and two JAIGEL columns (2.5H and 2H) from Japan Analytical Industry with chloroform as solvent.

Sample polymerisation was performed using a Dolan-Jenner Industries Fiber-Lite LMI White LED lamp at full power (780 lumen max) at a distance of 1cm for disk preparation and 2cm for film preparation from the samples to ensure uniform irradiation of all apertures.

The merocyanine content UV-Vis measurements were performed on a Perkin Elmer Lambda 750 UV-Vis-NIR spectrophotometer equipped with a 150mm integrating sphere containing a lead sulfide (PbS) and photomultiplier tube (PMT) detector. Between the measurement and exchanging the hydrochloric acid medium, the sample was allowed to stabilise for 15 minutes inside the UV-Vis spectrophotometer.

The molecular kinetic measurements of films were performed on a Varian Cary 50 Probe UV-Vis spectrometer with actuation of films carried out by illumination using a Dolan-Jenner Industries Fiber-Lite LMI LED lamp at full power (780 lumen max) at 6cm distance from sample. Assessment of the macroscopic swelling of gel disks were performed on an Aigo GE-5 digital microscope. Sample actuation was performed using a Dolan-Jenner Industries Fiber-Lite LMI LED lamp at full power (780 lumen max) at a distance of 4cm from samples. The area of the disks was measured using ImageJ software (See Figure S8). The same magnification was used for all hydrogel disks, allowing direct comparison using the pixel values.

Flow analysis and microfluidic valve testing was determined using a Fluigent L FRP flow meter (0-1000uL) and FRP flow-board. Water flow was provided using a constant head platform that was custom designed in-house. The actuating light source for the valve was a blue LED with peak emission around 450 nm (Kingsbright KA-3529AQB25Z4S).

Synthesis of 1,6-bromohexanol

1,6-bromohexanol was synthesised according to a procedure reported by Stumpel *et al.*^{S1}, reacting 1,6-hexanediol with hydrobromic acid. For analysis on TLC, a para-anisaldehyde staining agent was used.

¹H-NMR (400 MHz, CDCl₃, 25°C, TMS): δ = 3.66 (q, J = 6.4 Hz, 2H, HOCH₂), 3.42 (t, J = 6.8 Hz, 2H, BrCH₂), 1.96 – 1.80 (m, 2H, BrCH₂CH₂), 1.59 (p, J = 6.7 Hz, 2H, HOCH₂CH₂), 1.53 – 1.33 (m, 2H, CH₂ alkane), 1.27 (s, 1H, OH). ¹³C-NMR

(100 MHz, CDCl₃, 25°C, TMS): δ = 62.80 (HO-CH₂), 33.83 (Br-CH₂), 32.71 (Br-CH₂-CH₂), 32.53 (HO-CH₂-CH₂), 27.94 (CH₂ alkane), 24.95 (CH₂ alkane).

Synthesis of 6-bromohexyl acrylate

6-bromohexyl acrylate was synthesised according to a procedure reported by Stumpel *et al.*^{S1}, reacting 1,6-bromohexanol with acryloyl chloride.

¹H-NMR (400 MHz, 25°C, CDCl₃, 25°C, TMS): δ = 6.40 (dd, J = 17.3, 1.5 Hz, 1H, trans-HC=C), 6.12 (dd, J = 17.3, 10.4 Hz, 1H, H₂C=CH), 5.82 (dd, J = 10.4, 1.5 Hz, 1H, cis-HC=C), 4.16 (t, J = 6.6 Hz, 2H, ROCH₂), 3.54 (t, 0.8H, ClCH₂), 3.41 (t, 1.3H, BrCH₂), 1.93 – 1.84 (m, 1.3H, BrCH₂CH₂), 1.84 – 1.74 (m, 0.8H, ClCH₂CH₂), 1.69 (q, J = 6.7 Hz, 2H, ROCH₂CH₂), 1.54 – 1.35 (m, 4H, CH₂ alkane). Partial exchange of bromine by chlorine is observed in the NMR experiment. ¹³C NMR (100 MHz, CDCl₃): δ = 166.31 (C=O), 130.57 (CH=CH₂), 128.58 (CH=CH₂), 64.44 (OCH₂), 44.95 (ClC), 33.70 (BrC), 32.64 (BrCH₂CH), 32.48 (ClCH₂CH), 28.49 (OCH₂C), 27.83 (CH₂ alkane Cl), 26.55 (CH₂ alkane Cl), 25.33 (CH₂ alkane Br), 25.21 (CH₂ alkane Br).

Synthesis of Sp-6'-OH

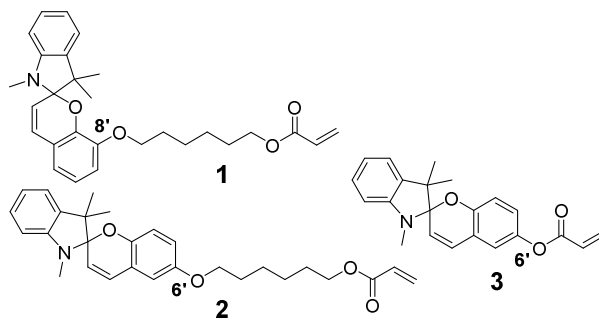
Sp-6'-OH was synthesised according to a procedure reported by Stumpel *et al.*^{S1} condensing 1,3,3-trimethyl-2-methyleneindoline with 2,5-dihydroxybenzaldehyde.

¹H-NMR (400 MHz, CDCl₃, 25°C, TMS): δ = 7.16 (t, J = 7.6 Hz, 1H, CH Ar), 7.06 (d, J = 6.5 Hz, 1H, CH Ar), 6.83 (t, J = 6.9 Hz, 1H, CH Ar), 6.76 (d, J = 10.2 Hz, 1H, NCCH=CH), 6.64 – 6.53 (m, 3H CH Ar), 6.51 (d, J = 7.7 Hz, 1H, CH Ar), 5.70 (d, J = 10.2 Hz, 1H, NCCH=CH), 4.36 (s, 1H, OH), 2.72 (s, 3H, NCH₃), 1.30 (s, 3H, CCH₃), 1.15 (s, 3H, CCH₃). ¹³C-NMR (100 MHz, CDCl₃, 25°C, TMS): δ = 148.65, 148.20, 136.81, 128.99, 127.55, 121.46, 120.48, 119.27, 119.00, 116.34, 115.59, 112.84, 106.73, 103.81, 51.64, 28.94, 25.85, 20.24. MALDI-ToF MS: m/z calcd for C₁₉H₁₉NO₂ (M+H)⁺: 294.15, found: 294.17.

Synthesis of SP-8'-OH

Sp-8'-OH was synthesised according to a procedure reported by Stumpel *et al.*^{S1} condensing 1,3,3-trimethyl-2-methyleneindoline with 2,3-dihydroxybenzaldehyde.

¹H-NMR (400 MHz, CDCl₃, 25°C, TMS): δ = 7.18 (t, J = 7.7 Hz, 1H CH Ar), 7.07 (d, J = 6.9 Hz, 1H, CH Ar), 6.90 – 6.81 (m, 2H CH Ar), 6.81 – 6.70 (m, 2H CH Ar and NCCH=CH), 6.64 (d, J = 7.3 Hz, 1H, CH Ar), 6.53 (d, J = 7.7 Hz, 2H, CH Ar), 5.69 (d, J = 10.3 Hz, 1H, NCCH=CH), 5.23 (s, 1H, OH), 2.75 (s, 3H, NCH₃), 1.30 (s, 3H, CCH₃), 1.18 (s, 3H, CCH₃). ¹³C-NMR (100 MHz, CDCl₃, 25°C, TMS): δ = 147.85, 143.11, 140.72, 136.43, 129.42, 127.65, 121.60, 120.30, 119.58, 119.26, 118.90, 118.03, 115.39, 106.89, 105.32, 51.72, 28.81, 25.93, 19.92. MALDI-ToF MS: m/z calcd for C₁₉H₁₉NO₂ (M+H)⁺: 294.15, found: 294.17.



Synthesis of 1

1 was synthesised according to a procedure reported by Stumpel *et al.*^{S1}, in which SP-8'-OH was used instead of SP-6'-OH. The crude product was pre-purified by subjection to a silica column using 10% ethylacetate in heptane, before injection in a recycle GPC column, using 256 and 360 nm as detection wavelengths. ¹H-NMR (400 MHz, CDCl₃, 25°C, TMS): δ = 7.12 (t, J = 7.6, 1H, CH Ar), 7.04 (d, J = 6.3 Hz, 1H, CH Ar), 6.84 (d, J = 10.2 Hz, 1H, NCCH=CH), 6.82 – 6.76 (m, 2H, CH Ar), 6.72 (d, J = 4.9 Hz, 2H, CH Ar), 6.47 (d, J = 7.7 Hz, 1H, CH Ar), 6.41 (dd, J = 17.3, 1.4 Hz, 1H, trans-HC=C), 6.13 (dd, J = 17.3, 10.4 Hz, 1H, H₂C=CH), 5.82 (dd, J = 10.4, 1.4 Hz, 1H, cis-HC=C), 5.70 (d, J = 10.2 Hz, 1H, NCCH=CH), 4.09 (t, J = 6.7 Hz, 2H, OCH₂), 3.89 – 3.65 (m, 2H, Ar OCH₂), 2.71 (s, 3H, NCH₃), 1.58–1.43 (m, 4H, CH₂ alkane), 1.32 (s, 3H, CCH₃), 1.29–1.05 (m, 4H, CH₂ alkane), 1.18 (s, 3H, CCH₃). ¹³C-NMR (100 MHz, CDCl₃, 25°C, TMS): δ = 166.32, 148.09, 146.20, 145.14, 136.92, 130.40, 129.45, 128.68, 127.33, 121.30, 120.16, 120.02, 119.58, 119.22, 118.98, 118.38, 106.62, 104.12, 70.75, 64.68, 51.37, 29.36, 28.95, 28.42, 25.74, 25.56, 25.36, 20.19. MALDI-ToF MS: m/z calcd for C₂₈H₃₃NO₄ (M+H)⁺: 448.25, found: 448.29. Yield: 50%

Synthesis of 2

2 was synthesised according to a procedure reported by Stumpel *et al.*^{S1}

Synthesis of 3

3 was synthesised according to procedure reported by Ziolkowski *et al.*^{S2,S3}

Substrate preparation

3x3 cm glass slides were cleaned in ethanol by sonication for 15 minutes and dried by a nitrogen flow. Subsequently the glass slides are treated in a UV-Ozone photoreactor (Ultra Violet Products, PR-100) for 20 minutes. The methacrylate glass is created by spincoating a 3-(trimethoxysilyl)-propyl methacrylate solution (1% v/v solution in 1:1 water-isopropanol) on the activated cleaned glass for 45 s at 3000 rpm and subsequently curing at 100°C for 10 minutes. The fluorinated glass substrates are made with the similar procedure, but using 1H,1H,2H,2H-perfluorodecyl-triethoxysilane solution (1% v/v in ethanol).

UV-Vis spectroscopy

The cut-off for all spectra's below ~340 nm is caused by absorbance of glass and PMMA.

According to Beer-Lambert's law (equation 1), the absorbance of a material is the product of the extinction coefficient, the pathlength and the concentration of a compound.

$$A = \epsilon c l \quad (\text{equation 1})$$

Where A is the absorbance, ϵ the extinction coefficient ($\text{l mol}^{-1} \text{cm}^{-1}$), c the concentration (mol l^{-1}) and l the pathlength (cm). Since the material swells and shrinks, the pathlength is varying, as well as the concentration of the compound. This would be problematic for a non-covalently attached film. However, the material that is used is confined in two dimensions since it is covalently attached to the surface (x-y direction). Therefore, it is assumed that the hydrogel only swells in the z-direction. Using this assumption simplifies the measurement, since the pathlength increase is inversely proportional to the concentration i.e. if the thickness increases with 10%, the concentration decreases with 10% in the z-direction.

Since both Sp and MC do not absorb at λ_{max} , the change in absorbance can be used to determine the concentration of McH^+ in the hydrogel (equation 2).

$$\% \text{McH}^+ = A(\lambda_{\text{max H}_2\text{O}}) / A(\lambda_{\text{max 1M HCl}}) \quad (\text{equation 2})$$

Spectral data and size measurements

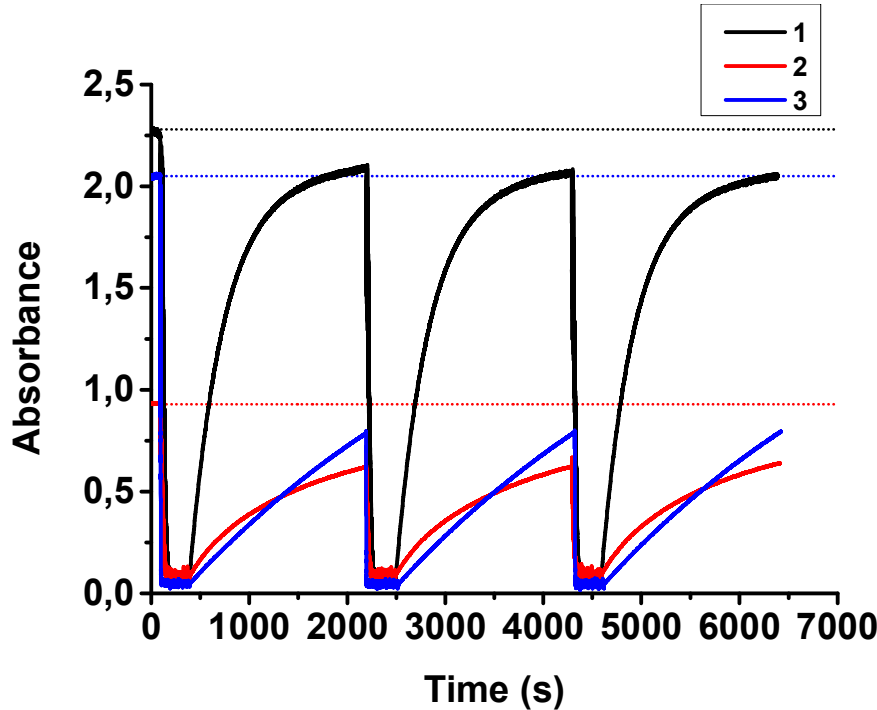


Figure S1. Isomerization kinetics of the hydrogel upon three consecutive illumination and relaxation runs. The absorbance intensity was measured at λ_{max} , 403, 467 and 425 nm for 1, 2 and 3 respectively. The dashed lines are used to indicate the initial absorbance maxima.

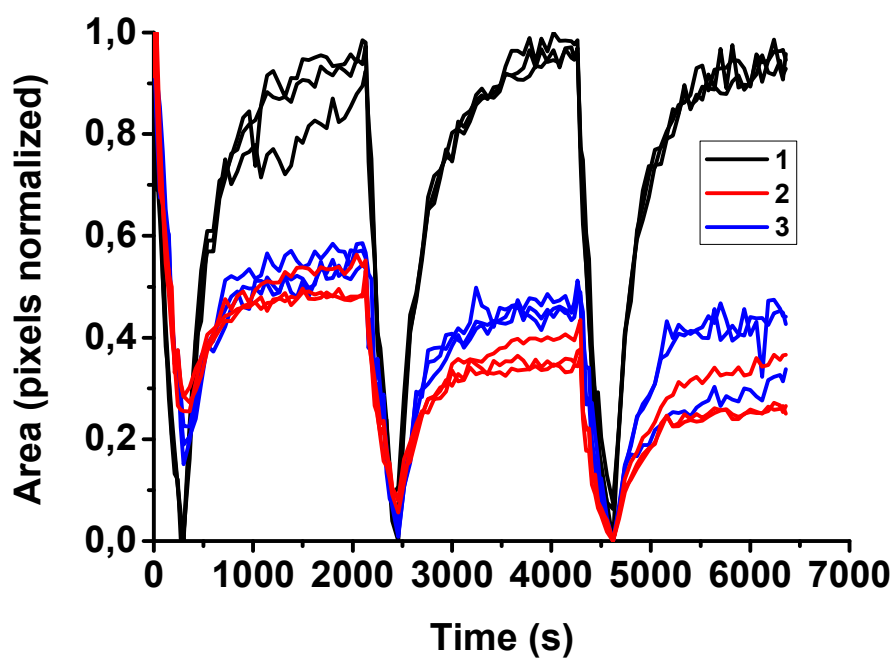


Figure S2. Three individual measurements of the volume changes of the three hydrogel disks that have been used to determine the average volume changes as shown in Figure 7.

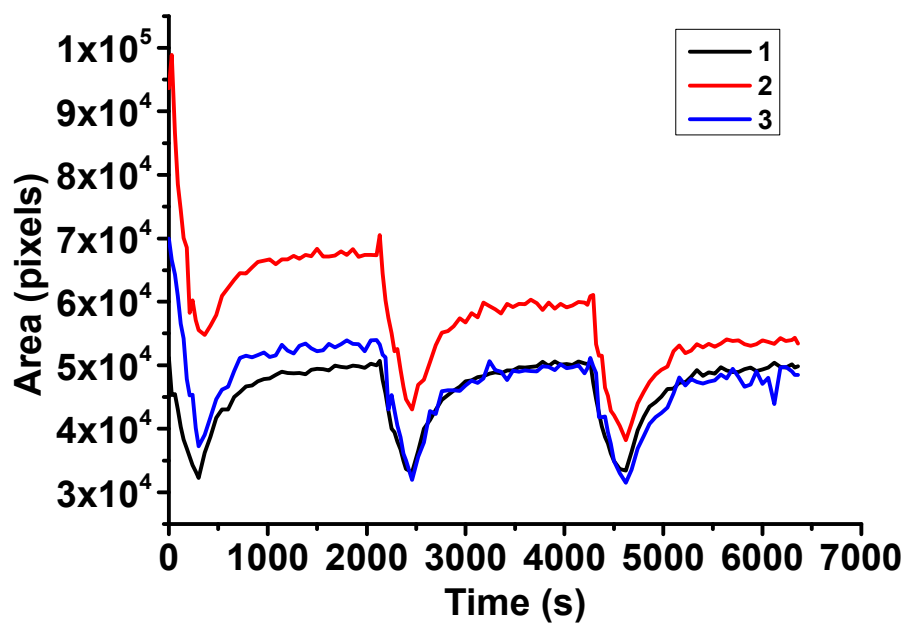


Figure S3. Raw average data (see also figure S2, 7) of the volume changes of the hydrogel disks upon three consecutive illumination and relaxation runs.

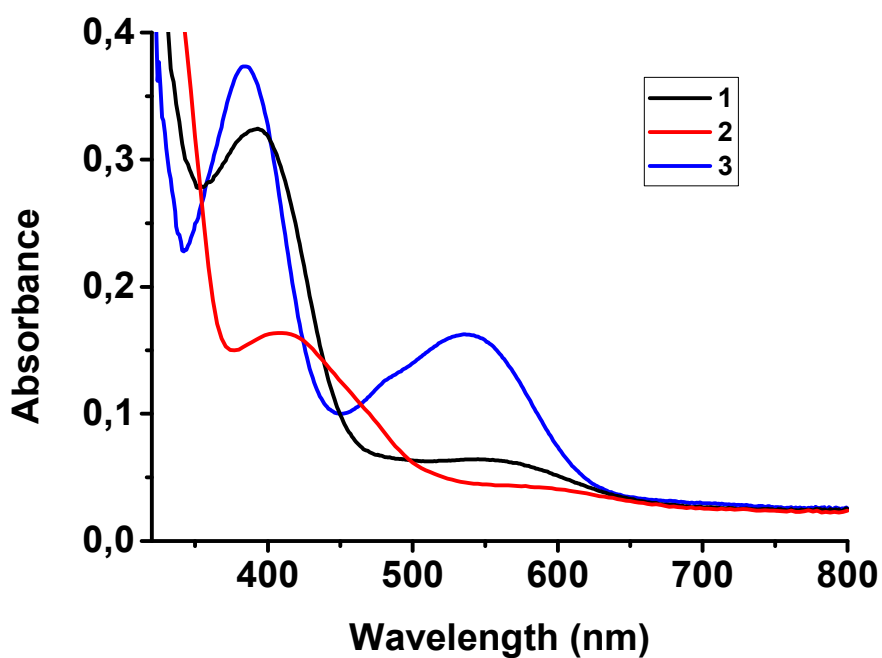


Figure S4. Absorption spectra of the surface attached hydrogels measured in 0.2M triethylamine.

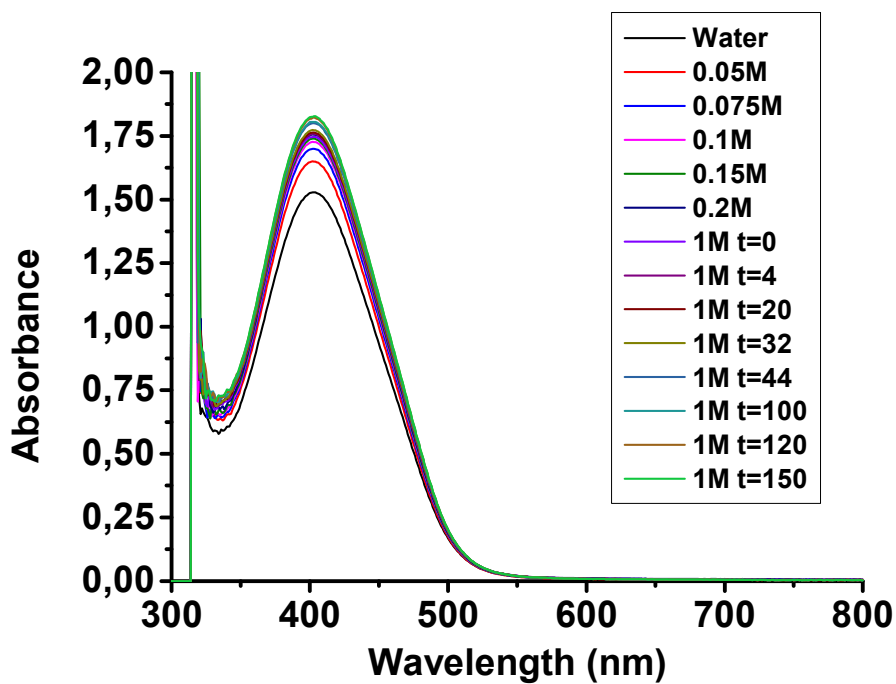


Figure S5. The absorption spectra of the surface attached gel containing derivative **1** at various concentrations of HCl, indicated by the molarity (M). At 1M, the material was allowed to equilibrate for 150 minutes indicated by t=150.

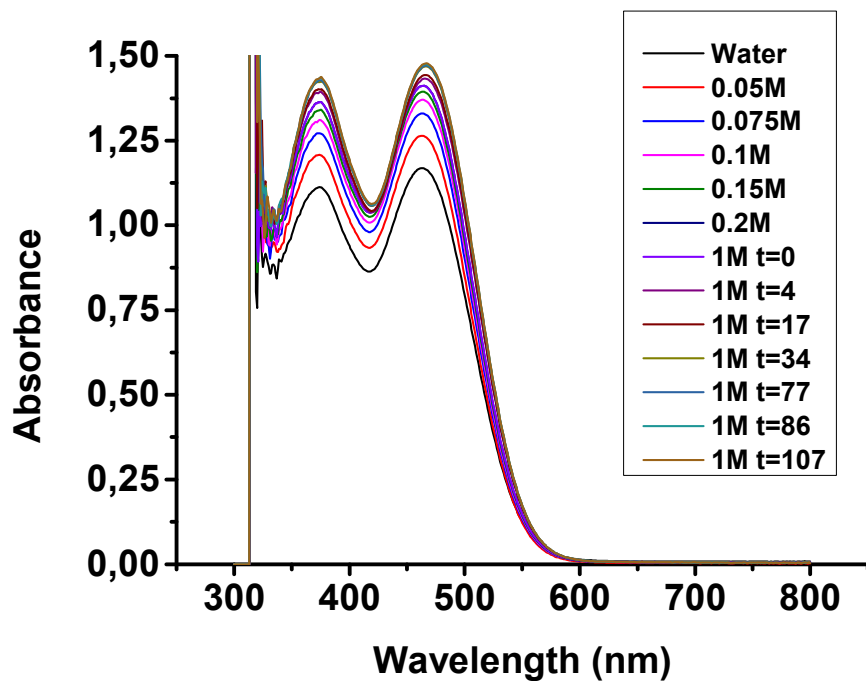


Figure S6. Absorption spectra of the surface attached gel containing derivative **2** at various concentrations of HCl, indicated by the molarity (M). At 1M, the material was allowed to equilibrate for 107 minutes indicated by t=107.

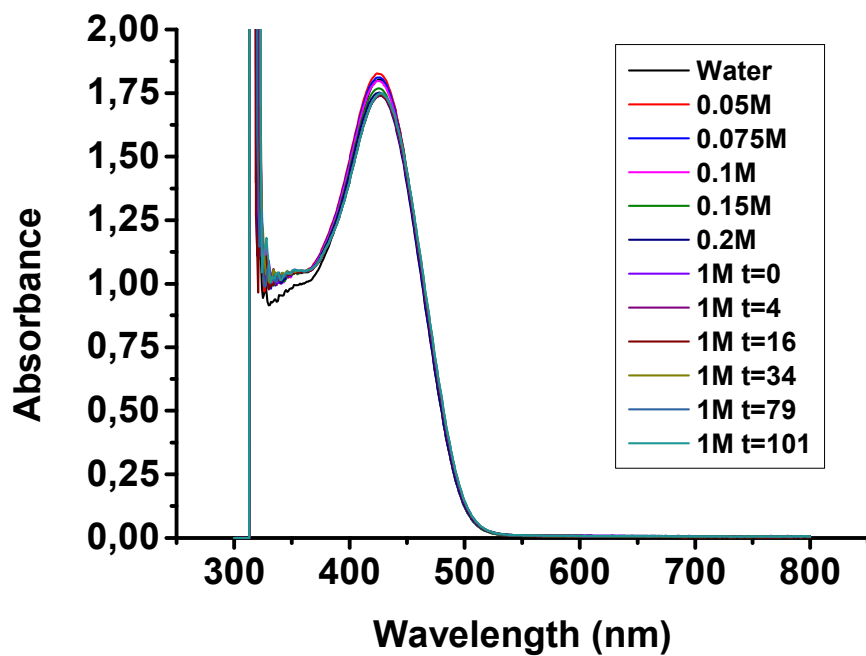


Figure S7. Absorption spectra of the surface attached gel containing derivative **3** at various concentrations of HCl, indicated by the molarity (M). At 1M, the material was allowed to equilibrate for 101 minutes indicated by t=101.

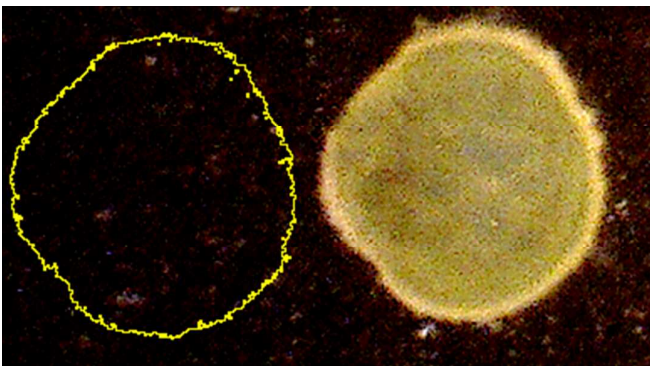


Figure S8. Example of the technique used for measuring the area of a hydrogel disk. The outline of each gel in a microscope photograph was drawn using ImageJ software. The resulting yellow contour (left, with gel removed for clarity) is the outline of the gel (right) and the number of pixels within this contour were counted to calculate the area of the disk.

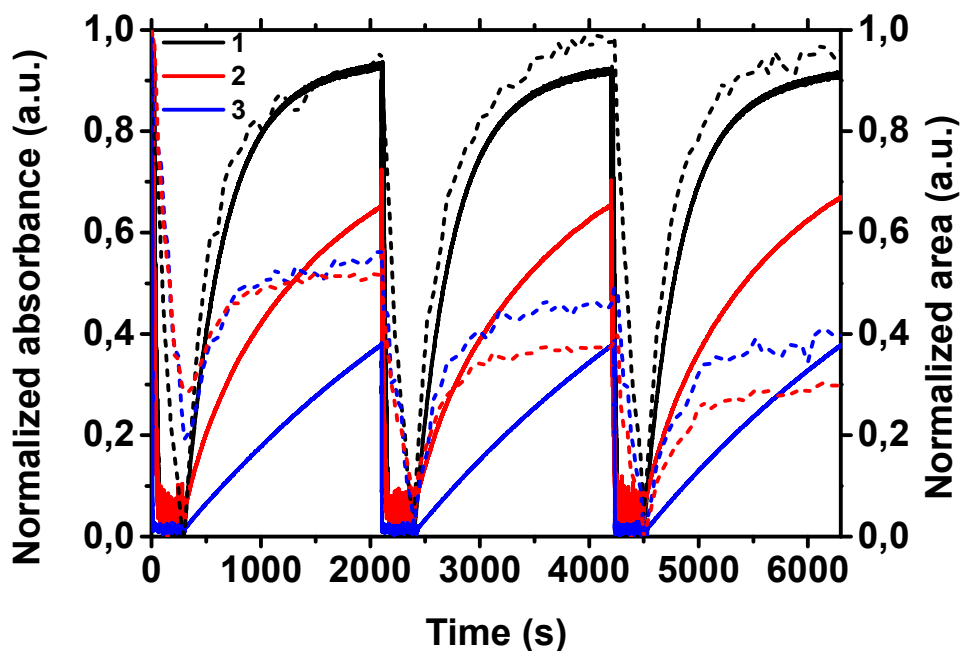


Figure S9. Normalized absorbance (dashed line) and area (solid line) of the hydrogel disk upon three consecutive illumination and relaxation runs.

REFERENCES

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- S2 Ziółkowski, B.; Florea, L.; Theobald, J.; Benito-Lopez, F.; Diamond, D. Self-protonating Spiropyran-co-NIPAM-co-acrylic acid Hydrogel Photoactuators. *Soft Matter* **2013**, *9* (36), 8754-8760.
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