Supporting Information

Molecularly imprinted polymer microspheres containing photoswitchable spiropyran-based binding sites

Tibor Renkecz,[‡] Günter Mistlberger,[‡] Marcin Pawlak,[‡] Viola Horváth,[†] and Eric Bakker[‡]*

[‡]Department of Inorganic and Analytical Chemistry, University of Geneva,

Quai E.-Ansermet 30, CH-1211, Geneva, Switzerland

[†]MTA-BME Research Group of Technical Analytical Chemistry, Hungarian Academy of Sciences–Budapest University of Technology and Economics, Szt. Gellért tér 4., H-1111, Budapest, Hungary

*E-mail address : Eric.Bakker@unige.ch

Synthesis route of SPMA.

1-(2-Hydroxyethyl)-2,3,3-trimethyl-3H-indolium bromide (**1**): A mixture of 2-bromoethanol (2.46 g, 1.39 mmol) and 2,3,3-trimethyl-3H-indole (2.61 g, 16.4 mmol) in 20 mL of dry acetonitrile was refluxed under N_2 atmosphere for 24 h. Then solvent was removed under reduced pressure, residue was suspended in 25 mL of hexane and the mixture was filtered. Obtained solid was then recrystallized from 35 mL $CHCl_3$ giving the product as a pinkish solid (2.75 g, 9.68 mmol, yield 59%). ^{1}H NMR (400 MHz, MeOD): δ = 7.93-7.91 (1H, m), 7.82-7.80 (1H, m), 7.68-7.65 (2H, m), 4.85 (3H, s), 4.72 (2H, t, J = 5 Hz), 4.08 (2H, t, J = 5 Hz), 2.92-2.89 (1H, m), 1.66 (6H, s); ^{13}C NMR (100 MHz, MeOD): δ = 198.80, 141.97, 141.06, 129.56, 128.88, 123.26, 115.23, 100.00, 58.16, 54.67, 50.29, 22.10, 15.60.

9,9,9a-Trimethyl-2,3,9,9a-tetrahydro-oxazolo[3,2-a]indole (**2**): Potassium hydroxide (0.92 g, 16 mmol) was added to water (50 mL) solution of **1** (2.75 g , 9.68 mmol)**.** Mixture was stirred at room temperature for 10 min and then it was extracted with Et_2O (3 x 20 mL). Organic phase was dried with magnesium sulfate and solvent was removed under reduced pressure, giving **2** as yellow oil (1.87 g, 9.17 mmol, yield 95%) which was used directly in the next step.

2-(3',3'-Dimethyl-6-nitro-3'Hspiro[chromene-2,2'-indol]-1'-yl)-ethanol (**3**): A solution of 2-hydroxy-5-nitrobenzaldehyde (2.297 g, 13.75 mmol) and **2** (1.87 g, 9.17 mmol) in 20 mL of ethanol was refluxed for 24 h. After cooling to room temperature, the precipitated solid was filtered, washed with small amount of ethanol and dried to afford desired product (2.62 g, 7.44 mmol, yield 81 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05 (1H, d, J = 3 Hz), 8.01 (1H, dd, J_1 = 9 Hz, J_2 = 3 Hz), 7.22 (1H, t, J = 8 Hz), 7.12 (1H, d, J = 7), 6.95-6.91 (2H, m), 6.78 (1H, d, J = 9 Hz), 6.70 (1H, d, J = 9 Hz), 5.91 (1H, d, J = 10 Hz), 3.86-3.71 (3H, m), 3.52-3.45 (1H, m), 3.39-3.34 (1H, m), 1.31 (3H, s), 1.22 (3H, s); ^{13}C NMR (100 MHz, CDCl3): δ = 160.52, 148.37, 142.25, 137.00, 128.22, 128.61, 126.57, 123.75, 123.30, 122.78, 120.38, 120.00, 116.28, 108.05, 107.79, 60.95, 54.25, 45.87, 22.65, 20.23.

1'-(2-Methacryloyloxyethyl)-3',3'-dimethyl-6-nitrospiro(2H-1benzopyran-2,2'-indoline) (**SPMA**): To a vigorously stirred solution of **3** (2.5 g, 7.10 mmol) and a catalytic amount of N,N-dimethylaminopyridine (0.12 g, 1 mmol) in 30 mL of chloroform, at 0 °C under an atmosphere of nitrogen triethylamine (862 mg, 8.52 mmol) and then chloroform solution (30 mL) of methacryloyl chloride (0.890 mg, 8.52 mmol) were added over a period of ca. 30 min. The reaction mixture was allowed to warm to room temperature overnight. It was then subsequently washed with 0.1 M aqueous HCl, 10% aqueous Na_2CO_3, water, and brine. The organic layer was dried over $MgSO_4$, filtered, and reduced in volume under reduced pressure to yield a purple sticky solid, which was purified on silica gel using hexane/EtOAc (6:1, v/v). Yield: 2.1 g (70%). IR ν_{max} 2965, 2927, 2869, 1716, 1610, 1578, 1518, 1480, 1458, 1378, 1361, 1335, 1295, 1271, 1162, 1125, 1089, 1025, 953, 912, 832, 808, 780, 746, 684, 630, 521 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$):δ = 8.06-8.02 (2H, m), 7.23 (1H, td, J = 8 Hz, 1 Hz), 7.12 (1H, dd, J = 7 Hz, 1 Hz), 6.94-6.91 (2H, m), 6.79-6.72 (2H, m), 6.10 (1H, s), 5.90 (1H, d, J = 10 Hz), 5.59-5.57 (1H, m), 4.32 2H, t, J = 6 Hz), 3.61-3.54 (1H, m), 3.49-3.42 (1H, m), 1.94 (3H, s), 1.30 (3H, s), 1.19 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.2, 159.1, 146.7, 141.1, 136.1, 135.7, 128.3, 127.9, 126.0, 125.9, 122.8, 121.8, 119.9, 118.5, 115.6, 106.8, 106.5, 62.6, 52.8, 42.5, 25.9, 19.9, 18.3.

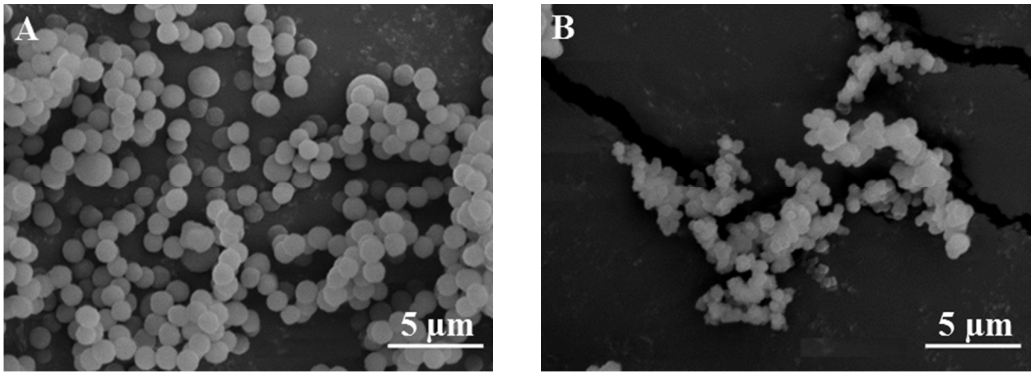

Figure S1. SEM images of molecularly imprinted (A) and non-imprinted (B) polymer microparticles containing MAA-EDMA without SPMA (polymer P1)

Experimental parameters of UV-Vis spectroscopy study and photoswitching experiments of the monomer solutions and polymer particle suspensions.

Spiropyran monomer solution and particle suspension were prepared in toluene and the UV-Vis absorbance spectra were recorded with a CCD-array detector (CCS200, Thorlabs) and a fast wavelength switchable xenon arc lamp as light source (Lambda DG-4, Sutter Instrument Company), both controlled with a LabView interface. The DG-4 wavelength switching device allowed the illumination with, for instance, UV-light for triggering photoreactions and with white light for the measurement of absorbance. The short time required for spectrum acquisition (< 0.1 s) every 10 s avoids undesired photoeffects due to the illumination during measurement. Repeated photoswitching cycles with consecutive UV and Vis irradiation were carried out and the absorbance of the MC form was recorded from the monomer solution and the particle suspension. For UV illumination a 365/20 nm band pass filter (F49-365 ZET Laser Clean UP, Chroma) was used for 160 s and for the visible light manipulation a 500 nm cut on long pass filter (FEL0500, Thorlabs) was applied for 300 s. A suspension of the spiropyran containing MIP microparticles in toluene was prepared and studied with constant agitation with a magnetic stirrer to prevent sedimentation using a 1.0 cm optical path length quartz cuvette at room temperature. Sample interrogation was carried out in a dark box to avoid ambient light.

First-order kinetic rate constants were calculated for the visible light induced back isomerization using the equation $\ln[A_t/A_o]= -kt$, where A_t is the absorbance value at time, A_0 is the maximum value reached after UV irradiation and k is the rate constant.

Photoisomerization properties of the MIP microspheres by absorbance measurements.

The photoswitching properties of the synthesized microparticles and the SPMA monomer were investigated by UV-Vis spectroscopy in cuvette experiments. Upon UV irradiation of 50 μM spiropyran methacrylate in toluene the absorbance peak of the open, merocyanine form appears with a peak maximum at 610 nm due to the conjugation between the two aromatic rings (Figure S2A) and the solution turns blue. Visible light illumination gradually decreases the absorbance peak of the merocyanine (Figure S2B) and the solution returns to colorless.

The same experiment was done with the spiropyran containing MIP microparticles (P6) in toluene suspension at 250 μg mL^{-1}. The background of the MIP microparticle suspension is elevated compared to the solution

spectrum due to light scattering of the particles, yet processable spectral data could be acquired. A similar, but somewhat broader absorbance peak was observed upon UV irradiation as in the monomer solution (Figure S3A). The absorbance peak decreased when visible light was used to switch the MC form back to SP (Figure S3B). This confirms the successful incorporation of the spiropyran monomer into the polymer matrix. Spiropyrans immobilized to polymer matrices tend to exhibit a hypsochromic (blue) shift in their UV-Vis spectrum compared to that in solution, indicating a more polar micro-environment.[1] We could not observe such a shift in case of the MIP microspheres which suggests that the methacrylate based polymer is rather apolar.

The back isomerization of the merocyanine form into spiropyran follows first order kinetics.[2] The evaluation of rate constants in the solution and in the polymer can indicate how the molecular photoswitching is affected by anchoring the spiropyran moiety to the solid phase. For the solution phase the rate constant was calculated as $1.5\pm0.3 \times 10^{-2}$ s^{-1}, while a decreased value of $4.9\pm0.2 \times 10^{-3}$ s^{-1} was obtained for the polymer particles. The decrease is attributed to the hindered ability of molecular motion of the merocyanine in the polymer matrix during photoswitching.

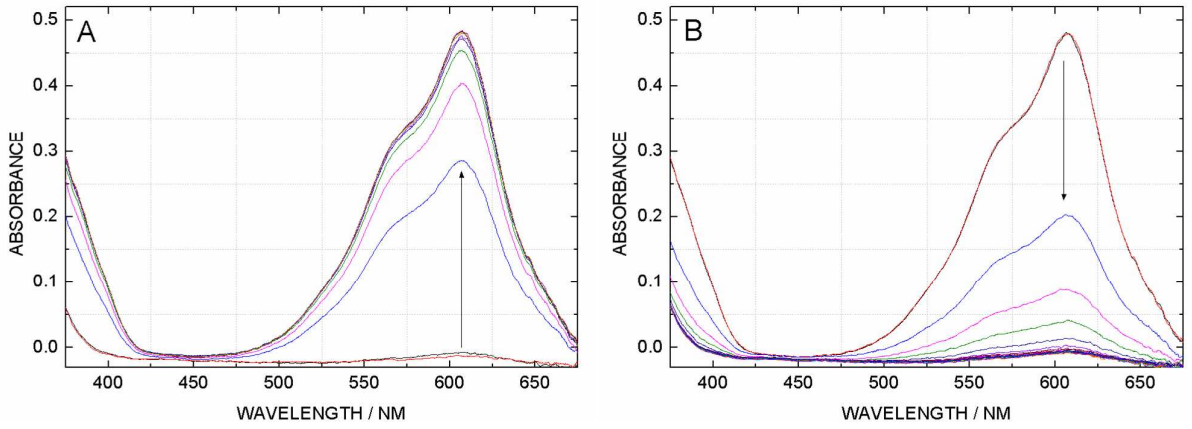

Figure S2. UV-Vis spectral changes with 10 s time increments of 50 µM SPMA in toluene irradiating with λ_{irr}= 365/20 nm UV light for 90 s (A) and with visible light (λ_{irr}= >500 nm) for 250 s after UV activation (B)

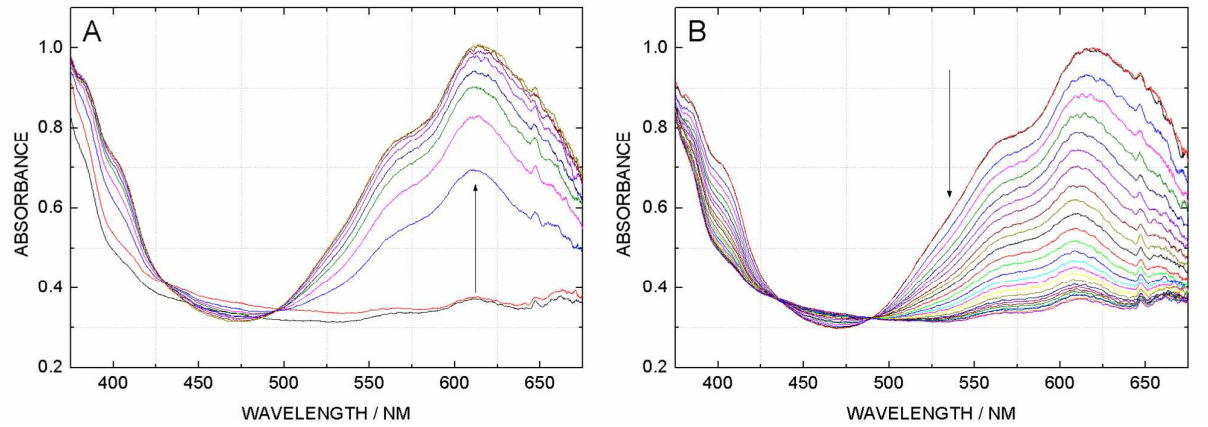

Figure S3. UV-Vis spectral changes with 10 s time increments of 250 μg mL^{-1} P6 MIP suspension in toluene irradiating with λ_{irr}= 365/20 nm UV light for 90 s (A) and with visible light (λ_{irr}= >500 nm) for 250 s after UV activation (B)

It is well-known that undesired photobleaching processes can occur when spiropyran is exposed to UV light for longer time periods. Therefore, repeated photoswitching cycles were applied to both systems to gain information about the stability of SPMA. SP–MC transformation was repeatedly triggered by photoswitching cycles of six UV and subsequent Vis light irradiation on 50 μM monomer solution and on the polymer particles in toluene. Relative absorbance values were calculated by dividing the measured absorbance with $A_{max.}$ In **Error! Reference source not found.**4, where the relative absorbance is plotted as a function of time of interrogation, the values of the monomer solution (red line) are only reduced to a small extent at the end of the consecutive cycles, which suggests a suitable photostability of the monomer.

In case of the suspension sample (black line) the relative absorbance values were calculated after subtracting the absorbance value of 250 μg mL^{-1} P1 (not photoswitchable) suspension, thus eliminating the contribution of particle scattering to the spectrum. It has to be noted that in the polymer matrix the open merocyanine form is already present to a measurable extent before UV activation indicated by an inital relative absorbance of 0.28, the particles were pale purple in their dry state. Polar ester groups, carboxyl and hydroxyl groups on the polymer can interact with the merocyanine moiety.[1] Hence, there is a slight shift in the SP→MC equilibrium due to the interactions between the open merocyanine form and the carboxyl and ester groups on the polymer backbone.

At the end of the sixth cycles the relative absorbance of merocyanine decreased to approximately 63% of its original value. In contrast to the literature[3] where the covalent attachment of 1% w/w spiropyran to a polymer matrix (PMMA) surface increased its photostability, the opposite effect was observed here. This might be related to the very high concentration of spiropyran monomer in the porous polymer matrix that may have resulted in the aggregation of merocyanine molecules and the loss of their photoswitching characteristics. Such an aggregation phenomenon was observed with polymer beads that were surface-modified with spiropyran units.[4] Furthermore, when we interrogated a SPMA solution with subsequent photoswitching cycles at elevated concentration (1 mM), the photobleaching of the spiropyran became conspicuous due to the abovementioned aggregation. Moreover, we have to add that in these cuvette experiments the constant motion of the particles in the stirred solution in front of the small illuminated area causes uncertainty in the repeated photoswitchability.

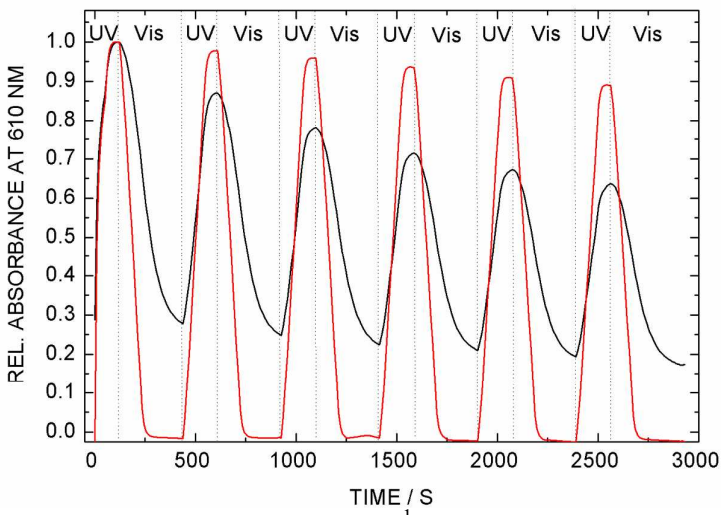

Figure S4. Photoswitching cycles of 250 μg mL^{-1} spiropyran containing MIP microsphere suspension (black line) and 50 μM SPMA (red line) in toluene (UV λ_{irr}= 365/20 nm, Vis λ_{irr}= >500 nm)

Method proposed by Rampey et al. for the calculation of additional parameters based on the Freundlich model:[5]

Affinity distribution:

$$N(K) = 2.303am(1-m^2)K^{-m} \qquad \text{(SEq1)}$$

where K is the affinity constant, calculated as the reciprocal concentration, *a* is the preexponential factor, *m* is the heterogeneity index, and N(K) is the number of binding sites with a given affinity.

Number of binding sites per gram of polymer:

$$N_{K_{\min}-K_{\max}} = a(1-m^2)(K_{\min}^{-m} - K_{\max}^{-m}) \qquad \text{(SEq2)}$$

Weighted average affinity constant:

$$K_{K_{\min}-K_{\max}} = \left(\frac{m}{m-1}\right)\left(\frac{K_{\min}^{1-m} - K_{\max}^{1-m}}{K_{\min}^{-m} - K_{\max}^{-m}}\right) \qquad \text{(SEq3)}$$

where $K_{\min} = \frac{1}{F_{\max}}$ and $K_{\max} = \frac{1}{F_{\min}}$. F values are between the range of experimental values. Thus, these calculations are limited and valid only in the experimentally determined concentration range.

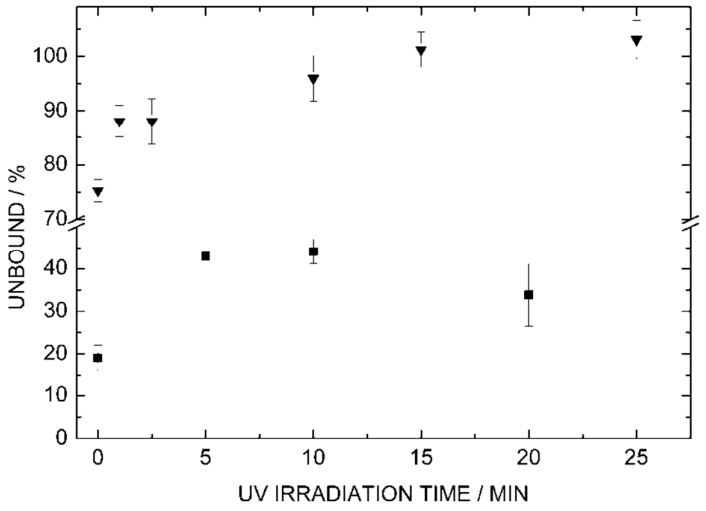

Figure S5. Release kinetics of the template as a function of the irradiation time measured on the photoswitchable terbutylazine imprinted polymer microspheres in acetonitrile (triangle) and toluene (square) (UV λ_{irr}= 365 nm, power= 4 W)

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