Photocontrolled self-assembly of azobenzene nanocontainers in water: light-triggered uptake and release of small molecules.

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1. Materials and procedures

Materials and general procedures

Commercially available compounds were reagent grade quality and were used without further purification. The ^{1}H and ^{13}C NMR spectra were recorded at 298 K in $CDCl_3$, DMSO-d_6, D_2O or CD_3OD with a Varian Mercury 400 MHz or with a Varian DDR 500 MHz spectrometer equipped with a 5-mm probe. Chemical shifts were calibrated using the internal $CDCl_3$ or benzene-d_6 resonances that were referenced to TMS. All chemical shifts are quoted using the δ scale and all coupling constants (J) are expressed in Hertz (Hz). ^{1}H-NMR spectra in D_2O were recorded with a PRESAT (Varian) pulse sequence to remove the signal of residual non-deuterated water.

Photophysical measurements

The experiments were carried out in air-equilibrated solution at 298 K unless otherwise noted. UV–vis absorption spectra were recorded with a PerkinElmer λ40 spectrophotometer and a Varian Cary 50 Bio spectrophotometer using quartz cells with path length of 1.0 cm, 0.2 cm or 0.1 cm. Luminescence spectra were performed with a PerkinElmer LS-50 or an Edinburgh FLS920 spectrofluorimeter equipped with a Hamamatsu R928 phototube. Lifetimes were measured by the above-mentioned Edinburgh FLS920 spectrofluorimeter equipped with a TCC900 card for data acquisition in time-correlated single-photon counting experiments (0.5 ns time resolution). Photochemical reactions were performed at room temperature on thoroughly stirred air equilibrated solutions by using a Helios Italquartz Polymer 125 medium pressure Hg lamp (125 W).

Photoisomerization experiments monitored by ^{1}H NMR were performed in air equilibrated DMSO-d_6 solutions irradiated directly inside the NMR tube. The selection of the desired irradiation wavelength (365 or 436 nm) was accomplished by the use of an appropriate interference filter. The number of incident photons in the solution experiments, determined by ferrioxalate actinometry in its micro version.[1] The E→Z photoisomerization quantum yield (λ_{irr}= 365 nm) was determined from the disappearance of the ππ* absorption band of the E-azobenzene unit of the reactant at low conversion percentages (<10%, extrapolation to t=0 was made). The fraction of light transmitted at the irradiation wavelength was considered in the calculation of the yields. The estimated experimental errors are 2 nm on the band maximum, 5% on the molar absorption coefficient and luminescence lifetime and 10% on the quantum yield.

Dynamic light scattering and surface tension measurements

DLS measurement were recorded with a Malvern Instruments DLS ZetaSizer Nano-ZS.

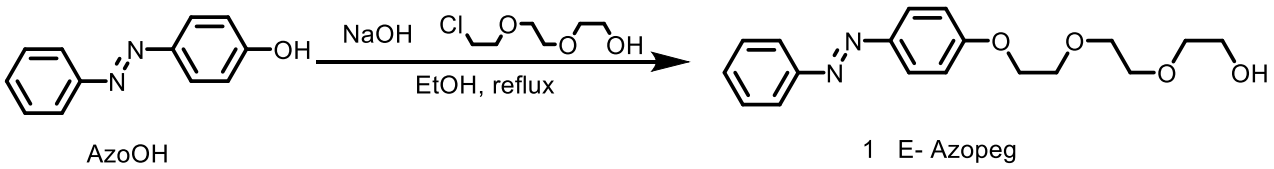
Surface tension measurement were recorded with the pendant drop method using a KSV CAM101 instrument under ambient conditions. The side profiles of drops were recorded for image analysis. The shape of the drop was recorded in a time range of 0–30 s, collecting images every 1 s.

Cryo-electron microscopy

Three microliters of a 2.2 mM solution of *E*-**AzoPeg** in H_2O/EtOH (95:5, v/v) was applied to lacey carbon films on 400-mesh copper transmission electron microscopy (TEM) grids. Excess liquid was blotted away with filter paper until a thin vicinal film remained. The liquid containing the fractions

contents was vitrified in liquid nitrogen and cooled with liquid ethane by plunge freezing. Electron micrographs of cryo-preserved samples were collected on an FEI Tecnai 12 (120 kV, LaB6 filament, FEI Company, Hillsboro, Oregon, United States) electron microscope equipped with a Gatan UltraScan 1000 (2K × 2K, Gatan, Pleasanton, California, United States) pixel CCD camera and a Gatan 626 cryo-holder. The nominal magnification was × 67 000, which results in an actual magnification of × 98 000 at the level of the CCD camera. The defocus range for the cryo-EM dataset was −1.0 to −2.0 μm.

2. Synthetic protocols and characterization of compounds



2-(2-(2-(4-(phenylazophenoxy)ethoxy)ethoxy)ethanol (1):[2,3] To a solution of 4-phenylazophenol (2.0 g, 10 mmol, 1 eq) in absolute ethanol (30 mL) was added NaOH (410 mg, 10 mmol, 1 eq) the mixture was stirred for 30 min until complete dissolution of NaOH. A solution of 2-[2-(2-chloroethoxy)ethoxy]ethanol (4.3 g, 25 mmol, 2.5 eq) in EtOH (5mL) was added dropwise. The solution was heated under stirring for 60 hours at 75°C. The solvent was evaporated under reduced pressure and the obtained red oil was purified by distillation in a Kugelrohr apparatus (Buchi) at 125°C and 0.5 mmHg. The product was obtained as an orange crystalline solid (2.5 g, 7.7 mmol, yield 76%).

^{1}HNMR (400 MHz, $CDCl_3$, TMS/ppm): δ 7.91 (d, J = 9.0, 2H), 7.87 (d, *J* = 7.0 Hz, 2H), 7.49 (t, J = 7.0 Hz 2H), 7.42 (t, 7.0 Hz 1H), 7.02 (d, J = 9.0 2H), 4.19 (m, 2H), 3.87 (m, 2H), 3.70 (m, 6H), 3.60 (m, 2H).

^{13}C-NMR (DMSO-d_6): δ 161.2, 152.7, 147.1, 130.4, 129.0, 124.7, 122.6, 114.8, 72.5, 70.8, 70.3, 69.6, 67.6, 61.7.

1) *Photochemistry and Photophysics: Concepts, Research, Applications*, V. Balzani, P. Ceroni, A. Juris, Wiley-VCH, 2014, ch. 12
2) F. Tian, D. Jiao, F. Biedermann, O. A. Scherman, *Nature Communications* **2012**, *3*, 1207
3) J. Liu, C. S. Y. Tan, O. A. Scherman, *Angew. Chem. Int. Ed.* **2018**, *57*, 8854.

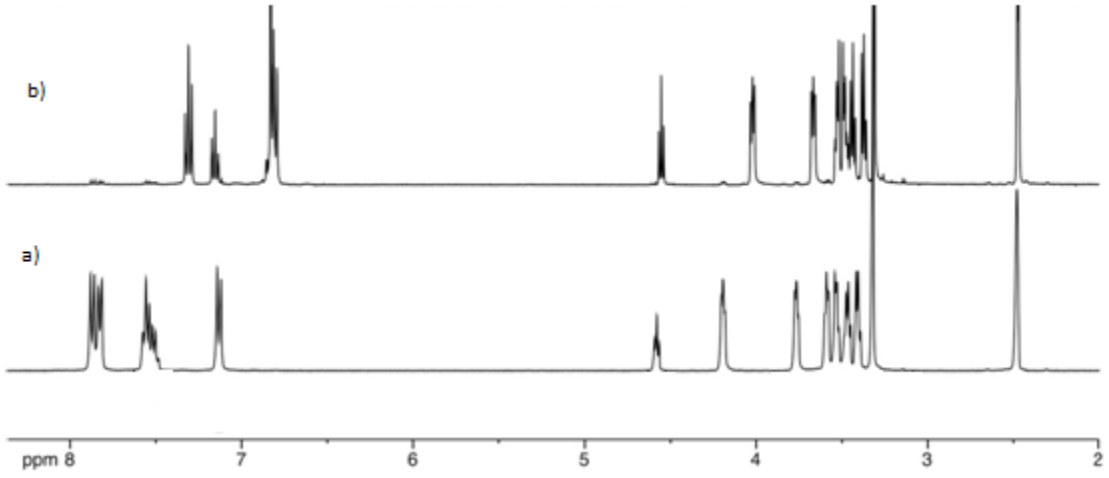


Figure S1 a) ^{1}H-NMR (400 MHz, DMSO-d_6, 278 K) spectra of a 3 mM solution of *E*-**AzoPeg** b) the same sample after irradiation with 365 nm light (photostationary state 99:1 Z/E isomer).

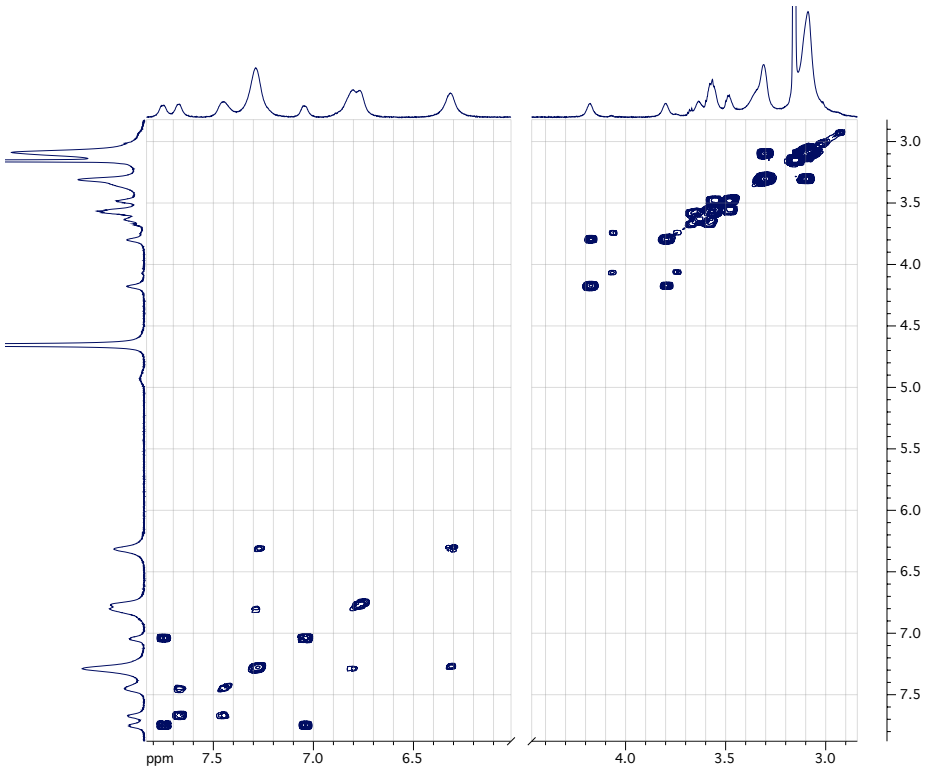


Figure S2 ^{1}H-^{1}H-COSY (500 MHz, D_2O/CD_3OD 95:5 v/v, 278 K) of a 3 mM solution of *E*-**AzoPeg**.

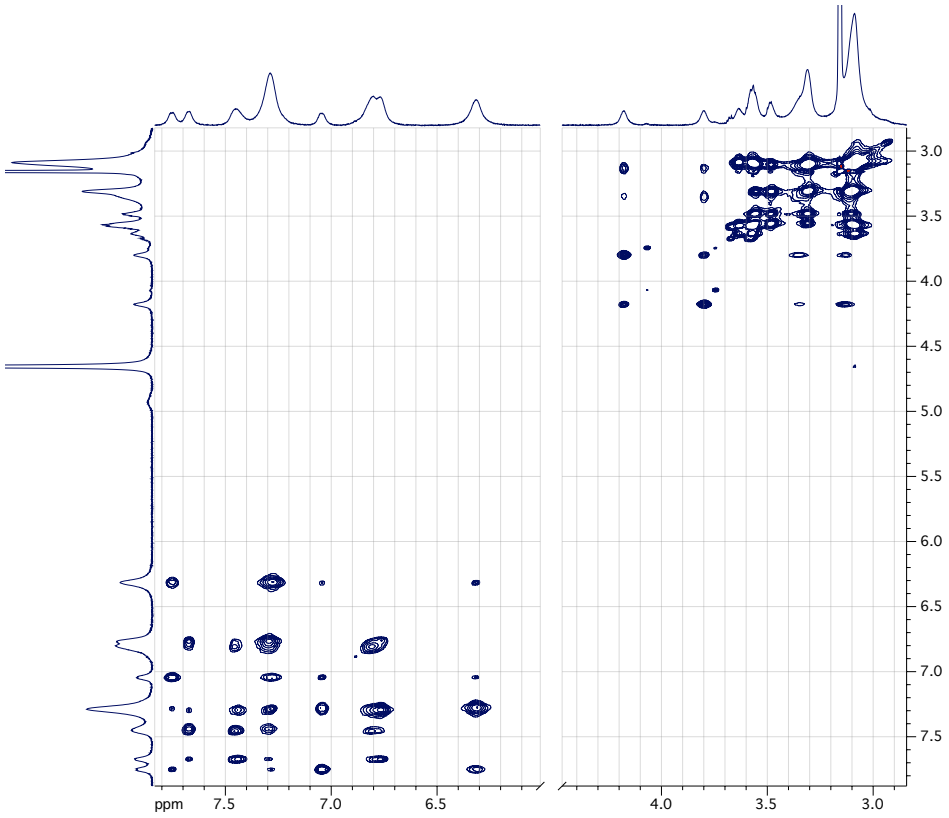


Figure S3 ^{1}H-^{1}H-TOCSY (500 MHz, D_2O/CD_3OD 95:5 v/v, 278 K) of a 3 mM solution of *E*-**AzoPeg**.

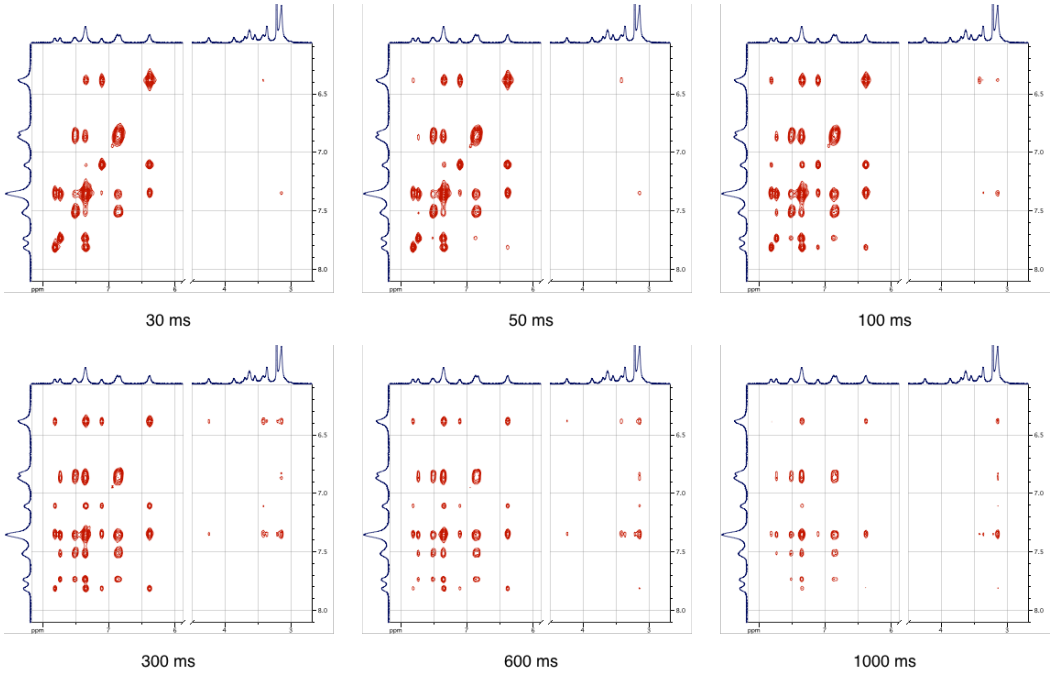


Figure S4 NOESY spectra (500 MHz, D_2O/CD_3OD 95:5 v/v, 278 K) of a 3 mM solution of *E*-**AzoPeg** at increasing mixing times showing exchange correlations and the buildup of NOEs correlations in the aggregated form followed by transfer of NOEs toward the free *E*-**AzoPeg** for long mixing times.

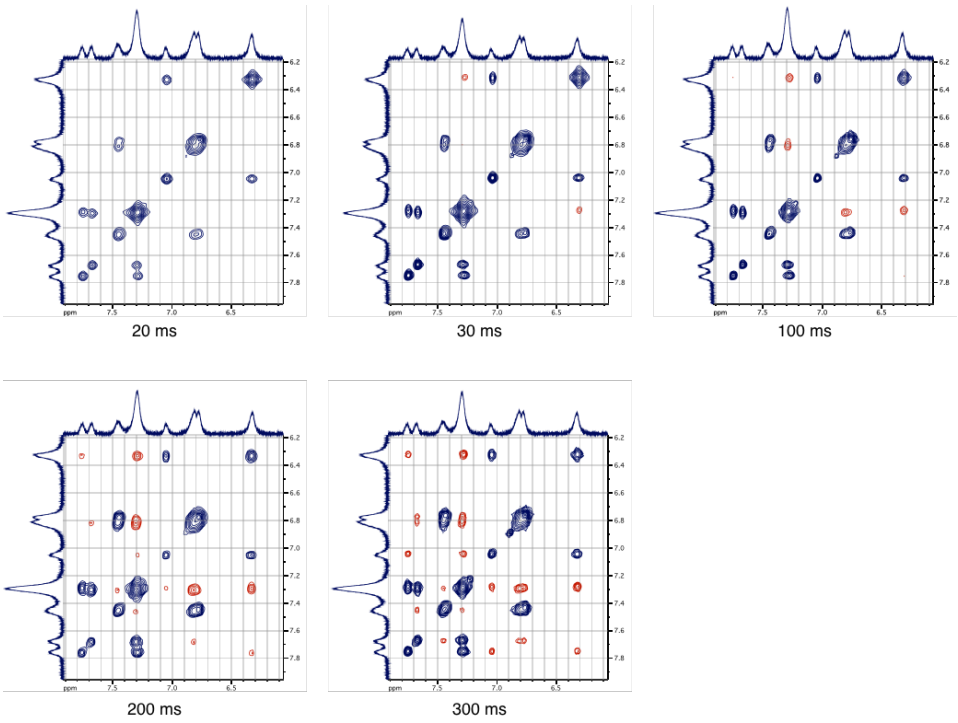


Figure S5 ROESY spectra (500 MHz, D_2O/CD_3OD 95:5 v/v, 278 K) of a 3 mM solution of *E*-**AzoPeg** at increasing mixing times showing exchange correlations (blue) and the buildup of NOEs correlations in the aggregated form followed by transfer of NOEs toward the free *E*-**AzoPeg** for long mixing times (red).

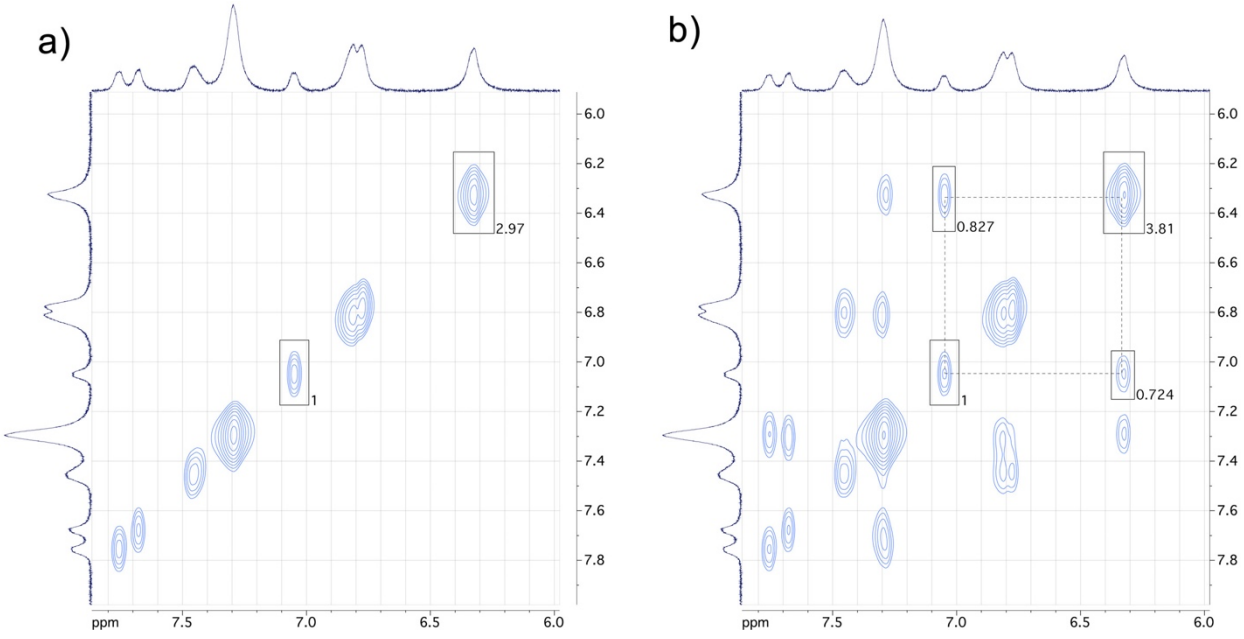


Figure S6 EXSY spectra (500 MHz, D_2O/CD_3OD 95:5 v/v, 278 K) of a 3 mM solution of *E*-**AzoPeg** with a mixing time of a) 0 ms and b) 20 ms. The solid boxes correspond to the exchange peaks between the protons of the free and aggregated form of *E*-**AzoPeg**. Rate constants were calculated using the software EXSYCalc developed by Mestrelab research.

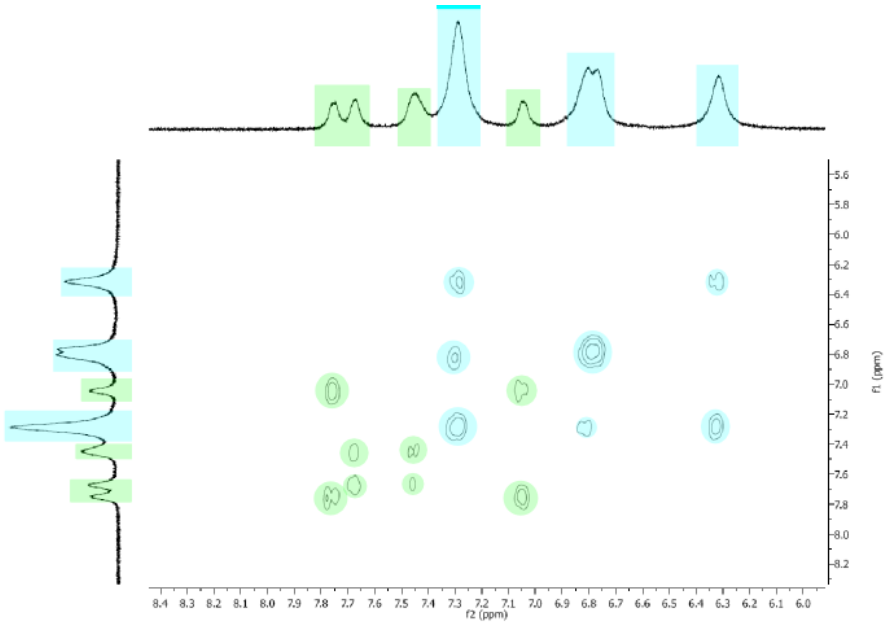


Figure S7 Aromatic region of the ^{1}H-^{1}H-COSY (400 MHz, D_2O/CD_3OD 95:5 v/v, 278 K) showing the presence of *E*-**AzoPeg** (green) and nano-aggregates (light blue) of *E*-**AzoPeg**.

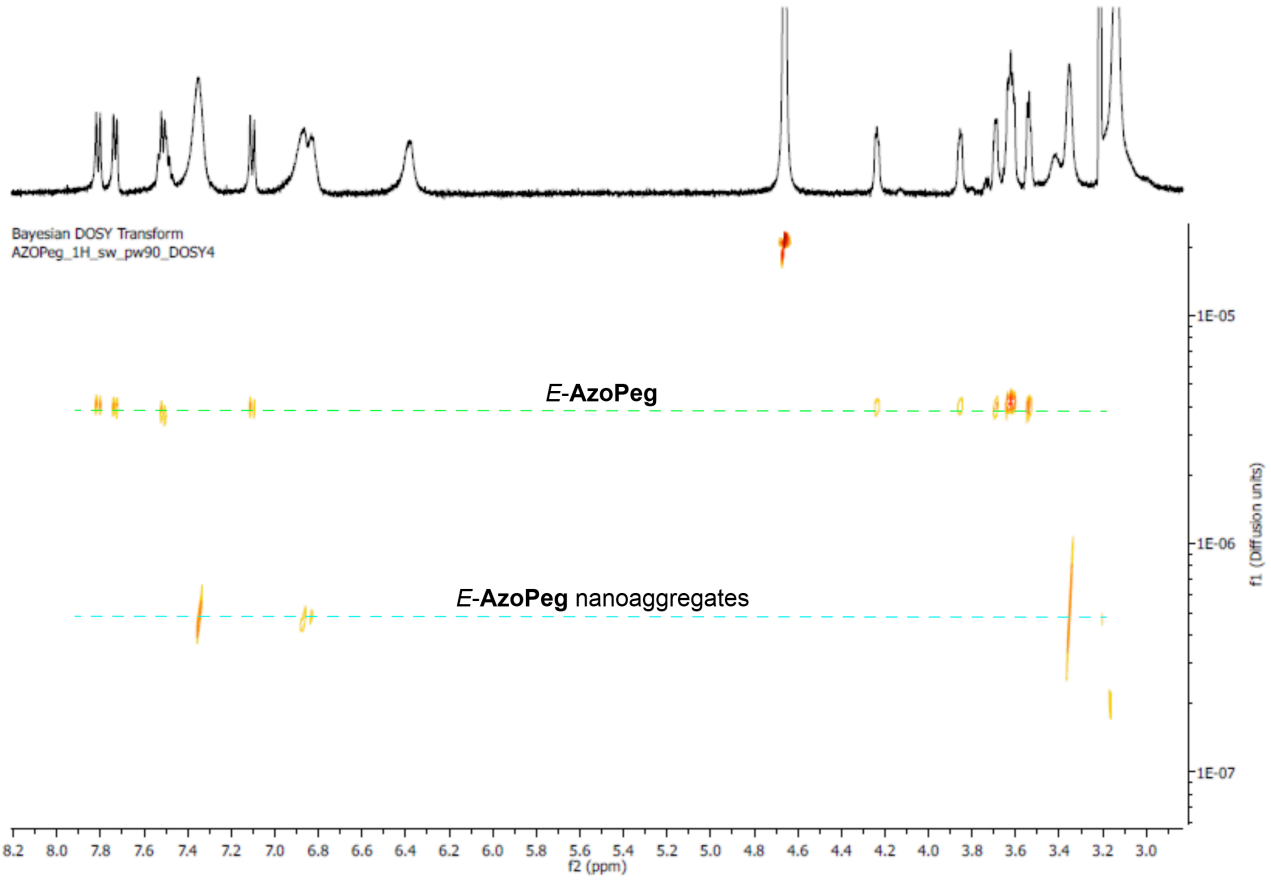


Figure S8 DOSY-NMR (500 MHz, D_2O/CD_3OD 95:5 v/v, 278 K) of *E*-**AzoPeg** showing the difference in diffusion rate between solubilized *E*-**AzoPeg** and nanoaggregates of *E*-**AzoPeg**.

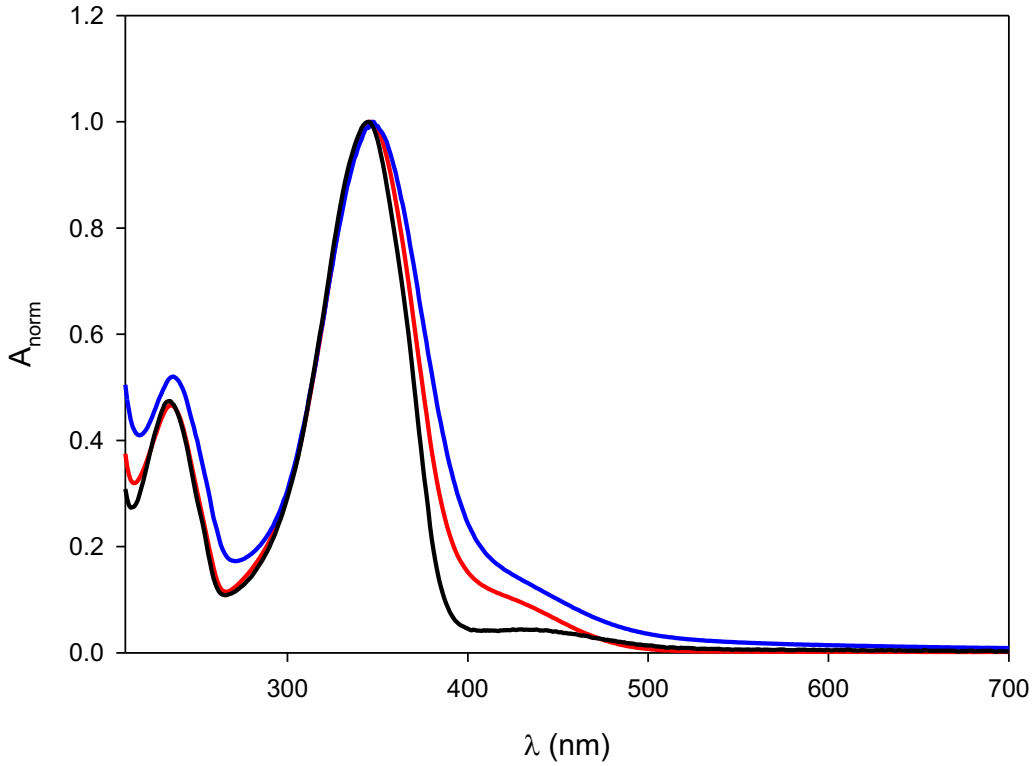


Figure S9 Normalized absorption spectra of a solutionof *E*-**AzoPeg** in H_2O (red), EtOH (black) and H_2O/EtOH 95:5 v/v (blue, nanoaggregates are present).

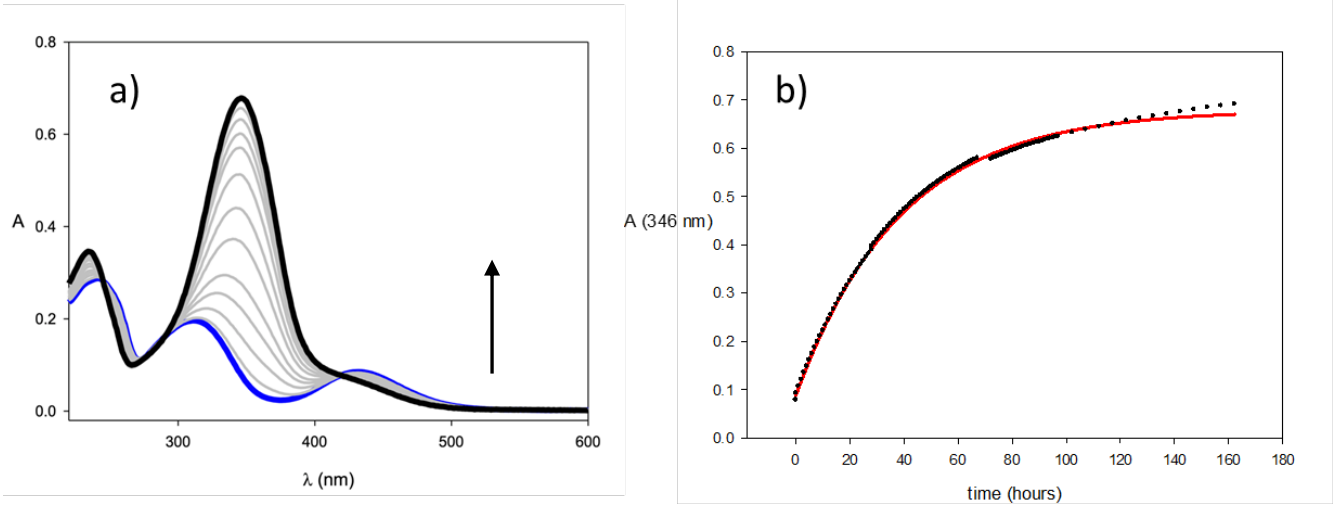


Figure S10 (a) Absorption spectral changes of an aqueous solution of *Z*-**AzoPeg** $3.03 \cdot 10^{-5}$ M at 35°C showing the kinetic of the thermal back reaction to *E*-**AzoPeg** (the blue trace represents the photostationary state after irradiation at 365 nm). (b) Absorbance changes recorded at 346 nm of an aqueous solution of *Z*-**AzoPeg** $3.03 \cdot 10^{-5}$ M at 35°C showing the kinetic of the thermal back reaction to *E*-**AzoPeg**. The red curve represents the fitting of the experimental data points according to a first order kinetic.

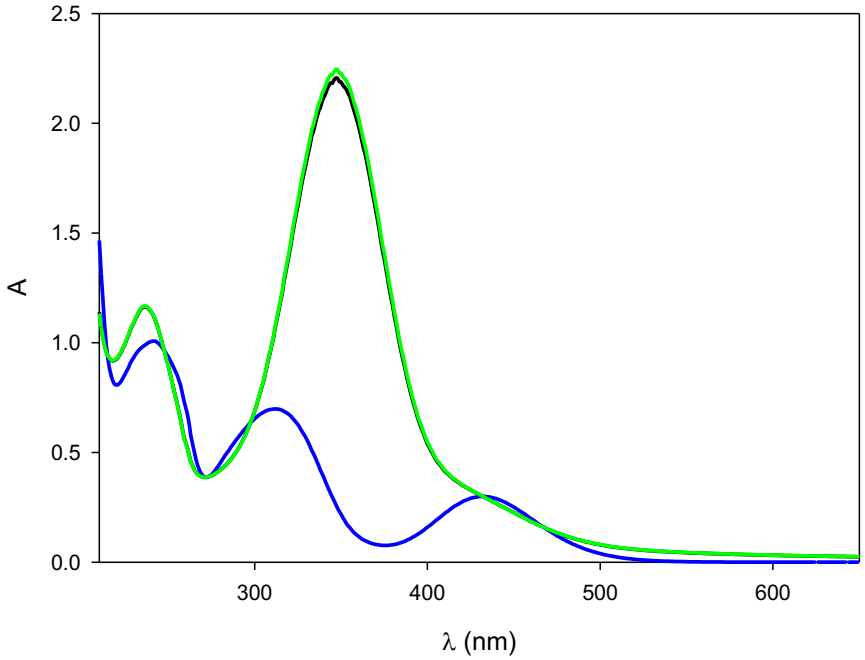


Figure S11 Absorption spectra of a solution of *E*-**AzoPeg** $1.04 \cdot 10^{-3}$ M in H_2O/EtOH95:5 v/v (black), at the photostationary state after irradiation with 365 nm light (blue) and after thermal back reaction to *E*-**AzoPeg** at 45°C (green) recorded in a 0.1 cm path length cuvette.

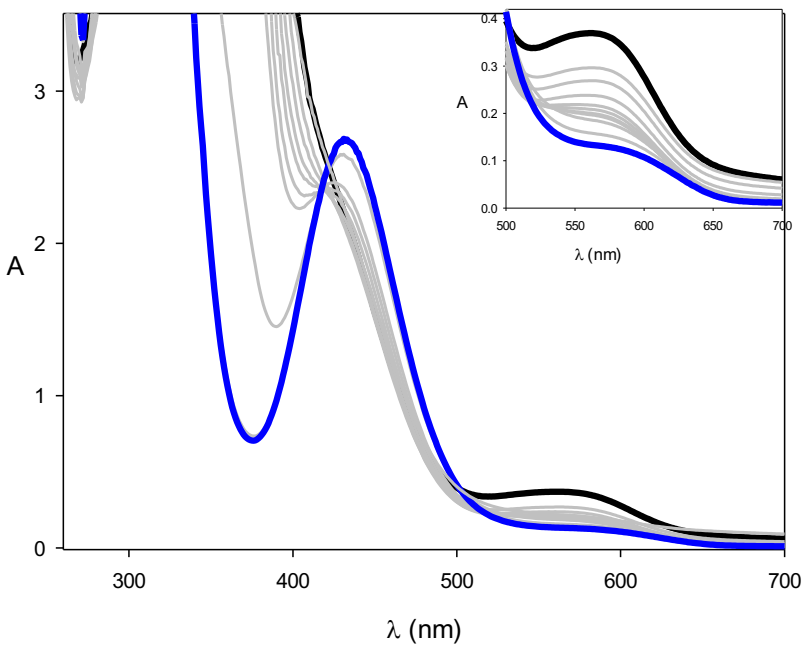


Figure S12 Absorption spectra of a solution of nanoaggregates of *E*-**AzoPeg** $9.5 \cdot 10^{-4}$ M in H_2O/EtOH 95:5 v/v loaded with Nile red $9.5 \cdot 10^{-6}$ M before (black) and after (blue) irradiation with 365 nm light.

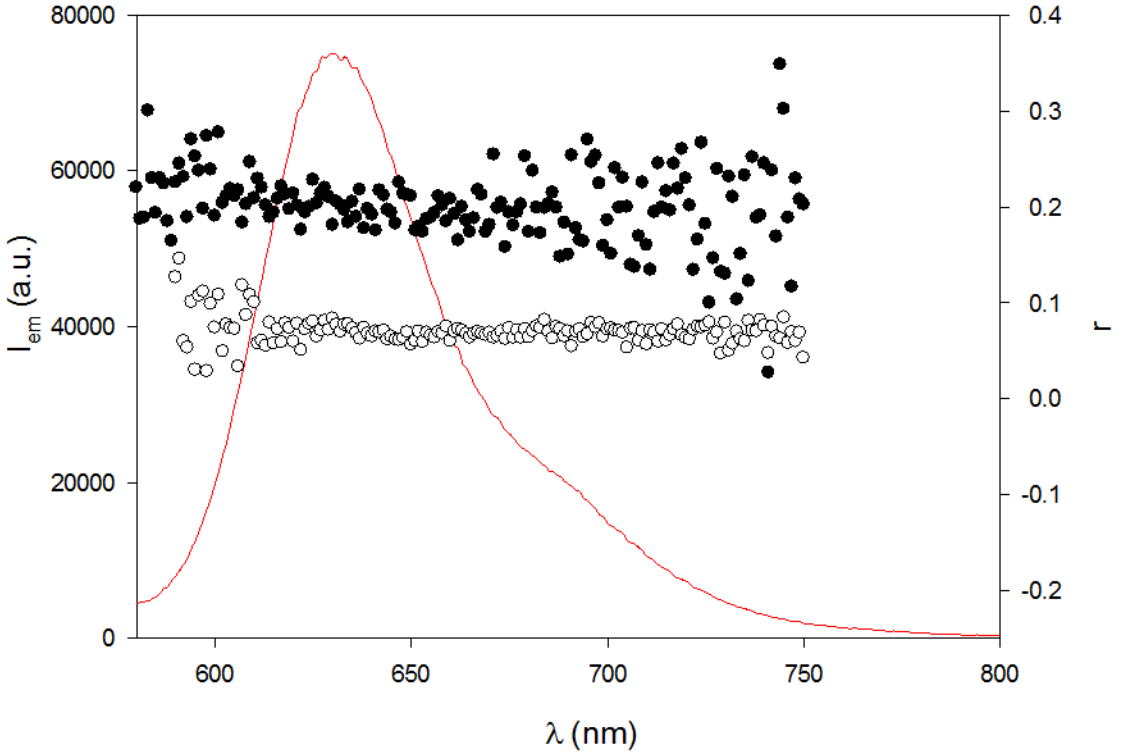


Figure S13 Fluorescence spectra of *E*-**AzoPeg** aggregates loaded with Nile red (red line). Nile red fluorescence anisotropy before (black circles) and after (white circles) irradiation with 365 nm light.

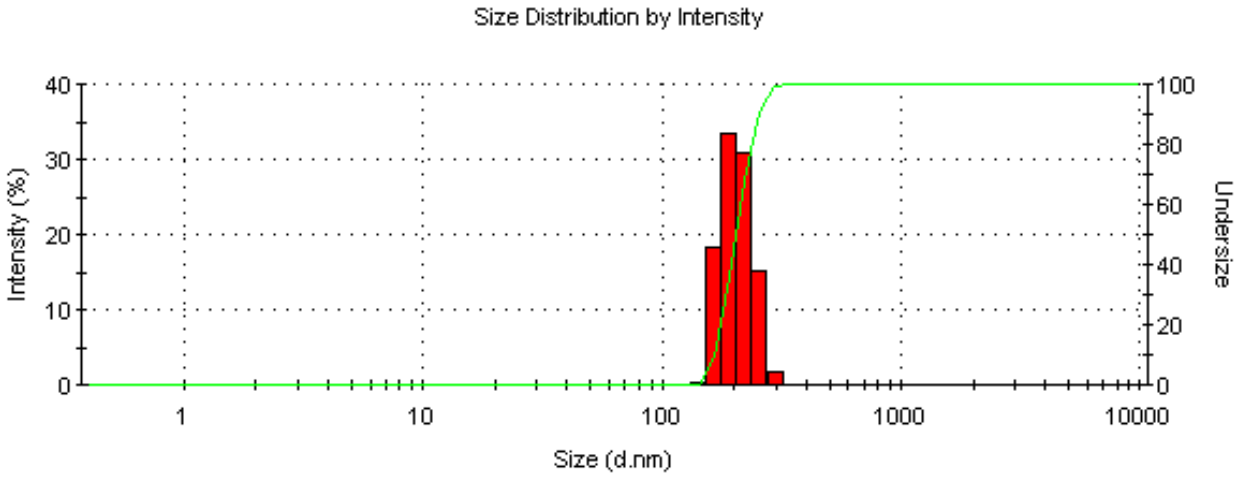


Figure S14 Hydrodynamic diameter distribution obtained by DLS of a solution of *E*-**AzoPeg** 2.3 mM in H_2O/EtOH 95:5 v/v and KCl 1.0 mM (d_h= 200 nm, PDI 0.047, 298 K)

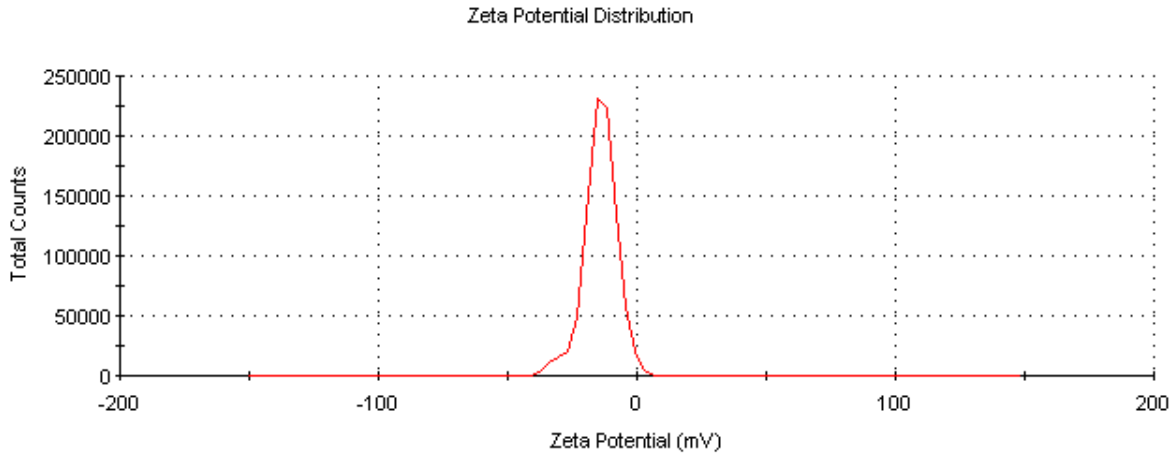


Figure S15 Zeta potential measurement of a solution of *E*-**AzoPeg** 2.3 mM in H_2O/EtOH 95:5 v/v and KCl 1.0 mM (zeta potential = -14.1 mV)

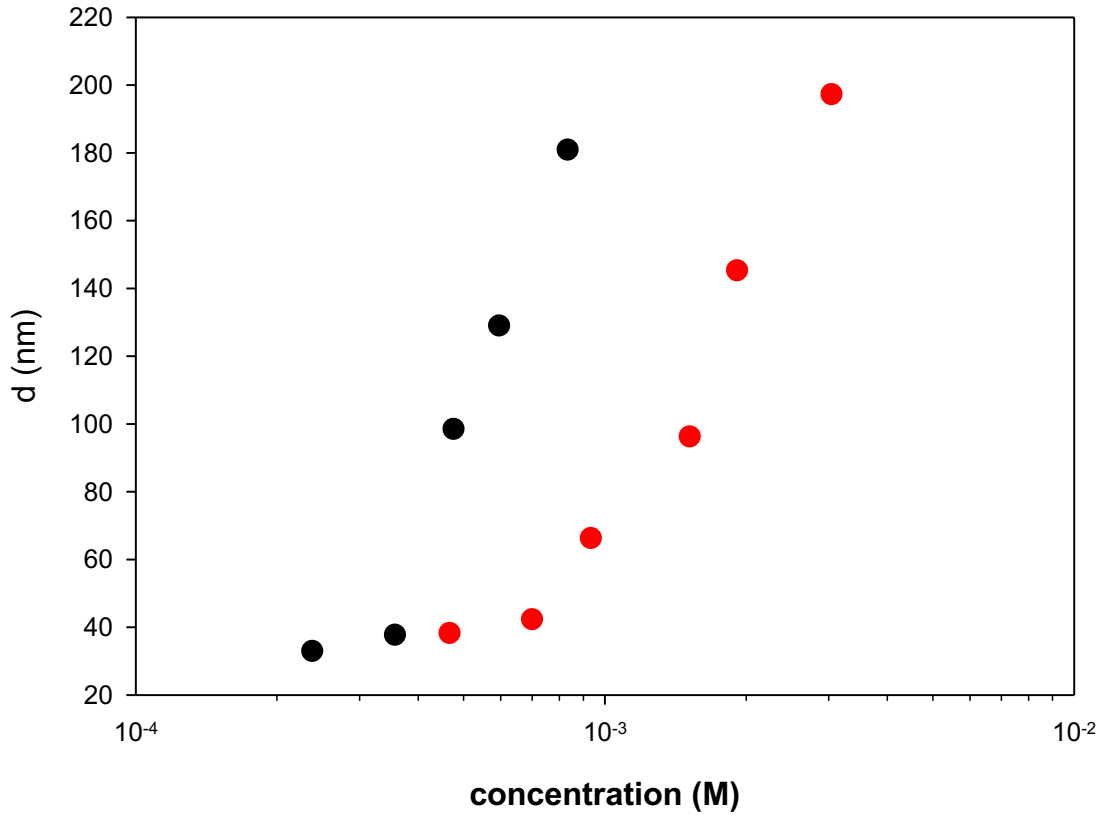


Figure S16 Hydrodynamic diameter obtained by DLS of solutions of *E*-**AzoPeg** at different concentration in H_2O/EtOH 95:5 v/v (red) and H_2O/EtOH 97.5:2.5 v/v (black)

Table S1 Critical aggregation concentration (CAC) value of *E*-**AzoPeg** as determined by different techniques.

Techniques	Solvent, ratio (v/v)	CAC (M)
NMR	DMSO-d_6/D_2O 5:95	3×10^{-4}
Surface tension measurement	EtOH/ H_2O 5:95	7×10^{-4}
UV-Vis	EtOH/H_2O 5:95	8×10^{-4}
DLS	EtOH/H_2O 5:95	5×10^{-4}