Supporting Information

Stimuli-Responsive Supramolecular Polymers from Amphiphilic Phosphodiester-Linked Azobenzene Trimers

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Experimental Procedures

Synthesis and characterization of compounds

All chemical reagents and solvents required for the synthesis have been purchased from commercial suppliers (Aldrich, Alfa Aesar, or TCI) and used without further purification. Water was used from a MilliQ system. Analytical thin-layer chromatography (TLC) was carried out using aluminum sheets precoated with silica gel 60 and fluorescent indicator. The silica gel for the column chromatography had 60Å pore size, 230-400 mesh. Air-sensitive reactions and manipulations have been carried out under Ar atmosphere using the standard Schlenk technique. Glassware was dried in an oven at 110 °C. All synthesized compounds were stored at 00C under Ar. NMR spectra were obtained on a Bruker AV 300 (300 MHz) spectrometer at 298 K. Mass-spectrometric data were obtained on Thermo Fisher LTQ Orbitrap XL using Nano Electrospray Ionization.

Spectroscopic measurements

UV-vis spectra have been measured in a cuvette with an optical path of 1 cm over the range of 200-550 nm on a Varian Cary-100 Bio-UV/VIS spectrophotometer equipped with a Varian Cary-block temperature controller. The molar extinction coefficient of AZB3 was determined using a value of ε_{355} = 24026 dm$^3$ mole$^{-1}$ cm$^{-1}$ in EtOH for a single azobenzene unit and multiplied three times, due to the presence of three azobenzene units in the trimer. The molar extinction coefficients of AZB3 at 355 and 315 nm are 72078 and 26436 dm$^3$ mole$^{-1}$ cm$^{-1}$ respectively.

| Azobenzene | λ (nm) | A | ε (dm$^3$ mole$^{-1}$ cm$^{-1}$) | C (mol/L) | l (cm) |
|------------|--------|---|-------------------------------|-----------|--------|
| E-Azobenzene | 355    | 0.728 | 24026                          | 3.03*10$^{-5}$ | 1      |
|            | 315    | 0.267 | 8812                           | 3.03*10$^{-5}$ | 1      |
**AFM measurements**

AFM imaging was performed under ambient air conditions with a Nanosurf FlexAFM (Nanosurf AG, Switzerland) instrument using 100x100 µm² scanning head. All measurements were carried out in tapping mode employing PPP-NCHR-W cantilevers from Nanosensors (resonance frequency 280 kHz, tip radius 10 nm) or Tap190Al-G from BudgetSensors (resonance frequency 190 kHz, force constant 48 N/m). The mica substrates (20x20 mm²) were attached to a steel base plate with Scotch tape and freshly cleaved before each new experiment. The protocol of deposition of supramolecular assemblies on a mica substrate followed a strategy developed initially for DNA. A mica surface was modified with 3-aminopropyltriethoxy silane (APTES-mica). The APTES changes the mica surface charge to positive due to amino-groups. Thus, the attachment of AZB3 is realized by electrostatic interactions between negatively charged phosphate-groups of azobenzene oligomers and positively charged amines of APTES-mica. Sample preparation: a 20 µL aliquot of a 5 µM azobenzene trimer stock solution (10 mM sodium phosphate buffer, pH = 7.2, 10mM sodium chloride, ethanol 10 vol%) was dropped on an APTES-modified mica plate (20 x 20 mm²). After an incubation time of 5 min, the modified mica sample was rinsed with 15 ml of MilliQ water (>18 MΩ cm, 3 ppb TOC) and dried in a gentle stream of Ar (CarbaGas) for 30 seconds. This experimental protocol enabled uniform distributions of supramolecular polymers (SPs) over more than 1 cm² of the mica surface.

**TEM measurements**

Experiments were performed on an FEI Morgagni 268 using an operating voltage of 80 kV. Sample preparation: a 10 µL aliquot of the 5 µM E-AZB3 solution obtained by any of the methods described above was placed on a carbon-coated grid (S160-3, 300 mesh Cu, AgarScientific). After 5 min, the remaining solution was blotted with a filter paper, and MilliQ water (5 µL) was added. After 1 min, the water was blotted, and 0.8 % aqueous uranyl acetate (2 µL) was added, which was blotted again after waiting for 30 sec. After repeating the uranyl staining procedure once again, the sample was used for the measurements.

**Dynamic light scattering experiments**

DLS measurements were carried out on a Zetasizer Nano instrument (Malvern Instruments).

**Photoirradiation experiments**

Photoirradiation experiments were performed under a UV lamp (8 W, CAMAG UV lamp, 12VDC/VAC) at 366 nm (for trans-to-cis photoisomerization) and under KESSIL PR160L-427nm (LED lamp, max 45W, 352mW/cm²) at 427 nm (for cis-to-trans photoisomerization). The sample solutions in 1 mL quartz cuvette were placed at a distance of 10 cm from the light sources.
Synthesis of an amphiphilic azobenzene trimer

Scheme S1. Solution-phase synthesis of an amphiphilic azobenzene trimer AZB3.

Compound AZB was synthesized according to the reported procedure.[2]

Synthesis of (E,Z)-3-((4-(((4-(3-hydroxypropoxy)phenyl)diazenyl)phenoxy)propyl acetate (AZB-Ac):

Acetic anhydride (75 mg, 0.75 mmol) was added dropwise to a stirred and ice-cold solution of AZB (250 mg, 0.75 mmol) in pyridine (10 mL), and stirring was continued at room temperature for 3 h. The completion of the reaction was monitored by TLC. After the complete conversion of the starting material, ethyl acetate was slowly added to the mixture. The resulting solution was washed with water and brine, then the solvent was evaporated under the reduced pressure. The residue was purified by flash chromatography (DCM/MeOH 10/0.1) on a silica gel column, to give 188 mg (67%) AZB-Ac as a pale-yellow powder.
$^1$H NMR (300 MHz, Chloroform-d) $\delta$ 7.88 (d, $J = 8.9$ Hz, 4H), 7.01 (dd, $J = 9.1$, 2.6 Hz, 4H), 4.30 (t, $J = 6.3$ Hz, 2H), 4.17 (dt, $J = 19.9$, 6.1 Hz, 4H), 3.89 (t, $J = 5.9$ Hz, 2H), 2.17 (t, $J = 6.2$ Hz, 2H), 2.13 (t, $J = 6.2$ Hz, 2H), 2.09 (s, 3 H).

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 171.13, 160.77, 147.09, 124.37, 114.69, 65.73, 64.65, 61.22, 60.03, 31.99, 28.57, 20.98.

HRMS for C$_{20}$H$_{24}$N$_2$O$_5$: calcd 372.1685; found 372.1763.

$^1$H NMR (300 MHz, CDCl$_3$) of AZB-Ac.

$^{13}$C NMR (75 MHz, DMSO) of AZB-Ac.
Synthesis of (E,Z)-bis(2-cyanoethyl)-(((diazene-1,2-diylbis(4,1-phenylene))bis(oxy))bis(propane-3,1-diyl)) bis(diisopropylphosphoramidite) (AZB-PAM2):

AZB (200 mg, 0.6 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL). After addition of DIPEA (400 μL, 2 mmol, 3.3 eq), a solution of 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (300 mg, 1.2 mmol, 2 eq). The mixture was stirred for 3 h. The solution was evaporated, and the residue was dissolved in hexane/EtOAc/NEt$_3$ 50/49/1 and purified by flash chromatography (hexane/EtOAc/NEt$_3$ 50/49/1), furnished 163 mg (37%) AZB-PAM2 as an orange oil.

$^1$H NMR (300 MHz, Chloroform-d) δ 7.88 (d, J = 8.9 Hz, 4H), 7.02 (d, J = 9.0 Hz, 4H), 4.18 (t, J = 6.1 Hz, 4H), 3.93 – 3.74 (m, 8H), 3.63 (dt, J = 10.1, 6.8 Hz, 4H), 2.64 (td, J = 6.4, 0.7 Hz, 4H), 2.15 (t, J = 6.4, 4H), 1.20 (m, 24H).

$^{13}$C NMR (75 MHz, CDCl$_3$) δ 160.99, 147.02, 124.32, 114.69, 64.77, 60.18, 59.95, 58.44, 58.19, 43.14, 42.98, 30.99, 30.89, 24.71, 24.65, 24.61, 24.55, 20.43, 20.34.

$^{31}$P NMR (122 MHz, DMSO) δ 147.76.

HRMS for C$_{36}$H$_{56}$N$_6$O$_6$P$_2$: calcd 730.3737; found 730.3791.

$^1$H NMR (300 MHz, CDCl$_3$) of AZB-PAM2.
$^{13}$C NMR (75 MHz, DMSO) of AZB-PAM.

$^{31}$P NMR (122 MHz, DMSO) of AZB-PAM.
Synthesis of (((E,Z)-diazene-1,2-diyl)bis(4,1-phenylene))bis(oxy))bis(propane-3,1-diyl) bis[3-(4-((E)-(4-(3-hydroxypropoxy)phenyl)diazenyl)phenoxo)propyl] bis(phosphate) (AZB3):

Under nitrogen atmosphere, a solution of AZB-PAM2 (40 mg, 0.055 mmol) in 1,2-dichloroethane (0.5 mL) was added to a solution of 5-(ethylthio)-1H-tetrazole (20 mg, 0.15 mmol) in 1,2-dichloroethane (1 mL). The reaction mixture was stirred at r.t. for 10 min until TLC (hexane/ EtOAc/NEt₃ 50/49/1) showed the starting material's disappearance. The solution of AZB-Ac (41 mg, 0.1 mmol) in 1,2-dichloroethane (0.5 mL) was then added, and the reaction mixture was stirred at r.t. for 2 h. tert-Butyl hydroperoxide (70% wt in water, 30μL) was added, and the reaction mixture was stirred for 20 min. Chloroform (50 mL) was added, the organic layer was washed twice with saturated NaHCO₃ and once with brine. The organic layer was collected, dried over MgSO₄, and filtered. After removing all solvents under vacuum, without further purification, AZB3-Ac2 was dissolved in 2M ammonia solution in MeOH (20 mL) under nitrogen atmosphere, and the orange reaction mixture was stirred at 50 °C for 48 h. The resultant solution was concentrated to dryness in vacuo, to give 21 mg (34.3%) of AZB3 as an orange powder.

The crude product was purified by reverse-phase HPLC (column: ResproSil 100 RP-C8,5 μm, Dr. Masch GmbH; HPLC: Shimadzu Schweiz GmbH); eluent A = Et₃NHOAc (100 mM, pH 7.2, with 10% MeOH); eluent B = CH₃CN; gradient 0 – 40% B over 2 min, 40 – 70% B over 15 min, then 70 – 100% B over 2 min. Gradient flow 1 mL/min. The pure compound was characterized by high-resolution mass spectrometry.

| Time (min) | Eluent A (%) | Eluent B (%) |
|-----------|--------------|--------------|
| 0         | 100          | 0            |
| 2         | 60           | 40           |
| 20        | 30           | 70           |
| 22        | 0            | 100          |
| 25        | 0            | 100          |
| 27        | 100          | 0            |
| 30        | 100          | 0            |

The solution of AZB3 was collected at 14.5 min elution time. After the injection of the AZB3 solution (collected at 14.5 min) into the HPLC, four peaks can be observed (as shown on the HPLC trace diagram below). Peaks with elution times at 14.5, 10, 7.5, and 6 min can be attributed to the different forms of AZB3: namely E,E,E-AZB3, E,E,Z-AZB3, E,Z,Z-AZB3 and Z,Z,Z-AZB3. Oligomers that contain the cis-isomer in the structure are more hydrophilic due to the higher dipole moment of the chromophore than the trans-isomer. Therefore, the elution times of Z-containing trimers are lower. Based on the relative intensities of the signals, the trimer exists as a mixture of E,E,E-AZB3, E,E,Z-AZB3, E,Z,Z-AZB3 and Z,Z,Z-AZB3 isomers in 47%, 39%, 11%, and 3% ratio, respectively. Mass spectra of all the peaks confirm the molecular composition of AZB3. HPLC trace diagrams of the compounds that contain three azobenzene moieties in the structure have been previously reported, show a similar distribution of the isomers in the mixture, and in good agreement with the obtained data.[3,4]
HPLC traces of AZB3 monitored at 340 nm.

Mass-spectra of AZB3: calculated 556.1854; found 556.1863.
Figure S1. A,B) AFM images of E-AZB3 supramolecular polymers obtained by fast cooling of the solution (10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride with 10 vol% of ethanol); C,D) AFM images of 2D nanosheets found in a 1-week old E-AZB3 solution.

Figure S2. AFM images of E-AZB3 nanosheets prepared by cooling (0.1°C per minute) of the solution (10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride with 10 vol% of ethanol).
Figure S3. TEM images of E-AZB3 prepared by slow cooling (0.1°C per minute) of the solution (10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride with 10 vol% of ethanol).
Figure S4 (continued on next page)
Figure S4. **Top**) Comparison of the supramolecular polymer formation curves recorded upon cooling from 70 to 20 °C with 0.1 °C per minute cooling rate of an aqueous E-AZB3 solution with different ethanol volume fractions in the absence of light source; **Bottom**) UV-vis absorption spectra of E-AZB3 SPs at 20 °C (black line) and after UV-light irradiation at 366 nm for 60 min (blue line) of aqueous solution (10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride) with 10, 12, 15, and 17 vol% of ethanol respectively; [E-AZB3]: 5 µM, alongside the representative AFM images of the aggregates, resulted after UV-light irradiation of the solution (blue line).
Figure S5. AFM images alongside the height profile of the **Z-AZB3** aggregates after photoisomerization of **E-AZB3** SPs solution (10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride, ethanol 15 vol%).
Figure S6. Dynamic light scattering (DLS) measurements showing the particle size distribution (hydrodynamic diameter, $D_H$) of Z-AZB3 nanostructures in aqueous medium (10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride, ethanol 15 vol%) at 25 (black) and 75°C (red); [Z-AZB3]: 5 µM.

Figure S7. UV-Vis absorption spectra of Z-AZB3 in ethanol at 20 °C (black line) and after addition of the aliquot to aqueous medium (10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride, ethanol 15 vol%) at 20 °C (blue line); [Z-AZB3]: 5 µM.
Figure S8. AFM images alongside the height profile of the Z-AZB3 aggregates obtained upon addition of an aliquot of the photoisomerised ethanol solution upon irradiation at 366 nm, to an aqueous medium (10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride, ethanol 15 vol%).
Figure S9. Z-to-E photoisomerization at 427 nm (A) using the instrumental setup shown in (B); C) evolution of the UV-Vis absorption spectrum: irradiation of Z-AZB3 (stage 1, black line) leads to appearance of non-assembled E-AZB3 (stage 2) and further to the spectrum of assembled E-AZB3 (stage 3); spectra were recorded after every 30 seconds of irradiation; D) AFM images of the supramolecular structures corresponding to stages described in (C): toroids (stage 1), ill-defined aggregates (stage 2) and multi-layered nanosheets (stage 3). Conditions: [Z-AZB3]: 5 µM; 10 mM sodium phosphate buffer, pH = 7.2, 10 mM sodium chloride, ethanol 15 vol%
Figure S10. AFM images of multilayered supramolecular polymers formed after the reversible photoisomerization from Z-AZB3 to E-AZB3 in aqueous medium upon visible light irradiation.

References

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