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Block copolymer aggregates with photo-responsive switches: Towards a controllable supramolecular container

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ABSTRACT

Herein, we present a concept of combining host–guest chemistry with block copolymer self-assembly to fabricate an inner cross-linking block copolymer aggregate with photo-responsive switches on the basis of the reversible interaction between azobenzene and β-cyclodextrin, which can serve as a controllable supramolecular container to load and release guest molecules reversibly. The inner cross-link makes the block copolymer aggregates exhibit good stability, and the aggregates can keep their spherical morphologies during photo-irradiation treatment. When the switches are in on-state, cyclodextrins can bind with hydrophobic pyrene molecules; and when the switches are in off-state, pyrene molecules will get away from the cyclodextrins. The photo-controllable switches embedded in the aggregates endow this new supramolecular container with the capability to load and release guest molecules reversibly without structure disruption. It is anticipated that this line of work may open an avenue for fabricating new polymeric containers which can be used for controllable molecular transfer and catalysis.

1. Introduction

Cyclodextrins are perfect unimolecular containers for binding many kinds of small guest molecules [1–8], and this binding behaviour can be scaled-up when a great number of cyclodextrins are grafted on polymers [9–11]. Among the guest candidates, azobenzene moiety can change from trans-form to cis-form reversibly under photo stimuli, resulting in distinct binding behaviour with cyclodextrin. For trans-azobenzene, it always binds well with α- or β-cyclodextrin, which bears six and seven glucose units, respectively. However, the interaction between cis-azobenzene and α- or β-cyclodextrin is weak because they are non-matched host–guest pairs. This unique host–guest interaction has been extensively used to construct controllable supramolecular assemblies [12–17] as well as molecular machines [18–24]. Different from supramolecular entities by small building blocks [25,26], block copolymer aggregates, profiting from their well-defined fascinating self-assembly structures [27–34], are effectively developed as containers to load drugs or catalysts because of their high loading capacity in aqueous solution [35–42]. Moreover, disassembling and reassembling the aggregates by external-stimuli [43–54] can extend the block copolymer containers in incorporating substrates reversibly, which will endow the block copolymer aggregates with potential applications in drug target-releasing and catalytic fields. Nevertheless, the stimuli-responsive dynamic assembly of polymeric containers will hardly recover the self-assembly structure but lead to the loss of block copolymers during reassembly process. Therefore, new supramolecular containers are being explored which may behave like natural enzymes that can bind with the substrates reversibly without any structural damage.

With the above thoughts in mind, in this article, we provide a model system by embedding photo-controllable binding switches based on the reversible interaction between azobenzene (Azo) and β-cyclodextrin (CD) into the interior of a cross-linked block copolymer aggregate. The switches are expected to be controlled reversibly by photo-irradiation, loading the substrates into block copolymer aggregates without the disruption of the assembled architecture.

2. Experimental section

2.1. General methods

UV–Vis spectra were obtained using a HITACHI U-3010 spectrophotometer and fluorescence spectra were collected by taking use of a HITACHI F-7000 spectrophotometer. ^{1}H NMR spectra were

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recorded on a JEOL JNM-ECA 300 (300 MHz) spectrometer. ESI-MS measurements were performed on a Thermo ELECTRON CORPORATION Finnigan LTQ. The inner cross-link degree of the block copolymer aggregates was determined by IR spectrum, using the integral of characteristic peaks of acryl amine bonds. The Fourier Transform Infrared (FT-IR) spectrum was collected on a Bruker IFS 66 V instrument. To prepare the IR sample, drops of block copolymer aggregate solution were cast onto one side of a CaF_2 slide, and the water was removed via a vacuum pump. High-pressure mercury lamp with an optical fiber (RW-UVA 05-100, purchased from Shenzhen Runwing Mechanical & Electrical Co., Ltd., China) with an intensity of 900 mW/cm^2 was used as the irradiation light source for photoisomerization of azobenzene. The two band-pass filters were of the wavelengths 365 ± 10 and 450 ± 10 nm, which can be used to produce the UV light with wavelength at 365 nm and visible light with wavelength at 450 nm, respectively. Cryo-Transmission Electron Microscopy (Cryo-TEM) samples were prepared in a controlled environment vitrification system (CEVS) at 25 °C. A micropipette was used to load 5 μL sample solution onto a lacey support TEM grid, which was held by tweezers. The excess solution was blotted with a piece of filter paper, resulting in the formation of thin films suspended the mesh holes. After waiting for about 10 s to relax any stresses induced during the blotting, the samples were quickly plunged into a reservoir of liquid ethane at its melting temperature (−183 °C). The vitrified samples were then stored in liquid nitrogen until they were transferred to a cryogenic sample holder (Gatan 626) and examined with a JEM 2200FS TEM (200 keV) at about −174 °C. The phase contrast was enhanced by underfocus. The images were recorded on a Gatan multiscan CCD and processed with DigitalMicrographs. Transmission electron microscopy (TEM) was performed on a MODEL H-800 Electron microscope operating at an acceleration voltage of 100 kV. To prepare TEM samples for the study of the size and the morphology of the aggregates, a drop of the dilute aqueous solution was deposited onto a copper grid, which had been pre-coated with a thin film of polyvinyl formal and then coated with carbon. Two minutes after the deposition, the excess aqueous solution was blotted away with a strip of filter paper. This process was repeated several times to ensure that there was enough sample absorbed on the grid. All the samples were stained with 0.5% phosphotungstic acid hydrate before observed under TEM. Electrochemical measurements were performed using a potentiostat (Autolab PGSTAT12, The Netherlands). Electrochemistry was carried out in a conventional three-electrode glass electrochemical cell.

2.2. Materials

The reagents for atom transfer radical polymerization (ATRP) including PEG polymer with hydroxyl group as one terminated group (M_n 1900), 2-bromo-2-methylpropionyl bromide, 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA) and tert-butyl acrylate (tBA) were purchased from Alfa Aesar. For PEG polymer, it was dried in refluxing toluene to get rid of trace amount of water inside. The catalyst used for polymerization, CuBr, was treated with 30% (volume percentage) acrylic acid aqueous solution to remove copper(II), dried in vacuum and then kept under Ar. Pd/C (palladium, 5% in activated carbon powder) was purchased from Aldrich and used as catalyst to prepare 6-amino-6-deoxy-cyclodextrin. β-cyclodextrin was recrystallized in water twice and dried before use. Triethylamine was dried by NaOH before use. Other reagents and solvents were all purchased from Beijing Chemical Reagent Company.

2.3. Synthesis of amino-terminated azoC4 (**4**)

As shown in Fig. 1, 4-phenylazo-phenol (**1**) (0.5530 g, 2.79 mmol), which was synthesized according to our previous work [12], was

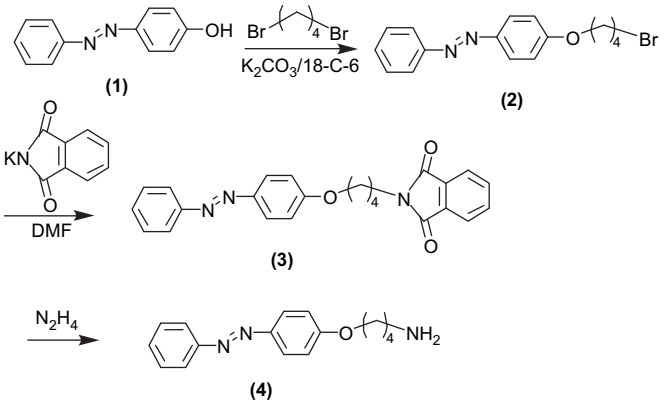


Fig. 1. The synthetic route for amino-terminate azobenzene.

added to 1,4-dibromobutane (5.800 g, 27.9 mmol) in acetone solution. After K_2CO_3 (1.5410 g, 11.17 mmol) and a trace amount of 18-C-6 was added, the solution was stirred and refluxed for 24 h under Ar protection. Then the solvent was removed and the residue was purified by silica gel column chromatography (eluent: petroleum ether to dichloromethane). The evaporation of the solvent gave a yellow powder (**2**) in 65% yield. ^{1}H NMR (solvent, $CDCl_3$) characterization: δ 7.91–7.85 (q, 4H); δ 7.52–6.99 (m, 3H); δ 7.01 (d, 2H); δ 4.09 (t, 2H); δ 3.51 (t, 2H); δ 2.10 (m, 2H); δ 1.99 (m, 2H). MS: $[M+H]^+$ calculated, 332.05, and found, 333.06.

Compound (**2**) (1.000 g, 3.00 mmol) and phthalimide potassium salt (0.6667 g, 3.64 mmol) dissolved in 20 mL DMF were stirred at 70 °C under Ar protection for 8 h. After solvent was removed by a vacuum pump, the residue was purified by silica gel column chromatography (eluent: dichloromethane) to afford (**3**) in 95% yield. ^{1}H NMR (solvent, $CDCl_3$) characterization: δ 7.92–7.83 (m, 6H); δ 7.19 (m, 2H); δ 7.50 (m, 3H); δ 7.00 (d, 2H); δ 4.08 (t, 2H); δ 3.79 (d, 2H); δ 1.90 (m, 4H). MS: $[M+H]^+$ calculated, 400.17, and found, 400.19.

Compound (**3**) (0.9700 g, 2.43 mmol) was dispersed in ethanol and then hydrazine monohydrate (0.1418 g, 2.84 mmol) was added. The cloudy solution became clear after heating at 80 °C for five minutes and the solution was kept at the same temperature under stirring for 8 h. Removal of the solvent afforded a yellow powder which was purified through extraction by dichloromethane against water three times. The pure AzoC4 (**4**) in 98% yield can be obtained after evaporation of the organic layer. ^{1}H NMR (solvent, $CDCl_3$) characterization: δ ($CHCl_3 = 7.26$): δ 7.94–7.86 (q, 4H,); δ 7.56–7.43 (m, 3H); δ 7.03 (d, 2H); δ 4.08 (t, 2H); δ 2.80 (t, 2H); δ 1.84 (m, 2H); δ 1.65 (m, 2H); δ 1.31 (s, 2H). MS: $[M+H]^+$ calculated, 270.15, and found, 270.09.

2.4. Synthesis of azobenzene grafted PEG–PAA

The precursor block copolymer poly(ethyleneglycol)-block-poly (acryl acid) (PEG_{43}–PAA_{153}) was synthesized by atom transfer radical polymerization (ATRP) [55,56]. The molecular weight of PEG–PtBA measured by gel permeation chromatography is 1.42×10^4 g/mol (polystyrene as the standard) and polydispersity index is 1.19. Azobenzene groups can be covalently introduced onto PAA chain of the block copolymer through the condensation reaction of amino groups with carboxylic acid groups. The graft quantity can be controlled by variation of the molar ratio between AzoC4 and carboxylic acid groups.

2.4.1. Low azo-graft PEG–PAA-g-$Azo_{5\%}$

After PEG_{43}–PAA_{153} (50.0 mg) was dissolved in PBS buffer (pH 7.4) at room temperature, 1-ethyl-3(3-dimethyl aminopropyl)

carbodiimide (EDC) (30.7 mg, 0.160 mmol) and N-hydroxysuccinimide (NHS) (18.4 mg, 0.160 mmol) were added quickly. After slow addition of AzoC4 (7.0 mg, 0.026 mmol, in 1 mL DMF), the condensation reaction was carried out under Ar protection for 48 h. Afterwards, the reaction solution was enclosed into a semipermeable bag and dialyzed against water for 5 days to remove the small molecules. During this process, the water was exchanged twice every day, and for the first three times $NaHCO_3$ aqueous solution was used to avoid the formation of N-hydroxysuccinimidyl esters with PAA chain. The final grafting yield is nearly 100% according to ^{1}H NMR measurement and the molar percentage of azobenzene on PAA segment was calculated as 5%.

2.4.2. High azo-graft PEG–PAA-g-Azo$_{22.5\%}$

Synthetic procedure was the same to that of low azo-graft PEG–PAA-g-Azo$_{5\%}$. The amount of AzoC4 was increased to make sure that more azobenzene will be grafted onto the polymer chain. The final graft percentage of azobenzene on PAA block was also estimated to be 22.5% by ^{1}H NMR.

2.4.3. CD-graft PEG–PAA-g-CD$_{5\%}$

6-Amino-6-deoxy-β-cyclodextrin [57] was grafted on PAA segment by the similar method to prepare azobenzene-grafted copolymer. Estimated by ^{1}H NMR, the CD grafting percentage was found as 5%.

2.5. The preparation of cross-linked block copolymer aggregates

2.5.1. The aggregate P-Azo/CD1

PEG–PAA-g-Azo$_{5\%}$ aqueous solution (4 mL, 0.4 mg/mL) was mixed with PEG–PAA-g-CD$_{5\%}$ aqueous solution (6 mL, 0.4 mg/mL) under stirring. The mixture, in which the molar ratio of azobenzene groups to CD groups was nearly 1:1, was kept stirring at room temperature for 1 h in dark. Aqueous $CaCl_2$ solution (0.02 M, 120 μL) was added dropwise into the mixture and the solution was kept stirring for another 8 h. Then 2,2′-(ethane-1,2-diylbis(oxy))diethanamine (0.02 M, 80 μL), a spatula-tipfull EDC and NHS were added to the solution to cross-link the PAA chains. After the cross-link reaction was carried out for 3 days, the solution was enclosed into a semipermeable bag and dialyzed against water for 3 days to remove the unreacted small molecules. Afterwards, the dialysis was performed in ethylenediamine tetraacetic acid (EDTA) solution to remove Ca^{2+} binding with carboxylate groups from the aggregate. Finally, the solution was diluted to 25 mL and used for further measurement.

2.5.2. The aggregate P-Azo/CD2

This aggregate was prepared via the assembly between PEG–PAA-g-Azo$_{22.5\%}$ and PEG–PAA-g-CD$_{5\%}$. The preparation process is the same with that of P-Azo/CD1. The mass concentration of original PEG–PAA-g-CD$_{5\%}$ and PEG–PAA-g-Azo$_{22.5\%}$ was controlled at 0.40 mg/mL and 0.84 mg/mL, respectively. In this way, the molar concentration of CD in P-Azo/CD2 is the same with that in P-Azo/CD1, but the ratio of azobenzene to CD is increased to 6.6:1.

2.5.3. The aggregates P-CD and P-Azo

P-CD refers to the aggregate produced by the above method only using PEG–PAA–g-CD$_{5\%}$ with no azobenzene in this aggregate. P-Azo refers to the aggregate formed by PEG–PAA-g-Azo$_{5\%}$ only, and certainly, there are no CD groups in this aggregate.

3. Results and discussion

3.1. The building blocks for fabrication of the block copolymer aggregates

As illustrated in Fig. 2, two kinds of block copolymers are involved to prepare the aggregates with photo-controllable switches. One is poly (ethyleneglycol)-block-poly (acryl acid) (PEG_{43}–PAA_{153}) grafted with 5% β-CD on PAA segment; the other one is PEG_{43}–PAA_{153} grafted with 5% or 22.5% azo on PAA segment.

To graft azobenzene moieties onto the PAA segment, we chose the condensation reaction of amino groups of (**4**) with carboxylic acid groups under the catalysis of EDC/NHS in PBS buffer solution with pH 7.4. The synthetic procedure of amino-terminate azobenzene (**4**) is outlined in Fig. 1 of the Experimental section. Through controlling the molar ratio of the reagents, two different azobenzene-grafted block copolymers were obtained: PEG–PAA-g-Azo$_{5\%}$ and PEG–PAA-g-Azo$_{25\%}$, as shown in Fig. 2a and b, respectively.

6-Amino-6-deoxy-β-cyclodextrin was applied to prepare the CD-grafted block copolymer PEG–PAA-g-CD$_{5\%}$ as show in Fig. 2c. The grafting method is similar to the preparation of azobenzene-grafted copolymer. The grafting percentage of CD on PAA was finally defined by ^{1}H NMR.

3.2. Preparation of the cross-linking block copolymer aggregates and the photoisomerization of azobenzene in the aggregates

The block copolymer aggregates (P-Azo/CD1) were prepared according to the method proposed by Bronich et al. [58] as shown in Scheme 1. Firstly, PEG–PAA-g-CD$_{5\%}$ and PEG–PAA-g-Azo$_{5\%}$ were aggregated together through the association of carboxylate groups with Ca^{2+}, in which the molar ratio of cyclodextrin (CD) to azobenzene (Azo) is about 1:1. It should be noted that the block copolymers can hardly form any regular aggregates in the absence of Ca^{2+} because of the electrostatic repulsion between carboxylate groups. Secondly, the interior of the aggregates was cross-linked by 1,2-ethylenediamine through the catalysis of EDC/NHS. Thirdly, Ca^{2+} was removed by EDTA and the aggregate solution was dialyzed against water to get rid of unreacted small molecules. Finally, the solution was diluted to the required concentration. As indicated

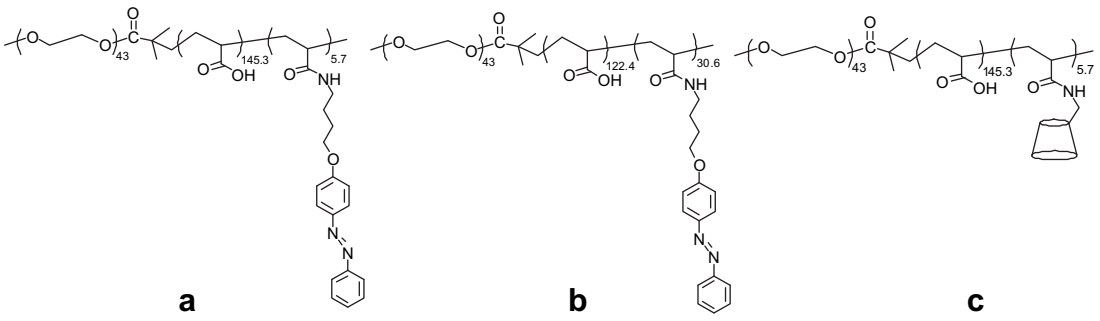
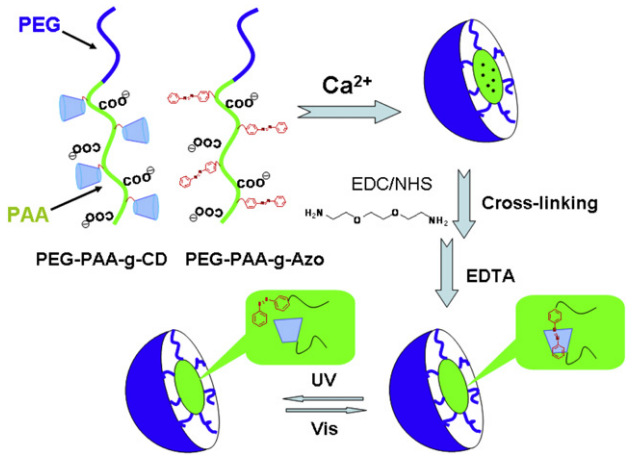


Fig. 2. The polymers used to fabricate block copolymer aggregates: (a) PEG–PAA-g-Azo$_{5\%}$; (b) PEG–PAA-g-Azo$_{22.5\%}$; (c) PEG–PAA-g-CD$_{5\%}$.



Scheme 1. Preparation of the cross-linked block copolymer aggregates with photo-controllable switches.

by Cryo-TEM, spherical aggregates are formed after this three-step procedure.

The photoisomerization of azobenzene can easily take place in P-Azo/CD1 aggregates. As shown in Fig. 3a, upon irradiation with UV light of 365 nm, the absorption band at around 342 nm decreases remarkably against irradiation time, concomitantly the absorption band around 440 nm increases slightly. The absorption bands at around 342 nm and 440 nm are ascribed to π–π^* and n–π^* transitions of azo, respectively. The change of the absorption bands induced by UV irradiation is indicative of the photoisomerization of azo from trans- to cis-form. When irradiated by visible light of 450 nm, the π–π^* absorption increases again with the slight decrease in the n–π^* absorption, indicating that the photoisomerization of Azo undergoes a change from cis- to trans-form. Moreover, the photoisomerization of Azo in P-Azo/CD1 can take place reversibly by alternating UV and visible light irradiation, as shown in Fig. 3b. It should be noted that similar photoisomerization behaviors of Azo are also found in P-Azo/CD2 which is composed of Azo and CD with the molar ratio of 6.6:1, and P-Azo which contains Azo but no CD. The easy photoisomerization of azobenzene in the aggregates permits that the switches based on the interaction between Azo and CD can respond to photo-stimuli.

The spherical aggregates of P-Azo/CD1 are clearly observed by Cryo-TEM, as shown in Fig. 4a. The size of the aggregates extends from 100 nm to 300 nm. There is no big difference for the Cryo-TEM observations before and after photo-irradiation, indicating that the aggregates are of good stability. This property is different from the previously reported azobenzene-containing block copolymer aggregates which can be destroyed by the photoisomerization of azobenzene moieties [59,60]. Deducing from FT-IR data of P-Azo/CD1 casting on CaF_2 slide, the inner cross-linking degree of the free carboxylate groups is estimated to be 5.7%, as seen in Fig. S2, which should be the plausible explanation for the enhanced stability of the aggregate. In addition, TEM has also been employed to indicate the formation of spherical aggregates and no big difference of the TEM images before and after UV irradiation supports furthermore the stability of the aggregates, as shown in Fig. 4c and d. On the basis of TEM, it seemed that spherical aggregates of P-Azo/CD1 could look like vesicles structure, but it is not true when considering the Cryo-TEM observation. The different contrast of TEM may be caused by the deformation of aggregates under high vacuum.

3.3. Reversible loading behaviour of the block copolymer aggregates

Since CD binds with trans-Azo much more strongly than with cis-Azo, the cavities of CDs within the aggregates should be occupied dominantly by trans-Azo, instead of cis-Azo. We wonder if it is possible that some substrates, which can also bind with CD, can be controllably loaded into the aggregates through the photoisomerization of Azo. To confirm this hypothesis, we chose pyrene (Py) as a probe, whose fluorescence is sensitive to the polarity of its environment [61–63]. The emission intensity ratio I_1/I_3 of Py at 373 nm (I_1) and 383 nm (I_3) indicates the different polarity of the surrounding environment. Although there is no exact criterion to estimate what value of I_1/I_3 indicates polar or nonpolar environment for pyrene, the change of I_1/I_3 ratio in the fluorescence emission of pyrene can be used to indicate the polarity variation of the environment. Strong emission at 383 nm with low I_1/I_3 value indicates low polarity of the environment. As shown in Fig. 5b, emission of Py at 383 nm is clearly observed in P-Azo/CD1 after UV irradiation, and I_1/I_3 was measured as 1.229, indicating that Py is in low polar environment.

We assume the low polar environment for Py should be provided by CDs because cis-Azo does not bind well with CDs. To prove this assumption, we introduced adamantane ethanol (Ad), which can bind with CD more strongly than Py or Azo does [64,65], into P-Azo/CD1 to occupy the cavities of CDs. As shown in Fig. 5d, the emission of Py at 383 nm disappeared almost completely and I_1/I_3 increased to 1.419, indicating that CDs binding with Ad do not provide low-polar environment to Py anymore. However, when Azo is in trans-form, emission of Py at 383 nm can also be seen, as shown in Fig. 5a, and I_1/I_3 is calculated as 1.078. Upon addition of Ad, the emission at 383 nm was diminished and I_1/I_3 increased. Like in cis-P-Azo/CD1, the environment polarity change of Py in the presence of Ad suggests that CDs in trans-P-Azo/CD1 can still bind

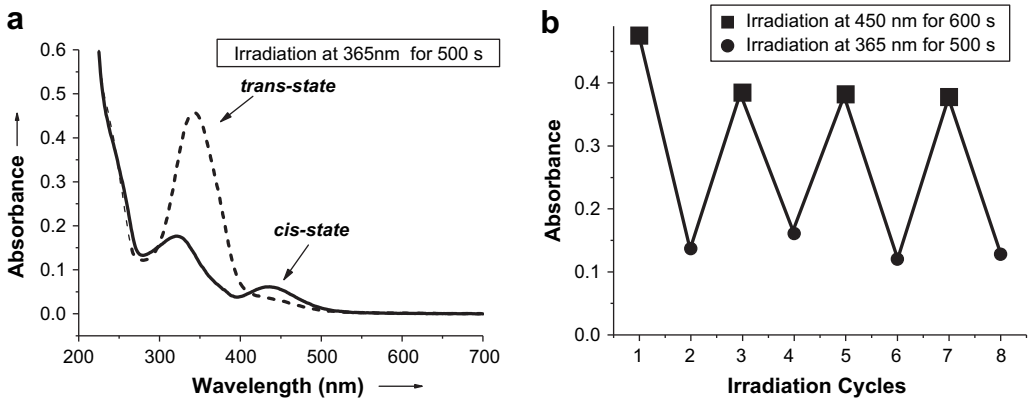


Fig. 3. (a) UV spectra for photoisomerization of Azo in P-Azo/CD1; (b) The photoisomerization cycles of UV absorbance of P-Azo/CD1 at 342 nm.

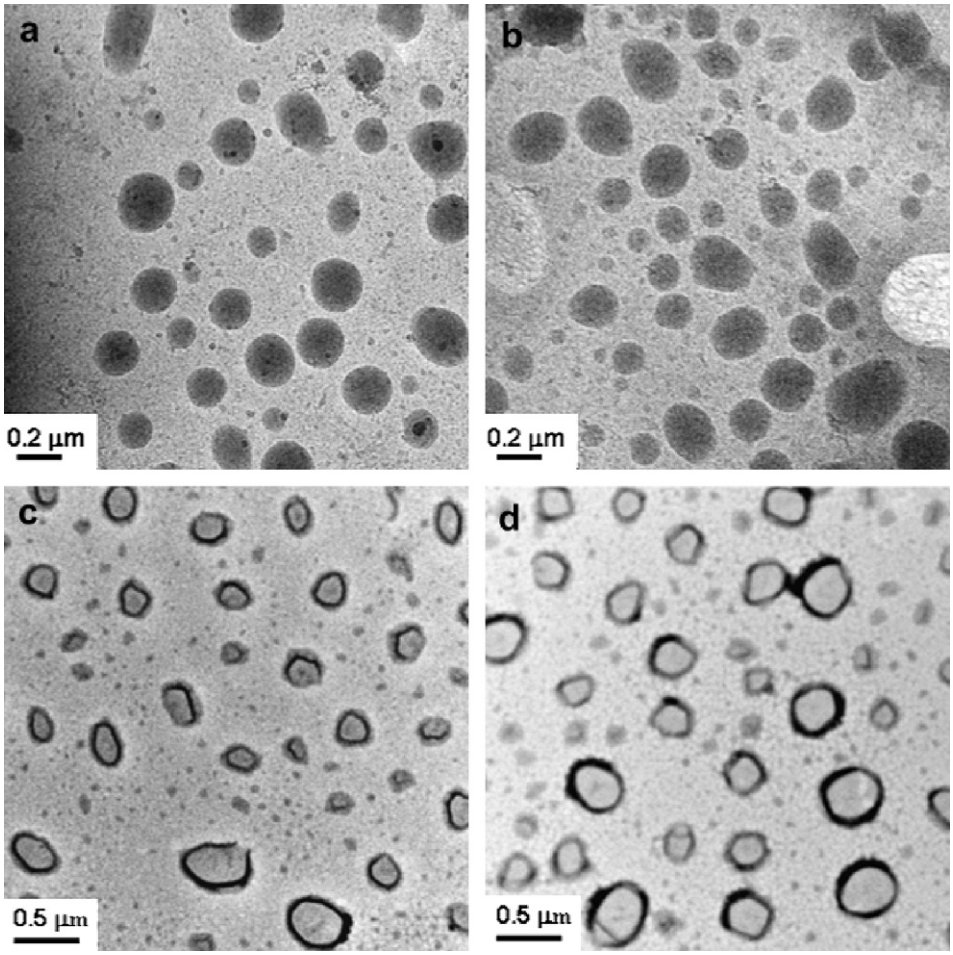


Fig. 4. Cyro-TEM observation of cross-linked aggregates P-Azo/CD1 (a) before UV irradiation; (b) after UV irradiation. TEM observation of cross-linked aggregates P-Azo/CD1 (c) before UV irradiation; (d) after UV irradiation.

with Py. It is easily understood that CDs bind with Py when Azo is in cis-form, but why do CDs still show binding behavior to Py when Azo is in trans-form? There are two possibilities: one is that CD binds with Py more favorably than with trans-Azo, but this is not the case because the binding constant between Py with β-CD ranges from 44 to 675 $M^{-1}$and the binding constant between trans-Azo with β-CD is more than 1000 M^{-1}; the other one is that the amount of Azo is not high enough to occupy most cavities of CDs

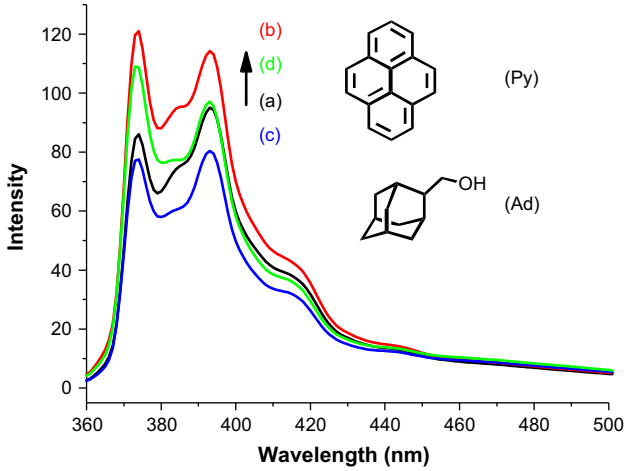


Fig. 5. Fluorescence spectra of Py in P-Azo/CD1 excited at 339 nm (a) before; (b) after UV irradiation for 500 s; (c) in the presence of excess Ad before; and (d) after UV irradiation for 500 s. In the fluorescence measurements, Py (20 μL, 0.1 mg/mL, in acetone) was dispersed in 3 mL of block copolymer aggregate solution. 50 μL of Ad ethanol solution with the concentration of 5 mg/mL was added afterwards.

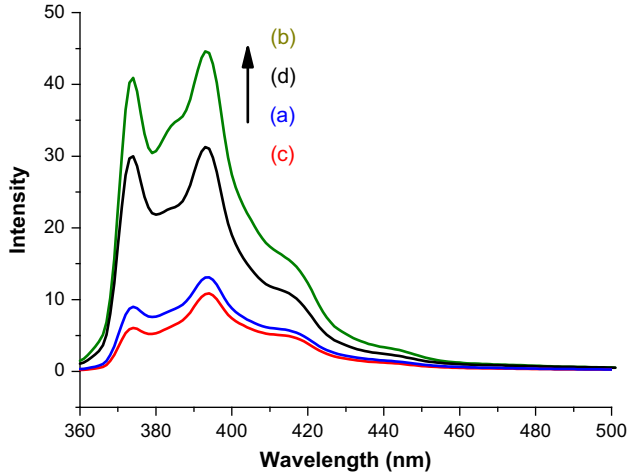


Fig. 6. Fluorescence spectra of Py in P-Azo/CD2 excited at 339 nm (a) before; (b) after UV irradiation for 500 s; (c) in the presence of excess Ad before; and (d) after UV irradiation for 500 s. The fluorescence measurement condition and Ad addition are the same to P-Azo/CD1.

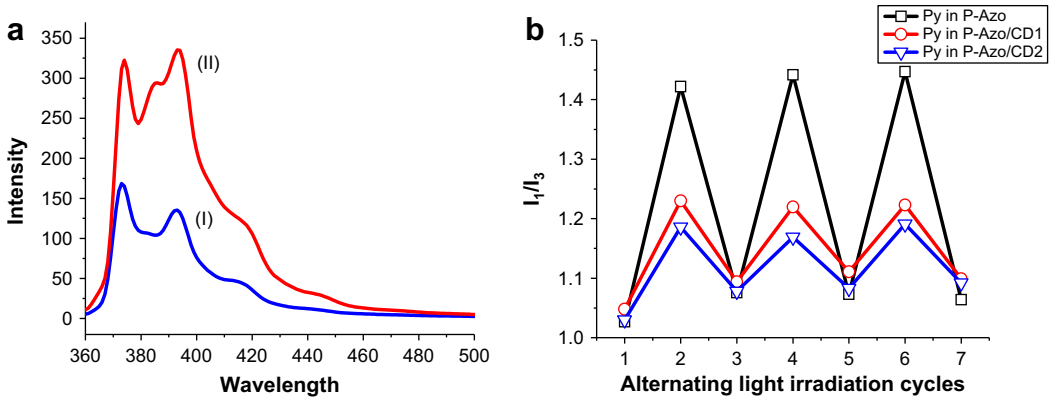


Fig. 7. (a) The fluorescence of Py excited at 339 nm (I) in pure water; (II) in P-CD solution. (b) The reversible environmental polarity of the block copolymer aggregates by photo-irradiation. 20 μL of Py acetone solution (0.1 mg/mL) was dispersed in 3 mL solution of block copolymer aggregates.

[66]. In addition, one point should be noted here is that fluorescence of Py can be partly quenched by trans-Azo, which induced the fluorescence intensity of Py to be lower in trans P-Azo/CD1 than that in cis P-Azo/CD1.

To address the issue if few CDs bind with Py in the presence of excess trans-Azo within the aggregates, we prepared another block copolymer aggregate (P-Azo/CD2) composed of PEG–PAA-g-$CD_{5\%}$ and PEG–PAA-g-$Azo_{22.5\%}$, in which the molar ratio of Azo to CD was 6.6:1 but the molar concentration of CDs is the same with that in P-Azo/CD1. The association between CD and azobenzene depends on their concentration and the binding constant between them. It is estimated that 45% CD can be associated with trans-azobenzene according to the binding equation. When Py was added into cis-P-Azo/CD2 solution, as shown in Fig. 6b, the emission of Py at 383 nm can be well observed and I_1/I_3 was 1.176. Like in cis-P-Azo/CD1, the emission of at 383 nm disappeared and its I_1/I_3 in cis-P-Azo/CD2 reached 1.336 in the presence of Ad. The increase of I_1/I_3 of Py in cis-P-Azo/CD2 upon addition of Ad supports the fact that CDs in cis-P-Azo/CD2 can provide low-polar environment to Py when Azo is in cis-form. As shown in Fig. 6a, Py in trans-P-Azo/CD2 does not display clear emission at 383 nm, but I_1/I_3 was still as low as 1.027. Although we do not fully understand the reason for the low I_1/I_3 value which may result from the fluorescence quenching of Py by trans-Azo, we confirm that CDs in trans-P-Azo/CD2 do not bind with Py because the emission spectrum of Py shows almost no change upon addition of Ad and I_1/I_3 is measured nearly the same with that before Ad addition. These results indicate that enough trans-Azo in the aggregates can compete to bind with CDs rather than Py. In other words, CDs in P-Azo/CD2 can bind with Py under control through the photoisomerization of Azo.

To further confirm that it is the photoisomerization of Azo that controls the loading of Py into CDs in the aggregates, we prepared the other two aggregates by employing the method of template and cross-linking, P-CD and P-Azo which were formed by only PEG–PAA-g-$CD_{5\%}$ or PEG–PAA-g-$Azo_{5\%}$, respectively. As expected, CD within P-CD can bind with Py very well. As shown in Fig. 7a, comparing with Py dissolved in pure water, Py in P-CD solution displays clear emission at 383 nm and its I_1/I_3 is as low as 1.067, indicating that P-CD provides non-polar environment to Py and this is the evidence that empty CDs in P-CD bind well with Py. However, the binding behaviour for P-CD with Py cannot be controlled by photo-irradiation for the absence of Azo in the aggregates. As to P-Azo, there is no CD in the aggregates, so Py should not bind with the aggregates reversibly by host–guest interaction. As seen in Table 1, we propose that the low I_1/I_3 value of Py in trans-P-Azo is also caused by the fluorescence quenching. As shown in Fig. 7b, I_1/I_3 values of Py in the aggregates are reproduced well by alternating UV and visible light irradiation, thus supporting the fact that the block copolymer aggregates with photo-switches can show reversible binding with Py by photo-stimuli.

To clarify the release of the guest molecules from the aggregates when Azo rebinds with CD after visible light irradiation, we have chosen an electro-active molecule, ferrocene carboxylic acid (FcCOOH), as another probe to observe its binding behaviour with P-Azo/CD2 in different conditions. Since the interior of the aggregates is cross-linked partly, there should have some free carboxylic acid groups left in the aggregates. Herein, in the presence of EDC/NHS, a layer of P-Azo/CD2 can be covalently attached on the surface of Au electrode which is decorated with amine groups by

Table 1
Polarity of the block copolymer aggregates probed by Py.

I_1/I_3	P-Azo/CD1	P-Azo/CD1 (Ad)	P-Azo/CD2	P-Azo/CD2 (Ad)	P-CD	P-CD/Ad	P-Azo
Before UV	1.078	1.274	1.027	1.010	1.057	1.339	1.027
After UV	1.229	1.419	1.176	1.336	–	–	1.442

I_1 and I_3 are the emission intensity at 373 nm and 383 nm, respectively.

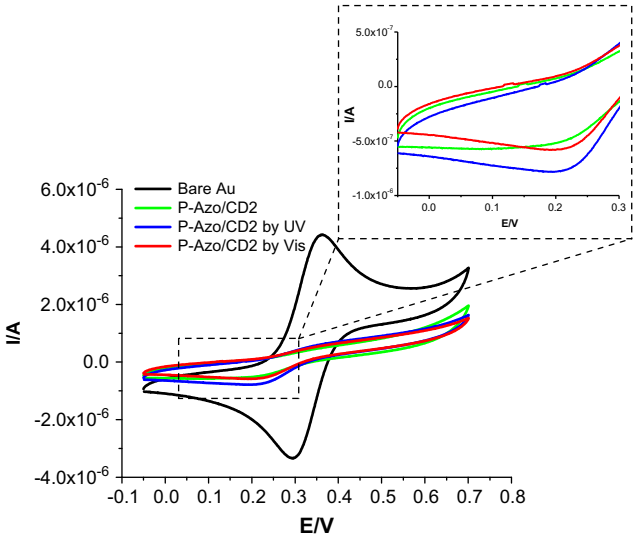


Fig. 8. The cyclic voltammetry (CV) of ferrocene carboxylic acid (FcCOOH) performed on Au electrode modified with P-Azo/CD2. [FcCOOH] = 1 mM. The CV measurements are carried in phosphate buffer solution with pH7.2, and total [PO_4^{3-}] is 0.1 M. The scan rate is 100 mV/s, and the reference electrode is Ag/AgCl.

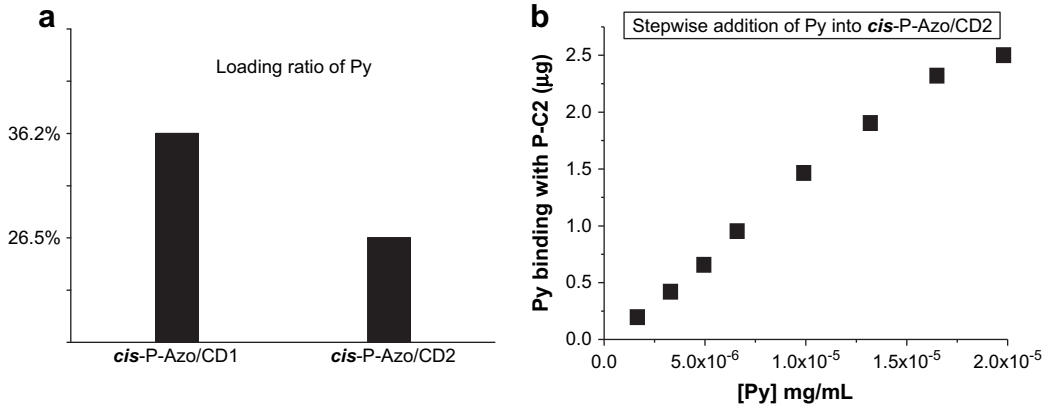


Fig. 9. (a) Loading amount of Py in cis-P-Azo/CD1 and cis-P-Azo/CD2. 20 μL of Py acetone solution (0.1 mg/mL) is dispersed in 3 mL of polymeric aggregate solution. (b) Binding amount of Py with cis-P-Azo/CD2 versus stepwise addition of Py. The fluorescence spectra are measured upon stepwise addition of concentrated Py acetone solution.

cystamine. As shown in Fig. 8, the cyclic voltammetry signal of FcCOOH decreases dramatically on the electrode modified with P-Azo/CD2 rather than on bare Au electrode. This variation indicates the successful modification of P-Azo/CD on Au electrode and this layer prevents FcCOOH from being close to the Au surface. However, after UV irradiation, the increase of reducing current of FcCOOH at 0.21 V implies that FcCOOH can be loaded into the aggregates when the switches are on-state. Moreover, by further visible light irradiation, the reducing current of FcCOOH at 0.21 V goes back, supporting our assumption that the guest molecules are released from the aggregates to some extent.

3.4. The loading amount of pyrene into block copolymer aggregates

A feasible approach to define the loading amount of Py into block copolymer aggregates is through the variation of I_1/I_3 intensity ratio [67,68]. Thus the weight ratio for Py binding with CD versus whole Py in the solution can be estimated by the following equation:

$$\frac{[\mathrm{PyCD}]}{[\mathrm{Py}_0]} = \frac{R_0 - R}{R_0 - R_1}$$

where [PyCD] and [Py_0] are the concentrations of Py binding with CD and whole Py in the solution, respectively. The parameters R_0 and R_1 denote the I_1/I_3 ratio in pure water and CD solution in which all Py molecules are bound with CD. R is the measured ratio in different solution with confined CD concentration. R_0 was detected as high as 1.576 when Py was dispersed in water with the concentration of 2×10^{-6} M. Although R_1 cannot be measured directly, the following applicable linear equation provides $R_0 - R_1$ from the Y-axis intercept.

$$\frac{1}{R_0 - R} = \frac{1}{K(R_0 - R_1)[\mathrm{CD}]} + \frac{1}{R_0 - R_1}$$

The plot of $1/(R_0 - R)$ versus 1/[CD] gave a straight line and $R_0 - R_1$ was found as 0.662. To confirm that CDs in the interior of block copolymer aggregates are free for Py loading, Azo is kept in cis-form by UV irradiation in advance. Finally, the weight ratios for Py binding with cis-P-Azo/CD1 and cis-P-Azo/CD2 are measured as 36.2% and 26.5%, respectively.

What is the relationship between the loading amount and the concentration of substrates? In our work, the driving force for Py loading is based on the host–guest interaction between Py and CD. Therefore, the binding behavior of Py with CD in the aggregates is in equilibrium and the loading amount will depend on Py concentration. More Py will likely be bound into the aggregates at a higher Py concentration. To confirm this assumption, P-Azo/CD2 was taken as an example to observe its loading ability upon stepwise addition of Py. As shown in Fig. 9b, when the concentration of CD in 3 mL cis-P-Azo/CD2 solution is kept at 3.4×10^{-5} M, the amount of Py binding with free CD in the interior of cis-P-Azo/CD2 increases gradually by increasing concentration of Py in the solution. When the concentration of Py reaches 1.9×10^{-5} M, the loading amount of Py into the block copolymer is as high as 2.5 μg. For the poor solubility of Py and fluorescence self-quenching of Py, much higher concentration for Py cannot be tried further.

4. Conclusion

We have successfully fabricated stable block copolymer aggregates with photo-controllable switches that serve as supramolecular containers to load small guest molecules. Interestingly, supramolecular containers can be used to tune the loading and unloading of substrates reversibly without destruction of the cross-linked aggregates. In our future work, we plan to covalently introduce catalysts into the aggregates. In this way, it is anticipated that controllable catalysis could be realized. For example, when the switches are on, the substrates might be loaded to interact with catalysts, and when the switches are off, the products would be released.

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Appendix. Supplementary data

The supplementary data associated with this article can be found in the on-line version, at doi:10.1016/j.polymer.2009.08.005.

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