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Photoresponsive supramolecular strategy for controlled assembly in light-inert double-chain surfactant system

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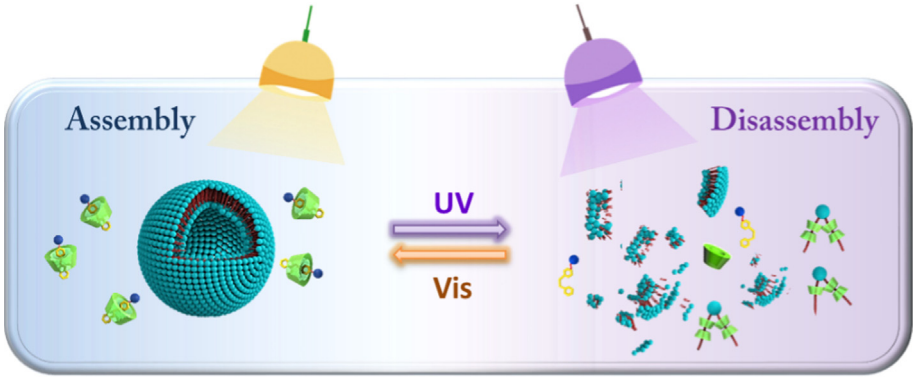
GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: One of the main advances in double-chain surfactant systems has been their progress from the construction of assemblies to the transformation application in medicine and material science, especially to the drug delivery systems. Thus, it is critical to develop stimuli-responsive assemblies based on double-chain surfactants. We predicted that reversible assembly switching can be achieved by manipulation of the ternary host-guest competitive complexation among β-cyclodextrin (β-CD), surfactants, and designed azobenzene (Azo).

Experiments: In this work, Azo was introduced into vesicles using supramolecular assembly strategy. Vesicles are formed only when Azo moieties are in *trans-form*. UV switching of Azo groups led to fast disruption of the Azo@β-CD complexes and relatively slow disintegration of the vesicles. With the alterative irradiation of UV and Vis light, the photoisomerization of azo group provokes the reversible disassembly and reassembly of vesicles.

Findings: This photo-responsive supramolecular strategy offered a controllable way to prepare responsive vesicles assembled from complex double-chain surfactants, such as phospholipids, which could be further used in drug delivery systems. This new perspective is instructive for the design and functional use of complex surfactants assembly. Importantly, the study results paved the way for the development of novel light-responsive assembly materials operating in aqueous media and biological field.

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1. Introduction

Double-chain surfactants with unique molecular geometry have been a study focus because of their potential application in the biomedical fields [1,2]. Phospholipids are one of typical double-chain surfactants. The pioneering observation conducted by Alec Banghams [3,4] about 60 years ago revealed that phospholipids could form closed bilayered structures in aqueous systems. Since then, liposomes have gradually become a promising material carrier from just an exotic object of biophysical research on account of good biocompatibility and biodegradability [5,6].

Generally speaking, double-chain surfactants are difficult to dissolve in water. Of all the types of liposome preparation, methods including hydration of lipids in aqueous buffer, freeze-thaw cycling, film hydration, reversed phase evaporation, normal phase integration, detergent depletion, pH adjustment and microfluidics processes are the most superb [7,8]. However, these preparation methods all require additional postprocessing steps such as solvent removal and sonication, which inevitably induce the heterogeneity and uncontrolled chemical and mechanical conditions [9,10]. In addition, the vesicles formed by such double-chain surfactants incline to arrange tightly and are difficult to be regulated by external stimulation. As a result, the development of a simple method for the preparation and regulation of lipid vesicles is of great significance to the understanding of the cell division mechanism and other life processes as well as designing novel functional materials. Therefore, smart molecular self-assemblies applied in the construction of reversible assemblies with switching biofunctions are arousing intense interest, and their basic units, i.e., the smart supramolecular structures, are responsive to such external stimuli, as pH [11–13], temperature [14–16], and chemicals [17–19].

Light has several additional merits as an external stimulus. It is a mild energy source, and produces no chemical waste [20]. Besides, it can be remotely operated with high spatial and temporal precision at the molecular level, which facilitates the exploration of the variations in mesoscopic phase behavior through the microscopic changes of molecular functional groups [21]. Azo is one of the most employed photo-responsive compounds for self-assemblies in light of the large photo-induced changes in its molecular geometry and physical properties [22–24]. To construct photo-controllable self-assemblies, a responsive group is introduced into the system [25]. However, such covalent practice often consumes a large amount of time and involves purification processes, leading to remarkably high preparation costs. Moreover, owing to biocompatibility, the poisonous solvents (e.g., chloroform) used inevitably in the incorporation of two phenyl rings of the Azo groups into the specific site of one molecule would confine the scope of assembly and the application of light control in the field of life science. It is a demanding and challenging task to introduce functional groups into self-assembly systems (especially insoluble ones, such as lipids) directly by noncovalent interactions.

Azo is known to bind to α-and β- CDs in the stable *trans*-form. Upon UV light irradiation, Azo is transformed into the unstable and bulkier *cis*-form and released from the β-CD cavity [26]. Given the fact that the light-responsive inclusion and exclusion can be reversibly isomerized by interchanging alternate UV and Vis irradiation, various photo-responsive self-assembling systems have been created, including molecular shuttles [27], micelles and vesicles [28–30], gels [26,31], and nanoparticles [32,33]. These systems can combine with and release drugs, proteins, etc. In our previous work, we reported a simple approach to constructing a general photo-responsive system with Azo@β-CD and amphiphilic aggregations formed by single-chain surfactants [24]. The binding capability of surfactants@β-CD is between that of *trans*-Azo@β-CD and *cis*-Azo@β-CD [24,34–37]. Intriguingly, this supramolecular photo-controlled mechanism enables the reversible control of the properties of complex amphiphilic molecular systems and functions as a supramolecular glue for drug delivery.

Herein, we reported the self-assembly of a ternary supramolecular system based on a light-responsive Azo@β-CD complex, which made the reversible formation and dissociation of double-chain surfactant vesicles possible. Egg yolk lecithin (Phosphatidylcholine (PC) is the main component, so PC was used in the following description of egg yolk lecithin related experiments) is a typical lipid selected as an assembly unit waiting to be regulated. It could assemble into vesicles by virtue of ultrasonic cell crasher. Designed Azo and β-CD operate as the photo switch. The introduction of host-guest complex *trans*-Azo@β-CD had no implications for PC vesicles, while β-CD was released into the bulk solution containing *cis*-Azo and formed new host-guest complexes PC@β-CD after UV irradiation, resulting in the collapse of the vesicles. The supramolecular assembly strategy allows for the photo regulation of the light-inert system, and also offers a straightforward alternative to the construction of vesicles formed by hardly soluble double-chain surfactants. Additionally, this smart regulation approach can be adapted to other double-chain surfactant assembly systems such as didodecyldimethylammonium bromide (DDAB), even though they are charged oppositely. With a hydrophobic layer, both PC and DDAB vesicles are able to function well as a drug carrier. Drug molecules are released from vesicle walls when the vesicles disintegrate owing to the photochemical conversion from *trans*-Azo to *cis*-Azo. However, under the action of isomerization induced by visible light, *trans*-Azo is formed again, leading to full restoration of the vesicles. The "photo-responsive insoluble vesicles" are expected to provide insights into the design and functional use of double-chain surfactant self-assemblies. They may also contribute to the development of more bioinspired controllable systems.

2. Experimental section

2.1. Materials

Egg yolk lecithin (AR) was purchased from AlfaAesar China (Tianjin) Chemistry Co., Ltd. Dexamethasone (DEX, 98%) was purchased from Macklin. Didodecyldimethylammonium bromide (DDAB, 98%) and β-Cyclodextrins (β-CD, >98%) were purchased from TCI. Doxorubicin hydrochloride (DOX, >98%) was purchased from Beijing Sehcda Technology Co. Ltd. All the organic solvents (AR) were from Beijing Chemical Works. Distilled water was purified through Milli-Q Advantage A10 Ultrapure Water System. The two Azo molecules used in our paper were synthesized in our laboratory. See Supporting Information for the specific synthetic route, ^{1}H NMR and ESI-MS results. One is Azobenzene-4-sodium benzoate (Azo-Ac for short, in Scheme S1), and another one is 1-[*p*-(phenylazo)benzyl]-pyridinium bromide (Azo-Py for short, in Scheme S2).

2.2. Sample preparation

All solutions were equilibrated at 25 °C for 24 h before further analysis. The UV light was generated by FC-100/F long-wave UV light (365 nm, 230 V, 50 Hz, and 0.9 A). The duration of irradiation was according to specific experimental requirements. Generally speaking, half an hour to an hour of UV irradiation is enough to achieve the disassembly of vesicles. After exposure to Vis light for 4 h, the vesicles can be reassembled again. In photo-responsive systems, the PC vesicles were a mixture of PC (0.6 g/L or 0.625 mM) with Azo-Ac@β-CD (2.5:2.5 mM) in aqueous solution, and the DDAB vesicle was a mixture of DDAB (0.5 mM) with Azo-Py@β-CD (1:1 mM) in aqueous solution. The samples after UV irradiation should kept away from light.

Assembly of PC vesicles: PC is insoluble in water. PC can form stable vesicles by ultrasonic cell crasher (Scientz Biotechnology Co. Ltd., JY92-Ⅱ) for 4 h. The bulk phase was an aqueous solution containing 5 wt% ethanol. The working mode of cell breaker is: titanium alloy probe 3 mm, ultrasonic power 200 W, interval time 5 s, ultrasonic time 5 s, working 99 times. 2.4 g/L PC aqueous solution was selected as stock solution.

Assembly of DDAB vesicles [38]: Desired amounts of DDAB and water were added into tubes, and a clear solution was obtained by ultrasonic cleaners (KQ300DE, water-bath type) for 2 h at 50 °C, where DDAB vesicles were formed. 10 mM DDAB aqueous solution was selected as stock solution.

2.3. Transmission electron microscopy observation (TEM)

Samples were observed using a JEM-2100 instrument (80 kV). The samples were prepared with negative-staining methods. Drops of samples were added onto 200-mesh copper grids coated with a formvar film and followed stained by uranium dioxane acetate saturated solution. For Cryo-TEM measurements, a piece of micro grid copper mesh is immersed in the sample solution, and then the redundant liquid is blotted off with filter paper to form a thin film on the copper mesh. The copper mesh with thin film was quickly frozen in liquid ethane and was observed in TEM at −183 °C.

2.4. Spectral measurements

UV–vis absorbance measurements were carried out on a UV-1800 SHIMADZU spectrophotometer (Hitachi. Ltd., Tokyo, Japan), and 1 mm quartz cuvets were used. All spectral measurements were recorded at 25 °C. In order to prevent photoisomerization, UV irradiated samples (under 1 h UV irradiation) need to be placed in the sample tank immediately in the dark for testing.

2.5. Dynamic light scattering (DLS):

An ALV/DLS/SLS-5022F light-scattering apparatus equipped with a 22 mW He-Ne laser operating at 632.8 nm was used to perform DLS experiments. All the samples were filtered through a 0.45 μm membrane filter of hydrophilic PVDF to remove dust before the measurements. The scattering angle was 90°. The autocorrelation function ($g^{(2)}(q, \tau)$) of the scattering light intensity was measured directly by dynamic light scattering instrument, and was further analyzed by CONTIN method [39]. The specific autocorrelation function was shown on the Methods section in Supplementary materials.

2.6. ^1H nuclear magnetic resonance (^1H NMR)

^1H NMR experiments were conducted on a Bruker ARX 500 MHz spectrometer with D_2O as the solvent at 25 °C. The concentration was consistent in all experiments. In the NMR experiment, the solvent water changed into D_2O. For PC vesicles system, the concentration of PC, Azo-Ac and β-CD is 0.625 mM, 2.5 mM and 2.5 mM, respectively; for DDAB vesicles system, the concentration of DDAB, Azo-Py and β-CD is 0.5 mM, 1 mM and 1 mM, respectively.

2.7. Isothermal titration calorimetry (ITC)

ITC measurements were performed in a TAM 2277–201 microcalorimetric system (Thermometric AB, Järfälla, Sweden) at 25 °C. To monitor the binding processes of the DDAB with β-CD, the sample cells were initially loaded with β-CD solution (2 mM), and then a certain concentration of DDAB molecule solution (5 mM) was injected consecutively into the stirred sample cell in portions of 10 μL via a 500 μL Hamilton syringe until the interaction process was completed. The system was stirred at 90 rpm with a gold propeller. Each ITC curve was repeated at least twice with a deviation of ±4%.

2.8. Drug loading

DOX and DEX were selected as drug modes in our experiment. Drug was loaded using an equilibrium dialysis method [40]. Stock solutions of DOX (10 mg/ml) were prepared in water. Stock solutions of DEX (5 mg/ml) were prepared in 1:1 water/ethanol mixed solvent. The stock solution of two drugs was added into the aqueous solution of Azo-Ac/β-CD, which was dropped into the aqueous solution of PC. The mixed system was thermostatically incubated in dark for 24 h at 25 °C to allow drug loading sufficiently. The solution was further transferred to a dialysisbag (MWCO = 3500) against water in the dark for 48 h to remove free drug molecules, PC, Azo, and β-CD. The encapsulation percentage of drug was determined by UV–Vis spectrophotometry at 480 nm for DOX and 280 nm for DEX in water according to the calibration curve of DOX with different concentrations. The calibration curve was acquired with different drug concentrations. Encapsulation efficiency were calculated according to the following equations:

$$\text{Encapsulation efficiency} = \frac{\textit{weight of loaded drug}}{\textit{weight of feeding drug}} \times 100\%$$

2.9. Drug release from PC vesicles in vitro

Dispersed drug loaded vesicles (~$C_{PC@Azo\text{-}Ac/\beta\text{-}CD}$ = 0.75 mM) were added to a dialysisbag (MWCO = 3500), which was then incubated in 80 mL phosphate buffer saline (PBS) at pH 7.4 at 37 °C in a water bath. At predetermined frequencies, 3 mL of incubated solution was taken out, and 3 mL of fresh PBS was added to refill the incubation solution to 80 mL. The amount of released drug was determined by measuring the UV–Vis absorbance of the solutions at 480 nm or 280 nm. For the UV light–triggered drug release measurements, the drug-loaded vesicles should be subjected to UV light irradiation for 10 min and 1 h before dialysis. Drug releasing efficiency was calculated according to the following equation:

$$\text{Drug releasing efficiency} = \frac{\textit{weight of released drug}}{\textit{weight of loaded drug}} \times 100\%$$

2.10. Cytotoxicity measurements in vitro

MTT assay was employed to measure the cell viability in *vitro* [41]. Hela cells were seeded into 96-well culture plates at a density of 5 x10^3 cells well and grown for 24 h. Then DOX-PC with and without Azo-Ac@β-CD carrier was added into 96-well culture plates. The concentration of PC carrier was in the range of 0-100 μg/mL. After 48 h incubation, the cell viability was measured at 540 nm by a micro plate reader. The following formula was used. Each assay was repeated six times.

$$\text{Cell viability} = \frac{A_{540\ nm}\ \text{for the treated cells}}{A_{540\ nm}\ \text{for the control cells}} \times 100\%$$

3. Results and discussion

3.1. PC vesicle

The economical and easily available egg yolk lecithin was chosen in our work. The major ingredient of egg lecithin is PC. PC is composed of one phosphate polar head and two hydrophobic tails, and one of the tails contains unsaturated double bonds. The cho-

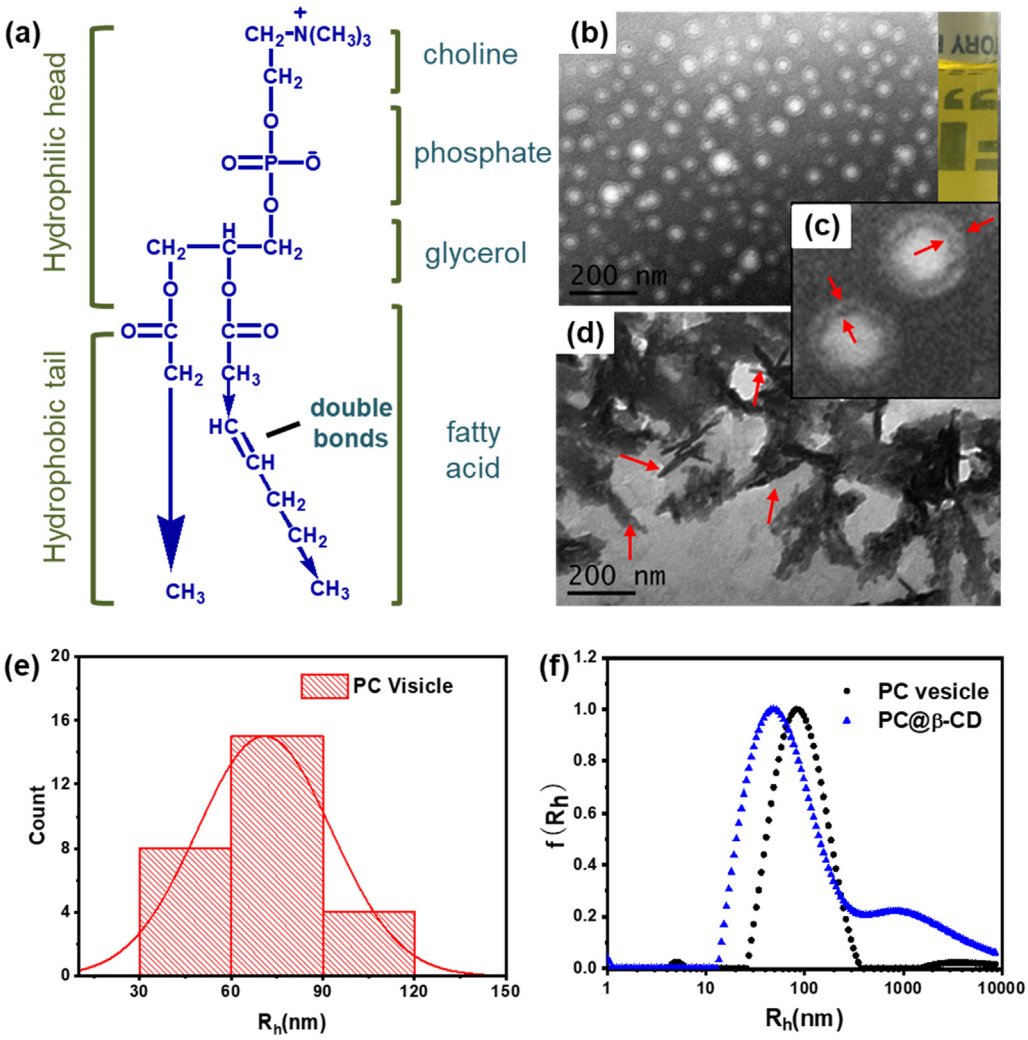

Fig. 1. (a) Structural Formula of Phosphatidylcholine (PC), TEM images of (b) PC vesicles (0.6 g/L), and the inset in Fig. 1b is the macrophotograph of PC solution, (c) larger version of Fig. 1b and (d) pin-shape structure after adding β-CD (PC: β-CD = 0.6 g/L : 2.5 mM); (e) The radius distribution of PC vesicles observed in the TEM image, Fig. 1b; (f) Dynamic light scattering result of PC vesicles before and after the addition of β-CD.

line group also present in the PC head group, as shown in Fig. 1a, which endows PC with a zwitterionic (dipolar) character. PC itself cannot disperse in aqueous solution, and it was processed into a transparent solution (the inset in Fig. 1b) with a small amount of ethanol (5 wt%) using an ultrasonic cell crasher. When dissolved in aqueous solution, the amphiphilic PC assembled into vesicles. The morphology and size distribution of the PC assembly were analyzed by transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements. Vesicles with a double-layer structure can be observed in Fig. 1b, 1 and Fig. S1, and the statistical average radius is around 70 nm according to their size distribution (Fig. 1e). An average hydration radius of 77 nm was obtained by DLS results in Fig. 1f, which was consistent with TEM results. It should be noted that the repeatability of the result is poor. DLS results of different batches of vesicles prepared by ultrasonic crasher are shown in Fig. S2.

After adding β-CD into PC stock solution, irregular rough pin-shaped aggregates were formed, as shown in the TEM images in Fig. 1d. The β-CD has a special structure of a hydrophilic exterior and a hydrophobic cavity [40]. The alkyl chain inserted into the hydrophobic cavity and forms an inclusion complex when β-CD was mixed with the PC stock solution, and it has been proved that an average of four β-CD molecules bind to each PC molecule with an enthalpy of reaction of 46 kJ mol^{-1} [42]. Besides, no precipitation was observed visually, even after the mixture was treated by high-speed centrifugation, proving that the inclusion compound was soluble. The interactions between β-CDs and phospholipids have been examined in prior studies [42,43]. Generally, the hydrophobic cavity of β-CD contains the carbon chain of PC, implying that lipid vesicles are dissolved and replaced by soluble β-CD–lipid complexes under a strong interaction between β-CDs and phospholipids. We can further infer from it that the formation of irregular structures in PC@β-CD system is a result of the complexation between PC and β-CD. Since β-CD is deemed as a water structure breaking reagent [44], it may interact with hydrated water molecules on the PC vesicle surface and slightly weaken the condensation forces of PC molecules, thereby affecting PC chain packing. According to the DLS results, the newly formed aggregate had hydration radius of 48.5 nm and 885.0 nm in Fig. 1f after inclusion with β-CD, demonstrating that new aggregates were formed by PC@β-CD complex. PC@β-CD systems exhibited slower decaying autocorrelation curves (Fig. S3), suggesting larger size particle. Also, the autocorrelation curves decayed and then fluctuated as the equilibrium time grows, indicating that the aggregate was not a classical spherical structure, which was consistent with the results of the radius distribution in Fig. 1f.

3.2. Photo responsiveness of the PC assembly

Azo-Ac (Scheme S1) was introduced into this light control system. The transformation of Azo-Ac molecules upon UV irradiation was monitored by UV–vis spectroscopy (Fig. S4). Driven by hydrophobic and van der Waals interactions, *trans*-Azo-Ac entered the cavity of β-CD and Azo-Ac@β-CD complexes were generated. However, when *trans*-form of Azo-Ac was converted to *cis*-form, β-CD was unable to include the bulky *cis*- form anymore due to the spatial mismatch. Subsequently, the light-induced disassembly and reassembly of the vesicles was investigated by analyzing the conversion between photo-responsive *trans*-Azo and *cis*-Azo complexes. The light-responsive ternary system is illustrated in Fig. 2a-c.

The PC assembly structure of this system was not affected by the presence of Azo-Ac@β-CD complexes. However, when Azo-Ac was converted into *cis*- form, a large number of "free" β-CDs were released due to the decreased binding force between Azo-Ac and β-CDs, and they preferentially bound to amphiphilic molecules, thereby altering the assembly behavior. The photo-regulation of amphiphilic self-assemblies can be achieved by alternating operation of Vis/UV irradiation.

Before UV light exposure, Azo-Ac was in *trans*-form and inserted into the cavity of β-CD. The inclusion of *trans*-Azo-Ac and β-CD was confirmed by ^{1}H NMR (Fig. 3), in which H-3 and H-5 peaks of β-CD moved towards the higher field by 0.13 ppm after β-CD was combined with Azo-Ac. When adding the Azo-Ac@β-CD to the PC system, the vesicle structure formed again without obvious damage, as suggested by the TEM results (Fig. 2d). According to the DLS results, the aggregate of the system has a hydration radius of 88 nm (Fig. 2g), which are same to those of the aggregate of the simple PC system.

The Azo-Ac@β-CD complexes underwent *trans–cis* photoisomerization. Before UV illumination, there is about 87% *trans*- content in the solution as calculated from ^{1}H NMR spectrum of aromatic protons in Azo group (Fig. 3). After UV illumination, the aromatic protons of the *cis*-Azo-Ac shift further upfield due to the magnetically anisotropic effect. The amount of *trans*- content was reduced to 38% while the *cis*- content can increase to 62%. Fig. 2h demonstrates a dramatic decline in the absorption of *trans*-Azo-Ac and concomitantly an absorption peak attributable to *cis*-Azo-Ac upon UV irradiation. Interestingly, the absorption of *cis*-Azo-Ac in the presence of β-CD was almost the same as that of pure *cis*-Azo-Ac, which suggested that the *cis*-form hardly interacted with β-CD (Fig. S5). Under this condition, β-CD caught the hydrophobic chain of PC tightly, destructed the vesicles and formed pin-shaped structures (Fig. 2e). This was further demonstrated by ^{1}H NMR results that the proton peaks ascribing to H-3 and H-5 in the cavity of β-CD and the hydrophobic tail moved towards higher field by 0.09 ppm (Fig. 3). The chemical shifting was probably due to the hydrophobic interaction. According to DLS measurements, the average particle size decreased to about 73 nm (Fig. 2g). The above observations also indicated the assembly structure transformation in response to light irradiation.

Azo-Ac was converted to the *trans*-form when the system was exposed to the visible light. Since the binding constant of PC@β-

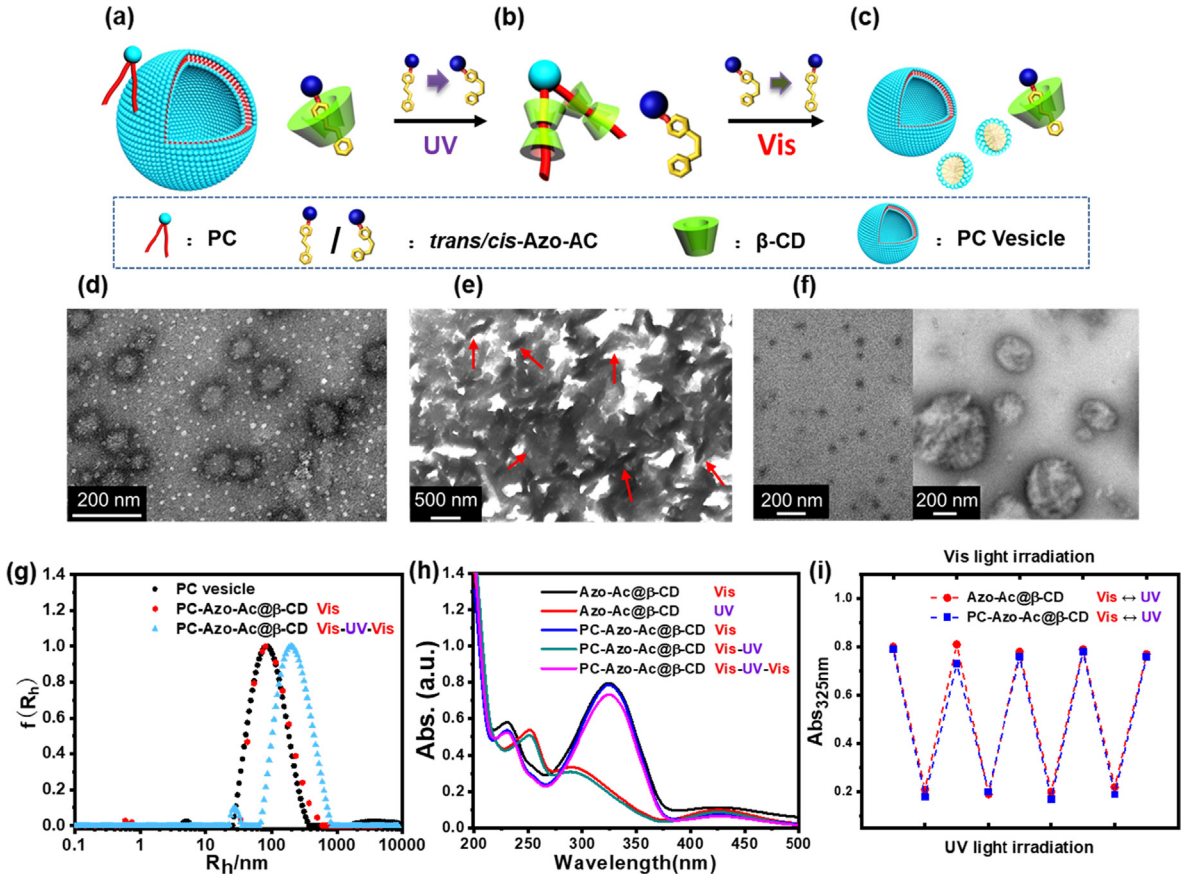

Fig. 2. (a-c) Schematic presentation of the PC vesicle triggered assembly and disassembly mediated by light-responsive formation and disruption of the complex formed between β-CD and Azo-Ac; TEM images: (d) PC vesicles with *trans*-Azo-Ac@β-CD; (e) the formation of broken vesicles (pin-like structure) upon UV irradiation; (f) the reformation of large and small vesicles upon UV irradiation and followed by visible light again; (g) DLS results of PC vesicles and PC vesicles with *trans*-Azo-Ac@β-CD upon alternative UV/Vis irradiation; (h) UV–Vis absorption spectra and (i) repeated absorbance (at 325 nm) of Azo-Ac@β-CD and PC-Azo-Ac@β-CD.

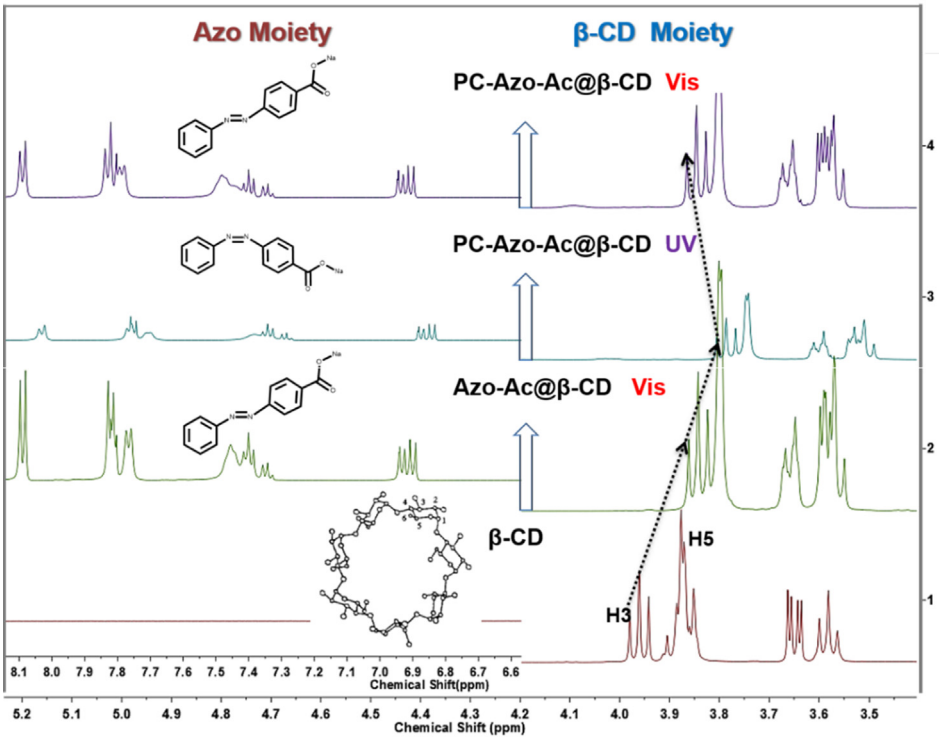

Fig. 3. Partial ^{1}H NMR spectra of β-CD, Azo-Ac, PC-Azo-Ac@β-CD before and after UV irritation, including the spectra of Azo and β-CD moiety.

CD was between that of *trans*- and *cis*-Azo-Ac@β-CD, *trans*-Azo-Ac made the ingress into the cavity of β-CD again and pushed PC out under the radiation of visible light during the host–guest competition. Peaks of H-3 and H-5 in β-CD in ^{1}H NMR moved towards the lower field and back to the original position of *trans*-Azo@β-CD without adding PC (Fig. 3). Interestingly, the pin-shaped structures were shifted into big vesicles and micelles upon Vis irradiation, as can be observed in TEM images (Fig. 2f). DLS results (Fig. 2g) also showed two types of corresponding peaks. When the PC system was radiated by the Vis light, the PC molecules in aqueous solution returned back to vesicles spontaneously without the help of the ultrasonic cell crasher and the co-solvent, and the light reversible control was achieved. The reversibility of the transformation of disassembly and reassembly triggered by UV/Vis light irradiation could be verified by UV–Vis spectra (Fig. 2i). From TEM images (Fig. S6), it was difficult to find the vesicles during TEM observation after the second UV irradiation, only some small fragments formed after the vesicles disintegrated; when exposed to Vis light again, the vesicles regenerated.

3.3. Photo responsive capture and release of drugs

Interestingly, the self-assembly of this host–guest system could be controlled by simply changing light irradiation to induce the ternary host–guest competition. The strength of the interactions in the *trans*-Azo-Ac@β-CD system is generally the strongest, followed by that in the PC@β-CD system and the *cis*- Azo-Ac@β-CD system successively [37,45,46]. The stimuli-responsive vesicles can be potentially employed to transport the entrapped drug and further trigger the drug release by light stimuli at the desired point.

To investigate whether PC vesicle could be used as a potential light-controlled drug release system, DOX and DEX were encapsulated inside the hydrophilic and hydrophobic region of vesicles, respectively. It was evident in TEM images that the PC vesicular structure remained intact after loading DOX and DEX (Fig. 4). DOX is an archetypal anticancer drug, and can be encapsulated in the hydrophilic region of vesicles. DEX is similar to cholesterol, which is hardly soluble in water and can interact with PC via the hydrophobic effect, having anti-inflammatory and anti-rheumatism effects [47,48]. According to encapsulation efficiency measurements, it is calculated that about 76.7% (w/w) of DOX and 67.5% (w/w) of DEX were encapsulated in the PC vesicles. These values are comparable to those in other co-assembled drug delivery systems [49]. The drug-loaded vesicles could be dissociated under UV irradiation. In Fig. 5a, about 22% entrapped DOX was released 10 min after UV irradiation, and a further 26.8% was released over time. Similarly, 38% of the loaded DEX was released after 10 min of UV irradiation, and the release rate reached 56.2% 1 h later (Fig. 5b). In contrast, there was no significant increase in the released amounts of drugs even after 10 h without UV light irradiation. Therefore, stimulating the drug release by UV light was expected to be exciting.

Due to the extremely small toxicity to cells (Fig. 5c), PC-Azo-Ac@β-CD vesicles are expected to be used as a potential biocompatible drug carrier in vitro. The release of DOX resulted in the decrease of the viability of Hela cells. According to Fig. 5d, the viability of the Hela cells after UV irradiation was obviously lower than that before irradiation, especially when the UV light was strong. It is noteworthy that the PC-Azo-Ac@β-CD could still form stable vesicles in DMEM (Dulbecco's modified eagle medium, a medium containing various amino acids and glucose) solution used to culture the cells (Fig. S7).

3.4. System extension: photo regulation of DDAB vesicle

It could be predicted that the photo-control and reconstruction of the system could be achieved by using a hydrocarbon surfactant comprising a hydrophilic head and two hydrophobic tails. Besides, the binding constant of the surfactant with β-CD should be between that of *cis*-Azo and *trans*-Azo with β-CD. PC belongs to a

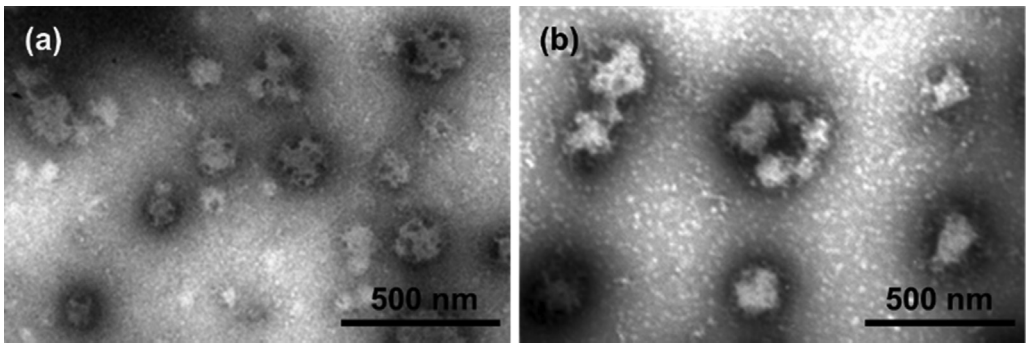

Fig. 4. TEM images of PC-Azo-Ac@β-CD vesicles loaded with (a) DOX and (b) DEX.

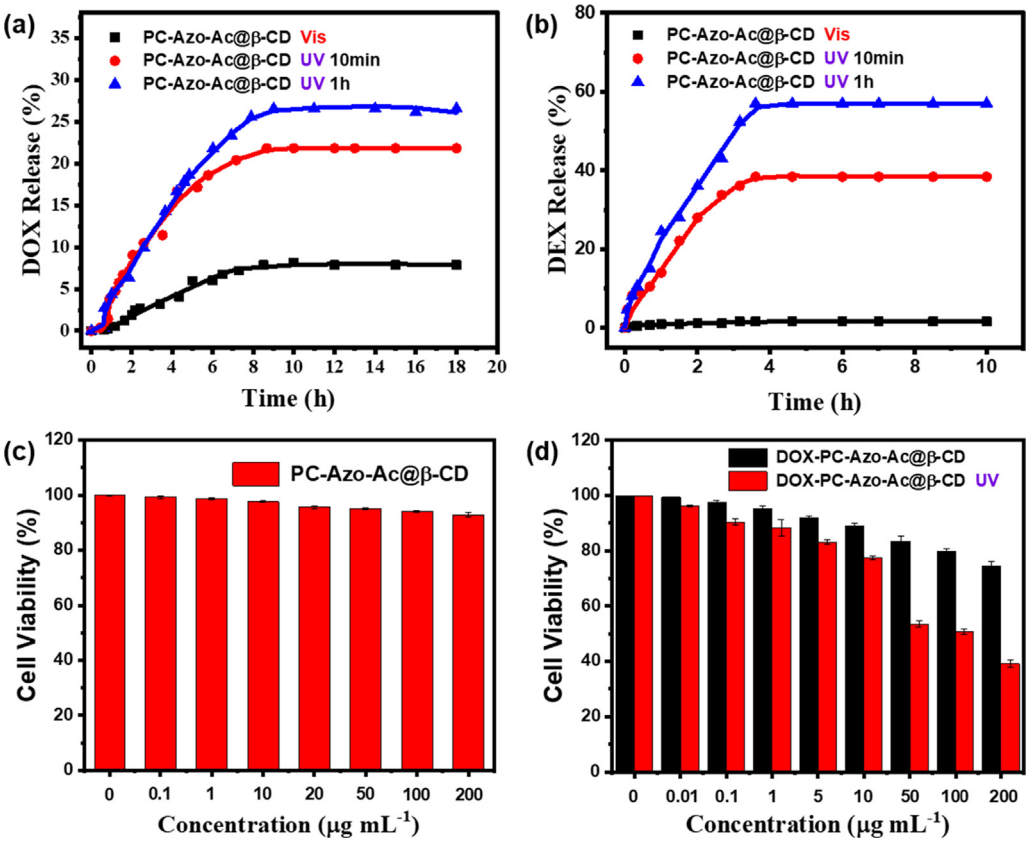

Fig. 5. (a) DOX and (b) DEX release curves of PC@β-CD/Azo systems under visible light (black), UV light for 10 min (red) and 1 h (blue) in PBS at pH 7.4 and 37 °C; (c) Cytotoxicity of different concentration of PC-Azo-Ac@β-CD vesicles to Hela cells after 48 h; (d) Cytotoxicity of different concentration of DOX-loaded vesicles under visible light (black) and UV light (red) for 10 min in vitro measured with Hela cells. Data were presented as the mean ± standard deviation (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

neutral double-tailed hydrocarbon surfactant. For further verification of the universal applicability of this blue print, we chose DDAB, a cationic double-tailed hydrocarbon surfactant (Fig. S8a) for the research of non-neutral systems. To avoid electrostatic interactions between DDAB and photo-responsive Azo, we synthesized Azo-Py (Scheme S2), a cationic Azo molecule.

After ultrasound treatment for 2 h, DDAB formed vesicles under 50 °C (Fig. S8b), and the diameters ranged from hundreds of nanometers to a few microns. According to ITC and mass spectrum results (Fig. S9), the binding ratio of DDAB and β-CD was 1:2. When β-CD was added to the DDAB system and the molar ratio between the two was 2:1, the alkyl chain of DDAB took over to the β-CD cavity, and the proton peaks moved towards higher field. Consequently, the vesicles were damaged, and only some fragments were observed (Fig. S10), which no longer had the characteristics of vesicles (clear edge and middle hydration layer). The above findings demonstrated the great value of the photo-controllable "Azo@β-CD" in the regulation of assembly and disassembly (the schematic illustration is shown in Fig. 6a). It was assumed that Azo-Py changed from *trans*-form into *cis*-form upon UV irradiation (Fig. S11). In visible light, the *trans*-Azo-Py occupied the cavity of β-CD due to the weak shielding effect. Peaks in the ^{1}H NMR spectra moved to the lower field (Fig. S12), and the vesicles formed by DDAB (Fig. 6b). Upon UV irradiation, Azo-Py left the hydrophobic cavity of β-CD, and meanwhile, β-CD bound to DDAB to form DDAB@β-CD complexes and break the vesicle structure. Only some irregular fragments of vesicles can be seen in Fig. 6c. Both proton peaks on the alkyl chain of DDAB and the aromatic group on

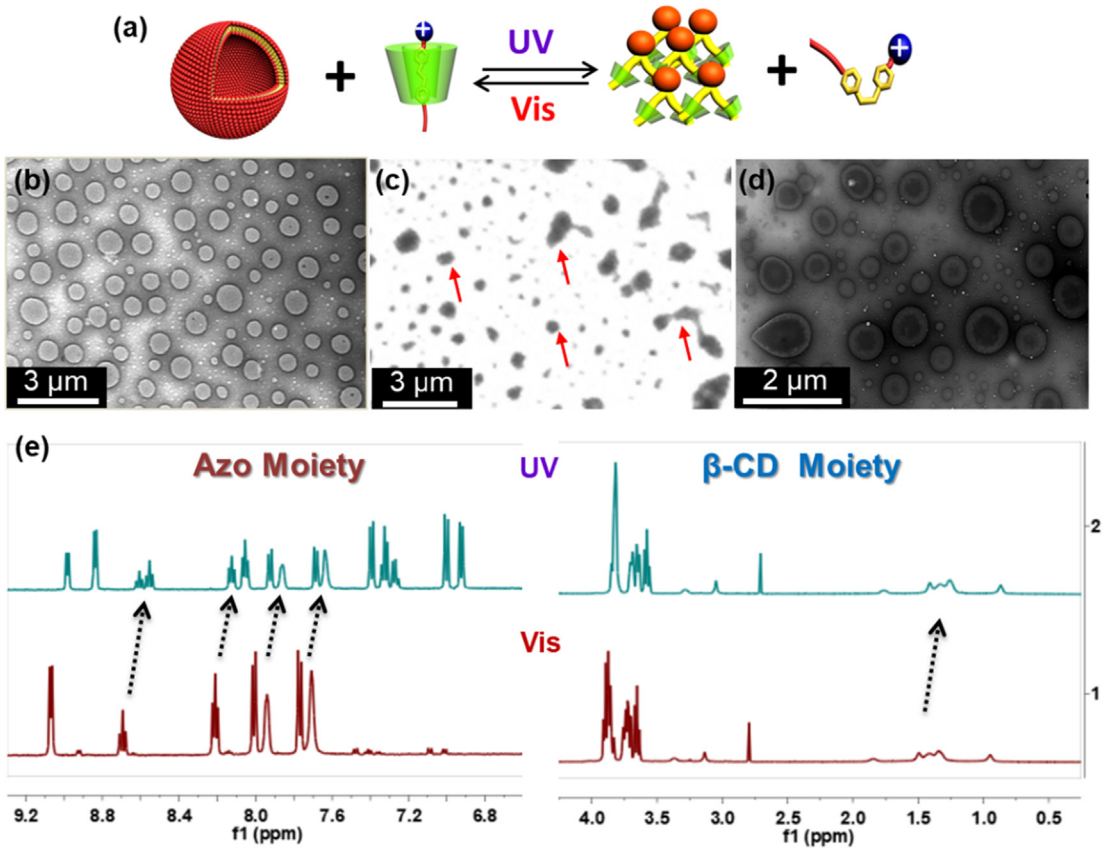

Fig. 6. (a) Schematic presentation of the DDAB vesicle triggered self-assembly and disassembly mediated by light-responsive formation and disruption of the complex formed between β-CD and Azo-Py; TEM images: (b) DDAB vesicles with *trans*-Azo-Py@β-CD; (c) the formation of fragments upon UV irradiation; (d) the reformation of DDAB vesicles upon UV irradiation and followed by visible light again; (e) Partial ^{1}H NMR spectra of DDAB-Azo-Py@β-CD before (red line) and after UV irritation (blue line), including the spectra of Azo and β-CD moiety. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Azo-Py moved towards the higher field again in the ^{1}H NMR spectra (Fig. 6e). When exposed to Vis light again, the *trans*-Azo-Py formed the complex with β-CD and vesicles formed as before.

In the DDAB vesicles system, the assembly and disassembly were governed by alternating irradiation with UV and Vis light, and β-CD@Azo-Py complexes were taken as the photoresponse control. In addition, the UV–Vis spectrum (Fig. S13) of the DDAB system manifested the transformation between the *trans*- and *cis*-isomerism before and after UV irradiation, indicating that Azo-Py was involved in the system and photoresponse occurred. In view of guest competition with β-CD between cationic surfactants and Azo, this photoresponsive supramolecular strategy provide a potential platform for tuning the antibacterial activity (Fig. S14).

4. Conclusion

In summary, we have validated the feasibility of the photo mediated regulation of vesicles assembled by double-chain surfactants. Generally, the covalent synthesis of photo-responsive assemblies is energy-consuming [28,50–52]. The photo-responsive supramolecular assembly proposed in this study, however, can be implemented directly from simple Azo molecules, commercially available double-chain surfactants, and inclusive pocket-β-CDs. This is based on the principle that the binding constant of surfactants@β-CD is between that of *trans*- and *cis*-Azo@β-CD [53,54]. In other words, it means that the conversion of different host–guest complexes can be manipulated subtly and precisely by alternating light irradiation, so as to realize the reversible formation and disintegration of vesicles formed by double-chain surfactants. Due to its good responsiveness, low cost and well biocompatibility, this system is a favorable candidate for drug delivery.

This strategy was previously used to regulate the assembly of single-chain surfactants [24,36]. Its application in complex double-chain surfactant systems has not been explored yet because the physicochemical properties and assembly rules of double-chain surfactants are completely different from those of single-chain surfactants. According to the surfactant packing parameter, double-chain surfactants with a larger hydrophobic volume are more prone to form vesicles than single-chain surfactants [55–57]. Currently, the regulation of the assembly of double-chain surfactant systems with low solubility still craves for external forces and appropriate methods. The introduction of β-CDs yields a supramolecular complex, where PC competes against Azo-Ac for combination with β-CD. The formation of inclusion complexes can greatly improve the solubility of PC in water, thus facilitating the system regulation. Under such host – guest competitive interactions, the self-assembled nanostructures can be altered. The excellent *in situ* assembly and disassembly of photo-responsive systems in aqueous media boosts their bio-related applications, such as drug delivery. Furthermore, this study provides an ingenious method for the preparation of double-chain surfactant vesicles with good dispersion and size uniformity, promoting the extensive application of double-chain surfactants in the field of amphiphilic self-assemblies. As illustrated above, we plan to enhance the control of functional complexity by introducing responsiveness to multiple stimuli.

CRediT authorship contribution statement

Xuejiao Wang: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Visualization, Writing - original draft. **Xuedong Gao:** Methodology, Formal analysis, Resources. **Xiao Xiao:** Methodology. **Shasha Jiang:** Methodology. **Yun Yan:** Writing - review & editing, Supervision, Funding acquisition. **Jianbin Huang:** Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcis.2021.02.084.

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