Electronic Supplementary Information

Multi-responsive drug release from hydrogen-bonding multilayers containing PEGylated nanoparticles and azobenzenes

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A. Materials.

(3-Mercaptopropyl)trimethoxysilane (MPMS, 95%), methoxyethylene glycol maleimide (MePEG, MW5000), Fluorescein 5(6)-isothiocyanate (96%), Acryloyl chloride (AC), 3-aminopropyltriethoxysilane (APTES) and rhodamine B (RhB) were purchased from Shanghai Aladin Co., Ltd. (China) and used directly. Dimethyl sulfoxide (DMSO), N,N′-Dimethylformamide (DMF), 1,4-dioxane and 1-Azobiscyclo-hexanecarbonitrile (ABCN) were obtained from Aldrich. DMF was azeotropically distilled with benzene for dehydration and then distilled under vacuum. DMSO and 1,4-dioxane were previously dried with molecular sieves. ABCN was recrystallized from methanol before use. All other chemicals were commercially available and used without further purification.

B. Preparation of Poly{2-[4-phenylazophenoxy]ethyl acrylate-co-acrylicacid} (PEAPE).

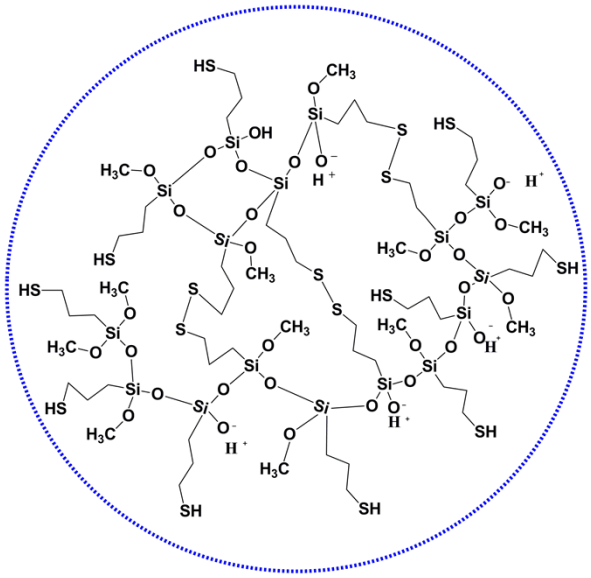
Preparation of Poly(acryloyl chloride) (PAC). PAC was prepared as described elsewhere.[1] Briefly, Acryloyl chloride (20 mL), dry 1,4-dioxane (20 mL), and ABCN (0.668 g) were added into a flask under N_2 protection. The flask was sealed and then heated in an oil bath (50 °C) for 14 h. The polymer was precipitated by adding petroleum ether (100 mL), collected by filtration, and washed twice with petroleum ether. The product was dried at 60 °C under vacuum for 48 h.

Preparation of PEAPE. PEAPE was synthesized according to the literature.[2] PAC (0.3 g, 0.0033 mol), triethylamine (0.56 mL, 0.0040 mol), and 2-[4-(4′-

ethoxyphenylazo)phenoxy]ethanol (whose amount was determined by the required degree of functionalization) were dissolved in anhydrous DMF (33 mL). The mixture was stirred at room temperature for 12 h under N_2 protection. Then suitable amount of water was added into the mixture and stirred for 10 min. The product was precipitated from HCl water solution (0.01 mol/L), collected by filtration, washed several times with water, and dried under vacuum. The polymer was further purified by dissolving in THF and precipitated from petroleum ether, collected by filtration, and washed twice with petroleum ether. The final product was dried at 70 °C under vacuum for 24 h. The degree of modification was determined to be 3 mol% by ^{1}H-NMR.

C. Preparation of PEGylated nanoparticles (NPs).

Thiolated NPs. Thiolated nanoparticles were synthesized according to the literature.[3] In brief, 1.5 mL (1.5766 g) of MPMS was mixed with 40 mL of DMSO and 1.0 mL of 0.5 mol/L aqueous NaOH and the resulting mixture stirred continuously using a magnetic stirrer. The reaction mixture was stirred for 24 h at 25 °C. The nanoparticles were purified by dialysis.



Scheme S1. Proposed schematic structure of thiolated NPs.

PEGylated NPs. Briefly, 50 mL of an aqueous dispersion of thiolated nanoparticles was mixed with 1 g of MePEG, and the reaction mixture was stirred for 9 h at room temperature. Similarly, the nanoparticles were purified by dialysis.

Thiolated NPs labeled with Fluorescein 5(6)-isothiocyanate (FITC). Briefly, 10 mg of FITC was mixed with 100 mL of thiolated nanoparticle dispersion. After 15 h of stirring the reaction mixture at room temperature, the nanoparticles were purified by dialysis. The fluorescently labeled thiolated nanoparticles were stored in a sealed vial wrapped in aluminum foil to avoid exposure to light. Fluorescently labeled nanoparticles were used to build multilayered materials using the above-described layer-by-layer deposition protocol.

PEGylation of fluorescently labeled NPs. FITC-labeling PEGylated nanoparticles were synthesized according to the literature.[3] Briefly, 1 g of MePEG was mixed with

50 mL of an aqueous dispersion of fluorescently labeled thiolated nanoparticles, and the reaction mixture was stirred for 9 h at 25 °C. Then the nanoparticles were purified by dialysis. The fluorescently labeled PEGylated nanoparticles were stored in a sealed vial wrapped in aluminum foil to avoid exposure to light.

Purification and Storage of NPs. All the nanoparticles were purified by dialysis against 5 L of Milli-Q water for 48 h (eight changes of water in total). Dialysis tubing (MWCO: 12-14 kDa, Isbio Ltd., China) was used for this purpose. The purified nanoparticles were stored in aqueous dispersions in sealed containers in a refrigerator with 4°C.

D. NPs Characterizations.

Raman spectra were recorded using laser Raman spectroscopy (RENISHAW, INVIA, Laser at 785 nm). Dynamic light scattering (DLS) and ζ potential measurements were carried out on dilute solutions of nanoparticles at 25 °C using a Nano-S Zetasizer (Malvern Instruments, United Kingdom). Each sample was analyzed at least four times, then the mean values of particle size, polydispersity index (PDI), and ζ potential were calculated. Prior to DLS studies, the dispersions of PEGylated nanoparticles were filtered through 0.45 μm filters (Shanghai, China). TEM images of thiolated nanoparticles and PEGylated nanoparticles were acquired using a JEM-2100 high-resolution electron microscope operating at an acceleration voltage of 200 kV. For sample preparation, the carbon-coated Cu grids were brought into contact with aqueous dispersions of nanoparticles for 60 s, followed by exposure to 1 wt% uranylacetate solution for 30 s and then dried off with a filter paper. All absorption and fluorescence measurements were performed on solutions in 1 cm^2 quartz cuvettes.

E. Synthesis of *α*-CD-rhodamine B (*α*-CD-RhB).

α-CD-RhB was synthesized according to previous reports.[4]

F. Inclusion complex formation and characterizations.

30 mg (1.05×10^{-3} mmol) PEAPE were dissolved in 60 mL H_2O. Before dissolving, the pH values of the solution were adjusted to an appropriate value by adding a few drops of NaOH dilute solution until PEAPE dissolved. After completely dissolving, the pH values of the solution were adjusted to be 2.60 by adding a few drops of HCl dilute solution. Then 16 mg (0.1116 mmol) *α*-CD-RhB was added under ultrasonic condition at room temperature. The mixtures were stirred overnight at room temperature and then dialysis against uncomplexed *α*-CD-RhB for 48 h in a dialysis tube. The host-guest interaction based on PEAPE and *α*-CD-RhB was measured with UV-vis spectroscopy.

G. Substrate preparation.

Quartz wafers were cut into small pieces (approximately 2 cm × 1.2 cm) using a diamond scribe. Each wafer was then immersed into a fresh piranha solution (30% H_2O_2/98% H_2SO_4, v/v =1:3; CAUTION: Piranha solution is a very aggressive, corrosive solution, and appropriate safety precautions should be utilized, including the

use of acid-resistant gloves and adequate shielding) and heated until no bubbles were released. The substrate was rinsed carefully with deionized water and dried with nitrogen. The cleaned quartz slide was treated in 2% (v/v) APTES/95% ethanol solution for 24 h, and subsequently dehydrated at 115 ^{o}C for 45 min to obtain the amino-silanized quartz wafer.

H. LbL film assembly.

A freshly treated quartz wafer was alternately dipped in the 0.5 mg/mL PEAPE solution (pH 2.60) and the 0.5 mg/mL dispersion (pH 3.00) of fluorescently labeled nanoparticles NPs each for 10 min and drying in air. After each dipping, the wafer was washed with enough Milli-Q water for 2 min. All subsequent layers were deposited by similar way. For comparison, PEAPE@α-CD-RhB/unlabelled NPs and PEAPE/FITC-labeling NPs multilayer coatings were also made by the LbL approach mentioned above onto quartz wafers under same conditions.

I. LbL film characterizations.

Surface morphology of LbL film was observed by using a Shimadzu SPM-9500J3 SPM Scanning Probe Microscope with AFM work mold in tapping mode in air. Surface hydrophilic/hydrophobic properties of LbL film was carried out on a Shanghai JC2000C1 contact angles test instrument. Absorbance of the film was characterized with a Shimadzu UV-2550 spectrometer. Surface fluorescence images were viewed on a fluorescence microscope (OLYMPUS IX71) with a Nikon camera at 400 × magnification and an exposure time of 800 ps. Film thickness was measured with a Tencor P-10 surface profilometer.

J. Multi-responsive drug release experiments.

Release under UV-irradiation. The UV irradiating light was from a high-intensity 365 nm UV lamp equipped with 5 in. diameter filter. The intensity of the lamp was 8000 μW/cm^2 at distance of 15 in. A 300 W xenon lamp equipped with a filter (455 nm) was used as visible light source. The sample was placed 14 cm away from the lamp. The surrounding temperature of the samples was controlled at 25 ^{o}C using a cold plate.

Surface patterning. we presented a pattern onto the LbL film surface via area-selective release. The procedures of pattern formation are described as follows. Briefly, a quartz wafer with as-prepared (PEAPE@a-CD-RhB/unlabeled NPs)$_{10}$ film was transferred into a cuvette with 25.0 mL Milli-Q water at room temperature. Next, a ‘butterfly’-shaped mask was also immersed in water and positioned on the film. Then, they were irradiated by UV light for 60 min, followed by a brief washing with Milli-Q water and drying in the air.

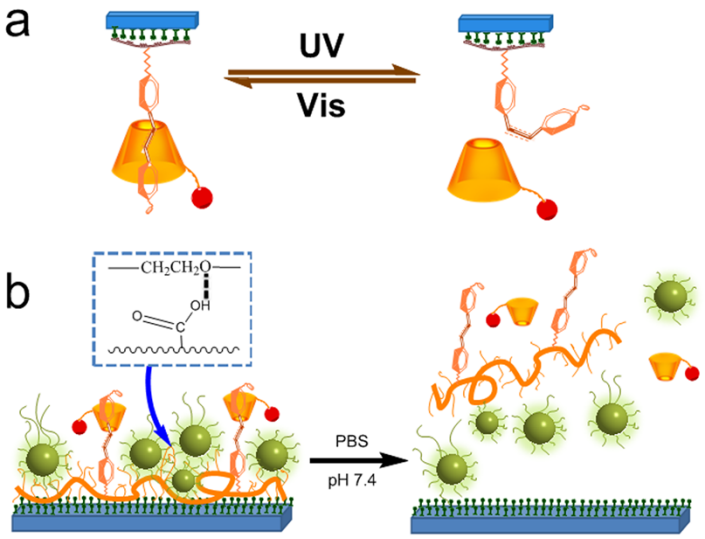
The reversibility of release/loading behavior. To examine the reversibility of releasing/loading behavior, a 6-bilayer film was transferred into 20 mL water and then irradiated by UV light. After 30 min of UV light irradiation, the sample was placed into a cuvette with 20.0 mL of α-CD-RhB aqueous solution (0.0037 mM) and then irradiated by visible light for another 15 min.

Release under physiological conditions. In order to address the capability of drug release of the loaded multilayers under physiological conditions, we incubated a 10-bilayer film in a phosphate buffered saline (PBS) solution under different pH conditions (pH = 7.4 and 10.4) at room temperature. The films were removed after regular intervals of time, washed with Milli-Q water. Drug release from the 10-bilayer film was followed by measuring the UV-Vis spectra of released drugs in corresponding PBS solution. The concentration of α-CD-RhB was calculated based on the calibration curves collected from UV-Vis absorbance (λ_{max} = 566 nm). Percentage of α-CD-RhB is calculated as:

$$\% \ \alpha\text{-CD-RhB} = \frac{c_0 - c_t}{c_0} \times 100 \text{Error!Error!}$$

C_0 is the maximum released concentration of α-CD-RhB in corresponding PBS solution, and C_t is the concentration of α-CD-RhB in corresponding PBS solution after time "t" of incubation. As illustrated in Fig. S10, the release rate is quick and remarkable as soon as the film is exposed to PBS for 3 min. This rapid release process is caused by two factors: first is the disassembly of H-bonded; second is the disassembly of host-guest system based on PEAPE and α-CD-RhB from NaCl-containing PBS solution. In case of the unique aspect of H-bonded self-assembly, the deconstruction of the film proceeds through rapid bulk disintegration rather than gradual surface erosion, as the originally smooth film becomes significantly rougher, nonuniform. We can conclude that this self-assembly method is reversible: multilayers could be selectively destroyed after creation.

Salt-responsive release. To study the ionic strength effect on the drug release rate, we immersed a 10-bilayer multilayer film in the NaCl aqueous solution with different concentrations. Indeed, the extraction rate of α-CD-RhB from the multilayer film is correlated with ionic strength.



Scheme S2. (a) Schmatic photoresponse of the host-guest system of azo group and α-CD-RhB. (b) Representation of hydrogen-bonding multilayers under physiological conditions for drug delivery.

Table S1. Characteristics of the NPs before and after PEGylation

sample	z size nm	PDI	ζ potential mV	SH group content μmol/g
Thiolated NPs	55.86±0.7	0.131	-38.0±2.3	252±125
PEGylated NPs	78.50±0.3	0.117	-8.03±1.1	15±1

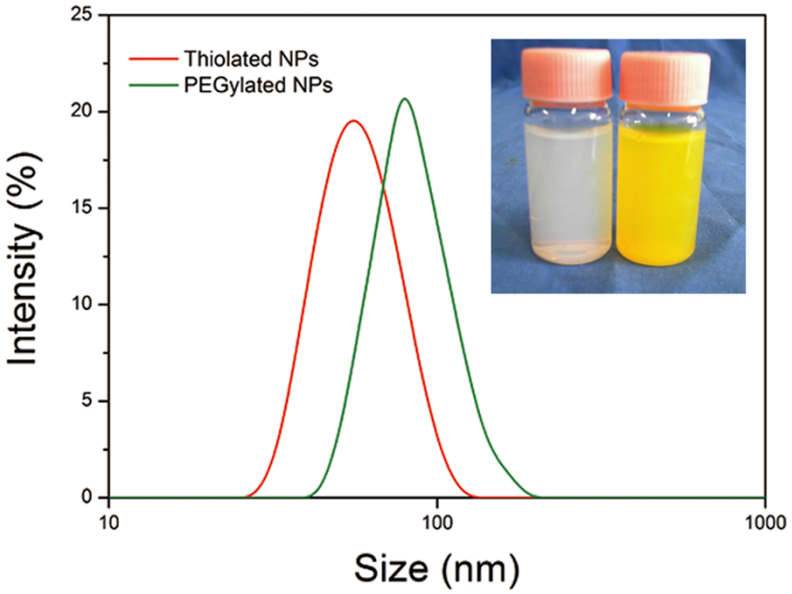


Fig. S1. Size distribution for nanoparticles formed from 0.2 mol/L MPMS in DMSO. Nanoparticle concentration in dispersion is 0.15 mg/mL. Insert: the photograph of thiolated nanoparticles (left) and PEGylated nanoparticles labeled by FITC (right).

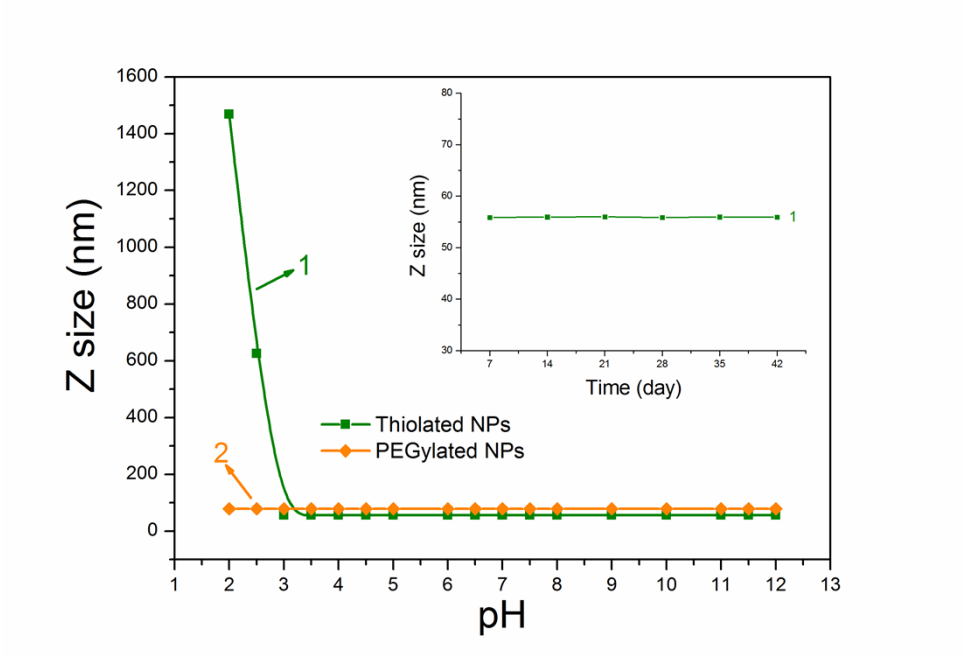


Fig. S2. pH-dependence of nanoparticle size in aqueous dispersion at room temperature. Insert: time-dependence of thiolated nanoparticle size in aqueous dispersion (0.18 mg/mL) at room temperature. Although NPs with a MePEG corona are known to have a tendency to form aggregates (small clusters of micelles), these PEGylated NPs are stable in dilute aqueous solutions (0.10 mg/mL) within 14 days in room temperature without agglomeration.

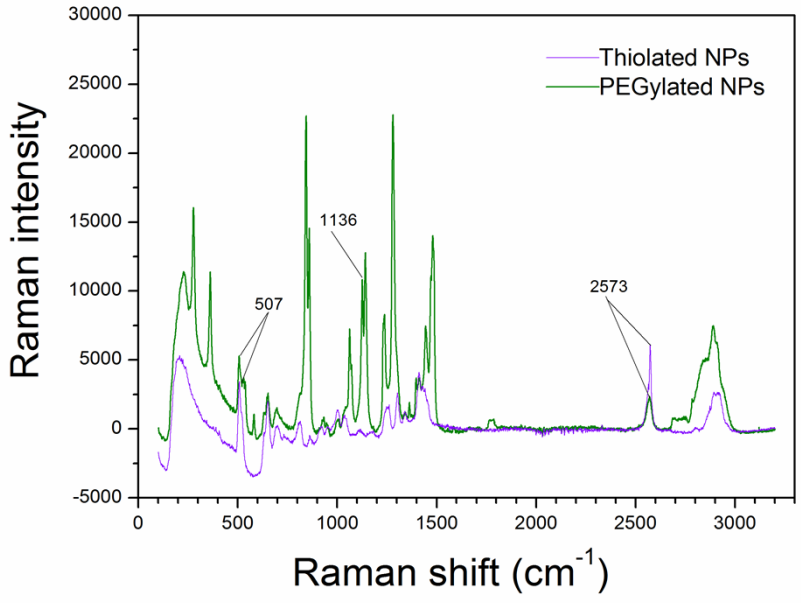


Fig.S3. Raman spectra of thiolated and PEGylated nanoparticles. Assignments: 507 cm^{-1} -ν(S-S), 1256-1456 cm^{-1}-δ(CH_2), 2573 cm^{-1} -ν(SH), and 2934 cm^{-1} -ν(CH_2). The peak at 1136 cm^{-1} responsible for $\upsilon(C\text{-}O\text{-}C)_{asym}$ in MePEG, appears in the spectrum of PEGylated nanoparticles. PEGylation results in a decrease in the intensity of the peak at 2573 cm^{-1}.

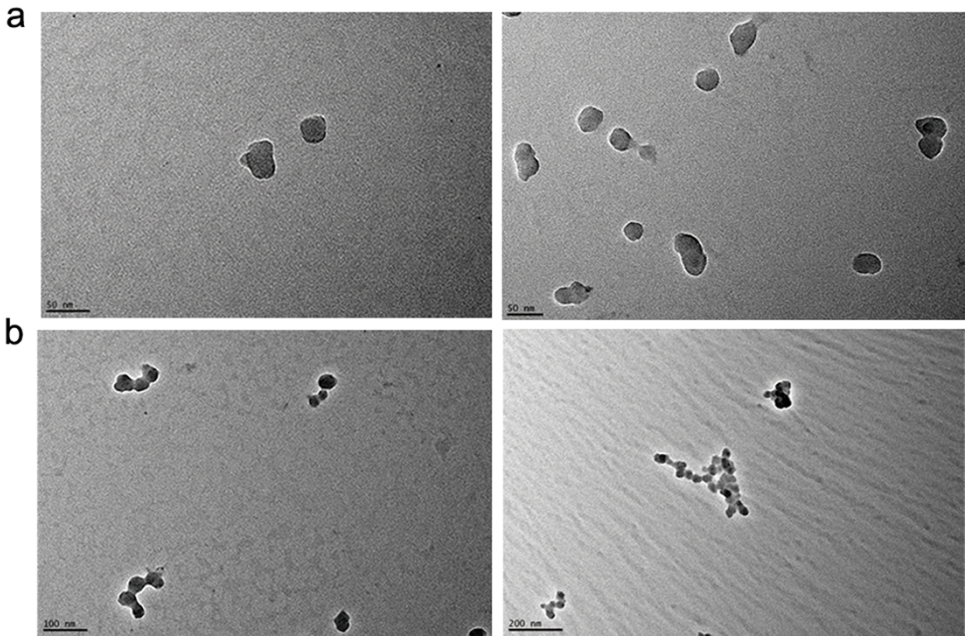


Fig. S4. TEM images of thiolated nanoparticles (a) and PEGylated nanoparticles (b).

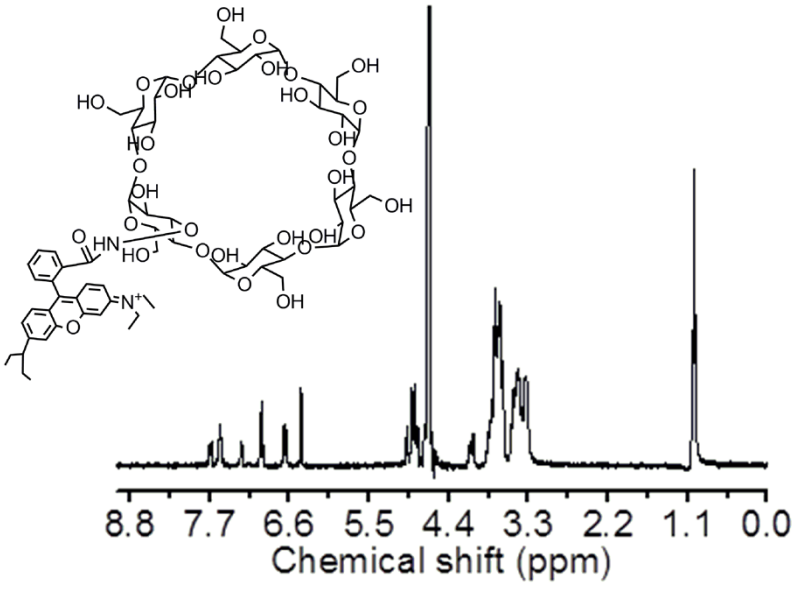


Fig. S5. The ^{1}H NMR spectra of α-CD-RhB.

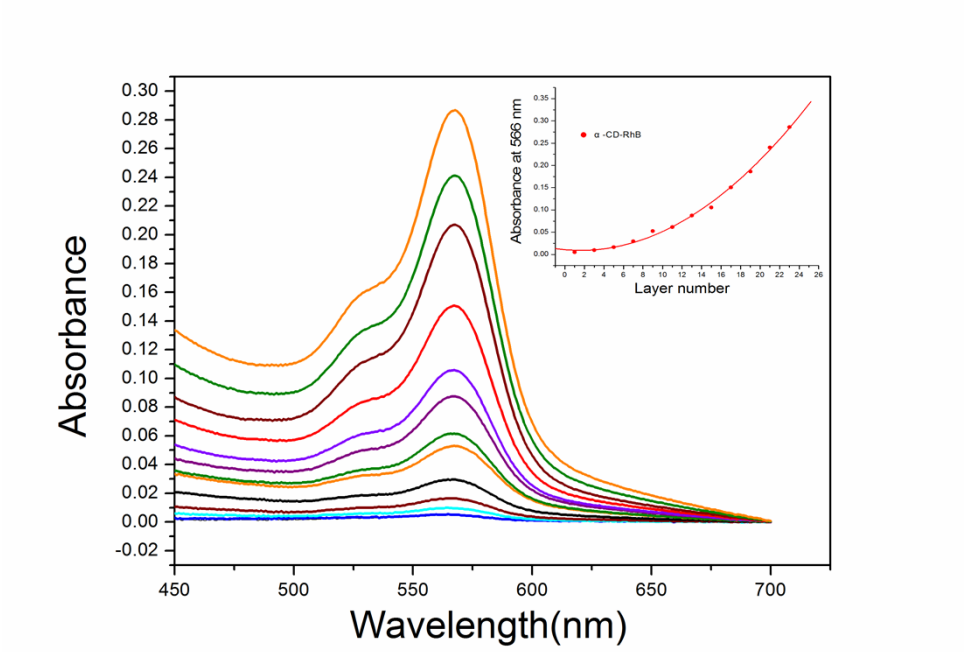


Fig. S6. UV-Vis absorption spectra of the films on a quartz wafer (from bottom to top: 1 to 23 monolayers). The absorbance maximum of *α*-CD-RhB in the film at 566 nm is taken as the reference for monitoring the film growth. Insert: The inset shows growth tendency of *α*-CD-RB.

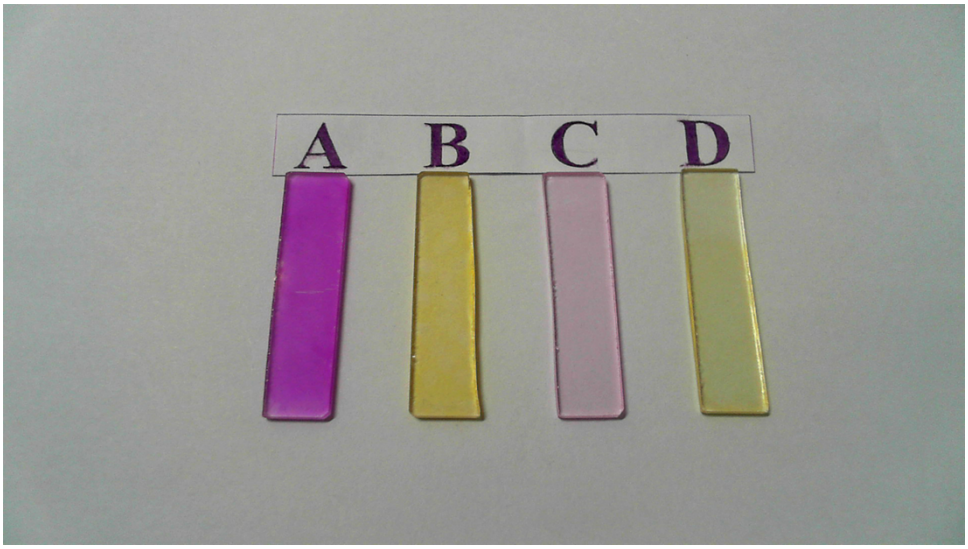


Fig. S7. Photographs of (PEAPE@*α*-CD-RB/unlabeled NPs)$_{15}$ (A), (PEAPE/labeled NPs)$_{15}$ (B) multilayers deposited on a quartz wafer. Photographs of (PEAPE@*α*-CD-RB/labeled NPs)$_{15}$ multilayers before (C) and after (D) UV irradiation.

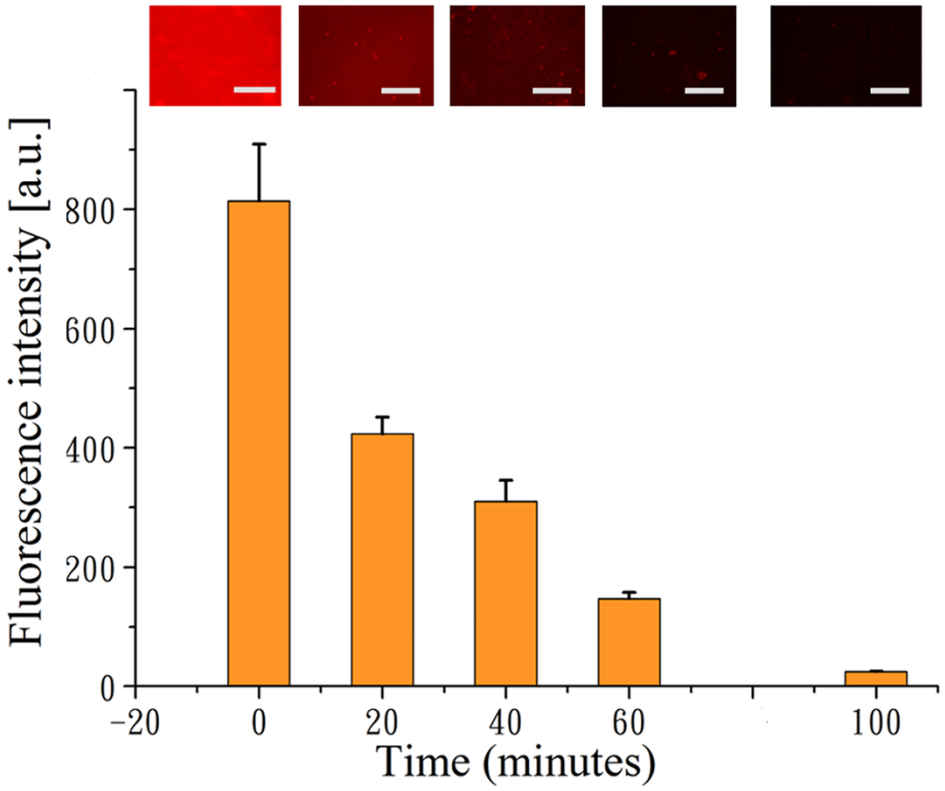


Fig. S8. Fluorescence levels of (PEAPE@α-CD-RhB/unlabeled NPs)$_{20}$ multilayers under UV irradiation. Inserts show fluorescent microphotographs (size bar is 100 μm).

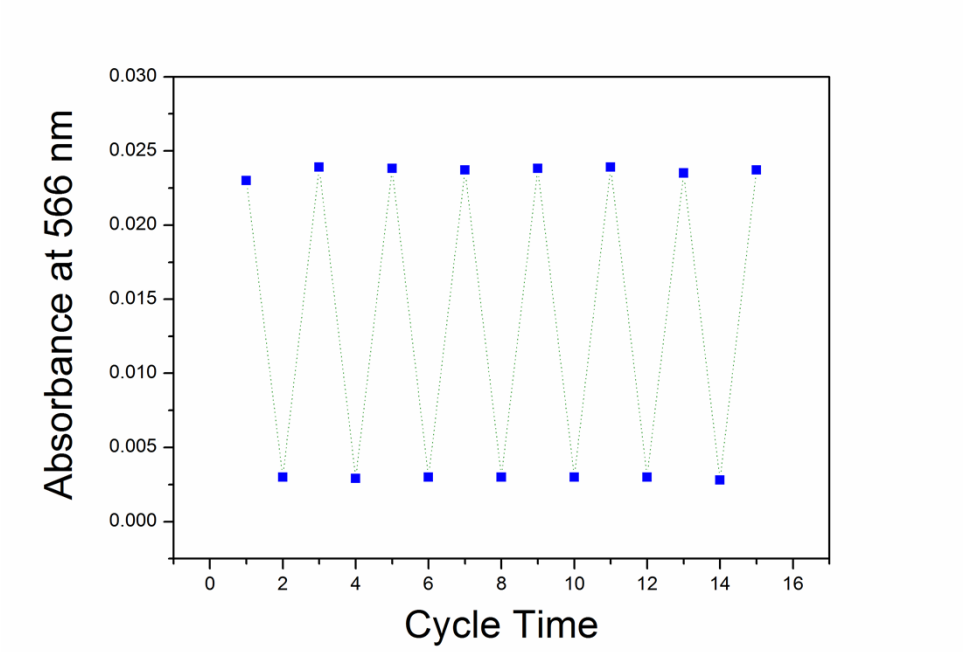


Fig. S9. The cyclic absorbance features of multilayer film at 566 nm as a result of the cyclic release and loading.

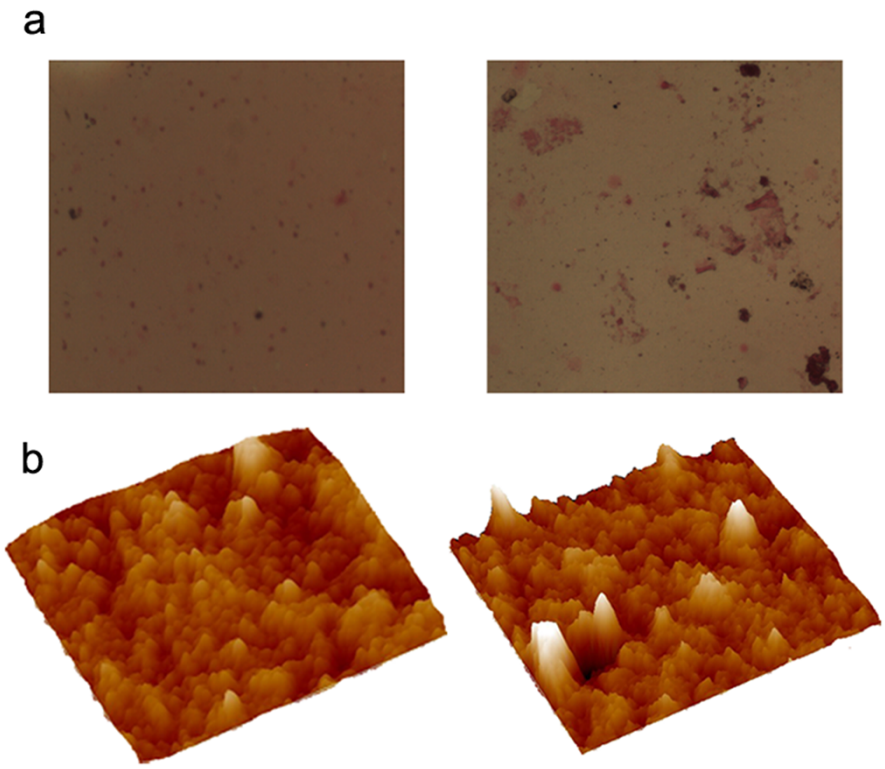


Fig. S10. Deconstruction of a hydrogen-bonding 10-bilayer film in PBS buffer at pH 7.4. (a) Opticalmicroscope and (b) AFM images of the film surface (left) before and (right) after incubation in PBS buffer for 3 min. The Z-scale in the AFM image is 280 nm.

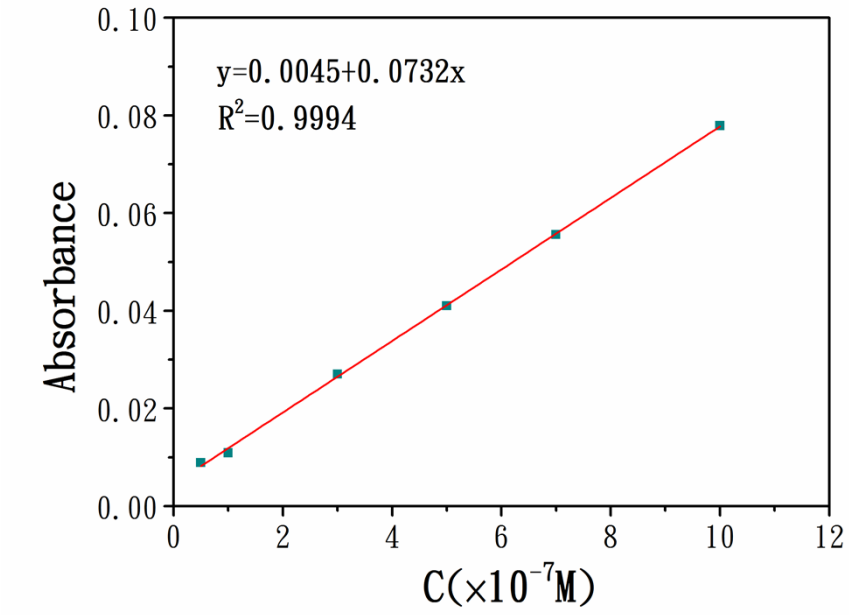


Fig. S11. The calibration curve of α-CD-RhB collected from UV-Vis absorbance at 566nm.

References

1. Y. S. Yang, G. R. Qi, J. W. Qian and S. L. Yang, *J. Appl. Polym. Sci.*, 1998, **68**, 665.
2. L. Wu, X. Tuo, H. Cheng, Z. Chen and X. Wang, *Macromolecules*, 2001, **34**, 8005.
3. (a) G. S. Irmukhametova, G. A. Mun, V. V. Khutoryanskiy, *Langmuir*, 2011, **27**, 9551; (b) G. S. Irmukhametova, B. J. Fraser, J. L. Keddie, G. A. Mun, V. V. Khutoryanskiy, *Langmuir*, 2012, **28**, 299.
4. W. Xiao, W.-H. Chen, X.-D. Xu, C. Li, J. Zhang, R.-X. Zhuo and X.-Z. Zhang, *Adv. Mater.*, 2011, **23**, 3526.