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# The Preparation of Azo-substituted Polyrotaxane End-capped with PNIPPAAm and Its Dual Stimuli-responsive Behavior for Drug Delivery Application

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# Abstract

Polyrotaxane-containing triblock copolymer (PRTBP) was synthesized via ATRP of NIPPAAm initiated by self-assembly of α-cyclodextrins (α-CD) with distal 2-bromopropionylend-capped poly(caprolactone) (Br-PCL-Br) in water at 25 ℃. After then, azobenzene group was introduced into the polyrotaxane block via the hydroxyl of threaded α-CD to get azo-substituted PRTBP (APRTBP). The structure was characterized in detail by $^{1}$H NMR, FTIR and WXRD techniques. Their thermal and light responsive property was demonstrated by turbidity measurements and UV spectrum respectively. Furthermore, the micelle with the diameter of around 100 nm was made from APRTBP in aqueous solution. The dynamic laser scattering (DLS) measurement demonstrated that the micelles contracted under UV irradiation and successively expanded under visible light which offers reversible on-off switch for controlling the micelle's drug release behavior. Consequently, the *in vitro* drug release curve which is similar with the pharmacokinetic curve of multiple dosing administrations was achieved when the UV and visible light was applied alternatively so as to simulate on-off state for drug release. It also showed the micelle possessed much higher release rate and higher cumulative release amount under UV irradiation than those under visible light. Furthermore, the low critical solution temperature (LCST) of NIPPAAm blocks in APRTBP can be used as another switch to trigger the drug release as implied by *in vitro* drug release test with doxorubicin (DOX) as model drug. Considering the interesting dual stimuli-responsiveness and its on-off switch like behaviors resulting from dual responsiveness, APRTBP shows great potential as candidate for controlled drug delivery systems and other possible biomedical applications.


Keywords: dual stimuli-responsive, polyrotaxane, drug delivery, azobenzene, poly(N-isopropylacrylamide) (PNIPPAAm)

## Introduction

Polyrotaxane(PR) where a number of cyclodextrin(CD) are threaded on the linear polymer chain and interlocked by terminal bulky stoppers is a typical supramolecular system which attracted tremendous attention in recent years since it was firstly reported by Harada and colleagues in the 1990s[1]. Subsequently, large number of PRs was synthesized by various effective and smart synthetic strategies during last two decades[2] because of its wide applications including molecular switch, information storage devices, light and electric sensor *etc.*[3] resulting from its unique structure and excellent properties. Furthermore, because of their excellent biocompatibility which was proved by so many biocompatible PR hydrogels prepared in recent years[4-13] as well as readily functionalization by a variety of synthetic strategies, PR's biomedical application has also become a hot spot.

However, compared with conventional covalent molecules and other supramolecules, the free movement and rotation of threaded CDs along polymer chain is the unique advantage for PRs, therefore the scientists designed and achieved many advantageous functions for PR by means of this unique advantage. Yui and colleagues designed a dynamic polyrotaxane surface which can mediate the cell-materials interaction via the motions of threaded CDs[14-15]. Ito created so called slide-ring gels with free movable network junctions by crosslinking the CDs of polyrotaxane which shows stretchability and swellability much larger than conventional chemical gels, and mechanical properties quite different from them[16-17]. Furthermore, Urayama et al. found a marked reversible on/off switching in the flow properties of the slide-ring gel membrane and it shows off-state when CDs distributed uniformly and moved freely and on-state when they form aggregation[18]. Meanwhile, some groups also developed various PR based nano-size assemblies such as micelle, vesicle and the like as drug carriers[19-23]. For instance, our group[24-26], synthesized PR based triblock copolymer as drug carriers. We found the threaded CDs with high mobility and the available multicovalent

carrier-drug interactions derived from CD's multiple hydroxyls provide much more choices for modulating drug loading efficiency and the drug release rate, which spontaneously inspired us to carry on further research on PR based drug carriers.

On the other hand, several groups including us developed a novel synthetic strategy that polyrotaxane is prepared by end-capping the pseudopolyrotaxane with polymer chain via atom transfer radical polymerization (ATRP)[27-33].With this strategy, a novel PR-based triblock polymers(PRTBP) was firstly synthesized via ATRP of NIPPAAm in this paper where the PNIPPAAm blocks were used not only as the end-capping stoppers, but also as stimuli-responsive moieties to introduce thermal responsiveness. After then, the light-responsive azobenzene group was incorporated into the threaded CD in order to endow light stimuli-responsiveness. Thus, the dual stimuli-responsive azo-substituted PR-based triblock polymer (APRTBP)which can form micelle in water and be used as drug carrier was constructed herein. As well known, azobenzene moieties show reversible molecular size change as well as conformation change under alternative UV and visible irradiation, and some literatures also reported the accumulation of the azo-moieties' volume change may induce the macroscopic morphology and volume change of the azo-based materials, for example, the bending of the film made from azobenzene compounds[34-36] and periodic expansion and contraction of hydrogel formed by azobenzene-containing polyrotaxanes[37-38]. But sometimes its conformation change may be hindered by restriction of main chain of the polymer, which suffered azo-substituted polymers for a long time and usually leaded to low trans/cis transition efficiency and slow light stimuli-responsive rate, and even suppressed the macroscopic morphology change. However, for APRTBP, since the azobenzene moieties were connected with CDs, their conformation and size change may be free of hindrance due to the free mobility of threaded CDs. Thus, it was supposed that the accumulation of azo-moieties' size change can induce the size change of whole APRTBP molecule and further lead to APRTBP micelle's morphology change under UV/vis irradiation. In other word, the contraction of azobenzene moieties under UV may result in the shrinkage of the APRTBP micelle

and vice versa under visible light. Consequently, this light sensitive contraction and expansion behavior may offer an on-off switch for the drug release of APRTBP micelles.

The light sensitive contraction and expansion behavior of APRTBP micelle was demonstrated by DLS measurement in this paper. And its thermal and light sensitive drug release behaviors were thoroughly investigated with DOX as model drug. It showed the LCST can be used as trigger to induce drug release and reversible on-off style release under alternative UV/vis irradiation. Furthermore, the on-off style release curve was found to be similar with the pharmacokinetic curve of multiple dosing administrations, which inferred the alternative light operation on APRTBP micelle may have the potential to simulate clinical multiple dosing administrations.

## Experimental

### 2.1. Materials and Measurement

α-CD was purchased from Wako Pure Chemical Industries, Ltd., Japan. N-Isopropylacrylamide (NIPPAAm) (Acros, Belgium) was purified by recrystallization from n-hexane. ε-Caprolactone (CL)was bought from Acros, Belgium. 2-Bromopropionyl bromide was bought from Alfa Aesar, USA. Triethylamine (TEA) (VAS Chemical Reagents Company, Tianjin, China) was refluxed with *p*-toluenesulfonyl-chloride and distilled under vacuum. Copper(I)bromide (Cu(I)Br) was prepared from $CuBr_2$, purified by stirring in acetic acid, washed with methanol and finally dried under vacuum before use. N,N,N',N'',N''-Pentamethyldiethylenetriamine(PMDETA), *p*-phenylazobenzoyl chlorideand stannous octanoate($SnOct_2$) were purchased from Sigmaaldrich, USA and used as received. 1, 4-Butanediol (BDO)was bought from Chemical Reagent Company, Beijing, China. Doxorubicin·HCl (DOX·HCl)was obtained from Beijing HuaFeng United Technology Co., Ltd, Beijing, China. All other reagents used were of analytical grade.

FTIR spectra were measured using Shimadzu IR Prestige-21 FTIR spectrometer at room temperature in the range of 4000–500 $cm^{-1}$ with a resolution of 2 $cm^{-1}$ and 20 scans. Powder samples were prepared by dispersing the samples in KBr and compressing the mixture to form disks.$^{1}$H NMR (400 MHz) spectra were recorded on a Bruker ARX 400spectrometer at room temperature using tetramethylsilane (TMS) as an internal standard. The Gel permeation chromatographic(GPC) measurements were carried out on Waters 2410 instrument at a flow rate of 1.0 ml/min. All the GPC data were calibrated by using polystyrene (PS) standards. The wide-angle X-ray diffraction (WXRD) measurements were conducted with powder samples using Philips X'Pert Pro diffractometer with an X'celerator detector in the reflection mode. The radiation source used was Ni filtered, Cu-Ka radiation with a wave length of 0.154 nm. The voltage was set to be 40 kV and the current 20 mA. Samples were mounted on a sample holder and scanned from 4.5°to 60°in 2θ at a speed of 5°/min.

The critical micelle concentration was measured by fluorescence spectrometer (Varian Cary Eclipse) with pyrene as the probe. TEM observation was carried on JEM-2010 under 200 KV. The Dynamic Laser Scattering (DLS) measurement was carried on D-63225 Langen/Hessen Laser Goniometer System, Germany.

**2.2. Preparation of PPR comprising Br-PCL-Br and α-CDs**

PCL was prepared according to previous reports[39].$^{1}$H NMR (δ, $CDCl_3$): 4.08 (t, -O-$CH_2$-, 2H), 3.70 (s, -$CH_2$-OH, 2H), 2.33(t, -$CH_2$CO-, 2H), 1.74~1.56 (m, -$OCH_2CH_2CH_2CH_2CH_2CO$, 4H), 1.40 (m, -$OCH_2CH_2CH_2CH_2CH_2CO$, 2H).$M_n$=6700, $M_n$ /$M_w$=1.53(determined by GPC using THF as eluent).

The distal 2-bromopropionyl end-capping PCL(Br-PCL-Br) was also prepared by literature[40]. $^{1}$HNMR (δ, $CDCl_3$): 4.10 (t, -O-$CH_2$-, 2H), 1.82~2.05 (d, -$CH$(Br)$CH_3$, 4H),2.25 (t, -$CH_2$CO-, 2H), 1.72~1.56 (m, -$OCH_2CH_2CH_2CH_2CH_2CO$-, 4H), 1.41(m, -$OCH_2CH_2CH_2CH_2CH_2CO$-, 2H) ppm.

0.3 g of Br-PCL-Br was dissolved in 50 mL acetone at 60℃, while 1.08 g of α-CD was dissolved in 20 mL deionized water. Then, the hot α-CD solution was added dropwise to the Br-PCL-Br solution. The resulting white suspension was stirred at60℃ for 3 h. After then, the suspension was ultrasonically treated for 20 min at 60℃and then allowed to cool to room temperature for standing overnight[27]. The white precipitate were collected by vacuum filtration, washed several times with cold water and acetone to remove free α-CD and PCL and dried.

**2.3 Preparation of PR-based triblock copolymers (ATRP procedure)**

NIPPAAm (144.5mg, 1.28mmol) and the PPRs(0.10g) were added to 5 mL of deionized water in a Pyrex reactor and then stirred overnight. After PMDETA (4.26mg，0.02mmol) was added, the reactor was quenched in liquid nitrogen. CuBr (3.66mg，0.02mmol)was added to the reaction mixture, followed by degassing three times. The reactor was sealed under vacuum, and the polymerization started and maintained at 25℃for 6.0 h[28]. The polymerization was terminated by breaking the Pyrex reactor. The product was purified by dialysis (MWCO 3500) in water for 2 days and lyophilization.

**2.4 Preparation of azobenzene substituted PR-based triblock copolymers**

The PR based copolymers (0.2g, 2μmol) was dissolved in 10 mL anhydrous N,N-dimethylacetamide(DMA), and LiBr powder (785mg, 6.4mmol) and 1 mL anhydrous triethylamine were added into the solution. 5 mL DMA of *p*-phenylazobenzoyl chloride(45.5mg, 93μmol) was also added dropwise. After then, the mixture was heated to 50 □ and reacted for 6 h. After reaction, the formed precipitation was removed by centrifuge, and the solution was precipitated in 200 mL ethyl ether to give the crude product. The product was further purified by dialysis in water for 2 days and lyophilized to give yellow solid product with 19.7%.

**2.5 Drug load and release test**

Hydrochloride was removed from DOX in base solution before drug

encapsulation. APRTBP (25 mg) and DOX (10 mg) was dissolved in 10 mL DMF. The DMF solution was placed in dialysis bag (MWCO 3500) and dialyzed with distilled water for 24 h. After dialysis, the solid component was removed from solution by centrifuge (4000 rpm, 5 min), and the DOX loaded nanoparticles were obtained by lyophilization. Drug loading content (DLC) and drug loading efficiency (DLE)were measured by UV spectrum and calculated according to the following equations. DOX content was determined based on the absorbance intensity at 480 nm according to the standard line obtained from a series of solutions with different DOX concentrations.

$$(1)\ \mathrm{DLC(wt.\%)} = \frac{\text{weight of loaded drug}}{\text{weight of polymer}} \times 100\%$$

$$(2)\ \mathrm{DLE(wt.\%)} = \frac{\text{weight of loaded drug}}{\text{weight of drug in feed}} \times 100\%$$

The aqueous samples solution(10 mL, 1 mg/mL) were firstly added into dialysis tubing and then placed into a flask containing 100 mL PBS solution. And the drug release behavior was measured under three different conditions as mentioned below. At selected time intervals, 2mL solution outside the dialysis tubing was taken out for UV-vis analysis (Hitachi U-2800). The release experiments were conducted in triplicate for each sample and the results presented were the average data.

The three release conditions were as following: (1) The samples were measured from 25 □ to 35 □ with interval of 1 □, respectively and the release solution was maintained for 15 min at each temperature before 2 mL measuring solution was taken. (2) Two parallel samples were measured under UV irradiation and visible light respectively during the whole releasing time. (3) The sample was measured under the alternative 1 h UV and visible light irradiation during the releasing time.

**2.6 Dual stimuli-responsive property measurements**

The thermo-responsive behaviors of the aqueous solutions of PR-based triblock copolymers were estimated by turbidity measurements using Hitachi UV-2800. The light transmittance was recorded at 500 nm of wave length after being heated for 10 min with a heating rate of 1.0 ℃/min. Values for the cloud point of PR-based triblock

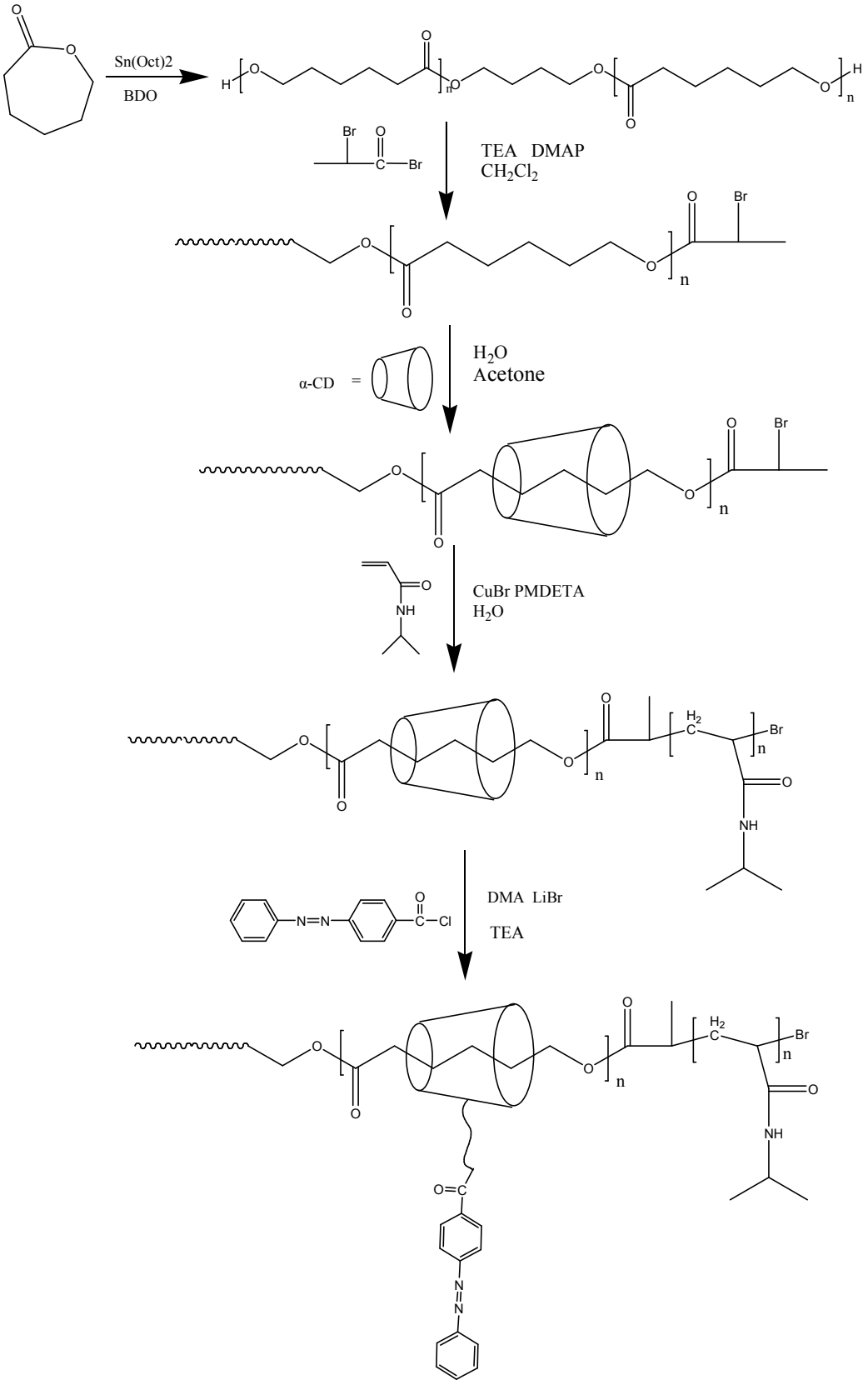
copolymers were defined as a temperature when the transmittance is 50%.

The light-responsive property was demonstrated by UV spectrum measurement. The sample's UV spectrum was firstly measured before UV irradiation, and then the sample was transferred into UV box and irradiated with UV light of 365 nm for predeterminated time. The sample's UV spectrum was measured again right after irradiation. The light-responsive property can be proved by the difference of the spectra before and after irradiation.

## 3 Results and Discussions

### 3.1 The preparation and characterization of APRTBP

The synthetic route of azo-substituted PR-based triblock polymer(APRTBP) was shown in Scheme.1. The PPR initiators were made by the self-assembly of bromo-terminated PCL and α-CDs in the mixture of acetone and water. After then, the ATRP of NIPPAAm was initiated by PPRs dispersed in water to form PR-based triblock copolymer. The synthetic results were listed in Table.1 and it showed the length of thermal sensitive PNIPPAAm blocks is tunable and around 15 α-CDs are locked on each PCL chain in PRTBP. However, the aqueous and imhomogenous ATRP in this paper is easy to manipulate and brings significant convenience for PR synthesis compared with other conventional end-capping method. Furthermore, this strategy not only fulfils the preparation of PRs end-capped with polymeric chains rather than bulky organic molecules, but gives birth to PR-based triblock copolymers which can endow PRs many advantages such as amphiphilic PRs for drug delivery system[25], stimuli-response PRs[30-31], PR-based brushes[32] and H-type polymers[33]. After then, the azobenzen group was introduced *via* the esterfication between *p*-phenylazobenzoyl chloride and α-CDs' hydroxyl and the results were shown depicted in Table. 2. It also showed the substitute amount of azobenzene moieties in APRTBP can be controlled by its feed amount in Table.2.




Scheme.1 the synthetic route of APRTBP

Table. 1 The synthetic results of PRTBP via ATRP

| sample | Molar composition PCL: α-CD:NIPPAAm | | Molecule weight and polydispersity | | | Yield/% |
|---|---|---|---|---|---|---|
| | Feed ratio | [a]Found ratio | [a]$M_n$/ g·mol$^{-1}$ | [b]$M_n$/ g·mol$^{-1}$ | $M_n/M_w$ | |
| PRTBP-1 | 1:35:50 | 1:13:240 | $4.8\times10^{-4}$ | $8.3\times10^{-4}$ | 1.32 | 45.3% |
| PRTBP-2 | 1:35:100 | 1:16:580 | $8.8\times10^{-4}$ | $12.4\times10^{-4}$ | 1.29 | 38.8% |
| PRTBP-3 | 1:35:200 | 1:15:880 | $12.1\times10^{-4}$ | $13.5\times10^{-4}$ | 1.41 | 29.7% |

a measured by NMR

b measured by GPC

Table 2 The synthetic results of APRTBP

| [a]Sample | Feed molar ratio Azo: α-CD | Found molar ratio Azo: α-CD($n_a$:n) | CMC/g ·ml$^{-1}$ | LCST |
|---|---|---|---|---|
| APRTBP-2.1 | 1:1 | 0.6:1 | $2.9\times10^{-2}$ | 32.2℃ |
| APRTBP-2.2 | 2:1 | 1.3:1 | $2.1\times10^{-2}$ | 32.8℃ |
| APRTBP-2.3 | 4:1 | 3.4:1 | $1.8\times10^{-2}$ | 32.9℃ |

a all samples were synthesized from PRTBP-2

The FTIR spectra of samples of pure α-CD,PCL, PRTBP-2 and APRTBP-2.2 were showed in Fig. 1. The absorption peak at 1720cm$^{-1}$ arisen from the PCL's carboxyl was seen in PRTBP. The vibration absorption peaks of the amide bond of PNIPPAAm at 1643 and 1547 cm$^{-1}$was appeared in both PRTBP and APRTBP. Moreover, the characteristic peaks of α-CD at 1029 and 1159 cm$^{-1}$ were also seen in the spectra of PRTBP and APRTBP. These results provided the clear evidences confirming the successful synthesis of PRTBP and APRTBP.

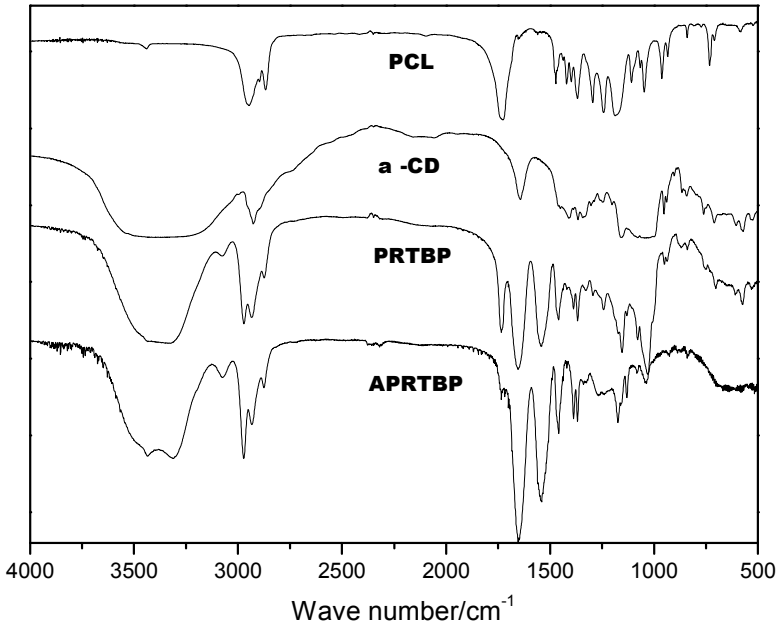



Fig.1 The IR spectra of PRTBP-2 and APRTBP-2.2

Furthermore, it was found that the carboxyl peak (1720cm$^{-1}$) became very tiny and can hardly be found in APRTBP compared with the one in PRTBP after the incorporation of azobenzene group. However, since the coverage ratio of α-CD in PRTBP is about 49%, half length of PCL chain was bare so that they can still show obvious carboxyl absorption peak at 1720 cm$^{-1}$ in IR spectrum. After the incorporation of azobenzene, the substituted α-CDs in APRTBP can move back and forward very fast along the PCL chain due to the destruction of channel-type crystallinity structure so that the carboxyl group may be shielded and weakened by the fast and free moving of azo-substituted α-CDs. Thus, the absorption peak of carboxyl group may red-shift and overlap with the NIPPAAm's characteristic peak at 1643 cm$^{-1}$ in APRTBP. Anyway, this result implied the mobility of CDs in APRTBP as well as successful preparation of APRTBP.

The NMR spectra were shown in Fig.2, and all the resonance peaks in the spectra of PRTBP and APRTBP can be well recognized after comparing with the spectra of pure

α-CD and PCL implying their successful preparation. The presence of CD's characteristic resonance peaks (O2,O3 and H1, δ=5.5, 4.8 and 4.5 ppm) in PRTBP and APRTBP indicated the threaded α-CD remained on the PCL so as to give mechanical interlocked rotaxane structure. The number of threaded α-CD(n)in PRTBP can be calculated from NMR spectrum according to following equation(3).

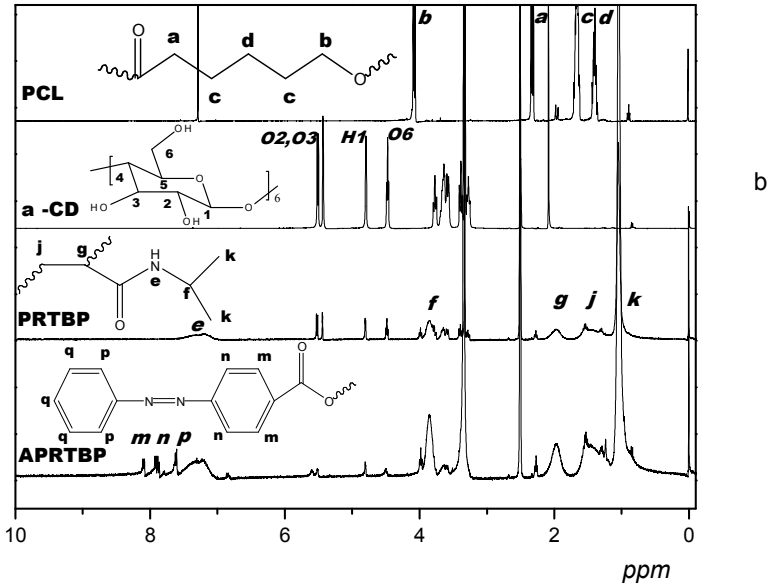
(3) $n=[2DP_{PCL}\times A_{4.81(H1)}]/6A_{4.07(b)}$

wherein $DP_{PCL}$ was the degree of polymerization of PCL and $A_x$ represents the integrated area under x ppm, and the results was shown in Table.1. Furthermore, the occurrence of several peaks assigned to PNIPPAAm(e, f, g, j, k) block in PRTBP and APRTBP after successful ATRP. The DP of NIPPAAm was calculated according to following equation(4) and the results were listed in Table.1.

(4) $DP_{NIPPAm}=[2DP_{PCL}\times A_{1.05(k)}]/6A_{4.07(b)}$

Finally, the group peaks ranging from δ= 7.6 to 8.2 *ppm* were assigned to azobenzene group and indicating the successful introduction of the azobenzene group. The number of modified azobenzene($n_a$) can be also calculated according Equation (5) by utilizing these peaks and the calculated results were depicted in Table.2.

(5) $n_a=[2DP_{PCL}\times A_{7.60\text{-}7.62(p)}]/2A_{4.07(b)}$




b

Fig.2 The NMR spectra of PRTBP-2 and APRTBP-2.2

The XRD results were depicted in Fig. 3. The strong diffraction peak at 2θ=20.4°which was not present in α-CD's XRD pattern and occurred in the PPR's pattern inferred the channel-type crystalline structure formed by threaded α-CD, whereas another two peaks at 22.7° and 23.2° corresponded to the crystallinity of PCL segments. These three peaks were all remained in the PRTBP's diffraction pattern implying the presence of polyrotaxane structure after PPR's end-capping reaction via ATRP although they became a little round and small due to the influence of amorphous PNIPPAAm block. However, the peak at 20.4°disappeared and a wide diffusion peak occurred instead in APRTBP's pattern, which indicated the destruction of channel-type crystalline structure resulting from the introduction of azobenzene group, and the destruction of crystallization may increase the movement ability of the α-CDs on the PCL chain. Moreover, PCL's two diffraction peaks were not observed either in APRTBP's pattern. This result was highly consistent with the result of IR and again implied the fast and frequent movement of azo-substituted α-CD on PCL chain may compensate the lack of full coverage and seems to be able to "shield" the whole PCL chain in APRTBP so that the crystallization of PCL was prevented.

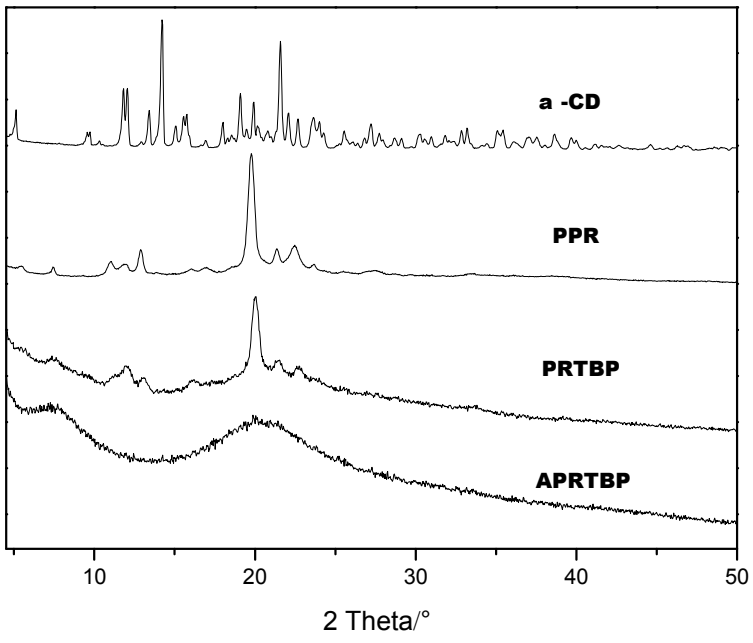
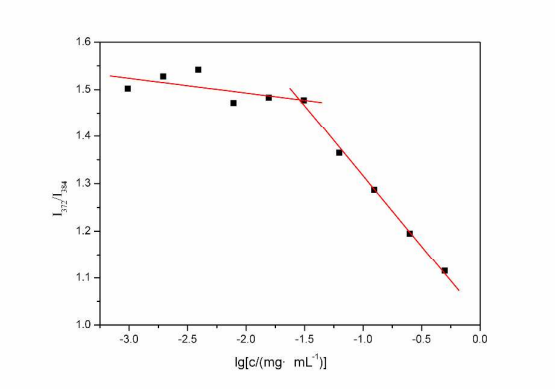


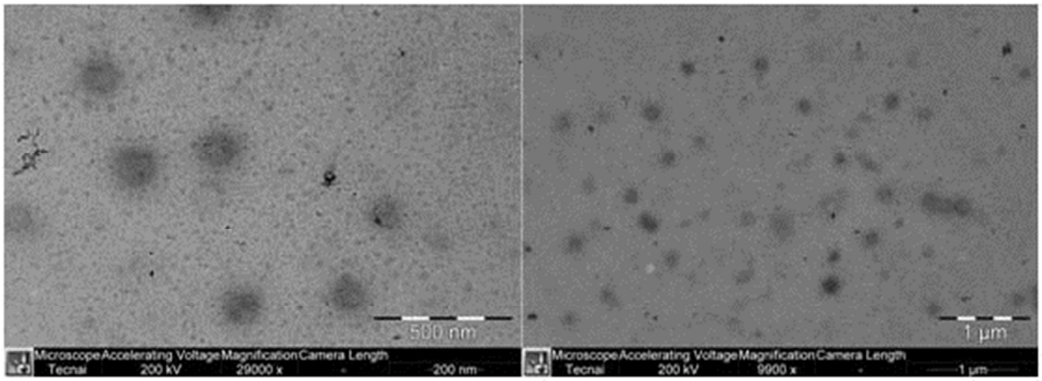

Fig.3 The XRD patterns of PRTBP-2 and APRTBP-2.2

### 3.2 The Dual stimuli-responsiveness properties of APRTBP

Fig. 4 showed the critical micelle concentration (CMC) measurement and TEM observation of APRTBP samples in water and the results of CMC measurement were available in Table.2.Thecrosspointin Fig.4a corresponded to CMC, and the micelles were formed above CMC concentrations. Subsequently, the formation of micelles was further demonstrated by TEM pictures in Fig.4b where the micelle with diameters of around 100nm was observed. However, the micelles based on block polymers are considered as promising drug carrier due to its ability to dissolve hydrophobic drugs and intrinsic sustainable release behavior. Furthermore, the micelles with size of 50-200nm are also believed to possess enhance permeability and retention(EPR) effect which is one of key points for the application of anti-tumor drug carriers. Thus, the prepared APRTBP micelle in this paper is a promising candidate for smart drug delivery systems.




a the CMC measurement of APRTBP-2.2




b TEM picture of APRTBP-2.2

Fig.4 the self-assembly property of APRTBP in water

The low critical solution temperature (LCST) measurement was shown in Fig. 5 and demonstrated the thermal responsive behaviors of APRTBP. As an amphiphilic block polymer, APRTBP is soluble in aqueous solution where PNIPPAAm blocks perform as hydrophilic segment. However, PNIPPAAm blocks would change to hydrophobic and precipitate suddenly from aqueous solution above their lower critical solution temperatures (LSCT) leading to dramatically changing of optical transmittance as well as the destruction of micelles. As can be seen in Fig. 5, the three samples all presented a sharp change of optical transmittance a little more than 32℃,the LCST of pure PNIPAAm[41] and close to human body temperature, which proved the thermal responsiveness of APRTBP. And the LCST could be used as a switch which can trigger the drug release of APRTBP micelle.

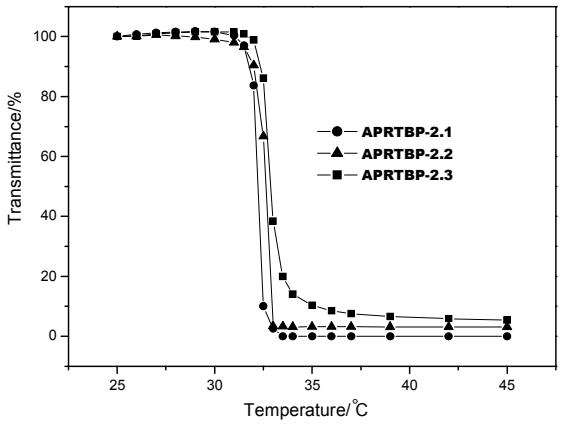
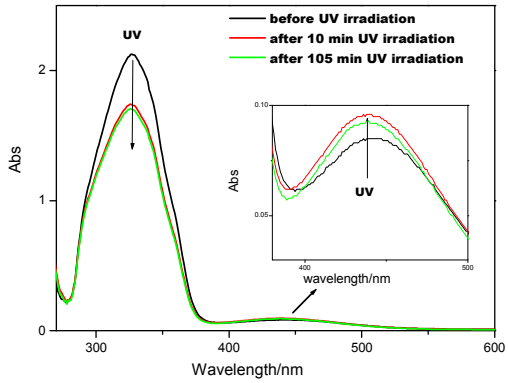



Fig.5 The LCST measurement of APRTBP

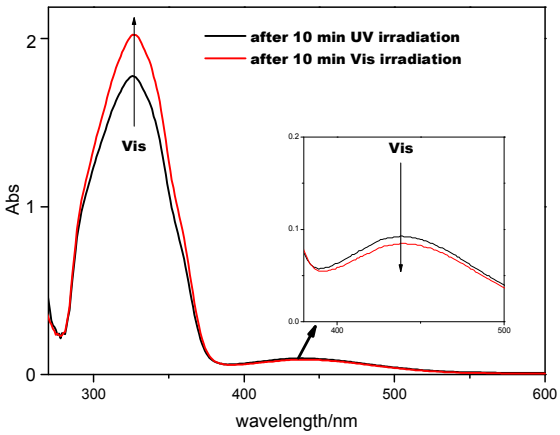
The light responsive behavior of APRTBP was investigated by UV-vis spectrophotometer. Fig. 6 showed their UV spectra under UV and visible irritation, respectively. The adsorption band at 330nm corresponding to the trans conformation of azobenzene group obviously decreased after irritation of UV light with wave length of 365 nm in Fig.6a, while the adsorption band at 440 nm (magnified figure in Fig. 6a)assigning to cis azobenzene increased, this implied the changing of azobenzene's conformation from trans to cis. And Fig.6b indicated this changing was reversible since the adsorption band at 330nm almost recovered to its original state (recover ratio: 91%, calculated from the height) after removing the UV light and being exposed

under visible light and so did the adsorption band at 440 nm(magnified figure in Fig. 6b)

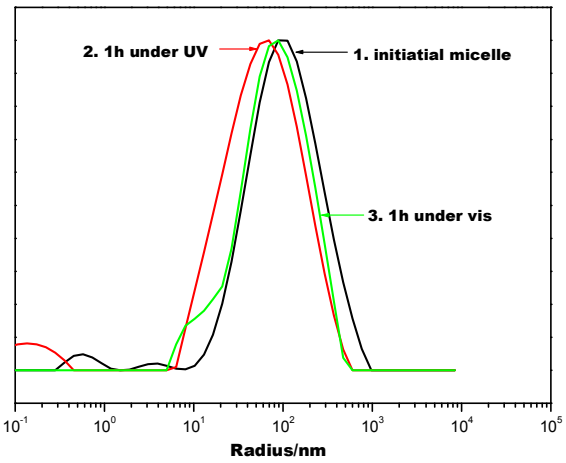
Furthermore, the light responsiveness of APRTBP micelle size was measured by DLS in Fig. 6c. Firstly, the initial micelle size before UV irradiation in Fig.6c seemed to be around 100 nm which is well consistent with the result of TEM observation. Secondly, it is the most important in Fig.6c that the micelle's size was found to decrease significantly from around 100 nm to around 60 nm after 1 h UV irradiation, while it expanded to nearly 100 nm again under 1 h visible light irradiation. These results clearly inferred the micelle shrinks upon UV irradiation and is able to expand reversibly by visible light. In other words, the micelle size shows significant photo responsiveness which has the potential to be used as on-off switch for smart drug delivery. When the micelle was loaded with some drugs, the UV irradiation may switch on the drug release since the shrinkage of the micelle could induce the release of encapsulated drugs and vice versa. There were some reports about the volume change of azo-based materials upon UV irradiation and their application as drug carriers. For example, Seki and colleagues reported an azobenzene substituted polyrotaxane hydrogel which underwent periodic expansion and contraction under the alternative UV and visible light irradiation[38]. Furthermore, He et al. also prepared azo-containing hydrogel and found different control drug release behaviors for *cis* and *trans* isomer state, respectively[37].




a The UV spectra of APRTBP-2.2 under UV irradiation




b The UV spectra of APRTBP-2.2 under Vis light irradiation




c The micelle's contraction and expansion under alternative UV/vis light

Fig. 6 light responsive property of APRTBP

However, the shrinkage of the micelle may be also due to the *trans/cis* conformation transition under UV irradiation as mentioned above, because the molecular length deformed from 9.0Å (trans) to 5.5 Å (cis), the molecular dipole movement changed from 0 D to 3.0 D when the azobenzene molecule changed from *trans* to *cis* conformation. It was supposed that the high mobility of CDs may also contribute to the fast response of APRTBP upon light stimuli and promote the *trans/cis*

conformation transition. However, the detailed mechanism will be further investigated in our following research. In addition, since APRTBP possesses both thermal and light responsiveness simultaneously, the APRTBP micelle also has two different switches correspondingly. Thus, if two switches were combined to manipulate the drug release, it may be able to create various kinds of smart, innovative and useful drug release behaviors which can meet various requirements in clinic.

**3.3 Drug encapsulation and in vitro releases of APRTBP**

The drug-loaded micelle was prepared by dialysis method and DOX as model drug can be encapsulated into the hydrophobic azo-substituted polyrotaxane core via hydrophobic-hydrophobic interaction. The DLC and DLE were 7.9% and 10.2%, respectively measured by UV-Vis spectrometer.

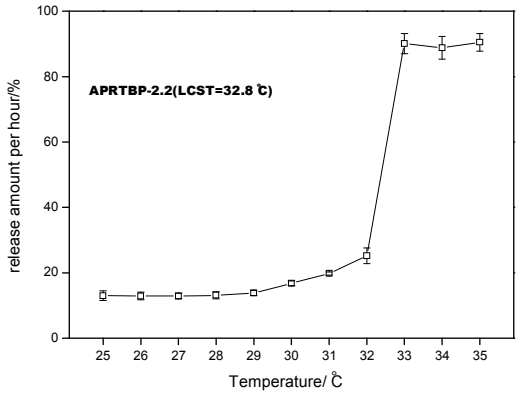
Nowadays, stimuli-responsive property was thought to be vital for the success of drug delivery system since it can improve the pharmaceutical effect and suppress the toxic side effect of the drugs at the most extend. It is divided into internal and external stimuli due to the type of stimuli source. The former usually utilizes the differences such as temperature, pH value differences between the sick site and healthy tissues *in vivo* to control the drug release. And the latter usually manipulate drug release by means of *in vitro* environment changes including light, magnetic field and the like. However, the manipulation ability on drug release behavior may be enhanced if these two strategies can be assembled on one micelle.

Thermal-responsiveness was typical internal stimuli way, and the first hour release amount of APRTBP micelle (APRTBP-2.2) under different temperature was measured and shown in Fig. 7a. It showed very small one hour release amount below LCST, the dramatically and suddenly increasing release amount above LCST. The small release below LCST was caused by diffusion effect. When the temperature reached LCST, the PNIPPAAm block changed from hydrophilicity to hydrophobicity and precipitated suddenly leading to the sudden collapse of the micelle and the burst release of the

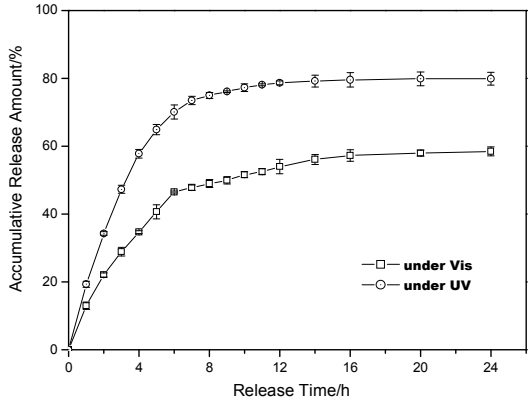
encapsulated drugs above LCST. Thus, the LCST can be used as a trigger for APRTBP to control the release of encapsulated drugs.

Light responsiveness as a typical external stimuli has drawn a great deal of attention since it provides the possibility for realizing remote and spatiotemporal drug release *in vitro* by tuning the wavelength, energy, and site of irradiation. Azobenzene was a typical light sensitive molecule and it underwent trans/cis transformation under UV irradiation. The light-responsive release behaviors of APRTBP micelle were shown in Fig. 7b and 7c. In Fig.7b, the sample under UV light showed much faster release rate and higher cumulative release amount than the one under visible light. This result was easy to explain since the DLS results showed the APRTBP micelles contract upon the UV irradiation.

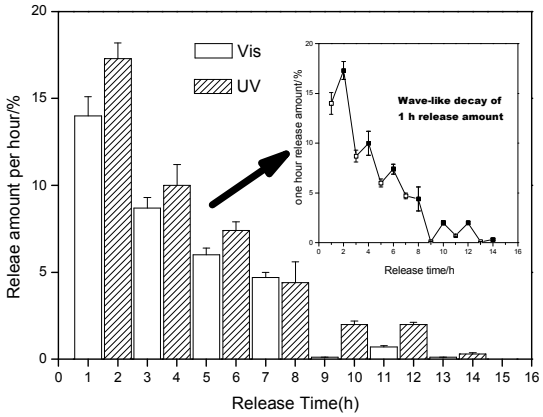
Furthermore, UV and Vis light was applied for 1h alternatively in Fig.7c, and the released amount per hour was depicted. On one hand, the on-off release behavior was observed since1h release amount under UV is always much higher than the corresponding 1 h release amount under visible light in each circle. It seems UV light switched on the drug release whereas the visible light switched “off” it. This phenomenon can also be well explained by DLS results since it showed the micelle contracted upon UV irradiation so that the encapsulated drugs may be squeezed out from the micelle and vice versa upon visible light. On the other hand, the curve of release amount per hour as a function of release time looks like the damped harmonic oscillating curve, which is similar with the pharmacokinetic curve of multiple dosing administrations. Thus, it inferred the alternative irradiation of UV and visible light on drug-loaded APRTBP micelles can mimic the multiple dosing administrations in clinic. Moreover, since the light is so easy to manipulate *in vitro*, the light responsiveness of APRTBP micelle is very promising to be used in smart DDS field.




a thermal-responsive drug release behavior of APRTBR-2.2




b light-responsive drug release behavior of APRTBR-2.2




c drug release behavior of APRTBP-2.2 under the alternative Vis and UV irradiation

Fig.7 the dual stimuli-responsive drug release behavior of APRTBP

Above all, APRTBP successfully assembled both internal/thermal and external/light stimuli property into one molecule and the thermal triggered drug release behavior, light sensitive on-off release behavior as well as simulating multiple dosing administrations can be obtained by virtue of dual stimuli-responsive properties of APRTBP. These clearly showed the promising potential for APRTBP in the field of smart DDS. However, although APRTBP as a supramolecular drug carrier shows its promising potential, there are still many works need to be done before it can be put into practice. For example, it was also reported that near infrared light instead of UV light is much more suitable for clinical usage because it penetrates the skin with fewer risk of damaging the cells and tissues in irradiated areas[42-43]. Thus some other near IR sensitive molecules such as 2-Diazo-1,2-naphthoquinone(DNQ) should be explored and used to replace azobenzene. And more important, the influence of CD's mobility on the light sensitivity should be further investigated. These further researches are carried on step by step in our lab as planned.

## 4 Conclusion

The polyrotaxane containing triblock polymer (PRTBP) was successfully prepared via ATRP reaction of NIPPAAm initiated with PPRs comprising bromo-terminated PCL and α-CDs in the presence of Cu(I)Br/PMDETA in aqueous solution. Then, azobenzene group was incorporated into the threaded α-CDs of PRTBP to formed azobenzene substituted PRTBP which was able to assemble into nano-size micelle in water. The DLS results showed the micelle contracted under UV irradiation and expanded upon visible light. After loaded with DOX, its *in vitro* drug release tests showed the LCST of APRTBP can be used as trigger to induce burst release indicating its thermal stimuli-responsive release property, while the light sensitive release property was also obtained because the sample under UV light showed much faster release rate and higher cumulative release amount than the one under visible light irradiation. Furthermore, it was found that the APRTBP micelle showed on-off style release behavior which can simulate multiple dosing administrations when the

UV and visible light was applied alternatively. Thus, APRTBP micelle shows the promising potential in controllable drug delivery system.

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