ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

A pH responsive micelle combined with Au nanoparticles for multi-stimuli release of both hydrophilic and hydrophobic drug

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1. Experiment Section

1.1 Materials.

All the chemicals were obtained commercially and used without further purification unless otherwise noted. The propargyl methacrylate (PMA) was obtained from Alfa Aesar (China) Chemical Co., Ltd. and N,N,N',N''-pentamethyl diethylene triamine (PMDETA) was purchased from TCI (Shanghai) Development Co., Ltd. The CuBr was obtained from Aladdin Industrial Inc. Other chemicals and all the organic solvents were obtained from Sinopharm Chemical Reagent Co., Ltd. Azodiisobutyronitrile (AIBN) was recrystallized with ethanol before use. The water used in the experiment was Milli-Q deionized. The entire DNA primers were purchased from Sangon Biotech (Shanghai) Co., Ltd. And the sequences were listed in Table S1.

<table>
<thead>
<tr>
<th>DNA name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA\textsubscript{72}</td>
<td>5'\textsubscript{-}N\textsubscript{2}-AGG CAC CAT CGT AGG TTT TTC CCA GGC ACC ATC GTA GGT TTT TCT TGC CAG GCA CAA ACC CCT AAC CCC-3'</td>
</tr>
<tr>
<td>DNA\textsubscript{36}</td>
<td>5'\textsubscript{-}N\textsubscript{3}-ACATCGCTACTTGATGACTTAAGTGAGCCACTGAA-3'</td>
</tr>
<tr>
<td>Complementary DNA</td>
<td>5'-SH-C6-GGG GTT AGG GTT TTG TGC CGA AGA AAA ACC TAC GAT GGT GCC TGG CAA GAA AAA CCT ACG ATG GTG CCT-3'</td>
</tr>
<tr>
<td>non-complementary DNA</td>
<td>5'-SH-C6-AGG CAC CAT CGT AGG TTT TTC TTG CCA GGC ACC ATC GTA GGT TTT TCT TGC CAG GCA CCA TCG TAG GTT TTT-3'</td>
</tr>
</tbody>
</table>

1.2 Methods.

The FT-IR spectrum was recorded on Nicolet 5700. The \textsuperscript{1}H NMR spectra was recorded on Bruker AV 300 Spectrometer. The gel permeation chromatography (GPC) was conducted on Polymer Laboratories PL-GPC220 GPC. THF was used as the mobile phase. The agarose gel electrophoresis was conducted using 2% agarose gel under 90 Volts. The image was taken by Kodak Gel Logic 112 equipped with carestream molecular imaging software. The transmission electron microscopy (TEM) was performed on JEOL JEM-2100 TEM. The DLS was conducted on Malven Zetasizer NanoZS equipped with laser at a wavelength of 633 nm. The UV-vis measurements were acquired on Agilent Technologies, Cary 60. The fluorescence measurements were acquired on OLYMPUS BX53 Fluorescence Spectrometer.

2 Synthesis of poly (Propargyl Methacrylate) (PPMA)

The poly (Propargyl Methacrylate) (PPMA) was obtained through solution polymerization\textsuperscript{1}. 12 mL 1,4-dioxane, 8.0 mg AIBN, 1023 \textmu L propargyl methacrylate were added into a 50mL three-necks bottle with reflux tube and a magnetic stirrer. The reactants were stirred under the protection of nitrogen and then heated to 65\textdegree C for six hours. Once the bottle cooled down to room temperature, the product was precipitated in methanol three times to
remove the unreacted monomers and then dried overnight in a vacuum oven at room temperature. The product was defined by FI–IR, \(^1\)H-NMR, GPC and MALDI-TOF spectrometer.

![FI-IR spectrum of PMA and PPMA](image1.png)

**Fig. S1** FI-IR spectrum of PMA and PPMA. The stretching vibration of C≡C (\(\nu_{C≡C}\)), ≡C-H (\(\nu_{≡C-H}\)) and C=C (\(\nu_{C=C}\)) occurred at 2130 cm\(^{-1}\), 3297 cm\(^{-1}\) and 1637 cm\(^{-1}\) respectively. Comparing to the blue curve of PMA, the \(\nu_{C≡C}\) at 1637 cm\(^{-1}\) disappeared as red curve showed. The absorptions of \(\nu_{C≡C}\) and \(\nu_{≡C-H}\) remained after the polymerization.

![\(^1\)H-NMR spectrum of PPMA](image2.png)

**Fig. S2** \(^1\)H-NMR spectrum of PPMA. (CDCl\(_3\), \(\delta\), ppm: 4.63(s, CH\(_2\)O), 2.52(s, C≡CH), 2.17-1.58 (m, C-CH\(_2\)-C), 1.26-0.88 (m, -CH\(_3\))).
Fig. S3 GPC chromatogram of PPMA. (Mn=30056, PDI=2.18).

Fig. S4 MALDI-TOF spectrum of PPMA. (Mass (Da) =24171.4).

3 Preparation of PPMA-g-DNA

The copper-catalyzed azide-alkyne (CuAAC) reaction\(^2\) was used to synthesize the PPMA-g-DNA. 3.1 mg PPMA was dissolved in 775\(\mu\)L DMF, then 20\(\mu\)L of the prepared solution was added into 625 \(\mu\)L DMF (1mM alkynyl), 32nmol ODN was dissolved in 16 \(\mu\)L \(H_2O\) (2 mM azide). Then, 490 \(\mu\)L mixed solvent was prepared of \(H_2O\):DMSO:IPA=2:3:1, and 10 \(\mu\)L PMDETA, 10 mg sodium ascorbate as well as 1.4 mg CuBr were added sequentially with intense shaking. Then, 20 \(\mu\)L PMMA solution, 16 \(\mu\)L ODN solution and 15 \(\mu\)L mixed solution were added together into a 0.5 mL tube, the reaction was proceeded at 55\(^\circ\)C for 3 hours. The PPMA-g-DNA was characterized by 2% agarose gel electrophoresis.
Fig. S5 Agarose gel electrophoresis of PPMA-g-DNA. Lane A is DNA ladder; lane B and D is DNA-g-PPMA; lane C is DNA alone. When the DNA was attached onto PPMA backbone, the moving speed of DNA was slowed because of the increment of molecular weight and volume.

4 Formations of PPMA-g-DNA Micelles, Morphology Transformation and Nile Red Encapsulation Experiments

Here, N, N-Dimethylformamide (DMF) was selected because of the following reasons. Firstly, DMF is also a favour solvent of PPMA. Secondly, THF is volatile, which is difficult to maintain the mixed solvent in a constant ratio during the self-assembling process. Lastly, former report\(^3\) confirmed that the block copolymer involving ionic segments tended to form spherical micelles in H\(_2\)O/DMF than in H\(_2\)O/THF resulted from the high dielectric constant of DMF.

To create a suitable environment for DNA-g-PPMA to self-assemble, the resulting solution was purified by a 10 kD Millipore’s Amicon Ultra-0.5 centrifugal filter device to remove Cu ion and other useless small molecules\(^1\). Simultaneously, the mixed solvent was replaced by pure water. Then, a certain volume of DMF was added until the volume ratio of DMF increased to 25 vol%.

**Formation of spherical micelles:** The pH of final solution was changed to 5 using acetic acid and then allowed to self-assemble at 15 °C for 5 days.

**Formation of nanofibers:** The pH of solution was changed to 5 using ammonia and then allowed to self-assemble at 15 °C for 5 days. 

**Spherical micelles transfer to nanofibers:** pH of the solution containing spherical micelles was changed to 8 and then allowed to self-assemble at 15 °C for 5 days.

Another approach is to heat the solution containing spherical micelles to 87 °C and staying for 10 minutes, followed by cooled down to 25 °C naturally.

**Nanofibers transfer to spherical micelles:** changing pH of the solution containing nanofibers to 5 and then allowing it to self-assemble at 15 °C for 5 days.
According the experimental results, we speculated that the transformation mechanism could be explained like this, DNA is pH sensitive and it tends to contract under acid condition. Moreover, many interactions, such as electrostatic interaction, hydrogen bonds and π-π stacking exist among DNA strands. When the solution pH was changed to 8, the DNA strands gradually stretch, which would destroy the mechanical balance, so the structure need reorganize to achieve a mechanical balance. In this process, the spherical might deform and gradually collide and fuse into nanofibers. Similarly, when adjusting the solution pH to 5, the broken mechanical balance forced the micelles to reform and restructure to achieve a balance.

Nile Red encapsulation experiments: 10 μL of the Nile Red in acetone (0.076 mM) was mixed with 20μL of spherical micelles solution and the acetone was evaporated overnight. Then, 5μL of the obtained solution was applied for the fluorescence spectroscopy observation. The fluorescence spectra were acquired on OLYMPUS BX53 Fluorescence Spectrometer and the excitation wavelength is 543 nm. The nanofibers were then observed using the similar protocol as above.

Fig. S6 TEM images of micelles. (A) and (C) are PPMA-g-DNA_{72} spherical micelles and nanofibers; (B) and (D) are PPMA-g-DNA_{36} spherical micelles and nanofibers.
The peak in black line was at 907.5 with the PDI of 0.166, the peak of red line was at about 77.02, and its PDI was 0.221.

Fig. 58 The intermediate state of the nanofibers formation. The sample was imaging by TEM after 2 days. Spherical micelles and nanofibers coexisted in the sample, which can reveal the possible assembling mechanism of nanofibers.

The intermediates were observed during the formation of nanofibers at pH 8. Former report confirmed that the block copolymer involving ionic segments tended to form spherical micelles in H2O/DMF resulted from the high dielectric constant of DMF. So the PPMA-g-DNA was prone to form spherical micelles at first. However, different from other common ionic segments, hydrogen bonds and π-π stacking also exists among DNA strands, which would drive the formed spherical micelles gradually transform to nanofibers as mentioned above, so we observed the co-existing of spherical micelles and nanofibers.
Fig. S9 Fluorescent images of DNA-g-PPMA micelles with Nile Red encapsulation under green light. (A) At pH=5, spherical micelles; (B) At pH=8, nanofibers.

1.2 mg Nile Red was dissolved in 1 mL acetone, then 20 μL solution was transferred to 3 mL H2O/DMF mixed solvent (VH2O/DMF=3) and 3 mL spherical micelles solution respectively for the next detection using fluorescence spectroscopy.

It is reported that Nile Red is non-fluorescent in aqueous solutions due to the formation of dimer or H-aggregates, but it is fluorescent in surfactant micelles. As shown in Fig. 2, the black line represented the fluorescence emission spectra of naked Nile Red. The red line belonged to the fluorescence emission spectra of Nile Red mixed with spherical micelles. We could find that the fluorescence intensity of Nile Red increased in spherical micelles, which because that Nile Red was encapsulated in hydrophobic PPMA, the core of spherical micelles. So we may draw the limited conclusion that the Nile Red was encapsulated in micelles.

Fig. S10 Fluorescence emission spectra of Nile Red in different states

Fig. S11 DLS results of different morphologies. Black and red lines represented spherical micelles at pH=5 while green and blue lines belong to nanofibers at pH=8. 1 and 2 means round 1 and round 2.
Fig. S12  (A) Spherical micelles were transformed to nanofibers by annealing. (B) The quenching experiment was done as control. Broken nanofibers twisted together, as well as lots of amorphous aggregates were formed.
5 Spherical Micelles Assembling with DNA Labeled Gold Nanoparticles and Hybridization

Preparation of 13 nm Au nanoparticles: The preparation of 13 nm Au nanoparticles and DNA labeled AuNPs according to the previous reports. 5 mL of 38.8 mM tisodium citrate was added rapidly into a stirred boiling aqueous solution containing 50 mL of 1 mM HAuCl4. The solution turned into black, purple and deep red in sequence within 2 minutes. After the solution was kept boiling and stirred for 15 min, it was naturally cooled down to room temperature. The concentration of gold nanoparticles (AuNPs) was 13 nM calculated by lambert-beer law. The extinction coefficient of $2.7 \times 10^8 \text{ M}^{-1} \text{ cm}^{-1}$ at 520 nm was used for the calibration.

Preparation of 13 nm Au nanoparticles modified with ssDNA: To activate the thiol-DNA, 5μL TCEP (100 mM) in Tris buffer (20 mM pH 7.3) was added to 20μL thiol-DNA (100 μM). The resultant solution was incubated for 1 h at room temperature. Then, the excess TCEP was removed using 3kD Millipore’s Amicon Ultra-0.5 centrifugal filter device. 500 μL of gold colloidal solution was added to the resulting solution above, followed by sonicating for 10 s and incubation for 20 minutes with shaking at room temperature. Then, the resultant solution was mixed with 0.1 M phosphate buffer (pH=7.2) and the final concentration of phosphate was maintained at 0.01 M, followed by sonicating for 10s and incubation for 20 minutes with shaking at room temperature. Finally, the salt aging process started, the concentration of NaCl increased to final concentration of 0.5 M using 2 M NaCl with the increment of 0.05 M NaCl every time and after each addition of NaCl, the mixed solution sonicated for 10 s and incubation for 20 minutes, then the solution was allowed to incubate overnight with shaking at room temperature. In order to remove the unacted DNA, the solution was centrifuged at 13,000 rpm for 20 min and then re-dispersed in pure water, the process repeated three times.
Fig. S13 (A) More TEM image of clusters formed by using complementary DNA labelled AuNPs; (B) More TEM image of control experiment using non-complementary DNA labelled AuNPs; (C) Isolated 13 nm AuNPs; (D) 13 nm AuNPs labeled DNA.

Fig. S14 UV-Vis spectroscopy of AuNPs at different states.

6 Drug Release

**pH triggered release of DOX:** 40 μL solution of carriers was mixed with 40 μL 1.0 mg/mL DOX and incubated for 2 hours, followed by centrifuging at 13200 rpm for 10 minutes. Then, the precipitation was re-dissolved in PBS buffer solution. The resulting solution pH was changed to 5.5. Immediately, the sample was divided equally into six parts. After the specific time, a sample was centrifuged at 13200 rpm for 10 minutes and supernatant was collected for measurement. The amount of doxorubicin released from carriers was determined by measuring the absorption intensity of the supernatant using UV-vis spectrometer. The
emission intensities were measured in the range of 400-600 nm. We evaluated the release rate of 100% by letting the system release DOX for more or less 72 hours and detecting the concentration of DOX in the solution.

**Exonuclease triggered release of DOX:** 50 μL solution of carriers was mixed with 50 μL 1.0 mg/mL DOX and incubated overnight, followed by centrifuging at 13200 rpm for 10 minutes and the precipitation was re-dissolved in PBS buffer solution and 50 μL 10 U/μL T7 exonuclease enzyme was added. Then the processing program was the same as the above.  

**Laser triggered release of DOX:** 50 μL solution of carriers was mixed with 50 μL 1.0 mg/mL DOX and incubated overnight, followed by centrifuging at 13200rpm for 10 minutes. Then, the precipitation was re-dissolved in PBS buffer solution. The processing program was the same as the above.

**Nile Red loaded and release:** 50μL solution carriers was mixed with 50 μL 0.076 mM Nile Red solution and incubated overnight. Then the processing program was the same as the above except for the emission intensities were measured in the range of 400-700 nm.

**Fig. S15** UV-Vis spectroscopy was used to determine the release of drug from the chosen carriers triggered by different stimulus. A-D represent the absorbance of DOX. (A) pH=5.5; (B) in the presence of enzyme; (C) laser; (D) Control (pH=7.4, without enzyme and laser). E and F represent the absorbance of Nile Red, (E) in the presence of enzyme; (F) Control.
8 References