Supporting Information

A Facile Mechanophore Functionalization of Amphiphilic Block Copolymer towards Remote Ultrasound and Redox Dual Stimulus Responsiveness

Rui Tong, Xili Lu, Hesheng Xia*

State key Laboratory of Polymer Materials Engineering, Polymer Research Institute, Sichuan University, Chengdu 610065, P. R. China, E-mail: xiahs@scu.edu.cn

1. Experiment Section

Materials

PEG \((M_n=2000)\), PPG \((M_n=2500)\), succinic acid (SA), thiodiglycolic acid (TDA), 3,3’-dithiodipropionic acid (DTPA), dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), DTT and pyrene were purchased from Sigma-Aldrich Chemical Company and used without further purification. Tetrahydrofuran (THF), dichloromethane (DCM), CaH\(_2\), methanol, ethanol, and sodium were purchased from Chengdu Kelong Chemical Reagents Institute. THF and DCM were dried by refluxing over sodium wire and CaH\(_2\) respectively, and distilled prior to use to remove the moisture and oxidative impurity. All chemicals and solvents were used as received unless stated.

Characterizations

FTIR analysis of the samples was performed on a Nicolet 560 Fourier transform infrared (FTIR) spectrometer. Proton nuclear magnetic resonance \(^1\)H NMR spectra were recorded at room temperature with a Bruker spectrometer operating at 400 MHz using CDCl\(_3\) as the solvent and tetramethylsilane as an internal reference. Molecular weight was measured with gel permeation chromatography (GPC, TOSOH, HLC-8320GPC) with THF as the eluent at a flow rate of 0.6 ml/min\(^{-1}\) at 40 °C. Molecular weight was calibrated with polystyrene standard. Dynamic light scattering (DLS) was performed on a Brookhaven BI-200 goniometer with vertically polarized incident light of wavelength \(\lambda = 532\) nm supplied by an argon laser operating at 200 mW and a Brookhaven BI-9000 AT digital autocorrelator. Measurements were made at 25.0 °C and at the detect angle of 90°. The autocorrelation functions from DLS were analyzed by using the non-negatively constrained least square algorithm (NNLS) method to obtain the diameter distributions. Micellar morphology was observed with Scanning Electron Microscopy (SEM, Inspect F, Fei Company, USA). Specimens for SEM observations were prepared by depositing several drops of micellar solutions onto silicon wafers and were dried by lyophilization. Steady-state fluorescence emission spectra of the micelle solutions were recorded on the 970CRT spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd). The excitation wavelength was 337 nm. The released percentages were estimated using the following formula: \(\%\text{release} = (I_0-I_t)/I_0\), where \(I_0\) and \(I_t\) is the fluorescence emission peak intensity recorded before and after HIFU or DTT treatment for \(t\) min, respectively. Transmittance of micelle solutions in the presence of DTT or under HIFU treatment were monitored at 500 nm with UV-vis spectrum (UV2300 II, Techcomp) at 37 °C. The Uv-vis transmittance and fluorescence emission spectra of micelle solutions after HIFU treatment were measured at 25 °C.

High intensity focused ultrasound apparatus

As we reported previously,\(^1\) the high intensity focused ultrasound apparatus comprises three main components: Arbitrary waveform generator (Agilent 33220A Function Generator), RF power amplifier (A150, Electronics & innovation) and acoustic lens transducer (H-101, Sonic Concept, USA). The acoustic lens transducer with a high acoustic focal pressure within a long focal volume of \(\phi 1.26 \text{mm} \times 11 \text{mm}\) and a geometric focal length of 62.6 mm was mounted at the bottom of a tank filled with water and the beams of ultrasound were pointed upwards and
focused on a special spot. The ultrasound power output can be adjusted in the range of 0~150 W and the frequency of ultrasound is 1.1 MHz. The focused beams of ultrasound can penetrate through latex membrane and act on the micelle solution in the glass cuvette reactor.

**Synthesis of the block copolymers**

![Diagram of Synthetic Route](image)

Scheme 1S Synthetic route to di-block copolymers of PEG-COO-SS-PPG, PEG-COO-S-PPG and PEG-COO-PPG.

**Synthesis of PEG-COO-SS-PPG:**
PPG (4 mmol, 10 g) and DTPA (20 mmol, 4.21 g) were dissolved in anhydrous THF (50 ml) in a 100 ml flask. The mixture was stirred for several minutes to obtain a clear solution. To this, DCC (20 mmol, 4.13 g) and catalyst amount of DMAP were added in. After 5 minutes, white precipitate appeared and the reaction mixture was stirred for another 48 hours at room temperature. The obtained reaction mixture was filtered to remove the precipitate N,N'-dicyclohexylure (DCU). The filtrate was concentrated by rotary evaporation. 100 ml of DCM was then added and the solution was filtrated again to remove the undissolved precipitate. The obtained clear solution was washed with 0.5 N HCl, saturated NaHCO$_3$ and saturated NaCl solutions for three times, and then dried over anhydrous MgSO$_4$. The solvent was then removed by evaporation and the obtained product PPG-DTPA was dried in vacuum at room temperature (~7.52 g, ~70%).

The as-synthesized PPG-DTPA (2.5 mmol, 6.73 g) was dissolved in 50 ml DCM. PEG (0.5 mmol, 1.00 g), DCC (2.4 mmol, 0.50 g) and catalyst amount of DMAP were then added in. The mixed solution was stirred for 48 hours at room temperature. After that, the solution was filtered and washed with 0.5 N HCl, saturated NaHCO$_3$ and saturated NaCl solutions for three times, and then dried over anhydrous MgSO$_4$. The solvent was then removed by evaporation and the obtained crude product was purified by silica gel column chromatography (using ethanol as the eluent to remove unreacted PPG-DTPA and other impurities, then using 10:1 DCM/ methanol to obtain the pure product, yield: ~1.51 g, ~64%). The control sample PEG-COO-S-PPG was also synthesized using the same procedure.

**Synthesis of PEG-COO-S-PPG:**
The control sample PEG-COO-S-PPG was synthesized using the same procedure. Typically, PPG (4 mmol, 10 g) and TDA (20 mmol, 3.00 g) were dissolved in anhydrous THF (50 ml) in a 100 ml flask. The mixture was stirred for several minutes to obtain a clear solution. To this, DCC (20 mmol, 4.13 g) and catalyst amount of DMAP were added in. After 5 minutes, white precipitate appeared and the reaction mixture was stirred for another 48 hours at room temperature. The obtained reaction mixture was filtered to remove the precipitate N,N’-dicyclohexylure (DCU). The filtrate was concentrated by rotary evaporation. 100 ml of DCM was then added and the solution was filtrated again to remove the undissolved precipitate. The obtained clear solution was washed with 0.5 N HCl, saturated NaHCO₃ and saturated NaCl solutions for three times, and then dried over anhydrous MgSO₄. The solvent was then removed by evaporation and the obtained product PPG-TDA was dried in vacuum at room temperature (~7.04 g, ~66.9%).

The as-synthesized PPG-TDA (2.4 mmol, 6.58 g) was dissolved in 50 ml DCM. PEG (0.5 mmol, 1.00 g), DCC (2.4 mmol, 0.50 g) and catalyst amount of DMAP were then added in. The mixed solution was stirred for 48 hours at room temperature. After that, the solution was filtered and washed with 0.5 N HCl, saturated NaHCO₃ and saturated NaCl solutions for three times, and dried over anhydrous MgSO₄. The solvent was then removed by evaporation and the obtained crude product was purified by silica gel column chromatography (using ethanol as the eluent to remove unreacted PPG-TDA and other impurities, then using 10:1 DCM/ methanol to obtain the pure product, yield: ~1.57 g, ~68%).

Synthesis of PEG-COO-PPG:

PEG-COO-PPG was also synthesized using the same procedure. PPG (4 mmol, 10 g) and SA (20 mmol, 2.36 g) were dissolved in anhydrous THF (50 ml) in a 100 ml flask and stirred for several minutes to obtain a clear solution. To this, DCC (20 mmol, 4.13 g) and catalyst amount of DMAP were added in. After 5 minutes, white precipitate appeared and the reaction mixture was stirred for another 48 hours at room temperature. The obtained reaction mixture was filtered to remove the precipitate N,N’-dicyclohexylure (DCU). The filtrate was concentrated by rotary evaporation. 100 ml of DCM was then added and the solution was filtrated again to remove the undissolved precipitate. The obtained clear solution was washed with 0.5 N HCl, saturated NaHCO₃ and saturated NaCl solutions for three times, and then dried over anhydrous MgSO₄. The solvent was then removed by evaporation and the obtained product PPG-SA was dried in vacuum at room temperature (~7.08 g, ~68.1%).

The as-synthesized PPG-SA (2.4 mmol, 6.50 g) was dissolved in 50 ml DCM. PEG (0.5 mmol, 1.00 g), DCC (2.4 mmol, 0.50 g) and catalyst amount of DMAP were then added in. The mixed solution was stirred for 48 hours at room temperature. After that, the solution was filtered and washed with 0.5 N HCl, saturated NaHCO₃ and saturated NaCl solutions for three times, and dried over anhydrous MgSO₄. The solvent was then removed by evaporation and the obtained crude product was purified by silica gel column chromatography (using ethanol as the eluent to remove unreacted PPG-SA and other impurities, then using 10:1 DCM/ methanol to obtain the pure product, yield: ~1.48 g, ~64.6%).

Figure 1S. FTIR spectra of PPG (A), PEG (B), PEG-COO-PPG (C), PEG-COO-S-PPG (D) and PEG-COO-SS-PPG (E).
Figure 2S. $^1$H NMR spectra and structural illustrations of PEG-COO-SS-PPG, PEG-COO-S-PPG and PEG-COO-PPG.

Figure 3S. GPC traces of PPG, PPG-DTPA, PPG-TDA, PPG-SA, PEG-COO-SS-PPG, PEG-COO-S-PPG and PEG-COO-PPG.
Preparation of blank micelle solutions

The obtained block copolymer was firstly dissolved in the THF. The copolymer micelles were formed by adding phosphate buffer solution (PBS) dropwise to the solution. Typically, 50 mg of PEG-COO-SS-PPG was dissolved in 10 ml of THF, and then 50 ml of PBS was added drop by drop under vigorous stirring to induce the aggregation of hydrophobic PPG blocks forming the micelle core. After that, another 50 ml PBS was added rapidly to stabilize the micelle morphology. THF was removed by evaporation at 40 °C for 24 hours. The initial polymer concentration was 0.5 mg ml\(^{-1}\). PEG-COO-S-PPG or PEG-COO-PPG micelle solution was also prepared by the same procedure.

Preparation of micelle solutions containing pyrene

The micelle solutions containing pyrene were prepared using a similar procedure to the blank micelle solutions. For instance, pyrene was firstly dissolved in THF with an initial concentration of 0.5 mg ml\(^{-1}\), and then 50 mg of PEG-COO-SS-PPG was introduced to 10 ml of pyrene/THF solution, 50 ml PBS was then added dropwise to induce the formation of the micelles and simultaneously the encapsulation of pyrene. After that, another 50 ml PBS was added rapidly to stabilize the micelle morphology. THF was allowed to evaporate by heating to 40 °C for 24 hours. The unencapsulated pyrene was deposited and then removed by filtration using 450 nm filters. PEG-COO-S-PPG/pyrene and PEG-COO-PPG/pyrene micelle solution was also prepared using the same procedure.

HIFU treatment of polymer micelles

Typically, 5 mL micelle solution was placed into glass cuvette reactor, which was sealed by latex membrane and immersed in a water tank (37 °C). The focused beams of ultrasound penetrate through latex membrane and act on the micelle solutions. In all HIFU treatment experiments, the focal spot of the beams was set at the center of the solution. After HIFU treatment for a certain time at a certain power output, the cuvette reactor was removed from the water tank and the sample was taken out for characterization. The fluorescence intensity of the copolymer micelles before and after HIFU treatment was detected after filtered with 450 nm filter.

DTT treatment of polymer micelles

DTT were directly added into the micelle solutions and stirred for few seconds and then kept in a water bath at a certain temperature for some time. The micelle solution after DTT treatment for different time was characterized by SEM, DLS and fluorescence analysis. The fluorescence intensity of the copolymer micelles before and after DTT treatment was detected after filtered with 450 nm filter. For PEG-COO-SS-PPG micelles, the precipitate obtained after DTT treatment was washed with deionized water for three times and characterized.

2. HIFU-induced cleavage of PEG-COO-SS-PPG, PEG-COO-S-PPG and PEG-COO-PPG

Table 1S. The number average molecular weight (\(M_n\)), weight average molecular weight (\(M_w\)) and polydispersity index (\(D_p\)) of PEG, PPG, PEG-COO-SS-PPG, PEG-COO-S-PPG and PEG-COO-PPG.

<table>
<thead>
<tr>
<th></th>
<th>PEG</th>
<th>PPG</th>
<th>PEG-COO-SS-PPG</th>
<th>PEG-COO-S-PPG</th>
<th>PEG-COO-PPG</th>
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<tr>
<td>(M_n)</td>
<td>1600</td>
<td>4100</td>
<td>5900</td>
<td>5800</td>
<td>5400</td>
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<tr>
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<td>8400</td>
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<td>7800</td>
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<tr>
<td>(D_p)</td>
<td>1.05</td>
<td>1.45</td>
<td>1.43</td>
<td>1.41</td>
<td>1.44</td>
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Table 2S. Peak area ratio of PEG-COO-SS-PPG micelles before and after HIFU treatment. (70 W, 10 min, calculated from \(^1\)H NMR)

<table>
<thead>
<tr>
<th></th>
<th>a/c</th>
<th>h/c</th>
<th>g/c</th>
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<tbody>
<tr>
<td>before HIFU</td>
<td>104.3</td>
<td>57.8</td>
<td>31.7</td>
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<tr>
<td>after HIFU</td>
<td>788.5</td>
<td>406.2</td>
<td>221.4</td>
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</table>

Table 3S. Peak area ratio of PEG-COO-S-PPG micelles before and after HIFU treatment. (70 W, 10 min, calculated from \(^1\)H NMR)

<table>
<thead>
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<th></th>
<th>a/c</th>
<th>h/c</th>
<th>g/c</th>
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<tbody>
<tr>
<td>before HIFU</td>
<td>128.3</td>
<td>65.5</td>
<td>33.3</td>
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<tr>
<td>after HIFU</td>
<td>929.4</td>
<td>446.9</td>
<td>231.5</td>
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</table>
Table 4S. Peak area ratio of PEG-COO-PPG micelles before and after HIFU treatment. (70 W, 10 min, calculated from $^1$H NMR)

<table>
<thead>
<tr>
<th></th>
<th>a/c</th>
<th>h/c</th>
<th>g/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>before HIFU</td>
<td>135.3</td>
<td>73.9</td>
<td>39.1</td>
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<tr>
<td>after HIFU</td>
<td>560.4</td>
<td>324.7</td>
<td>163.5</td>
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</table>

Figure 4S. $^1$H NMR spectra of PEG-COO-SS-PPG, PEG-COO-S-PPG and PEG-COO-PPG micelles before (A) and after (B) HIFU treatment. (70 W, 10 min)

After HIFU treatment, peak intensity of chemical shift at ~4.2 (c) decreases for the three copolymers (PEG-COO-SS-PPG, PEG-COO-S-PPG and PEG-COO-PPG). Peak areas were calculated for peaks a, c, g and h and peak area ratios were listed in Table 2S, 3S and 4S. The results show that for three micelles, the peak area ratios of a/c, h/c and g/c greatly increase after HIFU treatment. Chemical shift of peak c belongs to the resonance of protons in methylene groups of the terminal repeat units of PEG connected with ester groups (-CH$_2$-O-CO-), so the decrease in the intensity of peak c indicates the cleavage of ester groups.
The molecular weight change for PEG-COO-S-PPG and PEG-COO-PPG is similar with that for PEG-COO-SS-PPG (Figure 2c), which supports the cleavage of ester bond under HIFU treatment. The $M_w$ of both PEG-COO-S-PPG and PEG-COO-PPG decreases with HIFU time. Two new peaks corresponding to PPG and PEG appear in the longer retention time region after HIFU treatment for 10 min. This is because the ester bonds in the molecules are cleaved by HIFU in aqueous solution. After 20 min, the new peaks become more obvious, indicating more molecules are cleaved.
PEG-COO-SS-PPG/pyrene micelle solution was heated at different temperature for 30 min and then cooled to room temperature and the fluorescence intensity was detected. The fluorescence intensity was detected after cooling because PEG-COO-SS-PPG copolymer has a thermal-induced phase transition at this temperature range. Figure 8Sa shows the fluorescence intensity does not change after heating. The blank PEG-COO-SS-PPG micelle solution was also heated for 30 min at 85 °C and dried for GPC characterization. Figure 8Sb shows the retention time of the copolymer does not change before and after heating.

3. HIFU-induced disruption of PEG-COO-SS-PPG and PEG-COO-S-PPG micelles
4. DTT-induced disruption of PEG-COO-SS-PPG/pyrene micelles

Figure 10S. (a, b) DLS curves and (c, d) SEM images of PEG-COO-SS-PPG/pyrene micelles (a, c) before and (b, d) after DTT treatment.

Compared with blank PEG-COO-SS-PPG micelles, a slight increase in the mean diameter (~37 nm) could be detected after the encapsulation of pyrene. After DTT treatment, PEG-COO-SS-PPG/pyrene micelles were also disrupted.

5. DLS curves and SEM images of PEG-COO-S-PPG micelles after DTT treatment

Figure 11S. DLS curves of (a, a’) blank PEG-COO-S-PPG and (b, b’) PEG-COO-S-PPG/pyrene micelles (a, b) before and (a’, b’) after treatment in the presence of 10 mM DTT for 80 min.
Figure 11S shows that the mean diameters of blank PEG-COO-S-PPG and PEG-COO-S-PPG/pyrene micelles are ~35.5 nm and ~39 nm, respectively. After 80 min in the presence of 10 mM DTT, the diameters and diameter distributions of micelles nearly do not change. This is because no disulfide bond exists in this copolymer and the micelles could not be disrupted under the reductive environment. SEM images also show the same results (Figure 12S).

Figure 12S. SEM images of (a, a’) blank PEG-COO-S-PPG and (b, b’) PEG-COO-S-PPG/pyrene micelles (a, b) before and (a’, b’) after treatment in the presence of 10 mM DTT for 80 min.

Reference