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ARTICLE

Synthesis, Characterization, Micellization and Application of Novel Multiblock Copolymers with the Same Compositions but Different Linkages

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Several novel multiblock copolymers (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ with the same compositions but different linkages were constructed, and their micellization and application were studied. Firstly, the precursor HO-PEO-*b*-PS-*b*-PEO-OH was prepared by sequential LAP and ROP mechanisms, and the precursor Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl was achieved by the following modification procedure. Subsequently, using the efficient Glaser coupling reaction, the multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ was synthesized and the dyine groups enbeded in mainchain was modified by Thiol-yne reaction to give multiblock copolymer [PS-*b*-PEO-PEO-(OH)$_4$]$_s$. Also, by efficient Williamson reaction, the multiblock copolymer (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ was obtained. Finally, the micellar morphology formed from the synthesized copolymers were investigated and compared by DLS and TEM measurements, and the *in vivo* distribution of micelles was also studied by loading with fluorescent probe. The results revealed that, under the same conditions, the multiblock copolymers can form micelles with different sizes. Due to the hydrophobicity of introduced dyine groups and PS segments, the smaller size of micelles can be fromed and traverse BBB, which might gave a therapeutic application in the treatment of brain disease. However, the hydrophilicity of acetal and hydroxyl groups gave the similar effect as that of PEO segment, and the larger size of micelles are formed.

Introduction

In the past twenty years, with the innovative presentation and rapid development of various "living" / controlled polymerization mechanisms and efficient coupling reactions, plenty of polymers with complicated topologies and multiple compositions have been constructed by certain synthetic routes.[1-3] Correspondingly, the successful synthesis of these polymers further convenienced the related researches in polymer theory and polymer material field due to their unique physical properties and potential applications.[4-6] Among various synthesized polymers, the amphiphilic polymers are mostly focused because their unique self-assembly aggregates, polymeric micelles, provide some promising to alter the pharmacokinetic profile of drugs, reduce off-target toxicity and side effects, and enhance the therapeutic efficiency.[7-12] Polymeric micelles with small size are distinctively poised to efficiently deliver chemotherapeutics because the small size allows them to utilize the enhanced permeation and retention (EPR) effect, which describes the preferential accumulation of micelle particles in tumor tissue via leaky blood vessels.[13]

Up to now, the self-assembly behaviour of amphiphilic copolymers have gained great attentions and progresses from enormous researchers.[14-19] The morphologies of formed copolymeric micelles strictly depend on both compositions and topologies of copolymers. For example, the morphologies of micelles formed from multiblock copolymer [poly(caprolactone)-*b*-poly(ethylene oxide)]$_n$ [(PCL-*b*-PEO)$_n$] (PCL-*b*-PEO)$_n$ could be globules, fibers, and worms in certain selective solvents,[20] and those from diblock copolymer (PEO-*b*-PCL) were typical spherical particles.[21] Actually, the self-assembly behaviours of amphiphilic di- or triblock copolymers have been widely investigated,[22-24] however, the related study on multiblock copolymers is still rather limited. The largest obstacle is the difficulty to find some versatile routes to the required multiblock copolymers.

Typically, there are two approaches used to synthesize multiblock copolymers: (a) by sequential addition of monomers to a precursor contained multiple initiator units,[25] (b) by coupling reaction between reactive building blocks.[26] Comparatively, the latter approach (b) is widely used because the parameters of building blocks can be well controlled. The mostly adopted coupling reactions are copper(I)-catalyzed

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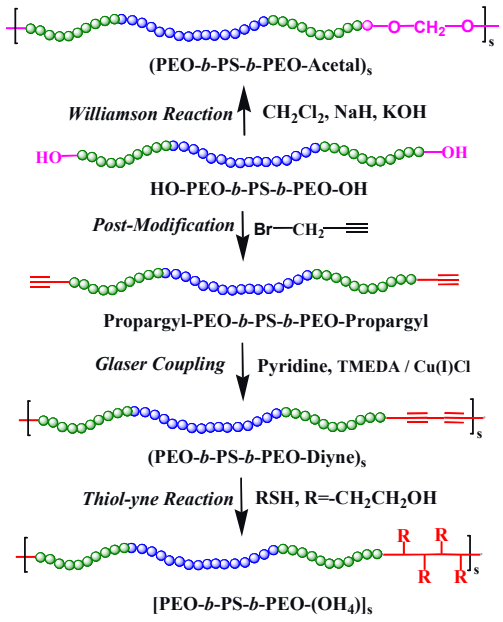
azide-alkyne cycloaddition (CuAAC) "Click" chemistry,[27,28] atom transfer radical coupling (ATRC) reaction,[29] and so on. For example, the amphiphilic multiblock copolymer [poly(acrylic acid)-*b*-polystyrene]$_n$ [(PAA-*b*-PS)]$_n$ was constructed by CuAAC "Click" chemistry of α-azide, ω-alkyne heterofunctional diblock copolymers.[30] Similarly, the multiblock copolymer [poly(isoprene)-*b*-polystyrene]$_n$ [(PI-*b*-PS)$_n$] was also successfully synthesized using the "Click" chemistry.[31] However, in approach (b), a precursor with defined azide and alkyne groups at each end must be firstly and selectively synthesized when CuAAC "Click" chemistry is used, and the possible disproportionation termination and side reaction on the formed carbon radicals also must be avoided in ATRC reaction.[32,33] Thus, finding an efficient and convenient coupling reaction is indeed the key point for multiblock copolymers.

Recently, the classic Glaser coupling reaction between alkyne groups has been evoked and found its application in polymer science, which provides an access to functional material with 1,3-conjugated structures.[34-39]. This reaction has the advantage of convenient introduction of alkyne groups onto polymeric precursor, mild operation under oxygen atomosphere and yield with high efficiency. As another classic and efficient coupling reaction, the Williamson reaction between active hydroxyl groups has also been applied in polymer science to construct polymers with acid-sensitive linkages.[40] This reaction also has the advantage of convenient character because dichloromethane (CH_2Cl_2) is simultaneously used as coupling agent and solvent in the presence of KOH and / or NaH. In our previous works, these two coupling reactions have been successfully used as an efficient cyclization method to cyclic polymers,[41-46] which have been actually confirmed with versatility and potential applications in polymer science.

Herein, considering the above limitations in synthesis, self-assembly behaviour and applications of multilblock copolymers, series of novel multiblock copolymers (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ with the same compositions but different linkages are firstly realized by controlled living anionic polymerization (LAP) and ring-opening polymerization (ROP) mechanisms, and the efficient Glaser reaction, Thiol-yne reaction and Williamson reaction are also adopted (**Scheme 1**). Subsequently, the micelles formed from these multiblock copolymers are also investigated and used as targeted delivery of fluorescent probe. The effects of linkages on self-assembly and their delivery behaviour are focused and compared.



Scheme 1. The illustration of synthetic route for multiblock copolymers with the same compositions but different linkages.

Experimental

Materials

Styrene [St, 99 %, Sinopharm Chemical Reagent Co. (SCR)] was washed with 10 % NaOH aqueous solution and followed by water three times successively, dried over anhydrous $MgSO_4$ for 24 h and further dried over CaH_2 and distilled under reduced pressure before use. Ethylene oxide (EO, 99 %, SCR) was dried over CaH_2 and distilled before use. Naphthalene (AR, SCR) was purified by sublimation. Tetrahydrofuran (THF, 99 %, SCR) was refluxed and distilled from potassium naphthalenide solution. Propargyl bromide (99 %, Aldrich) and dichloromethane (CH_2Cl_2), cyclohexane were purified by direct distillation from CaH_2. *n*-Butyllithium (*n*-Bu$^-$Li$^+$, 1.6 M in hexane, J&K), 2-mercaptoethanol (ME, 98 %, Aldrich), N,N-dimethylformamide (DMF), N,N,N',N'-tetramethylethylenediamine (TMEDA, 98 %, Aldrich), sodium hydride (NaH, 60 % dispersion in mineral oil, SCR), 2,2-dimethoxy-2-phenylacetophenone (DMPA), 1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide (DiR iodide, Aladdin) and cuprous chloride (CuCl, 99 %, SCR) were used as received. N-Phenyl-1-naphthylamine (PNA, Alfa Aesar, 97%) was purified by recrystallization in ethanol for three times. Diphenylmethyl potassium (DPMK) with concentration of 0.75 mol/L was prepared as described elsewhere.[47] The initiator of lithium naphthalenide was prepared from naphthalene and lithium according to reference,[46] and the concentration of 0.75 mol/L was analyzed by titration using hydrochloric acid (0.10 mol/L). All other reagents and solvents were purchased from SCR and used as received except for special declaration.

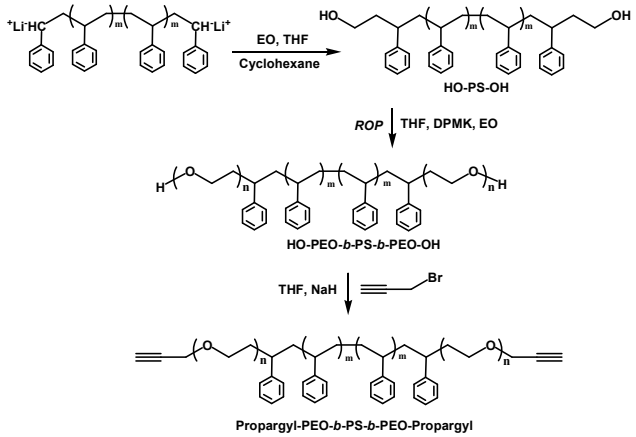
Measurements

Gel permeation chromatographic (GPC) analysis of polymers was performed in THF at 35 °C, with an elution rate of 1.0 mL / min on an Agilent 1260 equipped with a G1310A pump, a G1362A refractive index detector, and a G1314A variable wavelength detector. One 5 μm LP gel column (500 Å, molecular range 500-2×10^4 Da) and two 5 μm LP gel mixed bed column (molecular range 200-3×10^6 Da) were calibrated by PS standards. The absolute molecular weight of polymers was performed by GPC measurement through three Waters Styragel columns (pore size 10^2, 10^3 and 10^4Å), calibrated by PS standards, and equipped with three detectors: a DAWN H ELEOS (14-154°) (Wyatt multiangle laser light scattering detector, He–Ne 632.8 nm), ViscoStar (Wyatt), and Optilab rEX (Wyatt). ^{1}H NMR spectra were recorded on a Bruker (500 MHz) spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. The MALDI-TOF MS measurement was performed using a Perspective Biosystem Voyager-DE STR MALDI-TOF (matrix-assisted laser desorption / ionization time-of-flight) mass spectrometer (PE Applied Biosystems, Framingham, MA). The instrument was equipped with a nitrogen laser emitting at 337 nm with a 3 ns pulse width and working in positive mode. Matrix solution of dithranol (20 mg / mL), polymer (10 mg / mL) and cationizing salt of silver trifluoroacetate (10 mg / mL) in THF were mixed in the ratio of matrix: cationizing salt: polymer =10:1:2, and 0.8 μL of mixed solution was deposited on the sample holder (well-plate). The sizes of polymeric micelles in water were measured on a dynamic light scattering (DLS) instrument (Zetasizer Nano ZS90. Malvern) at 25 °C, a He-Ne laser (633 nm, 4 mW) was used to detect the scattering, and the detection angle was 90°. Transmission electron microscopy (TEM) images of micelles were obtained by using a Tecnai G2 F20 S-Twin electron microscope. Steady-state fluorescence (FLS) spectra of the samples were recorded on an Edinburgh Instruments 920 spectrometer, the emission intensity at 418nm was recorded to determine th critical micelle concentration (cmc) where the λ_{ex} was 340 nm. Fluorescent images were collected using the Clairvivo OPT (SHIMADZU Corporation, Kyoto, Japan) with a 735 nm single laser, and the exposure time was 5 s for each image.

Synthesis of Triblock Copolymer HO-PEO-*b*-PS-*b*-PEO-OH

In order to achieve the triblock copolymer HO-PEO-*b*-PS-*b*-PEO-OH, the precursor of difunctional α,ω-hydroxyl polystyrene (HO-PS-OH) was firstly prepared (**Scheme 2**). According to our previous works,[46] the precursor HO-PS-OH was synthesized by LAP of St monomers initiated with lithium naphthalenide and the following end-capping reaction with excess ethylene oxide agent. In a typical procedure, cyclohexane (700 mL), styrene (50.0 mL, 0.43 mol) and THF (6.0 mL) were sequentially introduced into a 1.0 L ampule. After the traced impurities in ampule was consumed by *n*-Bu$^-$Li$^+$, the needed lithium naphthalenide solution (35.0 mL, 24.9 mmol) was charged rapidly. Subsequently, the system was stirred at 25 ℃ for 1.0 h, and EO agent (6.30 mL, 125 mmol) was injected to cap the living –C$^-$Li$^+$species, and the formed –O$^-$Li$^+$ species were finally terminated with acidic methanol (0.1 mol/L HCl in CH_3OH). After all the solvents were evaporated, the product was revovered by precipitation into methanol for three times and dried under vacuum at 45 ℃ till to a constant weight. Yield: 44.8 g (98 %) ^{1}H NMR ($CDCl_3$) δ (ppm): 1.07-2.15 ($C_6H_5CHCH_2$-), 3.23-3.46 (-CH_2OH), 6.30-7.23 (-C_6H_5). $M_{n,GPC}$=3,000 g/mol, PDI=1.09, $M_{n,(MALDI\text{-}TOF\ MS)}$=2,500 g/mol.

Using the above HO-PS-OH as macroinitiator, the triblock copolymer HO-PEO-*b*-PS-*b*-PEO-OH was prepared by ROP of EO monomers (**Scheme 2**). Typically, the dry HO-PS-OH (10.0 g, 3.0 mmol) was dissolved in 200 mL THF and charged into a 500 mL dry ampoule, and the calculated DPMK solution (4.0 mL, 3.0 mmol) was added dropwise by a syringe under magnetic stirring. Then the ampoule was placed into an ice bath and the cold EO (25.0 mL, 0.49 mol) monomer was charged quickly, and the solution was heated to 60 °C and stirred for 96 h. Finally, the solution was terminated by acid methanol (0.1 mol/L HCl in CH_3OH). After the solvents were evaporated, the copolymers were precipitated into cold petroleum ether (30-60°C) slowly for three times and dried under vacuum at 45 °C till to a constant weight. Yield: 29.8 g (95 %). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.46-3.83 (-CH_2CH_2O-), 6.33-7.23 (-C_6H_5). $M_{n,GPC}$=11,200 g/mol, PDI=1.10, $M_{n,NMR}$=9,300 g/mol.

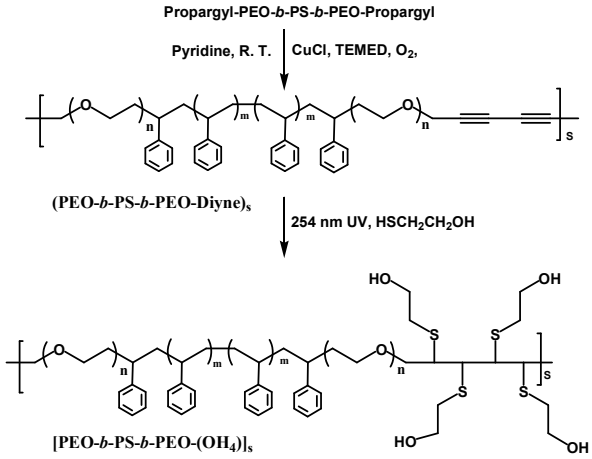


Scheme 2. The synthetic procedure for copolymer Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl.

Synthesis of Multiblcok Copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ by Glaser Coupling Reaction

The multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ was prepared using the functional Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl with terminal propargyl groups at both ends as precursor (**Scheme 3**). Typically, the precursor Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl was synthesized by modification of hydroxyl groups on HO-PEO-*b*-PS-*b*-PEO-OH with propargyl bromide. First, HO-PEO-*b*-PS-*b*-PEO-OH (3,000 g/mol, 30.0 g, 10.0 mmol) was added into a 500 mL round bottom flask and dried by azeotropic distillation with toluene. After the HO-PEO-*b*-PS-*b*-PEO-OH was again dissolved in dry THF (300 mL), NaH (2.4 g, 100.0 mmol) was added in three batches. Then the system was placed into an ice bath, propargyl bromide (3.0 mL, 38.3 mmol) was added dropwise within 2.0 h and the reaction was continued for another 48 h. Finally, the THF solvent was removed by evaporation, and the product was extracted with CH_2Cl_2, and then the organic layer was dried over $MgSO_4$ before purification by precipitation into cold petroleum ether (30°C -60°C) for three times. The obtained Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl was dried under vacuum at 45 °C till to a constant weight. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.45 (–C≡C*H*), 3.47-3.80 (–OCH_2CH_2O–), 4.20 (–OCH_2C≡CH), 6.33-7.23 (-C_6H_5), $M_{n,NMR}$=9,400 g/mol.

For the multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ with diyne groups as linkages (**Scheme 3**), Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl (6.00 g, 2.0 mmol), pyridine (75 mL), CuCl (0.32 g, 3.18 mmol), and TMEDA (1.60 mL, 10.49 mmol) were sequentially added into a 250 mL round bottom flask. Then the flask was filled with oxygen and maintained at 25 °C for 120 h. Finally, the solution was concentrated and the crude product was purified by passing through a neutral alumina column using CH_2Cl_2 as eluent to remove the copper catalyst. After the product was concentrated and recovered by precipitation into cold petroleum ether (30°C-60°C) for three times, the obtained (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ was dried under vacuum at 45 °C till to a constant weight. ^{1}H NMR ($CDCl_3$) δ(ppm): 3.46-3.85 (-OCH_2CH_2O-), 4.20 (-CH_2C≡CCH_2-), 6.31-7.24 (-C_6H_5). $M_{n,GPC}$=62,500 g/mol, PDI=1.55, $M_{w,MALLS}$=110,000 g/mol.



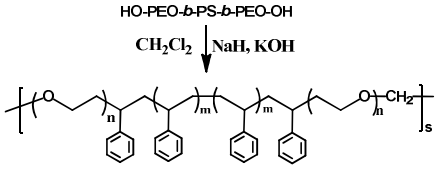
Scheme 3. The synthetic procedure for multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ and [PS-*b*-PEO-(OH)$_4$-PEO]$_s$.

Synthesis of Multiblcok Copolymer [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ by Thiol-yne Addition Reaction on (PEO-*b*-PS-*b*-PEO-Diyne)$_s$

The [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ was obtained by Thiol-yne addition reaction between mercaptoethanol agent and diyne groups on (PEO-*b*-PS-*b*-PEO-Diyne)$_s$. Typically, (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ (3.10 g, 0.05 mmol alkyne groups), DMPA (15.00 mg, 0.06 mmol), ME (2.0 mL, 27.0 mmol) and 45 mL DMF were charged into a 50 mL quartz glass ampoule and purged with nitrogen. Then the system was irradiated under UV (254 nm) for 24 h. After the evaporation of DMF solvent under reduced pressure, the crude product was dissolved in CH_2Cl_2 and precipitated into cold petroleum ether (30°C-60°C) for three times, and the obtained [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ was dried under vacuum at 45 °C till to a constant weight. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.06 (-SCH_2CH$_2$OH), 3.92 (-SCH$_2$CH_2OH), 3.34-3.43 (-CH$_2$CH(S)CH-), 3.45-3.83 (-OCH_2CH_2O-), 6.33-7.27 (-C_6H_5).

Synthesis of Multiblcok Copolymer (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ by Williamson Reaction

The mulitblock copolymer (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ with acetal groups as linkages was prepared by Williamson reaction using the above HO-PEO-*b*-PS-*b*-PEO-OH as precursor (**Scheme 4**). Typically, the precursor HO-PEO-*b*-PS-*b*-PEO-OH (0.50 g, 0.04 mmol) dried by azeotropic distillation with toluene was dissolved in 11.0 mL dry CH_2Cl_2 in a 50 mL round bottom flask. Then NaH (0.10 g, 4.17 mmol) and potassium hydroxide (KOH, 1.00 g, 17.86 mmol) were added, and the reaction was carried out at refluxed temperature for 72 h. After the reaction system was evaporated and extracted with CH_2Cl_2 /H_2O for three times, the CH_2Cl_2 phase was concentrated and precipitated into cold petroleum ether (30-60°C) for three times. The obtained (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ was dried under vacuum at 45 °C till to a constant weight. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.47-3.80 (-OCH_2CH_2O-), 4.65-4.80 (-OCH_2O-), 6.35-7.23 (-C_6H_5). $M_{n,GPC}$=64,000 g/mol, PDI=1.40.



Scheme 4. The synthetic procedure for multiblock copolymer (PEO-*b*-PS-*b*-PEO-Acetal)$_s$.

Determination of Critical Micelle Concentration (*cmc*)

According to literature,[48] the PNA was used as fluorescence probe to measure the *cmc* values of copolymers. First, 50 μL acetone solution of PNA (0.001 mol/L) was added into 25 mL water to give the concentration of PNA with 2 × 10^{-3} mmol/L. Then, different amounts of of copolymer solutions in THF were added into the above water containing PNA ([PNA] = 2 × 10^{-3} mmol/L), and the concentration of block copolymer were finally modulated from 5.0 × 10^{-5} to 7.0 × 10^{-2} mg/mL for FLS measurement. All fluorescence spectra were recorded at 25 ^{0}C.

Observation of Micellar Morphologies of Copolymers with Different Linkages

The micelles fromed from copolymers HO-PEO-*b*-PS-*b*-PEO-OH, (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ were prepared according to the following procedure. For example, the copolymer (40 mg) was firstly dissolved in 5.0 mL acetone, and then this solution was added dropwise into 6.0 mL deionized water under stirring. Subsequently, the mixture was evaporated under reduced pressure to remove the acetone solvent. Finally, an ultrathin carbon network was dipped into the obtained aqueous solution of copolymer, and the samples were subjected to a freeze drying procedure and the TEM images were obtained at 200 kV on a Tecnai G2 F20 S-Twin electron microscope.

Study on *in vivo* Distribution of Micelles from Copolymers with Different Linkages

The micelles contained DiR agent were prepared using the similar procedure as above, except that the DiR was firstly added and dispersed in acetone phase of copolymers before their addition into deionized water. Typically, 40 mg copolymer was firstly dissolved in 5.0 mL acetone, and then 20 μL DiR (10 mg/mL in ethanol) was added into the organic phase and vortexed. Subsequently, the organic phase was added dropwisely into 6.0 mL deionized water under stirring. After the acetone solvent was removed by evaporation, only water was remained and the DiR loaded micelles (33.3 μg / mL) were obtained.

The obtained micelles were then used to study *in vivo* distribution of copolymers. Typically, the nude mice were intravenously injected with 200 μL of DiR loaded micelles. After 4.0 h, the mice were anesthetized using isoflurane in oxygen and put into the chamber. For *in vivo* imaging, the mice were sacrificed by cervical dislocation, and major organs including hearts, livers, spleens, lungs, kidneys were excised. The fluorescent images of tissues were photographed and the near-infrared fluorescence signal intensity in different tissues was measured.

Results and discussion

Synthesis and Characterization of Copolymer HO-PEO-*b*-PS-*b*-PEO-OH

In this contribution, in order to synthesize multiblock copolymers with as much as possible PS and PEO segments, the precursor of triblock copolymer HO-PEO-*b*-PS-*b*-PEO-OH with defined terminal functionality and controlled compositions should be firstly ensured, and the formation of possible diblock copolymer PEO-*b*-PS and homopolymer PS should be largely avoided. Thus, the sequential LAP and ROP mechanisms with excellent controllability were adopted to synthesize the triblock copolymer HO-PEO-*b*-PS-*b*-PEO-OH.

Firstly, the difunctional living $^{+}$Li-PS-Li^{+} species grew from lithium naphthalenide were carefully end-capped with EO agent. Once the EO agent (eight-fold to living species) was added into the red system of $^{+}$Li-PS-Li^{+} solution, one could observe that the color of system was changed into light yellow immediately, which meant that the alkoxides (-O-Li^{+}) was actually formed. Furthermore, the high degree of aggregation of lithium alkoxides compared to other alkali metal alkoxides in hydrocarbon media rendered them unreactive toward further oligomerization of EO agent at the chain end. Additionally, different from the previous works on the functionalization of poly(styryl)lithium (PS^{-}Li^{+}) with propylene oxide,[49] styrene oxide,[50] 1-buteneoxide,[51] 3,4-epoxy-1-butene[52] and ethoxyethyl glycidyl ether[53] agents, the EO agent without any substituted group tend to lead the less side reactions and provided a high functionalization efficiency on living species and the HO-PS-OH with high purity was yield.

The successful LAP of St monomers and the followed end-capping reaction was evidenced by the monomodal peak and symmetrical GPC curve with a narrow molecular weight distribution (PDI=1.09) (**Fig. 1A**). The ^{1}H NMR spectrum of synthesized HO-PS-OH was monitored and shown in **Fig. 2**. Except for the characteristic resonance signals of aromatic protons (-C_6H_5) on PS segment ascribed at 6.29-7.25 ppm, the resonance signal appeared at 3.23-3.46 ppm corresponded to protons (-CH_2OH-) confirmed the successful introduction of EO agents onto PS end. As a direct and efficient method, the HO-PS-OH was also measured by MALDI-TOF MS and the accurate information was collected (**Fig. 3**). The peak at m/z=2487.7Da was attributed to the HO-PS-OH [$HOCH_2CH_2$-(C_8H_8)$_{22}$-$CH_2CH_2OH.Ag^{+}$], and the spacing between adjacent peaks was 104.0 Da, which was the mass of St unit. In the whole and expand MALDI-TOF mass spectrum, the absence of any sub-peak confirmed that only the functionalized HO-PS-OH was actually achieved.

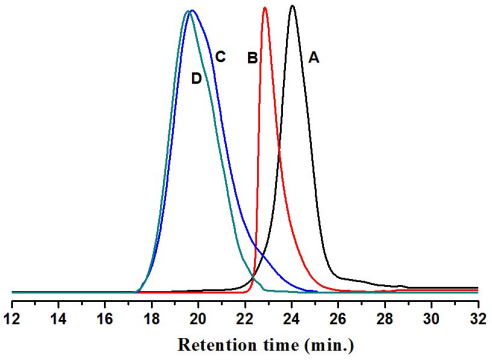


Fig. 1 GPC traces of polymers HO-PS-OH (A, M_n=3,000 g/mol, PDI=1.09), HO-PEO-*b*-PS-*b*-PEO-OH (B, M_n=11,200 g/mol, PDI=1.10); (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ (C, M_n=62,800 g/mol, PDI=1.55, (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ (D, M_n=64,000 g/mol, PDI=1.40).

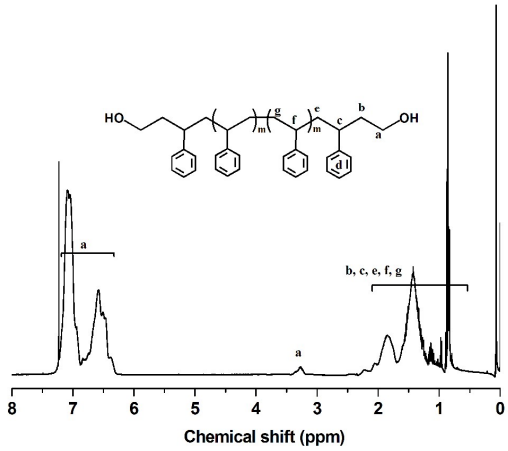


Fig. 2 ^{1}H NMR spectrum of precursor HO-PS-OH (in $CDCl_3$).

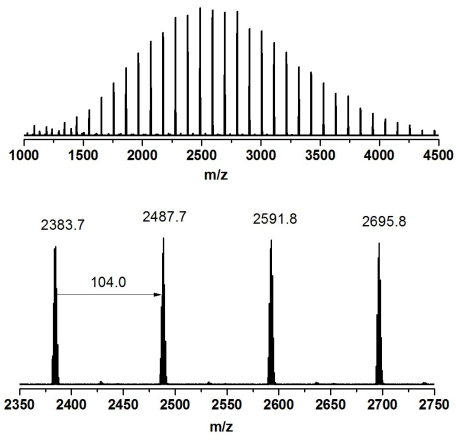


Fig. 3 The MALDI-TOF mass spectrum of HO-PS-OH precursor.

Then, the triblock copolymer HO-PEO-*b*-PS-*b*-PEO-OH were achieved by ROP of EO monomers using HO-PS-OH as macroinitiator. From **Fig. 1B**, it could be observed that the GPC trace of obtained HO-PEO-*b*-PS-*b*-PEO-OH was given with monomodal peak and low PDI. The composition of triblock

copolymer was further verified by ^{1}H MNR spectrum. As shown in **Fig. 4A**, the characteristic resonance signal of aromatic protons (-C_6H_5) on PS segment at 6.33-7.23 ppm and that of methylene protons (−CH_2CH_2O−) on PEO segment at 3.45-3.83 ppm were all well discriminated. Thus, the HO-PEO-*b*-PS-*b*-PEO-OH has been smoothly synthesized and its structure can be actually confirmed.

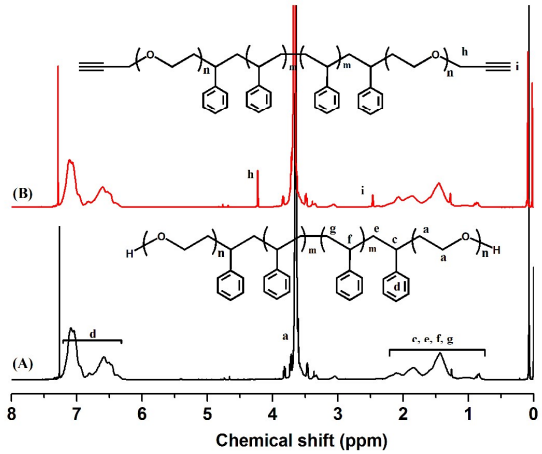


Fig. 4 ^{1}H NMR spectra of HO-PEO-*b*-PS-*b*-PEO-OH (A), Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl (B) (in $CDCl_3$).

Synthesis and Characterization of Mutilblock Copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$

The functional Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl was prepared by end group transformation of HO-PEO-*b*-PS-*b*-PEO-OH with propargyl bromide in the presence of NaH. From ^{1}H NMR spectrum of Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl (**Fig. 4B**), the characteristic resonance signals attributed to alkynyl proton (-C≡C*H*) and methylene protons (-OCH_2C ≡ CH) on propargyl group were discriminated at 2.43 and 4.21 ppm, respectively.

As the description in previous section, the Glaser coupling between alkyne groups has been widely used in polymer science, and was found with good yields at room temperature and under an air atmosphere.[45,46] Herein, the efficient Glaser coupling reaction was again used for our multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$. The GPC trace of the coupled product with a high PDI was shown in **Fig. 1C**, which was rather accorded with the characteristics of a step-growth polymerization mechanism. Also, the absolute molecular weight ($M_{w,MALLS}$=110,000 g/mol) of (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ could be derived by GPC measurement equipped with a multi-angle laser light scattering detector. From the above $M_{w,MALLS}$ and the molecular weight ($M_{n,NMR}$=9,400 g/mol) of Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl, the degree of Glaser coupling (DG) reaction could be calculated as 11.7. On the other hand, different from the ^{1}H NMR spectrum of Propargyl-PEO-*b*-PS-*b*-PEO-Propargyl (**Fig. 5A**), there was almost no obvious change except that the signal of alkynyl protons (–C≡C*H*) at 2.43 disappeared from the ^{1}H NMR spectrum of (PEO-*b*-PS-*b*-PEO-Diyne)$_s$. Thus, the GPC trace and ^{1}H NMR spectrum comprehensively confirmed the Glaser coupling reaction was successful.

Subsequently, the [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ was obtained by efficient Thiol–yne addition reaction between 1,3-diyne structures and excess 2-mercaptoethanol agent, in which the DMF was used as solvent and DMPA was used as photoinitiator under 254 nm UV irradiation. From the ^{1}H NMR spectrum of [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ (**Fig. 5B**), the characteristic resonance signal attributed to methylene protons (-OCH_2C≡C-) connected to the triple bonds disappeared completely, which confirmed that all the 1,3-diyne groups have been successfully transformed into hydroxyl groups.

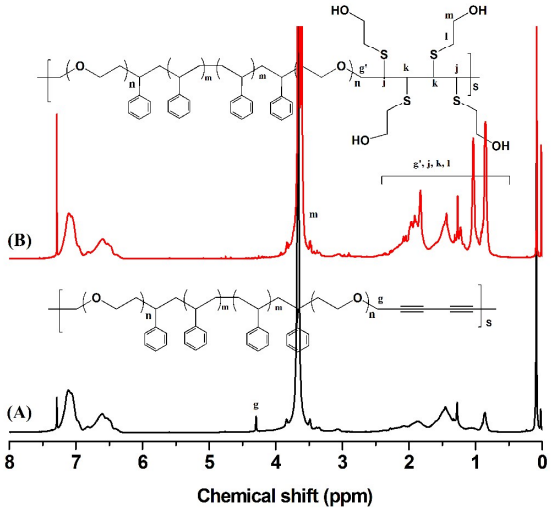


Fig. 5 ^{1}H NMR spectra of (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ (A) and [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ (B) (in $CDCl_3$).

Alternatively, based on the high efficiency and advantages of Williamson reaction, the high molecular weight multiblock copolymer (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ was also synthesized by coupling reaction between active hydroxyl groups at the end of HO-PEO-*b*-PS-*b*-PEO-OH. From the GPC result for (PEO-b-PS-b-PEO-Acetal)$_s$, a monomodal peak was also achieved. From ^{1}H NMR spectrum for (PEO-*b*-PS-*b*-PEO-Acetal)s (**Fig. 6**), except for the characteristic resonance signals for methylene protons 3.47-3.80 ppm (-CH_2CH_2O-) on PEO segment, 6.35-7.23 ppm (-C_6H_5-) on PS segment, the signals of new formed acetal protons (-OCH_2O-) after coupling were also discriminated at 4.65-4.80 ppm, which gave the information that the Williamson reaction was actually successful.

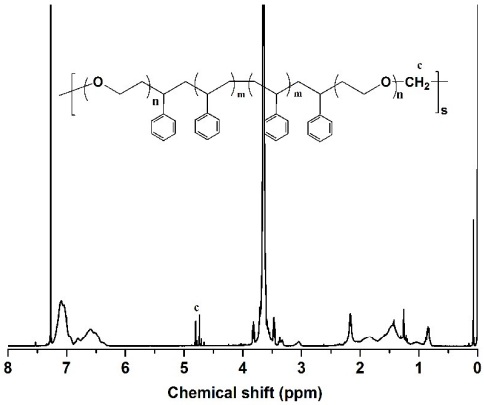


Fig. 6 ^{1}H NMR spectrum of multiblock copolymer (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ (in $CDCl_3$).

Investigation of Micellar Morphology of Multiblock Copolymers with Different Linkages

The amphiphilic polymers consisting of hydrophilic and hydrophobic segments can self-assembly into core-shell structure in selective solvent, such as water.[3,31] Herein, the micelles formed from multiblock copolymers (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, [PS-*b*-PEO-PEO-(OH)$_4$]$_s$, (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ and the precursor HO-PEO-*b*-PS-*b*-PEO-OH were all well examined by FLS, DLS and TEM measurements, and the results were further compared.

The *cmc* values of multi- or triblock copolymers in aqueous phase were firstly determined by fluorescence technique using PNA as probe. Typically, the PNA was a suitable fluorescent probe in terms of reproducibility because it bears high fluorescence activity in nonpolar environments, and it could be very easily quenched by polar solvents such as water.[54, 55] The relationship of the fluorescence intensity ratio (I/I_0) of PNA as a function of the concentration of copolymers aqueous solution was plotted in **Fig. 7**. It was found that I/I_0 increased sharply when the concentration exceeded a certain value, which proved PNA probe was incorporated into the hydrophobic core of micelles. Therefore the intersection of two straight lines with a value of 3.26×10^{-3} mg/mL was determined to be the *cmc* of triblock copolymer HO-PEO-*b*-PS-*b*-PEO-OH. Similarly, the multiblock copolymers with the same compositions but with different linkages were measured with the increased *cmc* values, i.e., 6.48×10^{-3} mg/mL for (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, 5.56×10^{-3} mg/mL for [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and 5.77×10^{-3} mg/mL for (PEO-*b*-PS-*b*-PEO-Acetal)$_s$. Obviously, the triblock colpolymer HO-PEO-*b*-PS-*b*-PEO-OH gave the lowest *cmc* value, and the multiblock copolymers [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ showed the modest and almost the same *cmc* values. However, the (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ was endowed with the highest cmc value. These *cmc* values preliminarily reflected that the topologies and copositions actually had some effect on their self-assembly behaviour.

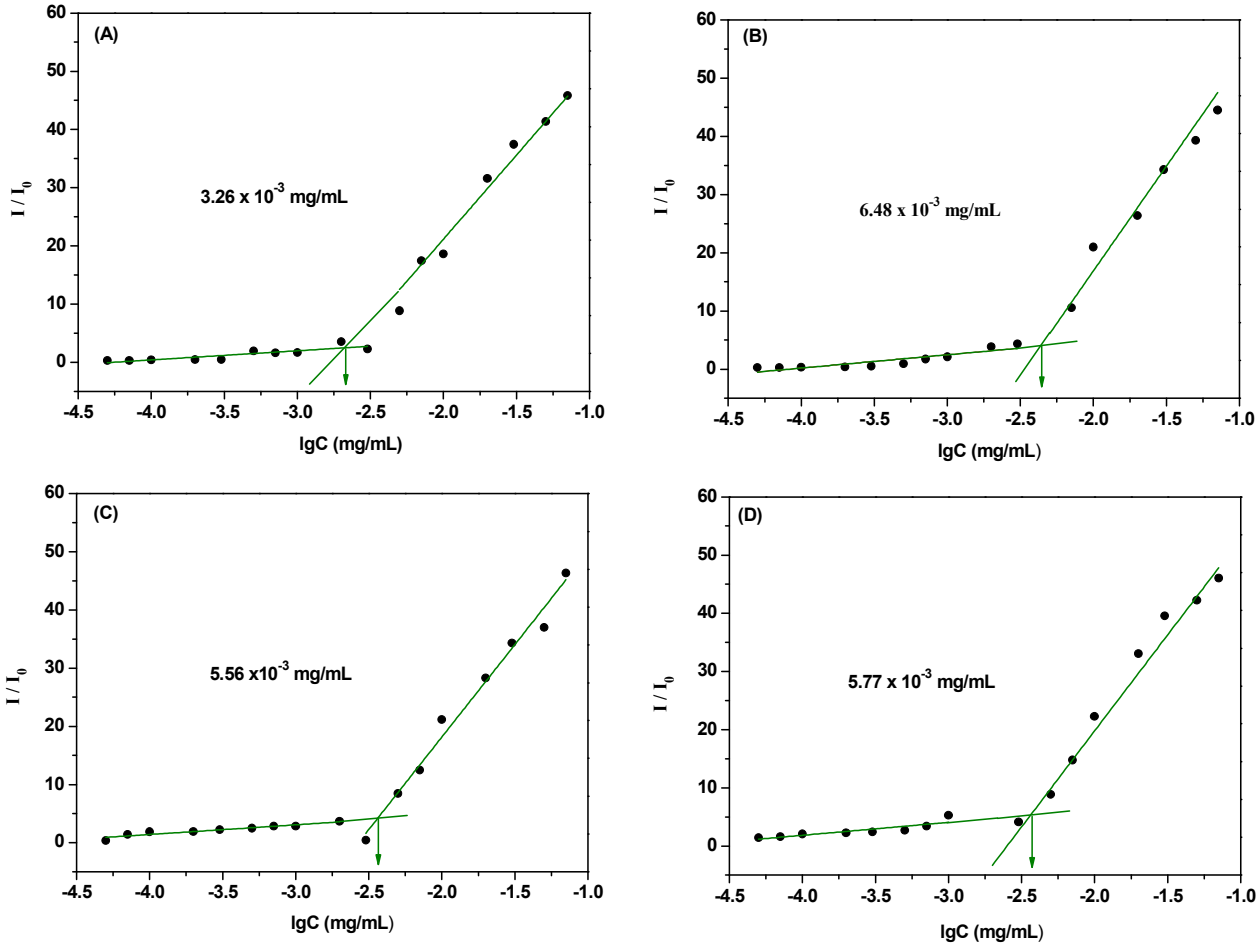


Fig. 7 The dependence of fluorescence intensity ratio (I/I_0) of PNA fluorescence emission spectra on the concentration of copolymers (A: PEO-b-PS-b-PEO, B: (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, C: [PS-*b*-PEO-PEO-(OH)$_4$]$_s$, D: (PEO-*b*-PS-*b*-PEO-Acetal)$_s$).

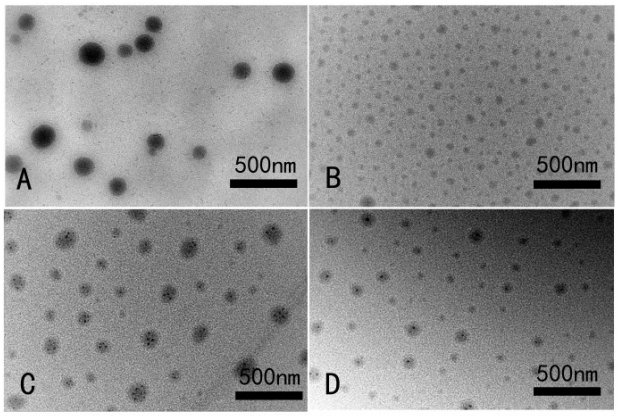


Fig. 8 The TEM images of micelles formed from copolymers HO-PEO-*b*-PS-*b*-PEO-OH (A), (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ (B), [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ (C) and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ (D).

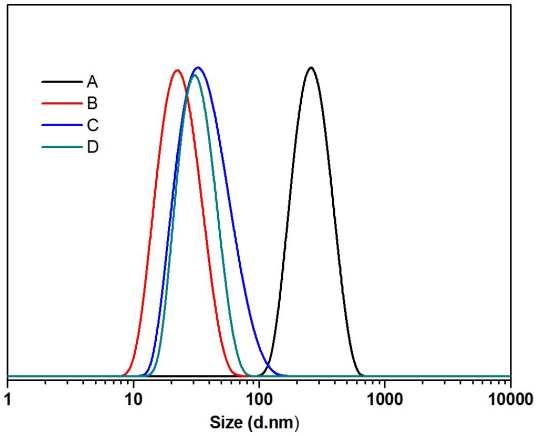
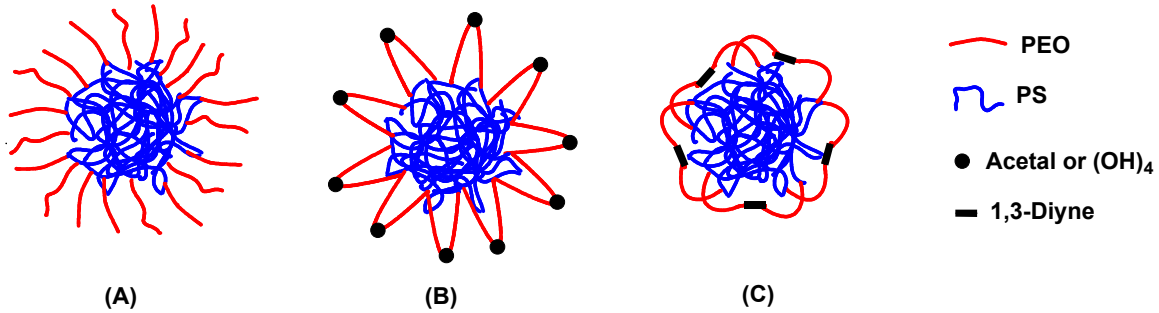


Fig. 9 The size distribution of micelles formed from copolymers PEO-*b*-PS-*b*-PEO(A), (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ (B), [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ (C) and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$(D).

Also, the mecillar morphology was also monitored by TEM measurement. As the illustrated TEM images in **Fig. 8**, all the samples formed the spherical core-shell micelles. Differently, the precursor of triblock copolymer HO-PEO-*b*-PS-*b*-PEO-OH formed the largest size of micelle, while the multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ gave the smallest size of micelle, and the multiblock copolymers [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ tend to aggregate into the medium size of micelles. Consistently, the DLS measurement also gave the same derivation of micelles on their sizes (**Fig. 9**). In detail, the average sizes of micelles formed from sample HO-PEO-*b*-PS-*b*-PEO-OH, (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ were monitored as 273nm, 24nm, 40nm and 34 nm, respectively.

Actually, three multiblock copolymer samples have the same compositions (molar ratio of EO to St units), but embedded with different linkages. The acetal and -OH$_4$ linkages tend to give the copolymers a hydrophilic character (similar to the contribution of the hydrophilic EO unit), while the 1,3-dyine linkages tend to bring some hydrophobic character to the copolymers. During the formation of micelles from multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, except the hydrophobicity brought by PS segment, the 1,3-dyine linkage would also contribute certain hydrophobicity. Thus, as the proposed mechanism for spherical micelles,[56,57] due to the high solubility of PEO segment in water, the hydrophobic dyine linkage embedded into polymer chain can not be completely buried in the hydrophobic core, but appears be oriented towards the hydrophilic corona. Also, because of the hydrophobicity of 1,3-dyine groups, the geometric tension of PEO around the core were reduced by the diyne groups in the middle of PEO segment (**Scheme 5**). Thus, the overall effect of hydrophilcity and hydrophobicity of (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ gave a small size of micelles. As for as the multiblock copolymers [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$, the embedded hydrophilic groups -OH$_4$ and acetal groups have the similar hydrophilicity to that of EO units, which would lead to a neglect effect on the micellization (**Scheme 5**). Additionally, compared with the above multiblock copolymers, the precursor HO-PEO-*b*-PS-*b*-PEO-OH gave the largest size of micelles, which again confirmed that the topology actually exert important function on their self-assembly behaviour.



Scheme 5. The proposed self-assemblied procedure for multi and triblock copolymers (A: HO-PEO-b-PS-b-PEO-OH, B: [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$, C: (PEO-*b*-PS-*b*-PEO-Diyne)$_s$).

Investigation of *in vivo* distribution of Micelles Formed by Multiblock Copolymers with Different Linkages

When the polymeric micelles were used in biomedical field, the micellar carrier was also hoped to contribute effective treatment of brain disease or diagnosis in some cases.[58] However, brain-targeted

delivery of drug or agent was usually hard to achieve due to the infiltrative nature of the blood-brain barrier (BBB).[59-61] The BBB protects foreign organisms and noxious chemicals by highly strengthened endothelial wall of vasculature system and controls the passage of drugs from the blood into the brain. Thus, one of the major obstacles for the brain targeted delivery is to overcome the BBB effect.[6265] Correspondingly, the screening of polymeric micelles with applicable size and morphology might be the key task. Thus, the *in vivo* distribution of micelles formed from the synthesized multiblock copolymers were also investigated and compared to screen an optimized system.

Generally, the administration *in vivo* was operated by intravenous injection, and the maximum distribution in the target nude mice was traced after 4 h of injection (**Fig. 10**). The distribution of the materials was detected by measuring the fluorescence probe loaded in micelles.[66,67] The *in vivo* result of mice revealed that the multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ was instantaneously transported through the BBB and well accumulated at the brain within 4 h, which demonstrated that the copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ might have much therapeutic application in the treatment of brain disease. On the contrary, the accumulation of micelles formed from copolymers [PS-*b*-PEO-PEO-(OH)$_4$]$_s$, (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ and HO-PEO-*b*-PS-*b*-PEO-OH gave the less amount at the brain. According to the above difference of self-assembly behaviour between several multi and triblock copolymers, one can speculate that the excellent permeability of (PEO-b-PS-b-PEO-diyne)s was mainly due to presence of the hydrophobic linkages of 1,3-diyne group and the multi-block structure, which collaboratively gave the proper size of micelle, and finally can overcome the BBB effect and realize an ideal drug delivery to the brain. Thus, the simple modification of certain linkages could be used to modulate the size of micelles and further control their *in vivo* distribution, which might bring some important reference to the application of multiblock copolymer in biomedical field.

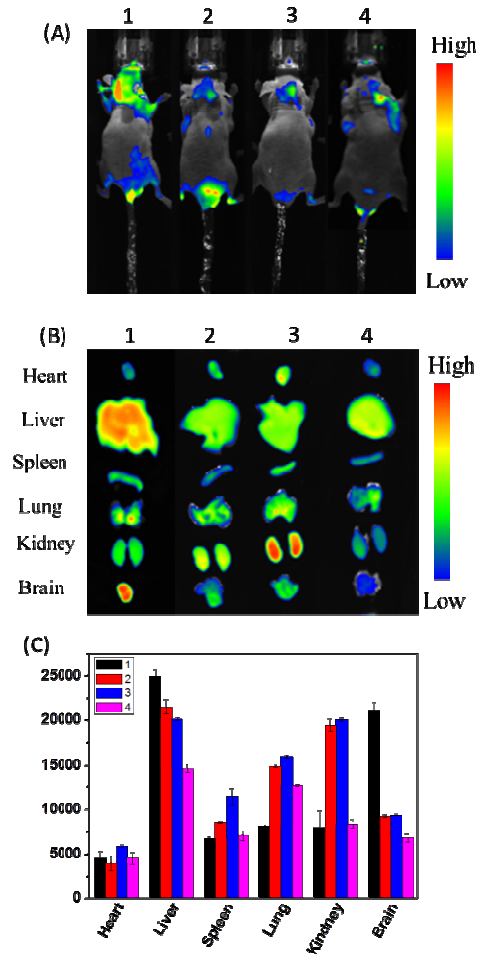


Fig. 10 Bio-distribution of DiR loaded micelles in nude mice (A), the optical images of organs of nude mice sacrificed (B), fluorescence intensity in organs (C) after administration at 4 h (1: (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, 2: [PS-*b*-PEO-PEO-(OH)$_4$]$_s$, 3: (PEO-*b*-PS-*b*-PEO-Acetal)s, 4: HO-PEO-*b*-PS-*b*-PEO-OH).

Conclusions

The multiblock copolymers (PEO-*b*-PS-*b*-PEO-Diyne)$_s$, [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ with the same compositions but different linkages were realized by controlled LAP and ROP mechanisms, and the efficient Glaser reaction, Thile-yne reaction and Williamson reaction were also adopted. The micellar morphology of micelles formed from the synthesized copolymers were also investigated by DLS and TEM measurements and compared. The formed micelles were further used to load the fluorescent probe to study their *in vivo* distribution. Under the same conditions, the precursor triblock copolymer HO-PEO-*b*-PS-*b*-PEO-OH formed the largest size of micelle, while the multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ gave the smallest size of micelle, and the multiblock copolymers [PS-*b*-PEO-PEO-(OH)$_4$]$_s$ and (PEO-*b*-PS-*b*-PEO-Acetal)$_s$ tend to aggregate into the medium size of micelles. Especially, the existed hydrophobic diyne groups would exert some important effect on the self-assembly behaviour of

multiblock copolymer (PEO-*b*-PS-*b*-PEO-Diyne)$_s$ and gave the smallest size of micelles, which can further traverse BBB and might gave a therapeutic application in the treatment of brain disease. Thus, we can concluded that the topologies of multiblock copolymers, even the simple modification of certain linkages, actually exert some certain influence on the self-assembly behavior and the possible targeted delivery, which might bring some important reference to the application of multiblock copolymer in biomedical field.

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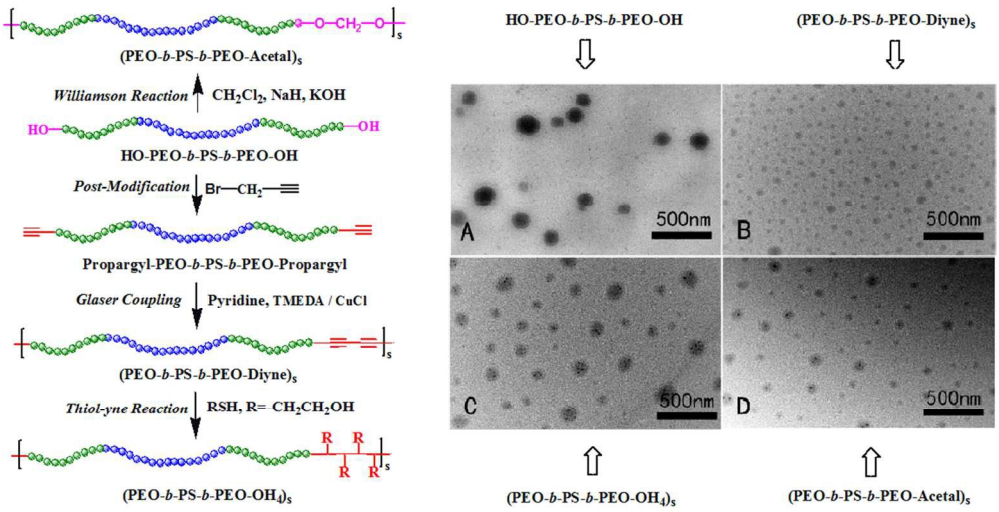
Notes and references

1 C. Zhang, Y. L. Yang, J. P. He, *Macromolecules,* 2013, **46**, 3985-3994.
2 H. F. Gao, K. Matyjaszewski, *J. Am. Chem. Soc.,* 2007, **129**, 6633-6639.
3 C. Wang, Y. T Kang, K. Liu, Z. B. Li, Z. Q. Wang, X. Zhang, *Polym. Chem.,* 2012, **3**, 3056-3059.
4 L. Sun, X. F. Ma, C. M. Dong, B. S. Zhu, X. Y. Zhu, *Biomacromolecules*, 2012, **13**, 3581-3591.
5 Y. Z. Lin, Y. F. Wang, J. Y. Wang, J. H. Hou, Y. F. Li, D. B. Zhu, X. W. Zhan, *Adv. Mater.,* 2014, **26**, 5137-5142.
6 L. Zhang, H. B. Zeng, Q. X. Liu, *J. Phys. Chem. C.,* 2012, **116**, 17554-17562.
7 O. J. Cayre, N. Chagneux, S. Biggs, *Soft Matter.,* 2011, **7**, 2211-2234.
8 J. Dai, S. D. Lin, D. Cheng, S. Y. Zou, X. T. Shuai, *Angew. Chem. Int. Ed.*, 2011, **50**, 9404-9408.
9 M. Zhang, M. Drechsler, A. H. E. Muller, *Chem. Mater.,* 2004, **16**, 537-543.
10 L. Chen, T. Y. Ci, T. Li, L. Yu, J. D. Ding, *Macromolecules*, 2014, **47**, 5895-5903.
11 S. Decato, T. Bemis, E. Madsen, S. Mecozzi, *Polym. Chem.,* 2014, **5**, 6461-6471.
12 C. Boyère, N. Duhem, A. Debuigne, V. Préat, C. Jérôme, R. Riva, *Polym. Chem.,* 2014, **5**, 3030-3037.
13 H. Maeda, T. Sawa, T. Konno, *J. Controlled Release,* 2001, **74**, 47-61.
14 N. Larson, H. Ghandehari, *Chem. Mater.,*2012, **24**, 840-853.
15 K. Wang, G. F. Luo, Y. Liu, C. Li, S. X. Cheng, R. X. Zhuo, X. Z. Zhang, *Polym. Chem.,* 2012, **3**, 1084-1090.
16 B. Y. Du, A. X. Mei, Y. Yang, Q. F. Zhang, Q. Wang, J. T. Xu, Z. Q. Fan, *Polymer*, 2010, **51**, 3493-3502.
17 T. Q. Chen, Y. P. Lu, T. Y. Chen, X. H. Zhang, B. Y. Du, *Phys. Chem. Chem. Phys.,* 2014, **16**, 5536-5544.
18 Y. P. Lu, T. Q. Chen, A. X. Mei, T. Y. Chen, Y. W. Ding, X. H. Zhang, J. T. Xu, Z. Q. Fan, B. Y. Du, *Phys. Chem. Chem. Phys.,* 2013, **15**, 8276-8286.
19 A. X. Mei, X. L. Guo, Y. W. Ding, X. H. Zhang, J. T, Xu, Z. Q. Fan, B. Y. Du, *Macromolecules*, 2010, **43**, 7312-7320.
20 J. Han, D. D. Zhu, C. Gao, *Polym. Chem.,* 2013, **4**, 542-549.
21 H. F. Lam, X. J. G, C. Wu, *J. Phys. Chem. B.,* 2007, **111**, 1531-1535.
22 A. Halperin, *Macromolecules*, 1991, **24**, 1418-1419.
23 Q. W. Zhang, J. Ye, Y. J. Lu, T. Nie, D. H. Xie, Q. L. Song, H. W. Chen, G. Z. Zhang, Y. Tang, C. Wu, Z. W. Xie, *Macromolecules*, 2008, **41**, 2228-2234.
24 G. Z. Zhang, F. M. Winnik, C. Wu, *Phys. Rev. Lett.,* 90.
25 H. Watanabe, Y. Matsumiya, T. Sawada, T. Iwamoto, *Macromolecules*, 2007, **40**, 6885-6897.
26 K. Sugiyama, T. Oie, A. A. El-Magd, A. Hirao, *Macromolecules*, 2010, **43**, 1403-1410.
27 C. Wu, Z. W. Xie, G. Z. Zhang, G. F. Zi, Y. F. Tu, Y. L. Yang, P. Cai, T. Nie, *CHEM. COMMUN.,* 2002, 2898-2899.
28 A. Touris, N. Hadjichristidis, *Macromolecules*, 2011, **44**, 1969-1976.
29 R. Nicolaÿ, L. Marx, P. H é mery, K. Matyjaszewski, *Macromolecules*, 2007, **40**, 9217-9223.
30 A. C. Greene, J. H. Zhu, D. J. Pochan, X. Q. Jia, K. L. Kiick, *Macromolecules*, 2011, 44, 1942-1951.
31 L. Z. Hong, F. M. Zhu, J. F. Li, T. Ngai, Z. W. Xie, C. Wu, *Macromolecules*, 2008, **41**, 2219-2227.
32 C. F. Huang, Y. Ohta, A. Yokoyama, T. Yokozawa, *Macromolecules,* 2011, 44, 4140−4148.
33 X. P. Wang, J. Huang, L. D. Chen, Y. J. Liu, G. W. Wang, *Macromolecules*, 2014, **47**, 7812-7822.
34 M. C. Baier, J. Huber, S. Mecking, *J. Am. Chem. Soc.,* 2009, **131**, 14267-14273.
35 H. Y. Gao, H. D. Wagner, D. Y. Zhong, J. H. Franke, A. Studer, H. Fuchs, *Angew. Chem. Int. Ed.,* 2013, **52**, 4024-4028.
36 (a) P. Siemsen, R. C. Livingston, F. Diederich, *Angew. Chem.* 2000, **112**, 2740-2767; (b) C. C. C. Johansson Seechurn, M. O. Kitching, T. J. Colacot, V. Snieckus, *Angew. Chem. Int. Ed.,* 2012, **51**, 5062-5085.
37 R. E. Martin, F. Diederrich, *Angew. Chem.,* 1999, **111**, 1440-1469.
38 (a) S. Shi, A. L. K, R. R. Tykwinski, *Angew. Chem., Int. Ed.,* 2006, **45**, 1034-1057; (b) H. Yun, T. C. Chou, H. Dong, Y. Tian, Y. M. Li, S. J. Danishefsky, *J. Org. Chem.,* 2005, **70**, 10375-10380.
39 (a) A. Stütz, G. Petranyi, *J. Med. Chem.,* 1984, **27**, 1539-1543; (b) A. Stütz, *Angew. Chem., Int. Ed. Engl.,* 1987, **26**, 320-328.
40 X. C. Pang, R. K. Jing, J. L. Huang, Polymer, 2008, **49**, 893-900.
41 X. C. Pang, G. W. Wang, Z. F. Jia, C. Liu, J. L. Huang, *J. Polym. Sic. Part A. Polym. Chem.,* 2007, **45**, 5824-5837.
42 Z. F. Jia, Q. Fu, J. L. Huan, *Macromolecules,* 2006, **39**, 5190-5193.
43 Z. F. Jia, Q. Fu, J. L. Huang, *J. Polym. Sic. Part A. Polym. Chem.,* 2006, **44**, 3836-3842.
44 Z. F. Jia, X. W. Xu, Q. Fu, J. L. Huang, *J. Polym. Sic. Part A. Polym. Chem.,* 2006, **44,** 6071-6082.
45 Y. N. Zhang, G. W. Wang, J. L. Huang, *Macromolecules,* 2010, **43**, 10343-10347.

46 B. Huang, X. S. Fan, G. W. Wang, Y. N. Zhang, J. L. Huang, *J. Polym. Sic. Part A. Polym. Chem.,* 2012, **50**, 2444-2451.
47 R. Francis, D. Taton, J. L. Logan, P. Masse, Y. Gnanou and R. S. Duran, *Macromolecules,* 2003, **36**, 8253-8259.
48 G. L. Lu, Y. J. Li, H. S. Gao, H. Guo, X. L. Zheng, X. Y. Huang, *J. Polym. Sic. Part A. Polym. Chem.,* 2013, **51**, 1099-1106.
49 R. P.Quirk, G. M. Lizarraga, *Macromolecules,* 1998, **31**, 3424-3430.
50 R. P. Quirk, H. Hasegawa, D. L. Gomochak, C. Wesdemiotis, K. Wollyung, *Macromolecules,* 2004, **37**, 7146-7155.
51 R. P. Quirk, Q. Ge, M. A. Arnould, C. Wesdemiotis, *Macromol. Chem. Phys.,* 2001, **202**, 1761-1767.
52 R. P. Quirk, D. L. Gomochak, C. Wesdemiotis, M. A. Arnould, *J. Polym. Sci., Part A: Polym. Chem.,* 2003, **41**, 947-957.
53 G. W. Wang, B. Hu, J. L. Huang, *Macromolecules*, 2010**, 43,** 6939-6942.
54 L. C. You, F. Z. Lu, Z. C. Li, W. Zhang, F. M. Li, *Macromolecules*, 2003, **36**, 1-4.
55 P. Xu, H. Tang, S. Li, J. Ren, E. Van Kirk, W. J. Murdoch, M. Radosz, Y. Shen, *Biomacromolecules*, 2004, **5**, 1736-1744.
56 Z. Tuzar, P. Kratochvil, *Surface & Colloid Sci.*, 1993, **15**, 1-88.
57 T. Nie, Y. Zhao, Z. Xie, C. Wu, *Macromolecules,* 2003, **36**, 8825-8829.
58 D. W. Hwang, S. J. Son, J. Jang, H. Youn, S. Lee, D. Lee, Y. S. Lee, J. M. Jeong, W. J. Kim, D. S. Lee, *Biomaterials,* 2011, **32**, 4968-4975.
59 J. A. Benitez, J. Segovia, *Curr. Gene. Ther.,* 2003, **3,** 127-145.
60 M. C. Lima, M. T. Cruz, A. L. Cardoso, S. Simoes, L. P. Almeida, *Curr. Drug Targets CNS Neurol. Disord.,* 2005, **4**, 453-465.
61 J. Y. Kim, W. Ⅱ. Choi, Y. H. Kim, G. Tae, *Biomaterials,* 2013, **34**, 1170-1178.
62 Y. C. Kuo, C. Yuan, S.Huang, *J Drug Target,* 2013, **21,** 730-738.
63 L. H. Liu, K. Guo, J. Lu, S. S. Venkatraman, D. Luo, K. ChyeNgd, E. A. Ling, S. Moochhala, Y. Y. Yang, *Biomaterials,* 2008, **29**, 1509-1517.
64 K. L. Kozielski, S. Y. Tzeng, B. A. H. D. Mendoza, J. J. Green, *NANO,* 2014, **8**, 3232-3241.
65 S. J. Son, D. W. Hwang, K. Singha, J. H. Jeong, T. G. Park, D. S. Lee, W. J. Kim, *J. Controlled Release,* 2011, **155**, 18-25.
66 Q. Y. Hu, G. Z. Gu, Z. Y. Liu, M. Y. Jiang, T. Kang, D. Y. Miao, Y. F. Tu, Z. Q. Pang, Q. X. Song, L. Yao, H. M. Xia, H. Z. Chen, X. G. Jiang, X. L. Gao, J. Chen, *Biomaterials,* 2013, **34**, 1135-1145.
67 Y. Liu, Y. Sun, C. Cao, Y. Yang, Y. Q. Wu, D. W. Ju, F. Y. Li, *Biomaterials,* 2014, **35**, 3348-3355.



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