pH-Responsive Vesicles from a Schizophrenic Diblock Copolymer[a]

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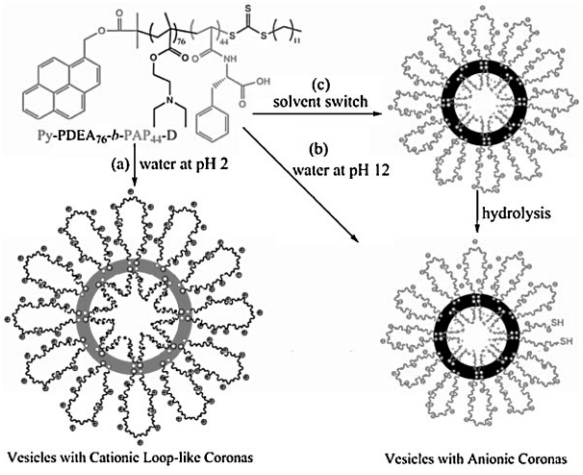
A novel tertiary amine and amino acid-based "schizophrenic" pH-responsive block copolymer, PDEA-*b*-PAP, has been synthesized by RAFT polymerization. By directly dissolving this copolymer in acid or basic aqueous solution block copolymer vesicles with switchable coronas and membranes were prepared. These novel assemblies were characterized using TEM, DLS, zeta potential, and SLS analysis.



Introduction

The self-assembly of amphiphilic block copolymers can afford a range of different nanostructures such as spherical micelles, vesicles, and cylinders.[1] The morphology or morphologies afforded upon self-assembly are influenced by a number of factors.[2,3] A suitable hydrophilic–hydrophobic balance in the amphiphilic polymer is required to form higher order structures and the morphology afforded depends on this ratio which is classically described as the dimensionless "packing parameter," p.[4] Amongst these nanostructures, polymer vesicles are of particular interest due to their hydrophobic membrane and hydrophilic corona, as well as their central hollow cavity, which has been proposed to facilitate their use as delivery vehicles in biomedical applications.[5,6] Compared to lipid-based vesicles, polymer vesicles have greater potential for advanced chemical functionalization and physiological application due to their improved stability and greater potential for chemical modification. Smart copolymer vesicles which respond to an environmental stimuli such as pH,[7] temperature,[8] or redox[9] have attracted much attention and have been reviewed recently.[10]

Of further interest is the synthesis and application of switchable or "schizophrenic" polymers which were first reported in 1998.[11] The term "schizophrenic block copolymer" was firstly coined by Armes and they recently reviewed this new sub-field.[12] The concept of "schizophrenic block copolymers" has now been extended to include copolymer chains that can directly self-assemble in pure aqueous solution in the absence of any organic cosolvent to form two distinct self-assembled structures such as micelles, cylinders, and vesicles. In each case, the individual blocks can be independently tuned to become either hydrophilic or hydrophobic by subtle adjustment of the solution temperature, solution pH, or ionic strength.[12] An initial example was that of a tertiary amine methacrylate-based AB diblock copolymer that was both pH- and salt-responsive and could form either A-core or B-core micelles in aqueous solution. In 2001, a second generation of schizophrenic copolymers were reported based on a poly(propylene oxide)-tertiary amine methacrylate diblock copolymer that exhibited both pH- and thermo-responsive behavior.[13a] Later in 2003, the Armes group reported a

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third generation which involved an ABC triblock copolymer which was capable of forming a ''trinity'' of micelles in aqueous solution at 20 °C simply by adjusting the solution pH. The cores of the three types of micelles were formed by hydrophobic forces, polyion complexation, and hydrogen bonding, respectively.[14] These novel materials form polymer micelles[13,15] or vesicles[16] with a switchable core and corona upon changing the solution pH,[16] temperature,[13] or ionic strength.[15]

Also of interest in the preparation of functional nanostructures are the incorporation and utilization of polypeptides.[17] Polymers with amino acid functionality and chirality (either in their main chain as polypeptides or in their side chain as polyamino acids) can enhance the possibility of the materials to form secondary and higher ordered structures.[18] A number of side chain amino acid-based monomers have been reported but we were especially interested in the *N*-acryloyl-L-phenylalanine (AP) monomer which was previously reported by Endo and coworkers in 2006 ($pK_a = 8.55$).[19] In this work they reported the reversible addition–fragmentation chain transfer (RAFT) polymerization of this monomer to afford PAP homopolymers. We have further explored the synthesis and polymerization of this monomer as we were interested in preparing a polymer which contained an amino acid functionalized (and hence chiral) domain. Of interest was the exploration of the responsive nature of a poly(amino acid)-*b*-poly(tertiary amino methacrylate) novel block copolymer. This new schizophrenic diblock copolymer was found to dissolve in pure water at different pH to form different vesicular nanostructures which we propose may have applications as responsive chiral membranes.

Experimental Part

Materials

All the reagents were purchased from Aldrich and used as received. Dialysis tubing was purchased from Medicell International Ltd with a molecular weight cut-off of 1 kDa. *S*′-1-dodecyl-(*S*′)-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (DDMAT) and benzyl biphenyl-4-carbodithioate (BBC) were synthesized according to the reported procedures.[20,21]

Characterization

THF GPC: the molecular weight distributions of polymers were assessed at 40 °C using a Polymer Laboratories PL-GPC50 Integrated GPC system equipped with a Polymer Laboratories micro-volume double piston pump (≈10 μL per stoke), a PLgel 5 μm Mixed-D column (300 mm × 7.5 mm), and a refractive index detector. The calibration was carried out using ten polystyrene standards with M_p values ranging from 580 to 377 400 Da. The eluent was THF containing 2.0% v/v TEA and 0.05% w/v BHT and the flow rate was 1.0 mL · min^{-1}. The data were processed using Cirrus GPC offline GPC/SEC software. DMF GPC: the molecular weight distributions of the polymers were also assessed at 40 °C using a Polymer Laboratories PL-GPC50 Plus Integrated GPC system equipped with a Polymer Laboratories pump, a Waters styragel HT 4 column, and a WellChrom K-2301 refractive index detector. The calibration was carried out using six poly(methyl methacrylate) standards with M_p values ranging from 1 900 to 790 000 Da. The eluent was DMF containing 5 mM NH_4BF_4 and the flow rate was 0.5 mL · min^{-1}. The data were processed using Cirrus GPC offline GPC/SEC software. ^{1}H NMR spectra were recorded using a Bruker AV 400 (400 MHz) spectrometer at ambient temperature using either $CDCl_3$, CD_3OD, $CD_3OD/CDCl_3$, D_2O/H_2O, D_2O, DCl/D_2O, $NaOD/D_2O$, or DMSO-d_6 as solvents. TEM images were obtained using a JEOL electron microscope operating at 200 kV equipped with a LaB6 gun and a Gatan digital camera. Freshly prepared aqueous PTA solution (1%, either at pH 1.8 or at 7.0 adjusted by NaOH) or 1.0% uranyl acetate solution were used to stain the nanostructures. The averaged size of vesicles was calculated based on examining at least 20 particles from at least six different TEM images. Cryo-TEM was performed on a Jeol 2010F TEM (200 KV FEG) with 4K Gatan Ultrascan camera and cryo capability. UV studies were performed using a Perkin Elmer Lambda 35 UV/Vis spectrometer. FTIR studies were conducted using Perkin Elmer Spectrum 100 FTIR spectrometer. Zeta potential and DLS studies were conducted at 25 °C using a Zetasizer Nano series instrument (Malvern Instruments). DLS studies of aqueous vesicles were conducted over a range of solution pHs at a fixed scattering angle of 173°. The data were processed by Cumulants analysis of the experimental correlation function and vesicle diameters were calculated from the computed diffusion coefficients using the Stokes–Einstein equation. Each reported measurement was the average of three runs. Static light scattering (SLS) studies were performed at 25 °C using a Malvern Autosizer 4800 Instrument. To produce the Zimm plot 20 angles between 30 and 150° and five different concentrations of vesicle solution were scanned and included in the calculation.

Synthesis of CTA and Polymers

Synthesis of Pyrene-DDMAT CTA, **1**

A flask with a magnetic stirrer bar and a rubber septum was charged with 1-pyrenemethanol (0.500 g; 2.11 mmol; 1.00 equiv.), DDMAT (0.807 g; 2.22 mmol; 1.05 equiv.), *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (EDCl · HCl; 0.457 g; 2.32 mmol; 1.1 equiv.), 4-dimethylaminopyridine (DMAP; 0.026 g; 0.20 mmol; 0.1 equiv.), and dichloromethane (10 mL). The reaction was conducted at room temperature for 48 h and purified by silica column using dichloromethane/hexane (1:1) as the solvent, 0.60 g of yellow product was obtained. Yield: 49%. ^{1}H NMR in $CDCl_3$ (400 MHz): δ(ppm) = 7.90–8.25 (m, Ar−H, 9H); 5.76 (*s*, Ar−CH_2−O−, 2H); 2.84–2.89 (*t*, −S−CH_2−CH$_2$−, 2H); 1.61 (*s*, −(OOC−C(CH_3)$_2$−S−, 6H); 1.00–1.50 (*m*, −S−CH$_2$−CH_2−CH$_3$, 20H); 0.78–0.83 (*t*, −CH$_2$−CH_3, 3H). ^{13}C NMR in $CDCl_3$ (500 MHz): δ(ppm) = 221.1 [S=*C*(S)S]; 173.0 [O=*C*(O)C]; 131.7, 131.1, 130.7, 129.7, 128.5, 128.0, 127.9, 127.7, 127.3, 126.0, 125.4, 125.3, 124.8, 124.5, 124.5, 123.2 (Pyrene); 66.4 (Py−CH_2−O); 56.0 (−OOC−(S)*C*(CH$_3$)$_2$); 36.8 (SCH$_2$CH$_2$); 31.9, 29.6,

29.5, 29.4, 29.3, 29.0, 28.8, 27.6, 22.67 ($H_2(S)C(CH_2)_{10}CH_3$); 25.4 ($-OOC-(S)C(CH_3)_2$); 14.1 (CH_3CH_2-).

The FTIR spectrum confirmed the characteristic bands: key signals (1) CH_3: 2 953 cm^{-1} (asymmetric stretch); 1 736 cm^{-1} (symmetric bend, umbrella); 1 463 cm^{-1} (asymmetric bend). (2) CH_2: 2 917 cm^{-1} (asymmetric stretch); 2 850 cm^{-1} (symmetric stretch); 1 454 cm^{-1} (scissoring bending). (3) Pyrene: 3 046 cm^{-1} (aryl CH stretch); 1 604, 1 596, and 1 588 cm^{-1} (symmetric ring stretch); 1 463 cm^{-1} (sideways ring stretch). (4) 1 736 cm^{-1} (C=O stretch). (5) Thiocarbonyl (C=S): 1 070–1 145 cm^{-1}.

Synthesis of *N*-Acryloyl-L-phenylalanine (AP) Monomer

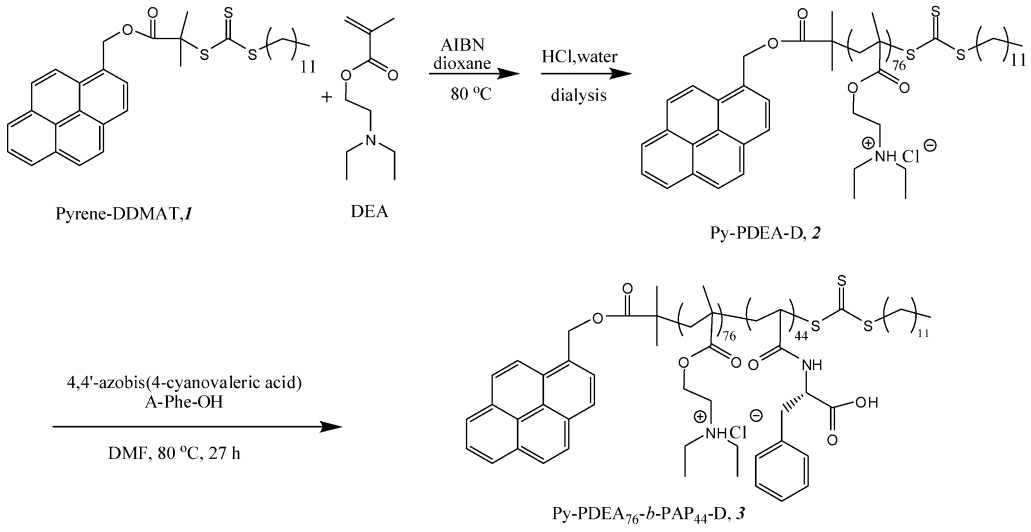
A literature method was modified to achieve higher conversion and product yield.[19] In a flask charged with sodium hydroxide (13.88 g; 0.340 mol) water (85.0 g) was added. After complete dissolution the solution was cooled using ice water. L-phenylalanine (28.66 g; 0.170 mol) was then added at room temperature and stirred for 30 min whilst cooled in an ice water bath. Into the lightly yellowish solution, acryloyl chloride (14.39 mL; 0.170 mol) was then added dropwise by syringe over 30 min. The reaction solution was initially homogeneous and after 80 min a precipitate was formed. Additional water (110 mL) was added again to form homogeneous yellowish solution. After 70 min, the solution was acidified to pH 2 by concentrated HCl solution and stirred and allowed to react for 1 h. Then the solution was filtered and the white precipitate was washed with 400 mL of acidic water (pH 1–2). The white precipitate was dried to afford a light yellow product (33.12 g). Yield: 87%. ^{1}H NMR in DMSO-d_6(400 MHz): δ(ppm) = 12.72 (*s*, $-COOH$, 1H); 8.42 and 8.43 (*b*, $-NH-$, 1H); 7.18–7.30 (*m*, Ar–H, 5H); 6.24–6.31 (*q*, CH_2=CH–, 2H); 6.02–6.08 (*q*, CH_2=CH–, 1H); 4.49–4.55 (*m*, $-HN-CH(CH_2-)COOH$, 1H); 2.87–3.13 (m, $Ar-CH_2-(HN)CH(CH_2)$-)COOH, 2H). ^{13}C NMR in $CDCl_3/CD_3OD$ (400 MHz): δ(ppm) = 173.4 ($-COOH$); 166.1 ($HN(H_2C=CH)C=O$); 136.1, 129.2, 128.3, 126.8 (Ar); 130.1 ($H_2C=CH-C=O$); 127.0 (H_2 C=CH–C=O); 53.4 ($HN-CH(CH_2)(COOH)$); 37.3 ($Ar-CH_2-C$).

The FTIR spectrum confirmed the characteristic bands: key signals (1) CH_2=CH: 3 080 cm^{-1} (weak, asymmetric stretch); 1 832 cm^{--1} (weak, $=CH_2$ out-of-plane bend overtone, twice compared with 914 cm^{-1}); 1 650 cm^{-1} (intense, C=C stretch); 914 cm^{-1} (weak, $=CH_2$ out-of-plane bend). (2) 1 708 cm^{-1} (C=O stretch). (3) 3 342 cm^{-1} (NH stretch). (4) COOH: 2 581 and 2 770 cm^{-1} (OH stretch of COOH dimer); 1 411 cm^{-1} (OH bend and asymmetric OH stretch); 1 250 cm^{-1} (combination of OH asymmetric stretch and OH bend); (5) CH_2: 2 921 cm^{-1} (asymmetric stretch); 2 855 cm^{-1} (symmetric stretch); 1 455 cm^{-1} (scissoring bend). (6) Aryl: 3 030 cm^{-1} (aryl CH stretch); 1 596 cm^{-1} (symmetric ring stretch); 1 497 cm^{-1} (a different ring stretch); 1 439 cm^{-1} (sideways ring stretch); 722 cm^{-1} (symmetric out-of-plane bending); 697 cm^{-1} (out-of-plane ring bending).

Reversible Addition–Fragmentation Chain Transfer (RAFT) Synthesis of PDEA Homopolymers

Polymer **2** (Py-PDEA$_{76}$-D) was synthesized to afford the macro-CTA for polymer **3**. The synthetic route is shown in Scheme 1. In a typical RAFT protocol, a flask with a magnetic stirrer bar and a rubber septum was charged with AIBN radical initiator (3.0 mg; 3.3 × 10^{-2} mmol), pyrene-DDMAT CTA (94.0 mg; 0.162 mmol), 2-(diethylamino)ethyl methacrylate (DEA) monomer (3.04 g; 16.2 mmol), and dioxane (8.12 mL). This solution was deoxygenated using a N_2 purge for 30 min before heating at 80 °C under a nitrogen atmosphere. The [DEA]/[pyrere-DDMAT]/[AIBN] relative molar ratios were 100:1:0.2. After 4.5 h the monomer conversion was calculated to be 76% by ^{1}H NMR spectroscopy. The final polymerization solution was diluted with acetone and 0.01 M HCl aqueous solution and then purified by dialysis against water at pH 2–4. A fine white powder was obtained after freeze-drying. ^{1}H NMR and FTIR spectra are shown in the SI [$\overline{M}_n$ (NMR) = 14 700 Da]. THF GPC: $\overline{M}_n$ = 16 500 Da; $\overline{M}_w/\overline{M}_n$ = 1.7 and DMF GPC: $\overline{M}_n$ = 29 400 Da; $\overline{M}_w/\overline{M}_n$ = 1.55.

A second PDEA homopolymer was synthesized, using DDMAT as a CTA, to afford a macro-CTA, C-PDEA$_{76}$-D which was subsequently chain extended to afford polymer **4**. $\overline{M}_n$ (NMR) = 14 400 Da; THF GPC: $\overline{M}_n$ = 21 200 Da; $\overline{M}_w/\overline{M}_n$ = 1.68 and DMF GPC: $\overline{M}_n$ = 49 600 Da; $\overline{M}_w/\overline{M}_n$ = 1.34.



Scheme 1. Synthesis of Py-PDEA-D copolymer, **2** and Py-PDEA-*b*-PAP-D diblock copolymer, **3**.

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Reversible Addition–Fragmentation Chain Transfer (RAFT) Synthesis of PDEA-*b*-PAP Diblock Copolymers

The synthetic route is shown in Scheme 1. In a typical RAFT protocol, a flask with a magnetic stirrer bar and a rubber septum was charged with 4,4′-azobis(4-cyanovaleric acid) radical initiator (2 mg; 7.0×10^{-3} mmol), protonated Py-PDEA$_{76}$-D macro-CTA (244 mg; 1.4×10^{-2} mmol), AP monomer (316 mg; 1.4 mmol), and DMF (8 mL). This solution was deoxygenated using a N_2 sparge for 30 min before heating at 80 °C under a nitrogen atmosphere. The [AP]/[Py-PDEA$_{76}$-D]/[4,4′-azobis(4-cyanovaleric acid)] relative molar ratios were 100:1:0.5. After 27 h reaction was purified by precipitation in ethyl acetate to form a white precipitate. THF GPC: $\overline{M}_n = 19\,700$ Da; $\overline{M}_w/\overline{M}_n = 1.27$ and DMF GPC: $\overline{M}_n = 33\,700$ Da; $\overline{M}_w/\overline{M}_n = 1.18$. By ^{1}H NMR, $\overline{M}_n = 27\,100$ Da. ^{1}H NMR and FTIR spectra of **3** are shown in SI, respectively.

Polymer **4** (C-PDEA$_{76}$-PAP$_{36}$-D) was synthesized according to the same procedure but with a different macro-CTA (C-PDEA$_{76}$-D) and a lower feed ratio of AP monomer to PDEA macro-CTA. The [AP]/[C-PDEA$_{76}$-D]/[AIBN] relative molar ratios were 50:1:0.5. After 24 h, the conversion was 71% as calculated by ^{1}H NMR spectroscopy. The polymer was purified by dialysis against water. $\overline{M}_n$ (NMR) = 22 200 Da. THF GPC: $\overline{M}_n = 21\,500$ Da; $\overline{M}_w/\overline{M}_n = 1.38$.

Preparation and Characterization of Vesicles

Copolymer **3** (100 mg, at varying copolymer concentrations) was dissolved into 100 g pure water at pH 2 at 80 °C for several minutes, then stirred at room temperature to form vesicles directly. At pH 12, the solution needed to be stirred for several hours to afford a clear blue nanostructure solution. The assemblies were characterized by ^{1}H NMR spectroscopy. After four months, the initial nanostructures still exist at pH 2 but do not exist at pH 12, as a result of different stabilities of trithiocarbonate groups in the vesicle membrane at low (stable) and high pH (unstable). The resultant nanostructures were characterized by DLS at different pH values and concentrations. The correlation functions fit well with the Cumulants analysis and the intensity-averaged vesicle size distributions are reasonably consistent with number and volume averaged size distributions. The resultant vesicles were stained by a range of different methods and viewed by TEM and cryo TEM.

Ellman's Assay

The presence of thiols in the polymers was determined using Ellman's reagent which was prepared by dissolving 5,5′-dithiobis(2-nitrobenzoic acid) in water and the pH was adjusted to 8.0 by 0.1 M NaOH to aid dissolution. The final concentration was 0.4 mg · mL^{-1} (1.0 mmol · mL^{-1}). To a 1.0 mL of vesicle solution at pH 12 (1.0 mg · mL^{-1}), 1 mL of Ellman's reagent was added followed by stirring for 15 min. The solution turned yellow. UV–Vis analysis was performed with absorbance monitored at 412 nm (with water and Ellman's reagent as the blank), a significant absorbance at this wavelength was observed. This confirmed the hydrolysis of the trithiocarbonate end group and production of a thiol. The same experiment was repeated for the vesicle solution at low pH, however the solution had a blue color and little absorbance was observed at ca. 412 nm compared to the control reaction.

Results and Discussion

Synthesis of CTA 1 and *N*-Acryloyl-L-phenylalanine (AP) Monomer

To synthesize the chiral diblock copolymer used in this work, a new fluorescent CTA was synthesized by the esterificaton of DDMAT[20] with pyrenemethanol. This afforded CTA **1** which was isolated in a 49% yield and fully characterized by ^{1}H and ^{13}C NMR spectroscopies and UV studies. An amino acid-based monomer, AP which was previously reported by Endo and coworkers in 2006 was utilized in this study.[19] Unfortunately in our hands this protocol afforded a very low yield of product, however upon modifying the experimental conditions by increasing the NaOH concentration the yield was increased to 87%.

Synthesis of Chiral Diblock Copolymers by Reversible Addition–Fragmentation Chain Transfer (RAFT)

To synthesize a PAP-based copolymer, first the homopolymerization of AP monomer by RAFT was investigated, as shown in Table 1 with DDMAT and BBC as CTAs. According

Table 1. Homopolymerization of AP by RAFT.

Run	CTA	Solvent	$R_{CTA/AIBN}$	Temp	M	Time	Con.	$\overline{M}_{n,th}$ [a)]	$\overline{M}_n$ [b)]	$\overline{M}_w/\overline{M}_n$
				°C	mol · L^{-1}	h	%	Da	Da	
1	DDMAT	Methanol	2:1	45	1.14	42	10	–	–	–
2	DDMAT	DMF	2:1	60	1.14	53	53	9 600	19 600	1.28
3	DDMAT	DMF	2:1	65	1.80	3	68	7 800	7 500	1.21
4	DDMAT	DMF	2:1	70	1.14	3	96	8 800	18 500	1.39
5	BBC	Dioxane	2:1	60	1.14	19	<5	–	–	–
6	BBC	Dioxane	2:1	90	0.61	3.5	96	25 600	16 500	1.21
7	BBC	Dioxane	5:1	90	0.61	24	20	–	–	–

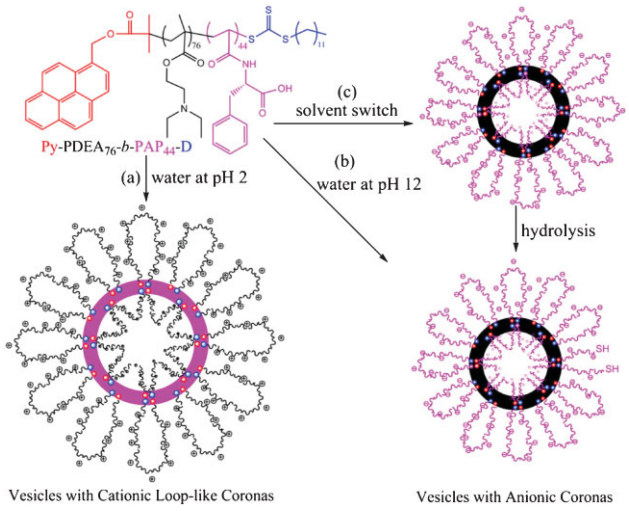
[a)]By conversion ^{1}H NMR spectroscopy; [b)]By THF GPC analysis.

to the best conditions reported in ref.,[19] the RAFT polymerization of AP in methanol at 45 °C using AIBN as the initiator was explored, using DDMAT rather than benzyl 1-pyrrolecarbodithioate as a CTA, but unfortunately, only 10% conversion was reached after 42 h and no further polymerization was found after prolonged reaction times (run 1). Following this we changed the solvent from methanol to DMF and increased the temperature from 45 to 60 °C. This afforded a controlled polymerization (run 2), however an induction period of around 6 h was observed. At 53% conversion the resultant polymer had a relatively narrow molecular weight distribution of 1.28. Upon slightly increasing the temperature to 65 °C a significant increase in the polymerization rate was observed with no detrimental effect on the polydispersity of the resultant polymer (run 3). Further increasing in the temperature to 70 °C further increased the polymerization rate with 96% conversion observed after 3 h, however the polydispersity had increased to 1.39 (run 4). We also explored the RAFT polymerization of AP monomer in dioxane using a dithioester, BBC, as the CTA at a CTA/AIBN ratio of 2:1. When the polymerization was run at 60 °C, the conversion was less than 5% after 19 h reaction (run 5) and no further polymerization was observed even after prolonged times. We increased the temperature to 90 °C and the conversions dramatically increased to 96% after 3.5 h and the polymers were well-defined ($\overline{M}_w/\overline{M}_n = 1.21$, run 6). Attempts to further improve the polymerization control at 90 °C by increasing the CTA/AIBN ratio (5:1), resulted in low conversions after 24 h.

As shown in Scheme 1, the chiral diblock copolymer, **3**, was synthesized by a two-step RAFT polymerization: first DEA was polymerized for 4.5 h using **1** and AIBN as the radical initiator in dioxane at 80 °C to form polyDEA, **2**, [$\overline{M}_n$ (NMR) = 14 700 Da; $\overline{M}_n$ (GPC, THF) = 16 500 Da, $\overline{M}_w/\overline{M}_n$ = 1.70; $\overline{M}_n$ (GPC, DMF) = 29 400 Da, $\overline{M}_w/\overline{M}_n$ = 1.55] and then an amino acid-based monomer, AP[19] was polymerized by RAFT using **2** (which had been protonated by HCl) as the macro-CTA and 4,4′-azobis(4-cyanovaleric acid) as the initiator in DMF at 80 °C for 27 h to form **3**, diblock copolymer [$\overline{M}_n$ (NMR) = 27 100 Da; $\overline{M}_n$ (GPC, THF) = 19 700 Da, $\overline{M}_w/\overline{M}_n$ = 1.27; $\overline{M}_n$ (GPC, DMF) = 33 700 Da, $\overline{M}_w/\overline{M}_n$ = 1.18]. The end group fidelity was determined by UV–Vis analysis and calculated to be 95 and 88% for polymers **2** and **3**, respectively.[22]

Self-Assembly of Py-PDEA-*b*-PAP-D Copolymers

The structure and proposed self-assembly of the chiral diblock copolymer, Py-PDEA$_{76}$-*b*-PAP$_{44}$-D (**3**) with a pyrene fluorescent terminal group is shown in Scheme 2. The direct dissolution of **3** in water at low pH (ca. pH 2) for several minutes at 80 °C forms large well-defined aggregates spontaneously with the hydrophobic PAP block, pyrene



Scheme 2. Self-assembly of **3** into vesicles with cationic coronas at pH 2 (route a) and anionic coronas at pH 12 by direct dissolution in water (route b) and solvent switch method (route c). Different colors represent different components: black for the PDEA which is hydrophilic and positively charged at low pH whereas above pH 8 it becomes hydrophobic; pink for PAP which is hydrophilic and negatively charged at higher pH whereas at lower pH it becomes hydrophobic; red for the hydrophobic pyrene (Py) at the end of PDEA and blue for the hydrophobic trithiocarbonate dodecyl group (D) at the end of the PAP. The flower vesicles at high pH undergo a rearrangement upon end group hydrolysis.

and the dodecyl terminal groups forming the membrane and with the positively charged PDEA forming the corona (zeta potential = +55.2 mV). This structure we propose has a flower-like vesicle morphology as at pH 2 the parent polymer has a BAB structure (with A representing the hydrophilic PDEA segment) which results in the looping of the hydrophilic segment since the hydrophobic pyrene end groups are forced to aggregate into the hydrophobic membrane.[23,24]

This self-assembly behavior was initially proposed by comparing the ^{1}H NMR spectra of the diblock in different solvents as shown in Figure 1. All the signals assigned to the copolymer and the end groups are clearly visible in a good solvent for both blocks (CD_3OD). However, when the copolymer is dissolved in D_2O/H_2O at pH 2, all the signals assigned to PAP block, pyrene, and dodecyl terminal groups are significantly attenuated. This is a result of the aggregation of the hydrophobic components in the vesicle membrane and as the signals from PDEA are still clearly visible this confirms, alongside the zeta potential data, the solvated PDEA corona in the vesicle structure.

Conversely, the direct dissolution of the block copolymer into water at higher pH (ca. pH 12) after several hours with stirring (at both room temperature and 80 °C), affords vesicles with the PDEA and pyrene forming the membrane while the coronas were composed of anionic PAP (zeta potential = −54.7 mV). This was confirmed by analysis of

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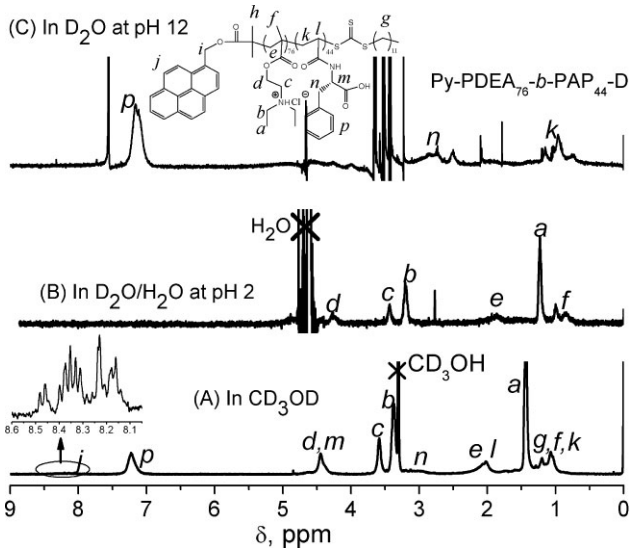

Figure 1. ^{1}H NMR spectra of Py-PDEA$_{76}$-*b*-PAP$_{44}$-D, **3**: (A) in CD_3OD, a good solvent for both blocks; (B) in D_2O/H_2O (1:9) at pH 2, the peaks from PDEA are visible whereas peaks assigned to PAP (such as *p* and *n*), and peaks assigned to pyrene (*j*) and DDMAT (*g*) are attenuated; (C) at pH 12, peaks from PAP (such as *p* and *n*) are visible whereas peaks from PDEA (*a*, *b*, *d*, *e*, and *f*), pyrene (*j*) are attenuated.

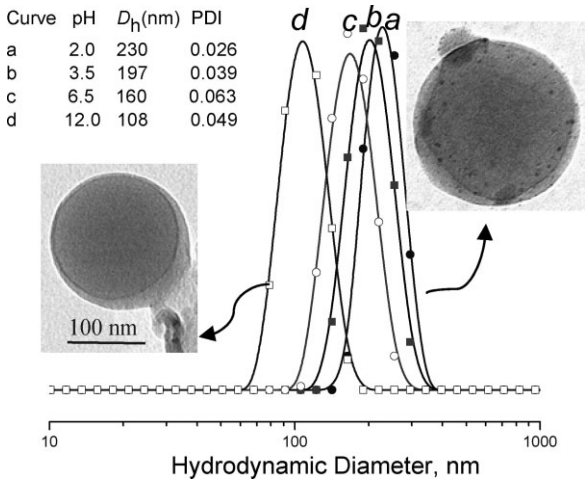

Figure 2. Z-averaged vesicle size distribution of Py-PDEA$_{76}$-*b*-PAP$_{44}$-D diblock copolymer vesicles by direct dissolution at different pHs determined by DLS analysis. See SI for correlation functions. The insets are TEM images of vesicles at pH 2 (right) and 12 (left) without staining.

the ^{1}H NMR spectrum of **3** (Figure 1C). It is proposed that at elevated pH the trithiocarbonate end group is prone to hydrolysis[24] (as confirmed by ^{1}H NMR analysis) hence transforming **3** to a BA-type copolymer. The hydrolysis starts as **3** dissolves in basic water and this competes with the fast self-assembly of the polymer chains into vesicles with looped coronas. Hence, initially a broad size distribution of particles (PDI = 0.180, 2 d of stabilization) results. After extended time (ca. 40 d) at high pH, we propose these looped vesicles transform via hydrolysis into conventional vesicles with well-defined sizes (108 nm, PDI = 0.049). Ellman's assay confirmed the presence of thiols in this vesicle solution at high pH. The self-assembly is proposed to be much faster than the copolymer dissolution and this was confirmed by nanostructure formation via a solvent switch method,[25] which immediately affords a transparent bluish solution with a narrower size distribution.

DLS studies of the vesicles at different pH are shown in Figure 2. Overall, the vesicle size decreases with an increase in solution pH. For example, the D_Hs are 230, 197, 160, and 108 nm at pH 2.0, 3.5, 6.5, and 12, respectively (all with very low polydispersities). This is consistent with a higher degree of protonation of the PDEA vesicle coronas in water at lower pH which leads to a stronger repulsive interaction and hence a bigger size. To explore the stability of trithiocarbonate groups in the vesicle membrane, the vesicles prepared at pH 12 or 2 were dried and re-dissolved in CD_3OD. ^{1}H NMR studies confirmed a significant amount of hydrolysis at pH 12 but little hydrolysis at pH 2 after 4 months (see SI for details). Furthermore, polymer **3** was treated with hexylamine and AP monomer to remove the trithiocarbonate end group[22] and then assembled in a similar manner to that reported for the parent polymer. By DLS analysis the observed nanostructures from the end modified polymer show little change in size or distribution (ca. 102 nm, PDI = 0.065) to those from the parent polymer.

Figure 3 shows the transmission electron microscopy (TEM) images of vesicles at pH 2. The number-averaged diameter was measured at ca. 170 nm. The puckering of the vesicles indicates a hollow structure (A), with the gray layer (B) showing the vesicle membrane and the black ring (B) showing the interactions between uranyl acetate and trithiocarbonate. It is noteworthy that although the hydrophobic volume of the end groups are relatively small compared to the overall volume of the diblock copolymer, they do greatly affect the self-assembly behavior. For example, at pH 2, the mass ratio of the hydrophilic PDEA (protonated) to the hydrophobic PAP is ca. 1.8 and in this regime spherical micelles would traditionally be expected to form.[10] However, we propose that flower-like polymer vesicles rather than spherical micelles form as a result of the contribution of the hydrophobic pyrene end group at the termini of the block copolymer. To confirm the effect of the pyrene end group, on the morphology of the resultant nanostructure, polymer **4** [C-PDEA$_{76}$-*b*-PAP$_{37}$-D, $\overline{M}_{n,GPC}$ = 21 500 Da, PDI = 1.38 (DMF); $\overline{M}_{n,NMR}$ = 22 200 Da], which is similar in structure to **3** but does not contain a pyrene end group was prepared (see Experimental Part for details). In **4** the end group is a carboxylic acid group which is hydrophobic at low pH (although less so than the pyrene ester end group). Self-assembly of **4** at pH 2 under identical

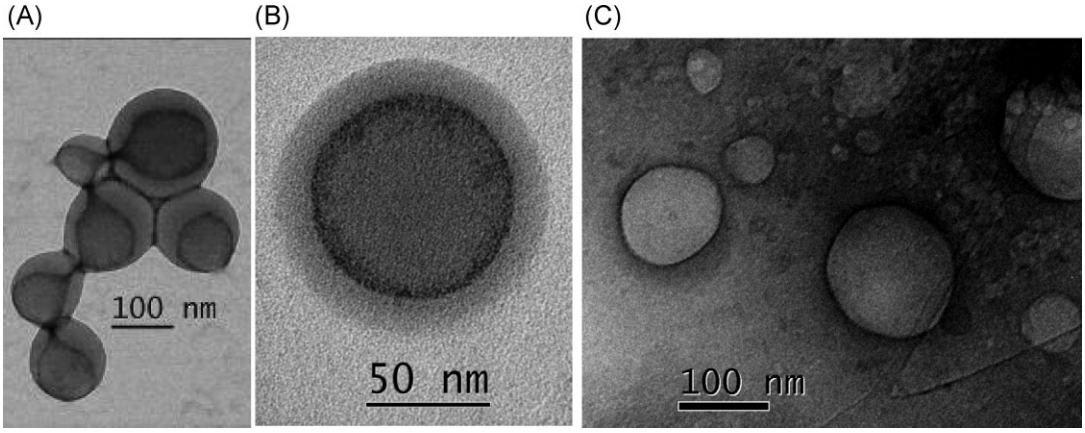

Figure 3. Traditional TEM images of vesicles prepared by self-assembly of Py-PDEA$_{76}$-*b*-PAP$_{44}$-D copolymer (**3**): (A, B) by directly dissolving the polymer in water at pH 2, then staining with uranyl acetate; (C) Cryo-TEM images of Py-PDEA$_{76}$-*b*-PAP$_{44}$-D diblock copolymer vesicles by directly dissolution in water at pH 12.

conditions to that described for **3** afforded much smaller nanostructures (ca. 21 nm) by DLS thus highlighting the significant effect of the pyrene end group on the self-assembly process.

Vesicles with looped anionic coronas at pH 12 prepared by the solvent switch method were supported by TEM analysis. The observed vesicle diameters (ca. 250 nm) were slightly smaller than the size determined by DLS. The vesicles prepared by directly dissolving the copolymer into alkaline solution (at pH 12) were observed to have a much smaller size ($D_H = 146$ and 108 nm before and after filtration, respectively) than those vesicles prepared by a solvent switch method ($D_H = 276$ nm). Cryo-TEM analysis confirmed the vesicular structure (see Figure 3C) and the observed vesicle sizes were ca. 100 nm, which compares well with the values observed by DLS analysis. This significant difference in size between the two vesicles prepared using different methods maybe due to the different structure (flower-like coronas vs. linear coronas), as well as possibly different vesicle-forming procedures.[26]

Static light scattering (SLS) data of vesicles by direct dissolution were analyzed by Zimm plot (Figure 4), where the concentration and the scattering angle were extrapolated to zero values. For vesicles at pH 2, the $\overline{M}_w$ was found to be 5.65×10^8 Da and the R_g 114 nm. For vesicles at pH 12 (without filtration), the $\overline{M}_w$ was 7.6×10^7 Da and the R_g 71 nm. Hence, the R_g/R_h values for both sets of vesicles were calculated to be 0.99 and 0.93 at pH 2 and 12, respectively, indicating a vesicle structure, rather than a solid sphere structure (which would have a predicted ratio of ca. 0.77).[27]

We explored the effect of increasing the solution pH on the resultant change in size of the vesicles by DLS analysis. We observed at pH 7.6–8.3 precipitation of the solution occurred, which prevented facile cycling between the extremes of pH. However, the vesicles could be reformed by further increasing the solution pH outside this range, however a broad distribution of particles were obtained (see SI). In addition, initial DLS experiments indicated that the CMC of these vesicles are low and in the region 0.001 mg · mL^{-1} for the high pH solution and 0.0005 mg · mL^{-1} for the low pH sample.

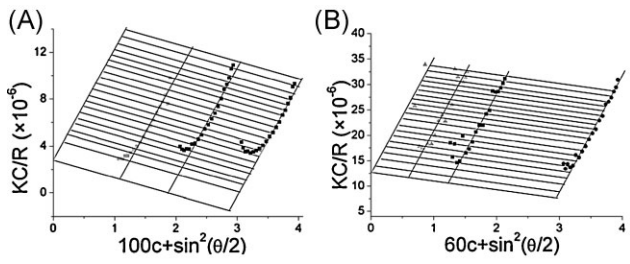

Figure 4. Zimm plot of vesicles by direct dissolution at pH 2 (A) and 12 (B).

Conclusion

In conclusion, polymer vesicles with a switchable corona and membrane were prepared by simple direct dissolution or solvent switching of a schizophrenic diblock copolymer in water at acidic and basic water, as proven by TEM, DLS, SLS, and ^{1}H NMR spectroscopy. The pyrene end group is essential for vesicle formation at low pH as the hydrophobic to hydrophilic ratio does not predict vesicle formation, and only polymer micelles are formed for a similar copolymer without the pyrene group. The vesicle coronas are looped due to two hydrophobic end groups in the copolymer to form flower-like vesicle nanostructures. The looped anionic coronas at higher pH eventually evolved into linear coronas with thiols at the chain end. This functionality may offer many post-modification possibilities for the nanostruc-

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tures such as thiolene or disulfide coupling chemistries. These vesicles may find application as a simple pH-switching probe and hence offer diverse possibilities in advanced material synthesis.

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