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Stimuli Responsive Triblock Copolymers by Chain-growth Polymerization from Telechelic Macroinitiators Prepared by a Step-growth Polymerization

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Krishna Dan and Suhrit Ghosh* [a]

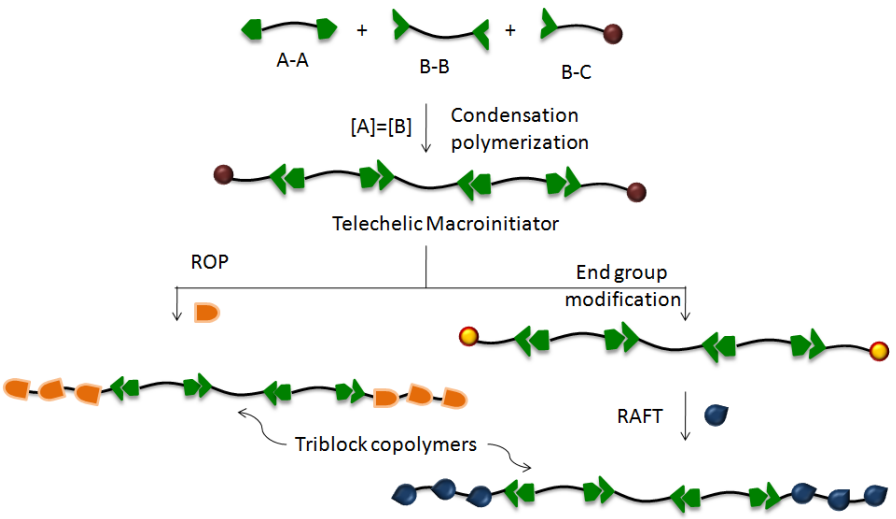
Various chain-growth polymerizations produce macromolecules of desired molecular weight with narrow distribution, allow incorporation of specific terminal groups and pendant functionalities and also enable synthesis of block copolymers. On the other hand the step-growth routes provide ample opportunities for functional diversity in the polymer backbone. Thus a strategy for synthesis of block copolymers involving both these synthetic routes in sequential fashion will be highly useful for making new polymers with structural diversity. This article describes such an approach for accessing stimuli responsive ABA type triblock copolymers. A telechelic polymer (TP) is prepared using a polycondensation between an A-A (A = acrylate) and a B-B (B= thiol) monomer in presence of a monofunctional impurity B-C (C = hydroxyl) by high yielding thiol-acrylate Michael addition reaction. A proper control of the stoichiometry between the three reactants ensures presence of the hydroxyl groups in both ends of the TP. Good corroboration between the theoretical and experimentally determined (by GPC and NMR) molecular weights suggests lack of any undesirable side reactions during the polycondensation. The two terminal –OH units are utilized as initiating sites for ring opening polymerization (ROP) of a cyclic lactide monomer in presence of a tin catalyst to produce an ABA type triblock copolymer. As the repeating unit of the "B" block contains an acid labile *β*-thiopropionate linker, the resulting block copolymer can be degraded by mild acid treatment. The free –OH groups at the both terminals of the TP are further linked to a chain transfer agent by an esterification reaction and the resulting macro CTA is used to polymerize *N*-isopropylacrylamide by RAFT polymerization producing an amphiphilic triblock copolymer that exhibits a lower critical solution temperature (LCST). Detail aggregation studies of the amphiphilic polymer by DLS, SLS, TEM and optical spectroscopy show formation of polymersome in aqueous medium which can encapsulate both hydrophobic and hydrophilic guest molecules.

INTRODUCTION

Self-assembly of block copolymers,[1] particularly the amphiphilic ones, have been a topic of intense research for more than last twenty years or so. Even though such polymers were investigated extensively much before that, in last two decades the research activities in this area have been tremendously boosted by the advancement in several controlled polymerization techniques. This has helped in exploring structural diversity of macromolecules [2] by the ability to incorporate diverse range of functional groups in a precisely defined location of a polymeric scaffold. More recently synthesis of functional polymers have reached another new dimension by the discovery of several new monomers and initiators containing highly reactive functional groups for facile post polymerization functionalization [3] using various high yielding reactions, commonly known as "click" chemistry. In this context, various thiol-mediated reactions have made a significant impact due to their facile reactions with different functional groups including disulfides, isocyanate, alkene, alkyne, maleimide, acrylate, epoxide and others.[4] However, the implications of such new developments in synthetic polymer chemistry have been mostly realized in chain polymerizations

while those made by step-growth routes have been benefited to a much lesser extent.[5] The reason could be that the ability to make macromolecules with pre-defined molecular weight, narrow distribution, and defined chain end make the controlled chain polymerizations more compelling to a synthetic chemist. However, the chain polymerization routes allow a limited freedom in tuning the nature of the polymer backbone due to the availability of only few family of monomers while step-growth routes offer much extensive structural variation in the polymer backbone by differing the chemical nature of the linker between the two functional groups either in a AB or A-A + B-B type condensation. It is also possible to control the molecular weight and incorporate specific functional groups at the chain ends in such polycondensation reactions by employing classical strategies such as carrying out the reaction with a stoichiometric imbalance of the two monomers or introducing a mono-functional impurity.[6] We envisioned such attempts to make new polymers by step-growth routes involving high-yielding "click" reactions would be highly promising to access new materials with desired functional properties.

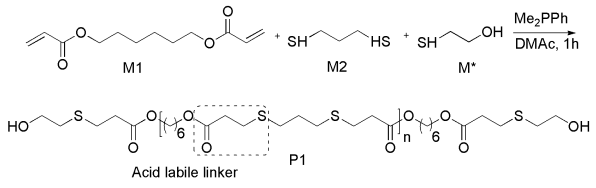
In the recent past we have shown[5c] synthesis of a telechelic polymer with free thiol groups at the both ends by an A-A+ B-B type condensation route using the high yielding Michael addition reaction between a di-thiol and a di-acrylate monomer mixed with a stoichiometric imbalance and in situ capping of the thiol at the chain ends by reacting them with an acrylate terminated polyethyleneoxide (PEO). This produces a ABA type amphiphilic triblock copolymer in a one-pot reaction. The presence of the β-thiopropionate functional groups in the polymer backbone makes this amphiphilic polymer a stimuli-responsive one which selectively degrades at a slow rate under mildly acidic pH.[7] This is particularly interesting in context of selective drug delivery in tumor cells because the pH is acidic compared to the healthy cells.[8] Although several other acid labile functional groups have been tested with similar objective,[9] most of them show a fast degradation in contrast to the β-thiopropionate linker which offers sustained release due to the slow degradation. These two features (easy synthesis and slow degradation selectively in acidic pH) together make the thiol-acrylate condensation an interesting option in context of synthesizing stimuli-responsive macromolecules. However, the synthetic methodology demonstrated in our earlier communication [5c] is limited to the fact that the entire polymer synthesis is dependent on this particular reaction and thus does not allow one to expand the scope of this in a wider domain. To achieve that, herein we have developed a different synthetic strategy (Scheme 1) for assessing telechelic polymers using a mono-functional impurity that contains another functional group (-OH) which remains benign during the course of polycondensation but can be utilized later as an initiating site to further carry out chain-polymerizations.[10] In this way we could synthesize two different types of ABA triblock copolymers by using either ring opening polymerization (ROP) or RAFT route to grow the peripheral polymer chains from the β-thiopropionate containing telechelic macroinitiator (Scheme 1). In this article we elaborate on the synthetic methodology and also stimuli-responsive polymersome formation by one of the block copolymers in aqueous medium.



Scheme 1: Synthetic strategy to access triblock copolymers using step-growth followed by chain polymerizations

RESULTS AND DISCUSSION

Synthesis of the telechelic polymer:



Scheme 2: Synthesis of **P1**

Synthetic scheme for the telechelic polymer **P1** is depicted in Scheme 2. Monomers A-A (M1), B-B (M2) and the mono-functional impurity [11] B-C (M*) were mixed together in 1.13: 1.0: 0.27 ratio and the polymerization was carried out for 1 h in presence of a dimethyl-phenyl-phosphine catalyst at rt. The resulting viscous solution was re-precipitated from excess Methanol to isolate the polymer **P1** as a colorless sticky solid. The ratio of M_1, M_2 and M^* were chosen in such a way so that $2N_{M1} = 2N_{M2} + N_{M^*}$. In this case, as the total concentration of the thiol group is equivalent to that of the acrylate, it is expected that the M^* and consequently free –OH groups will be present at both ends of the polymer. Based on the feed ratio of M1, M2 and M^*, the degree of polymerization can be estimated by equation 1.[5] Degree of polymerization

$$DP = \frac{1+r}{1+r-2rp}, \text{ where } r = \frac{N_{M1}}{N_{M2}+N_{M^*}} \qquad (1)$$

Where p = conversion; N_{M1}, N_{M2} and N_{M^*} are the mole fractions of M_1, M_2 and M^*, respectively.

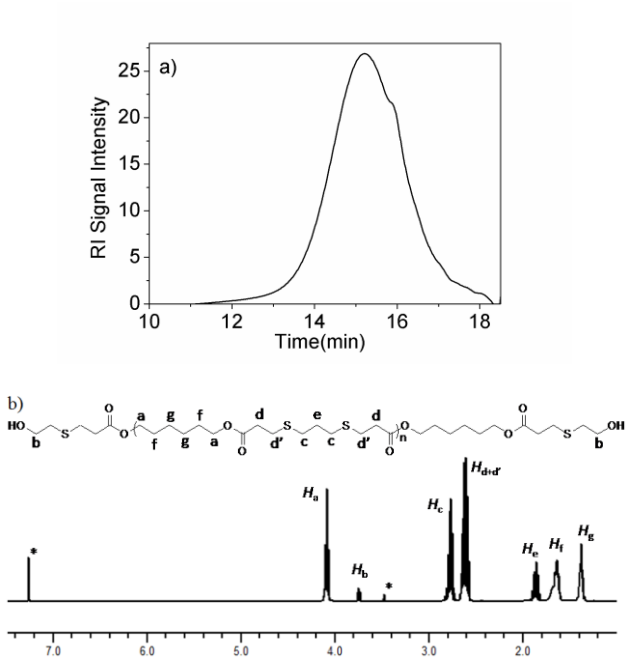


Fig. 1: (a) GPC traces of **P1** in THF; (b) ^{1}H NMR spectrum of **P1** in $CHCl_3$. * indicates residual solvent ($CHCl_3$ and Methanol) peaks.

By feeding these values and the conversion (calculated from the polymerization yield), the theoretically calculated molecular weight of **P1** = 3700 gmol^{-1} (Table 1) which reasonably matches with the observed value (M_n = 5000 gmol^{-1}, PDI = 1.2) obtained from GPC analysis (Fig. 1a).

P1 was further characterized by its ^{1}H NMR spectrum which is presented in Fig. 1b. Sharp peaks appear for most of the protons (as the molecular weight is not very high) and all of them can be assigned without much ambiguity as indicated in Fig. 1b. Notably the peak for the H_b corresponding to the methylene protons adjacent to the terminal –OH group is distinctly visible and thus from the ratio of the intensities of H_b and H_a (coming from the repeat unit) the molecular weight of the polymer can be estimated (Table 1) which corroborates well with that observed from GPC. Thus it reflects most of the polymer chains are terminated by M^* at both ends, failing to which the NMR estimated molecular weight is unlikely to match with the theoretically predicted value or the GPC numbers.

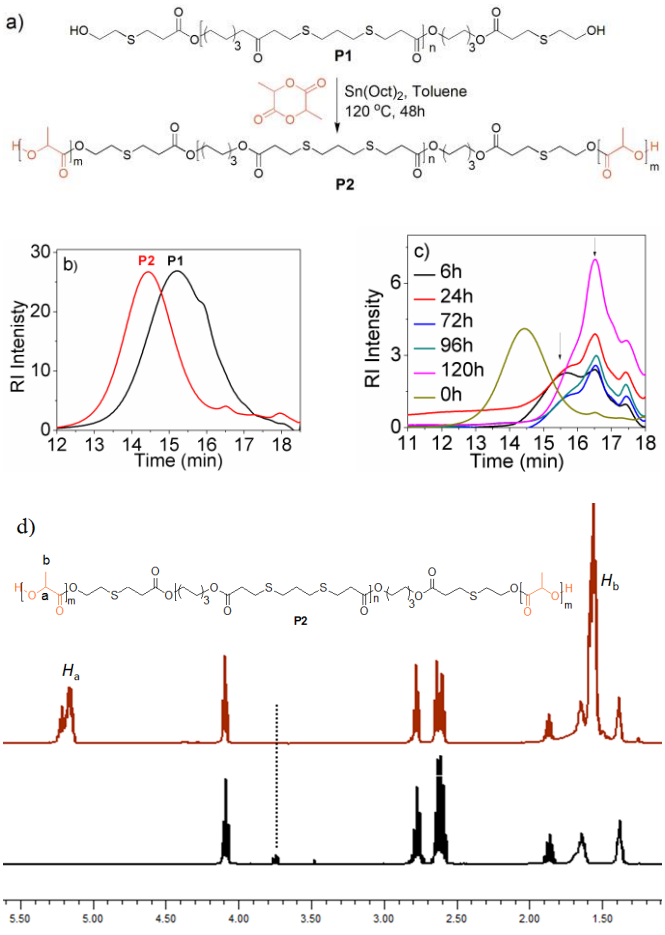


Fig. 2: (a) Synthesis of **P2**. (b) GPC traces of **P1** and **P2.** (c) GPC traces of acid digested products of **P2** in THF at various time intervals. (d) Selected region of the ^{1}H NMR spectra of **P1** (black) and **P2** (brown) in $CDCl_3$.

Table 1: Molecular weight of various polymers

Polymer	% Conv [a]	[b,c] $M_{n\,(theo)}$	$M_{n\,GPC}$	PDI	$M_{n\,NMR}$ [d]
P1	97	3700	5000	1.2	4300
P2	53	12000	13000	1.41	9600
P3	60	7700	11000	1.25	7800

[a] Estimated by gravimetric analysis; [b]$M_{n(theo)}$ of **P1** = (Degree of polymerization using equation 1 x repeat unit molecular weight x conversion); [c]$M_{n(theo)}$ of **P2** or **P3** = Molecular weight of **P1** + (Mole ratio of monomer/ macroinitiator) x repeat unit molecular weight x conversion; [d] Calculated from the ratio of integration of the peaks for chain end protons and repeat unit protons. For block copolymers, the NMR estimated molecular weight of **P1** is counted and accordingly the ratio of peaks from any of the repeat unit proton of the macroinitiator and that of the new block determines the molecular weight of the block copolymer.

Synthesis of a triblock copolymer from P1: The terminal -OH functionalities of the telechelic **P1** was utilized for initiating ring opening polymerization (ROP) of a cyclic lactide monomer (Fig. 2a).

The polymerization was carried out in presence of tin (II) 2-ethyl hexanoate [Sn $(Oct)_2$] catalyst in dry toluene at 120 °C for 48 h using the ratio of monomer/ **P1**/ $Sn(Oct)_2$ = 100: 1: 0.07. The resulting polymer was purified by precipitation from excess Methanol to get the desired triblock copolymer (**P2**) as a white solid in 53 % yield. GPC traces of **P2** and **P1** are compared in **Fig. 2b** which reveals the number-average molecular weight (M_n) of **P2** = 13000 $gmol^{-1}$ (PDI = 1.41). Assuming the new polymer represents the structure of **P2**, its theoretically estimated molecular weight (Table 1) appears to be very close to that obtained from the GPC indicating formation of the desired material. To further confirm the structure, its ^{1}H NMR spectrum is compared with that of **P1** (**Fig. 2d**). It can be seen that all the peaks for **P1** remain unaltered in the spectrum of **P2** suggesting the telechelic polymer **P1** survives during ROP. In addition, new sets of peaks appear (**Fig. 2d**) at δ = 5.17 ppm and 1.56 ppm for the H_a and H_b, respectively, of the polylactide backbone. Notably the small peak at δ = 3.75 ppm for the methylene protons of **P1** (**Fig. 1b**) at the chain ends is absent in the **P2** spectrum

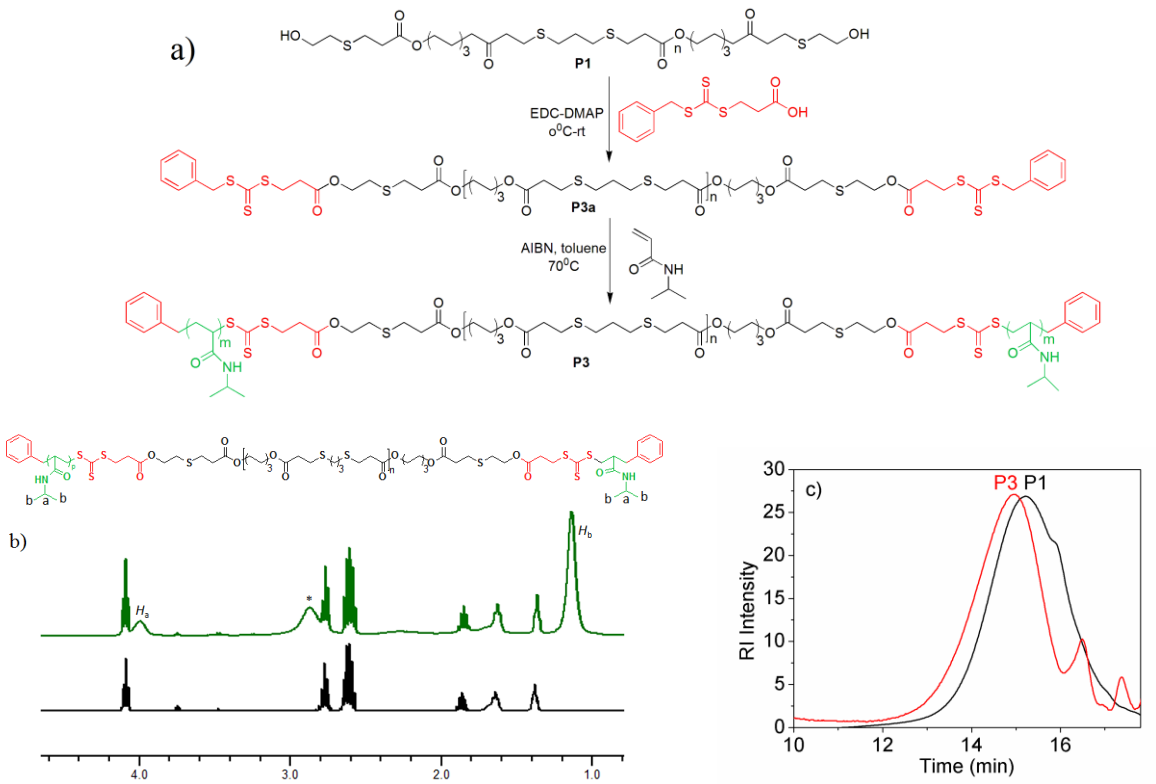


Fig. 3: (a) Synthesis of **P3** from **P1**. (b) Selected region of the ^{1}H NMR spectra of **P1** and **P3** in $CDCl_3$. (c) GPC chromatogram of **P1** and **P3**.

confirming complete conversion of the telechelic macroinitiator **P1** to the triblock copolymer **P2**. Formation of the block copolymer was also confirmed by the FT-IR spectrum of **P2** which shows a broad peak (1750 cm^{-1}) that appears to be the sum spectra of the carbonyl peaks of the two constituent polymers (Fig. S1). Interestingly **P2** represents a unique ABA triblock copolymer in which the middle block is an acid degradable one [12] owing to the presence of the *β*-thiopropionate ester linkage. Such triblock copolymers may show phase separated structures in solid state. Therefore they can be utilized to generate patterned surfaces[13] by selective removal of the one of the blocks by exploiting their acid-induced degradation. Thus we tested degradation of **P2** using 2% TFA in THF-Methanol (1:1) solvent mixture and compared the GPC traces of the products after different time intervals (Fig. 2c). It reveals degradation of **P2** even after 6 h and generation of low molecular weight polymeric/ oligomeric species. This does not necessarily mean incomplete degradation because even at complete disintegration of the acid labile "B" block, the polylactide block is expected to remain intact which is probably contributing to the appearance of the peak at ~ 16.5 min in the GPC chromatogram (Fig. 2c). The less intense hump at ~ 15.6 min that appears after 6 h and eventually disappears at a later stage possibly corresponds to the diblock copolymer produced by detachment of one of the beta-thiopropionate ester linker at the junction.

Synthesis and aggregation studies of a stimuli responsive amphiphilic triblock copolymer from P1: To further extend the utility of **P1** as a more versatile telechelic macroinitiator [14] the two terminal –OH groups were treated with a carboxylic acid functionalized chain transfer agent (CTA-COOH) by a standard coupling reaction in presence of EDC and DMAP (Fig. 3a) to produce the macro CTA (**P3**a, Fig. 3a). ^{1}H NMR spectrum of **P3**a (Fig. S2) shows absence of the peak for the methylene protons adjacent to the –OH suggesting complete conversion. Further new peaks are visible in the aromatic region as well as in the aliphatic zone corresponding to the various protons of the benzyl tri-thiocarbonate units present at the chain ends. **P3**a was used to polymerize N-isopropylacrylamide (NIPAM) monomer in presence of AIBN initiator under the RAFT polymerization technique to produce the amphiphilic triblock copolymer **P3** wherein the β-thiopropionate containing hydrophobic block is connected to the hydrophilic thermoresponsive poly-(NIPAM) blocks in both arms. From GPC (Fig. 3c), the number average molecular weight of **P3** was estimated to be 11,000 $gmol^{-1}$(PDI = 1.21) which corroborates well with the estimated value (Table 1). Furthermore in ^{1}H NMR of **P3** (Fig. 3c), the presence of the peaks at δ = 3.99 and 1.16 ppm confirms attachments of the NIPAM blocks. Based on the ratio of the integration of Ha and Hc (Fig. 3b), the molecular weight of **P3** can be estimated to be 7,800 $gmol^{-1}$ which can be considered as a good agreement with that calculated theoretically as well as estimated by GPC. FT-IR of **P3** also indicates the presence of a NIPAM block which contains the amide groups (Fig. S3). Amphiphilic character of the **P3** prompted us to examine its aggregation properties in water. A solution of **P3** in water (1.0 wt %) shows a bluish color (inset-Fig. 7) suggesting presence of nano-aggregates which can be confirmed by dynamic light scattering measurements showing a single peak (Fig. 4a) corresponding to average hydrodynamic diameter D_h (2 x R_h) = 200 nm. To gain better insight of the nature of these aggregates we also carried out static light scattering experiments.

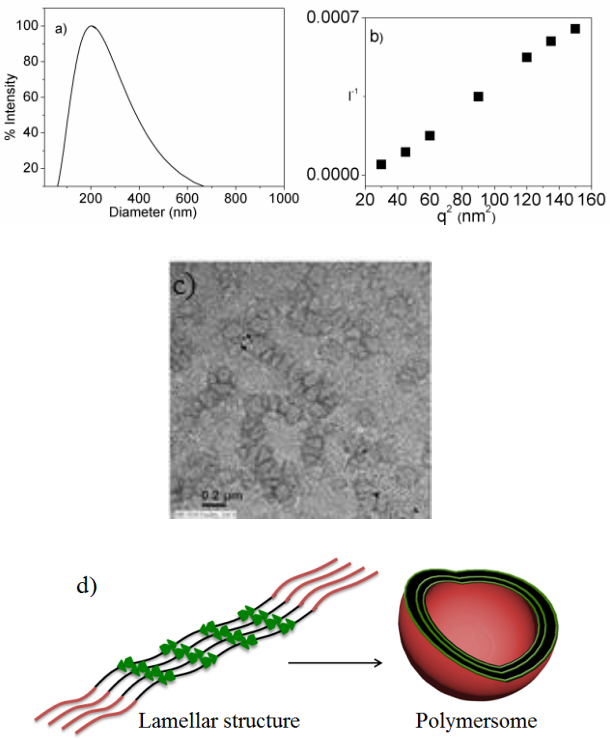


Fig. 4: (a) DLS and (b) SLS data of aqueous **P3** (1.0 mg/ ml) solution. (c) TEM images of **P3** aggregates. (d) A possible model depicting mode of assembly of **P3**.

The analysis of the concentration dependent scattering data using a partial Zimm plot (Fig. 4b) estimates the radius of gyration (R_g) to be 95 nm. The ratio of R_g/ R_h ~ 1.0 suggests the aggregates of **P3** are of hollow spherical nature.[15] Transmission electron microscopy (TEM) images of **P3** (Fig. 4c, S4) also show hollow near spherical aggregates with diameter in the range of 150-170 nm. The reduced size in the TEM compared to that observed in DLS can be attributed to the dehydration of the aggregates while preparing the samples for TEM experiments.[16]

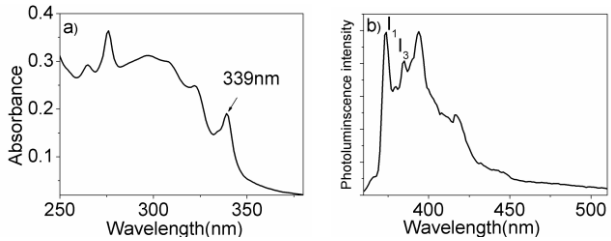


Fig. 5: (a) Absorption and (b) emission spectra of pyrene encapsulated in aqueous **P3** solution (1.0 mg/ ml). For the emission spectra λex = 337 nm.

Based on the light scattering and microscopic data it is proposed that the **P3** forms a lamellar structure by hydrophobic association of the middle block which folds and consequently forms a polymersome assembly (Fig. 4d).[15] Such structures are interesting in context of drug delivery [17] because of their ability to encapsulate both hydrophobic (in the membrane) as well as hydrophilic guest (in the water pool inside the aggregates) molecules. To test such possibilities dye loading experiments were carried out using a common spectroscopic probe pyrene which is hydrophobic in nature.

Absorption and photoluminescence spectra of pyrene treated **P3** solution are shown in Fig. 5. The absorption spectrum (Fig. 5a) reveals broad peak in the region of 380-250 nm due to the overlap of the absorption of **P3** and pyrene. However when it is compared with the spectrum of **P3** alone (Fig. S5), the encapsulation of pyrene becomes evident by the presence of an additional sharp peak at 339 nm. This is also supported by the emission spectra (Fig. 5b) which shows bands with fine structures. More importantly, the ratio of the intensities of the first (I1) and third (I3) vibrational bands is found to be 1.21 [18] which indicates dielectric of the local environment of pyrene is much less compared to that in water and thus confirms its encapsulation in the hydrophobic domain of the polymersome.

If the proposed polymersome assembly (Fig. 4d) indeed exists, then it should also be capable of encapsulating hydrophilic guests in addition to hydrophobic pyrene. To test this possibility, we examined encapsulation of Calcein in aqueous solution of **P3**. In this experiment, Calcein treated **P3** solution was subjected to dialysis for an extended period to ensure removal of any non encapsulated dye. The dialyzed solution exhibits characteristic absorption (Fig. 6a) and emission (Fig. 6b) bands of Calcein indicating encapsulation.

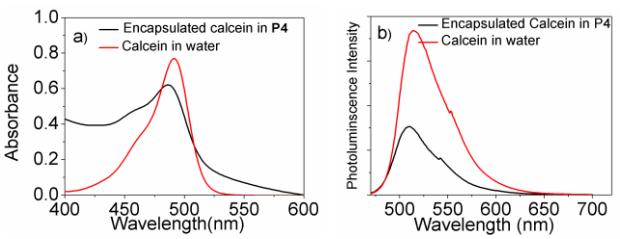
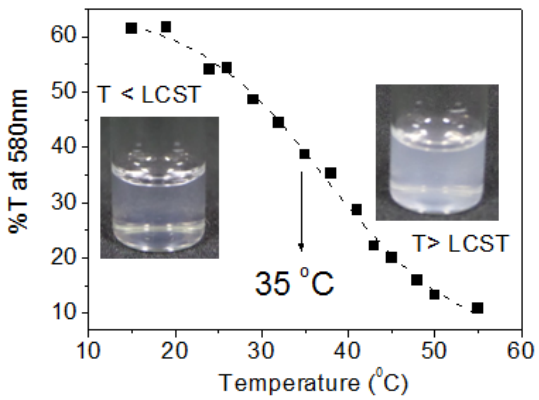


Fig. 6: (a) Absorption and (b) emission spectra of Calcein encapsulated in **P3** (1.0 mg/ ml) and the same dye in the absence of any polymer. Concentration of Calcein = 6 x 10^{-6} M.

The emission intensity of polymersome encapsulated Calcein solution when compared to the absorption normalized solution of the dye without any polymer; a reduced value is obtained (Fig. 6b) in the former case suggesting self-quenching for the polymer encapsulated dye due to their residence in the confined interior of the polymersome. [19] Further we examined the stimuli responsive properties of **P3** polymersome [20] as it contains the poly-(NIPAM) chains which are known to exhibit a lower critical solution temperature (LCST) [21] in aqueous solution. **Fig. 7** shows variation of the transmittance (at 580 nm) of an aqueous solution of **P3**. As the polymer itself does not absorb in this wavelength, a lowering of the transmittance indicates scattering from the sample as a result of macroscopic precipitation above LCST. It can be noticed that the transmittance is only ~ 60 % even at 15 °C confirming the presence of aggregated structures. However with increasing temperature it gradually decreases and reaches to ~ 10 % at 55 °C indicating macroscopic precipitation above LCST. Although from this plot it is possible to designate a transition temperature of ~ 35 °C which is close to the value of LCST of NIPAM, but intriguingly the transition is not sharp unlike most of the reported examples.[22] This can be attributed to the fact that this polymer is made from a macro CTA which was prepared by a condensation route and thus the distribution of molecular weight in this case is rather high compared to those obtained from controlled chain polymerizations. It is known in the literature that LCST depends on various structural parameters, one of which is the molecular weight.

Fig. 7: Variation of transmittance at 580 nm of an aqueous solution of



P3 (1.0 mg/ ml) as a function of temperature. The insets show images of the solution taken at ~ 20 °C (< LCST) and ~ 50 °C (> LCST)

In the present case due to broad distribution of molecular weight for the macro-CTA, the resulting triblock copolymer also contains polymer chains with wide distribution of hydrophobic/ hydrophilic ratio. This leads to the lack of a sharp transition for this particular thermoresponsive polymer.

Conclusion

In this article we have utilized a classical synthetic strategy to prepare telechelic macroinitiator using a step-growth polymerization between a di-thiol and di-acrylate monomer in presence of a mono-functional impurity containing an orthogonal functional group (-OH) which does not participate in the polycondensation reaction (thiol-acrylate Michael addition). A proper stoichiometry of the two monomers and the mono functional additive ensures the capping of the growing polymer chains by the monofunctional impurity in both ends. The terminal hydroxyl groups of the polymer were utilized for initiating ROP of a cyclic lactide monomer which produced an ABA type triblock copolymer. Due to the acid labile nature of

the β-thiopropionate linker in the backbone, the block copolymer could be disintegrated by mild acid treatment. Further functionalization of the terminal –OH groups with a functionalized CTA produced the telechelic macro CTA which was used to polymerize a NIPAM monomer using RAFT method producing an amphiphilic ABA triblock copolymer that showed thermoresponsive polymersome assembly in aqueous medium. Enormous progress has been made in making structurally diverse polymers in chain growth or step growth routes and their functional utilities have also been explored in different context. But examples of polymer synthesis employing both these techniques, as demonstrated in this article, are scarce.[10] To us it appears to be a very promising synthetic approach considering its possible impact of making new polymers those are structurally enriched by taking advantages of both step growth and chain growth techniques. Several interesting features of this study that includes (i) a generalized synthetic strategy for preparation of ABA triblock copolymer involving step growth followed by chain polymerization, (ii) utilization of the thiol-acrylate Michael addition reaction for polycondensation, and (iii) more importantly pH-responsive degradation of these polymers due to the presence of the acid labile β-thiopropionate linker make a strong basic for further utilization of this synthetic strategy to explore new stimuli responsive macromolecules for applications in the biomedical domain. Efforts in these directions are underway in our laboratory.

Experimental

Materials and methods: The reagents and solvents used here were purchased from Sigma Aldrich and other commercial sources and purified by standard methods.[23] 1H NMR spectra were taken from Bruker DPX-300 MHz NMR spectrometer and in each case the spectrum was calibrated using an internal standard TMS. UV/vis experiments were carried out in a Perkin Elmer Lamda25 spectrometer attached with a Peltier system for measuring the thermo-responsive property of the polymer. Dynamic Light Scattering (DLS) and Static light scattering (SLS) measurements were carried out in a BI-200SM goniometer (ver 2.0) instrument. TEM images were obtained from a JEOL-2010EX machine operating at an accelerating voltage of 100 KV. Fluorescence emission spectra were taken in a FluoroMax-3 spectrophotometer purchased from Horiba Jobin Yvon. Molecular weight of the polymer was determined by Waters gel permeation chromatography (GPC) equipped with a Waters 515 HPLC pump and a Waters 2414 refractive index (RI) detector using THF solvent. Molecular weight and polydispersity index (PDI) were calculated with respect to PMMA standards.

Synthesis of M1: A solution of freshly distilled acryloyl chloride (1.71 ml, 0.021 mol) in dry CH_2Cl_2 (4 ml) was drop-wise added to a ice-cold solution of hexane-diol (1.0 g, 0.00846 mol) and triethylamine (3.54 ml, 0.025 mol) in dry CH_2Cl_2 (12 ml) under inert atmosphere over a period of ~ 15 min. After the addition was over the reaction mixture was stirred at rt for another 12 h. The stirring was stopped and the reaction mixture was diluted with another 10 ml of CH_2Cl_2, the resulting mixture was washed with brine (4 x 15 ml) and passed through anhydrous Na_2SO_4. Solvent was evaporated under reduced pressure to get the crude product as colorless oil which was purified by column chromatography using silica gel as a stationary phase and CH_2Cl_2 as eluent to obtain the desired product as a colorless oil in 58 % yield. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ (ppm) = 6.40 (d, 2H), 6.11 (m, 2H), 5.79 (d, 2H), 4.15 (t, 4H), 1.68 (m, 4H), 1.42 (m, 4H).

Synthesis of P1: A solution of M1 (0.2 g, 0.884 mmol) M2 (0.0846g, 0.782 mmol) and M* (0.0165g, 0.211 mmol) in 130 µl degassed DMAc was placed in a reaction vessel under Ar atmosphere and cooled to ~ 0 °C by immersing it in an ice-bath. To this cold solution, 5.0 µl 0.1 wt % Me2PPh solutions in DMAc was added and then the polymerization was carried out in the same ice-bath over a period of 1 h under continuous flow of Ar gas. Then the viscous solution was diluted with 100 µl DMAc and precipitated from excess Methanol to get the polymer as colorless highly viscous liquid in 97 % yield. ^{1}H NMR (300MHz, $CDCl_3$, TMS): δ (ppm) = 4.09 (t, 52 H), 3.75 (t, 4H), 2.77 (m, 52H), 2.61 (m, 104 H), 1.86 (m, 26H), 1.64 (m, 52H), 1.38 (m, 52H).

Synthesis of P2: A solution of polymer **P1** (0.1g, 0.016 mmol), the cyclic lactide monomer (0.240 g, 1.6 mmol) and $Sn(oct)_2$ catalyst (0.9 mg, 0.0023 mmol) in 1.0 ml dry toluene was taken in a polymerization vessel which was stirred at 120 °C for 48 h under Ar atmosphere. Then the polymer was precipitated out from excess Methanol to obtain a white solid which was dried under vacuum to get **P2** in 53 %. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ (ppm) = 5.17 (m, 4H), 4.09 (t, 4H), 2.78 (t, 4H), 2.64 (m, 8H), 1.87 (m, 2H), 1.56 (m, 4H), 1.39 (m, 4H).

Synthesis of P3a: The carboxylic acid functionalized RAFT agent CTA-COOH (Fig. 3a) (0.0097 g, 0.0357 mmol), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (0.013 g, 0.0714 mmol), N, N dimethyl amino pyridine (0.0087 g, 0.0714 mmol) and hydroxylbenzotriazole (HOBt) (0.0096 g, 0.0714 mmol) were mixed together with 50 µl dry dichloromethane and the reaction mixture was cooled to 0°C by immersing it in an ice bath. To this, a solution of **P1** (0.1g, 0.0178 mmol) in 50 µl dry dichloromethane was added drop wise and then the reaction mixture was stirred at rt for 12 h under inert atmosphere. After that the reaction mixture was precipitated from excess Methanol to isolate **P3** as a yellow sticky sold mass which after drying was recovered 72 % yield. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ (ppm) = 4.10-4.07 (t, 4H), 2.79-2.74 (m, 4H), 2.65-2.57 (m, 8H), 1.88-1.84 (m, 2H), 1.66-1.57 (b, 4H), 1.40-1.36 (b, 4H); Additional peaks appear (Fig. S2) at δ (ppm) = 7.32 and 4.60 indicating the inclusion of the CTA at the chain end.

Synthesis of P3: P3a (0.072g, 0.0642 mmol), Azobisisobutyronitrile (AIBN) (0.5 mg, 0.0032 mmol) and *N*-isopropylacrylamide (0.072 g, 0.642 mmol) were dissolved in

0.3 ml degassed toluene and the solution was further degassed by purging Ar. Then it was placed in a pre-heated oil bath at 70 °C and stirred for 12 h under Ar atmosphere. The polymerization was stopped, cooled to rt and precipitated from diethyl-ether to get **P3** as a light yellow solid powder which was isolated in 60% yield. ^{1}H NMR (300MHz, $CDCl_3$, TMS): δ (ppm) = 4.09 (t, 4H), 3.99 (b, 1H), 2.81-2.73 (m, 4H), 2.65-2.58 (m, 8H), 1.90-1.83 (m, 2H), 1.66-1.62 (m, 4H), 1.25-1.20 (m, 4H,), 1.15 (b, 6H).

Degradation study with P2: 40 µl trifluoroacetic acid (TFA) was added to a solution of **P2** (4.0 mg) in 2 ml THF/ Methanol (1:1) solvent mixture and it was stirred at rt for extended time. At different time intervals, aliquots were taken out, the solvent was evaporated under reduced pressure and the pasty mass obtained was analyzed by GPC.

Pyrene encapsulation studies: A stock solution of pyrene (1.0 mM) in acetone was transferred to a vial and the solvent was removed by blowing air to obtain a thin film which was added with 1.5 ml aqueous **P3** solution (1.0 mg/ ml), sonicated for ~ 5 min and equilibrated for 4 h. After that the solution was filtered through a 0.45 µm hydrophilic membrane before spectral measurements.

DLS and SLS studies: For DLS and SLS measurements, aqueous solution of **P3** (1.0 mg/ ml) was prepared by directly adding water to the sample followed by sonication (~ 5 min) and filtration through a 0.45 µm size hydrophilic filter paper. In SLS studies, data were collected in seven different angles (30°, 45°, 60°, 90°, 120°, 135° and 150°) keeping the concentration of the solution same in all measurements. The radius of gyration (Rg) was estimated from partial Zimm plot using the equation shown below.

$$I^{-1} = C\,(1 + R_g^2 q^2 / 3)$$

Where $I = I^{'}.\,\mathrm{Sin}\theta$; $I^{'}$ = intensity of scattered light; θ = the angle of scattered light; C = a constant; R_g = the radius of gyration; q = magnitude of the scattering wave vector; $q = 4\pi n.\sin(\theta/2)/\lambda_o$ (n = refractive index of the of the liquid and λ_o = the wavelength of light in vacuum). From the slope of this plot one can estimate the R_g. As we are concerned about only the R_g (not molecular weight) SLS experiment was performed with only angular variation but no variation in the concentration.

TEM study: 10 µl of the aqueous **P3** solution that was prepared for DLS experiment was drop casted on a carbon coated copper grid which was air dried for overnight before TEM experiment was performed at an accelerating voltage of 100 KV.

LCST measurements: 2.0 ml of aqueous **P3** solution (1.0 mg/ ml) was placed in a quartz cuvette (l = 1.0 cm) and temperature of the sample was gradually increased from 15 °C to 55 °C using a peltier that was externally connected to the system. Transmittance at 580 nm was recorded at different temperature intervals. After a desired temperature is reached, 5 min equilibrium time was provided before the spectral measurement.

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Notes and references

[a] *Polymer Science Unit, Indian Association for the Cultivation of Science, 2A and 2B Raja. S.C. Mullick Road, Kolkata-700032, India.*
Fax: 91-33-2473-4971; Tel: 91-33-2473-2805;
E-mail: psusg2@iacs.res.in
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Entry for TOC only

Stimuli Responsive Triblock Copolymers by Chain-growth Polymerization from Telechelic Macroinitiators Prepared by a Step-growth Polymerization

Krishna Dan and Suhrit Ghosh*

Synthesis of stimuli-responsive ABA Tri-block copolymers using a step-growth followed by a chain-growth polymerization.