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Synthesis of Monodisperse Cylindrical Nanoparticles via Crystallization-Driven Self-Assembly of Biodegradable Block Copolymers

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Cover Letter

TITLE:

Synthesis of Monodisperse Cylindrical Nanoparticles via Crystallization-Driven Self-Assembly of Biodegradable Block Copolymers.

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KEYWORDS:

crystallization-driven self-assembly, ring-opening polymerization, reversible addition-fragmentation chain transfer polymerization, degradable polymers

SHORT ABSTRACT:

Crystallization-driven self-assembly (CDSA) displays the unique ability to fabricate cylindrical nanostructures of narrow length distributions. The organocatalyzed ring-opening polymerization of ε-caprolactone and subsequent chain extensions of methyl methacrylate and *N,N*-dimethyl acrylamide are demonstrated. A living CDSA protocol that produces monodisperse cylinders up to 500 nm in length is outlined.

LONG ABSTRACT:

The production of monodisperse cylindrical micelles is a significant challenge in polymer chemistry. Most cylindrical constructs formed from diblock copolymers are produced by one of three techniques: thin film rehydration, solvent switching or polymerization-induced self-assembly, and produce only flexible, polydisperse cylinders. Crystallization-driven self-assembly (CDSA) is a method which can produce cylinders with these properties, by stabilizing structures of a lower curvature due to the formation of a crystalline core. However, the living polymerization techniques by which most core-forming blocks are formed are not trivial processes and the CDSA process may yield unsatisfactory results if carried out incorrectly. Here, the synthesis of cylindrical nanoparticles from simple reagents is shown. The drying and purification of reagents prior to a ring-opening polymerization of ε-caprolactone catalyzed by diphenyl phosphate is described. This polymer is then chain extended by methyl methacrylate (MMA) followed by *N,N*-dimethyl acrylamide (DMA) using reversible addition−fragmentation chain-transfer (RAFT) polymerization, affording a triblock copolymer that can undergo CDSA in ethanol. The living CDSA process is outlined, the results of which yield cylindrical nanoparticles up to 500 nm in length and a length dispersity as low as 1.05. It is anticipated that these protocols will allow others to produce cylindrical nanostructures and elevate the field of CDSA in the future.

INTRODUCTION:

One-dimensional (1D) nanostructures, such as cylinders, fibers and tubes, have garnered increasing attention in a variety of fields. Amongst these, their popularity in polymer science is owed to their rich variety of properties. For example, Geng et al. demonstrated that filomicelles exhibit a tenfold increase in residence time in the bloodstream of a rodent model compared to their spherical counterparts, and Won et al. revealed that polybutadiene-*b*-poly(ethylene oxide)

fiber dispersions display an increase in storage modulus by two orders of magnitude upon crosslinking of the core during rheological measurements[1,2]. Interestingly, many of these systems are synthesized via the self-assembly of block copolymers, whether this be through more traditional methods of solvent switching and thin-film rehydration[3], or more advanced methods such as polymerization-induced self-assembly and crystallization-driven self-assembly (CDSA)[4,5]. Each technique holds their own advantages, however, only CDSA can produce rigid particles with a uniform and controllable length distribution.

Pioneering work by Gilroy et al. formed long polyferrocenylsilane-*b*-polydimethylsiloxane (PFS-PDMS) cylinders in hexanes and, when using mild sonication, very short cylinders with a low contour length dispersity (L_n). Upon the addition of a predetermined mass of diblock copolymer chains in a common solvent, cylinders of varying lengths with an L_n as low as 1.03 were synthesised[5,6]. Further work by the Manners group highlighted the high degree of control possible with the PFS system, which may be used to form remarkably complex and hierarchal structures: block-co-micelles, scarf shaped and dumbbell micelles to name a few[7,8]. Following these demonstrations, researchers investigated other, more functional systems for CDSA including: semi-crystalline commodity polymers (polyethylene, poly(ε-caprolactone), polylactide)[9–13] and conducting polymers (poly(3-hexylthiophene), polyselenophene)[14,15]. Armed with this toolbox of diblock copolymer systems that can be assembled quickly and efficiently, researchers have carried out more application-driven research in recent years[16]. Jin et al. have demonstrated exciton diffusion lengths in the hundreds of nanometers in polythiophene block copolymers and our group demonstrated the formation of gels from poly(ε-caprolactone) (PCL) containing cylindrical constructs[10,17].

Although it is a powerful technique, CDSA does have its limitations. The block copolymers must have a semi-crystalline component, as well as low dispersity values and high end group fidelities; lower order block contaminants may cause particle aggregation or induce morphology changes[18,19]. Due to these restrictions, living polymerizations are used. However, significant reagent purification, drying procedures and water/oxygen free environments are required in order to achieve polymers with the aforementioned properties. Attempts have been made to design systems that overcome this. For example, PFS block copolymers have been formed using click chemistry to couple polymer chains together[20]. Although the resulting cylindrical nanoparticles have demonstrated exemplary properties, the block copolymers are typically purified by preparative size exclusion chromatography and the synthesis of PFS still requires the use of living anionic polymerizations. Our group recently realized the living CDSA of PCL, the success of which revolved around using both living organobase-catalyzed ring-opening polymerizations (ROP) and reversible addition-fragmentation chain transfer (RAFT) polymerizations[10]. Although this method is simpler, living polymerizations are still required.

As the field is moving towards more application-driven research, and due to the problems associated with living polymerizations, it is believed that an outline of the polymer synthesis and self-assembly protocols will be advantageous to future scientific work. Thus, in this manuscript, the complete synthesis and self-assembly of a PCL-*b*-PMMA-*b*-PDMA copolymer is outlined. Drying techniques will be highlighted in the context of an organocatalyzed ROP of ε-caprolactone

and the subsequent RAFT polymerizations of MMA and DMA will be outlined. Finally, a living CDSA protocol for this polymer in ethanol will be presented and common errors in characterization data due to poor experimental technique will be critiqued.

PROTOCOL:

1. Drying of toluene

NOTE: If you have access to dry solvent towers, collect the toluene and degas by five freeze-pump-thaw cycles.

1.1. Dry 3 Å molecular sieves in a 250 mL Schlenk flask at 250-300 °C under vacuum for 48 h and transfer into a glovebox.

1.2. Dry two ampoules in the oven at 150 °C overnight and transfer them into the glovebox.

1.3. Transfer the activated molecular sieves into the two ampoules and remove from the glovebox.

1.4. Dry a two-neck round-bottom flask (RBF) and add 100 mL of toluene, the volume of which equals to, at most, half of the ampoule volume. Add 1.0 g of CaH_2 to the toluene and stir.

CAUTION: Be careful of H_2 release at this point. Always add CaH_2 under a steady flow of nitrogen to remove any H_2 build up in the flask.

1.5. Transfer the toluene into one of the ampoules containing the molecular sieves with a filter cannula and rest overnight.

1.6. Transfer the toluene into the last ampoule containing sieves with a filter cannula. Freeze-pump-thaw (5 cycles) the toluene and transfer into a glovebox.

2. Drying of the CTA-initiator/DPP

2.1. Add the chain transfer agent/initiator to a vial, securing with tissue paper.

2.2. Add 10 g of P_2O_5 into a desiccator. Place the vial above the powder.

2.3. Place the desiccator under dynamic vacuum for 8 h and static vacuum overnight.

2.4. Open the desiccator to agitate the P_2O_5. Resume the vacuum cycles for 5 days.

NOTE: The P_2O_5 may discolor or become clumpy if excess solvent/water is present. Replace the P_2O_5 if this is observed.

2.5. Backfill the desiccator with nitrogen and transfer to a glovebox.

3. Drying/Purification of ε-caprolactone

NOTE: For this section, all glassware and stirrer bars must have been dried in a 150 °C oven overnight prior to use. This will remove all water from the surfaces of the glass.

3.1. Add 100 mL of ε-caprolactone to a two-neck 250 mL RBF equipped with a stirrer bar and tap on the small neck.

3.2. Add 1.0 g of calcium hydride into the RBF, under a steady flow of nitrogen. Fit with a glass stopper and stir overnight at room temperature under a nitrogen atmosphere.

3.3. Dry the vacuum distillation equipment.

3.4. Attach the two-neck flask to a Schlenk line and purge by evacuating and filling with nitrogen three times. After purging, open the line to a steady flow of nitrogen.

3.5. Assemble the vacuum distillation equipment from the ε-caprolactone RBF, maintaining a steady flow of nitrogen to prevent water from entering the system. Attach the thermometer and seal in place.

3.6. Attach the adaptor to the Schlenk line. Remove the nitrogen flow and place the system under vacuum under this new connection.

3.7. Heat the ε-caprolactone at 60-80 °C, collecting the first 5.0 mL in the small RBFs and the rest in the two-neck RBF. Place the flasks in liquid nitrogen to condense the caprolactone effectively. Wrap the distillation equipment in cotton wool and foil to speed up the process.

3.8. Attach the Schlenk line to the collection flask and purge the line three times. Turn the line to nitrogen and open the tap. Add 1.0 g of calcium hydride to the flask, and a stopper, then leave under a nitrogen atmosphere stirring overnight.

3.9. Meanwhile, dispose of the excess calcium hydride by the dropwise addition of isopropanol, followed by 5.0 mL of methanol and then an excess of water once bubbling ceases. Rinse the glassware with acetone and place in the oven overnight.

3.10. Repeat the vacuum distillation again, without adding CaH_2 to the monomer once finished. Instead, transfer the caprolactone via cannula into an ampoule and transfer to the glovebox.

4. Ring opening polymerization of ε-caprolactone

4.1. Prepare stock solutions of initiator, catalyst and monomer. Weigh 0.10 g of diphenyl phosphate, 0.011 g of CTA-OH and 0.25 g of caprolactone into three separate vials. Add 0.5 mL

of toluene to each of the initiator and catalyst vials and gently agitate until the reagents are dissolved.

4.2. Mix the initiator and diphenyl phosphate stock solutions into one vial and add a stir bar.

4.3. Under moderate stirring, add the monomer into the initiator/catalyst vial. Fit the vial with a lid and stir for 8 h at room temperature.

4.4 After 8 h, remove the vial from the glovebox and immediately precipitate into an excess of cold diethyl ether dropwise.

4.5 Filter the white solid, dry and dissolve in 1 mL of tetrahydrofuran (THF). Precipitate twice more and dry thoroughly.

5. RAFT polymerization of methyl methacrylate and *N,N*-dimethylacrylamide

5.1. To remove the stabilizers from the dioxane and MMA, prepare several basic alumina plugs in Pasteur pipettes and filter the liquids into separate vials.

5.2. Weigh 0.5 g of PCL synthesized previously, 0.424 g of methyl methacrylate and measure 2 mL of dioxane into a vial and allow to dissolve.

5.3. Prepare a stock solution of pure azobisisobutyronitrile (AIBN, 10 mg in 1.0 mL) and pipette in 139 µL into the reaction mixture. Transfer to an ampoule equipped with a stir bar and seal.

5.4. Freeze-pump-thaw the solution three times. Backfill with nitrogen and place the ampoule in a preheated oil bath at 65 °C for 4 h.

NOTE: Do not heat the container with anything more than 30 °C before the freeze-pump-thaw cycles are complete, as this can cause the initiator to decompose.

5.5. To monitor conversion, remove the ampoule from the oil bath. Switch the cap for a suba seal under a flow of nitrogen, remove two drops and mix with deuterated chloroform. Run a proton spectrum on an NMR instrument.

5.6. Place the ampoule in liquid nitrogen until frozen and open the ampoule to air to quench the polymerization.

5.7. Precipitate the mixture dropwise into a vast excess of cold diethyl ether. Isolate by Buchner filtration and dry.

5.8. Take the polymer up in THF and precipitate twice more. Dry the polymer thoroughly and analyze by ^{1}H NMR spectroscopy and gel permeation chromatography (GPC).

5.9. Follow this procedure again, but with 0.5 g of PCL-PMMA, 1.406 g of DMA, 2.0 mL of dioxane and 111 µL of 10 mg.mL^{-1} AIBN in dioxane. Heat the polymerization at 70 °C for 1 h and precipitate the reaction mixture into cold diethyl ether three times.

6. Self-nucleation, seed generation and living crystallization-driven self-assembly

6.1. Place 5.0 mg of triblock copolymer into a vial and add 1.0 mL of ethanol. Seal the vial with a lid and parafilm and heat at 70 °C for 3 h.

6.2. Leave the vial to cool slowly to room temperature. Leave the solution to age at room temperature for two weeks. The solution will turn cloudy and will form a distinct layer at the bottom when fully assembled.

6.3. Dilute the 5.0 mg.mL^{-1} dispersion to 1.0 mg.mL^{-1}.

6.4. Place the dispersion in a sonication proof tube and place in an ice bath.

6.5. Insert the tip of the sonication probe into the middle area of the dispersion.

6.6. Sonicate the solution for fifteen cycles of 2 min at the lowest intensity, allowing to cool for 15 min before the next cycle.

6.7. Take an aliquot of the 1.0 mg.mL^{-1} seed dispersion and dilute to 0.18 mg.mL^{-1}.

6.8. Prepare a solution of unimer in THF at 25 mg.mL^{-1}. Add 32.8 µL into the seed dispersion and gently shake to allow full dissolution.

6.9. Leave the dispersion to age for three days with the lid slightly ajar so the THF can evaporate. This will produce cylinders of 500 nm in length if the starting seeds were 90 nm in length.

REPRESENTATIVE RESULTS:

PCL was analyzed by ^{1}H NMR spectroscopy and gel permeation chromatography (GPC). The ^{1}H NMR spectrum yielded a degree of polymerization (DP) of 50, by comparison of resonances at 3.36 ppm and 4.08 ppm, which correspond to the end group ethyl protons and the in-chain ester α-protons respectively (**Figure 1b**). This provided validation of the molecular weight values obtained by GPC where a single peak, with a dispersity value of 1.07, was observed with an M_n of 10,800 g.mol^{-1} (**Figure 1c**). A polymerization using reagents that had not been correctly dried yielded a product mixture which included oligomeric or low molecular weight PCL, as demonstrated by the trace which includes a low molecular weight tail (**Figure 1d**). This behavior is due to spurious initiation by water. By comparison, a properly dried polymerization that was left to react for 12h (that is, 4 h at conversions above 95%) gave a high molecular weight shoulder at 15,500 g.mol^{-1}, due to transesterification between polymer chains (**Figure 1e**).

The successive RAFT polymerizations were characterized by the same techniques. The ^{1}H NMR spectrum of the PCL-PMMA indicated a DP of 10 (of the PMMA block) by comparison of the PCL in-chain ester α-protons (4.08 ppm) and the methyl α-protons of PMMA (3.62 ppm, **Figure 2b**). The GPC trace displayed a unimodal peak (**Figure 2c**), however, when deliberately taken to too high conversions (>70%) a broadening of molecular weight and a high molecular weight shoulder was observed, most likely due to disproportionation side reactions (**Figure 2d**). The DP of the final block of PDMA was 200 upon comparison of the PCL in chain ether protons (4.08 ppm) and the DMA side chain methyl protons (2.93 ppm, **Figure 3b**). Again, the GPC trace was narrow and unimodal (**Figure 3c**). Upon repetition of the chain extension using impure PCL-PMMA, a low molecular weight shoulder appears (**Figure 3d**). This is a manifestation of a larger concentration of initiator in the polymerization, which results in a larger proportion of initiator derived chains being produced.

The self-nucleation process (the first step in a living CDSA) generated structures that were observed by transmission electron microscopy (TEM). Images collected after three days of aging displayed high-aspect ratio cylindrical particles accompanied by a sub-population of spheres (**Figure 4a**). The latter are unimer chains which have not yet grown onto the cylinders. Upon aging for a further ten days, a pure phase of cylinders was observed (**Figure 4b**). Sonication of the long cylinders caused them to fragment, yielding small cylindrical particles (seeds) with, upon examination of at least 300 particles by TEM, an average contour length of 90 nm with a dispersity of 1.15 (**Figure 4c**). These seeds were used to generate populations of cylinders with increasingly longer contour length by the simple addition of polymer chains (unimer) in a common solvent (**Figure 5b-g**). Interestingly, when L_N of the particles is plotted against the mass ratio of unimer to seeds, a linear trend is observed (**Figure 5i**). Further analysis of these particles by TEM indicates incredible uniformity over all the samples (**Figure 5h**).

Multiple issues can arise during living CDSA. Repetition of the self-nucleation process with a triblock copolymer that has a low molecular weight tail results in the observation of a population of plate-like structures (**Figure 6a**). If total sonication times exceed 30 min or cycle times are in excess of 2 min, the uniformity of the cylinders suffers greatly (**Figure 6b**). This is due to a small proportion of polymer dissolving from the particles (either due to the formation of extremely small, unstable particles, or through heating of the particle dispersion) and recrystallizing onto the remaining cylinders. Finally, the volume of common solvent added during the cylinder extension step can cause plate-like structures to be observed by TEM (**Figure 6c**).

FIGURE AND TABLE LEGENDS:

Figure 1: Typical results from a ring-opening polymerization of ε-caprolactone. (**a**) The reaction scheme of the synthesis of PCL_{50}, (**b**) the ^{1}H NMR spectrum displaying the resonances that are used to calculate DP and (**c**) a typical molecular weight distribution, (**d**) a molecular weight distribution of an ROP that contains trace water and (**e**) a molecular weight distribution of an ROP that has reacted for too long.

Figure 2: Typical results from a RAFT polymerization of methyl methacrylate. (**a**) The reaction scheme of the synthesis of PCL_{50}-$PMMA_{10}$, (**b**) the ^{1}H NMR spectrum displaying the resonances

that are used to calculate DP and (**c**) a typical molecular weight distribution of a good RAFT polymerization of MMA, (**d**) a typical molecular weight distribution of a RAFT polymerization of MMA that has been taken to too high conversion.

Figure 3: Typical results from a RAFT polymerization of *N,N*-dimethylacrylamide. (**a**) The reaction scheme of the synthesis of PCL_{50}-$PMMA_{10}$-$PDMA_{200}$, (**b**) the ^{1}H NMR spectrum displaying the resonances that are used to calculate DP and (**c**) a typical molecular weight distribution of a good RAFT polymerization of DMA, (**d**) a typical molecular weight distribution of a RAFT polymerization of DMA that was incorrectly purified in the previous step.

Figure 4: Preparation of triblock copolymer seed nanoparticles. TEM images of a 5 $mg.mL^{-1}$ dispersion of PCL_{50}-$PMMA_{10}$-$PDMA_{200}$ having aged for (**a**) three days, (**b**) two weeks and (**c**) after 15 x 2 min of sonication cycles. Scale bars are 500 nm, 100 nm and 1000 nm, respectively.

Figure 5: Living crystallization-driven self-assembly from seeds. (**a**) Scheme depicting the sonication and living CDSA of the triblock copolymer, (**b-g**) TEM images of the living CDSA up to 500 nm, (**h**) the properties of the particles and (**i**) the relationship between the average length of the micelles and seed/unimer mass ratio. Figure reproduced from Arno, M. C., Inam, M., et al. Precision Epitaxy for Aqueous 1D and 2D Poly(ε-caprolactone) Assemblies. *Journal of the American Chemical Society* **139**, (46)16980–16985 (2017).

Figure 6: Troubleshooting CDSA of the Triblock Copolymer. TEM images of structures (**a**) formed by CDSA of a triblock copolymer with a low molecular weight shoulder, (**b**) formed by the incorrect sonication of long cylinders and (**c**) formed by the addition of a high volume of common solvent to the seed dispersion.

DISCUSSION:

The synthesis and living CDSA of the triblock copolymer PCL_{50}-$PMMA_{10}$-$PDMA_{200}$ has been outlined. Although stringent conditions are required, the ring-opening polymerization of ε-caprolactone gave polymers with excellent properties that enabled the successful chain extensions of MMA and DMA. These polymers were successful in their self-seeding, obtaining a pure phase of cylindrical micelles, which were sonicated into seed particles of L_N 98 nm. Through simple addition of unimer, cylinders with average lengths ranging up to 495 nm were produced in a controlled manner. A triblock terpolymer is used over a diblock copolymer in this instance. This overcomes fragmentation issues when the cylinders are transferred to water. It has been previously reported that the incorporation of a stabilizing short block with a high glass transition temperature can prevent the cylinders from fracturing.

However, deviation from the protocols can result in polymers that are unfit for CDSA applications. For example, it is very important that the monomer must be added to the initiator/catalyst solution, not vice versa in ROP. This ensures that all initiation events occur within the same time window and a polymer of low dispersity is obtained. The importance of effective reagent drying procedures relative to the success of ring opening polymerizations has been continually outlined throughout this manuscript.

There are also common pitfalls encountered in RAFT polymerizations. Judging the conversion by time alone will result in an incorrect degree of polymerization. A multitude of factors can cause the kinetics to differ day to day (pump vacuum, volume of headspace and purity of initiator for example). Thus, it is recommended that when aiming for specific conversions, the polymerization is monitored by ^{1}H NMR spectroscopy throughout. Precipitations must be carried out with solutions containing 20 wt% of polymer or less, otherwise purification is not effective. Albeit simple, minor changes to the self-assembly protocol can induce significant loss of uniformity in the samples. For example, if the volume of the unimer solution is too high, THF can plasticize the crystalline core and induce a phase change to a plate like geometry. Similar artifacts can be observed if the concentrations of the unimer solution (> 100 $mg.mL^{-1}$) or the seed-dispersion (>5 $mg.mL^{-1}$) are too high.

This manuscript has highlighted the protocols and nuances of a variety of polymerization techniques in the context of CDSA, in the hope that others will be able to reproduce the results and continue research into this exciting field. The translation of these methods to other, more application-driven ideas is of paramount importance to both the authors and the scientific community at large.

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Figure 1

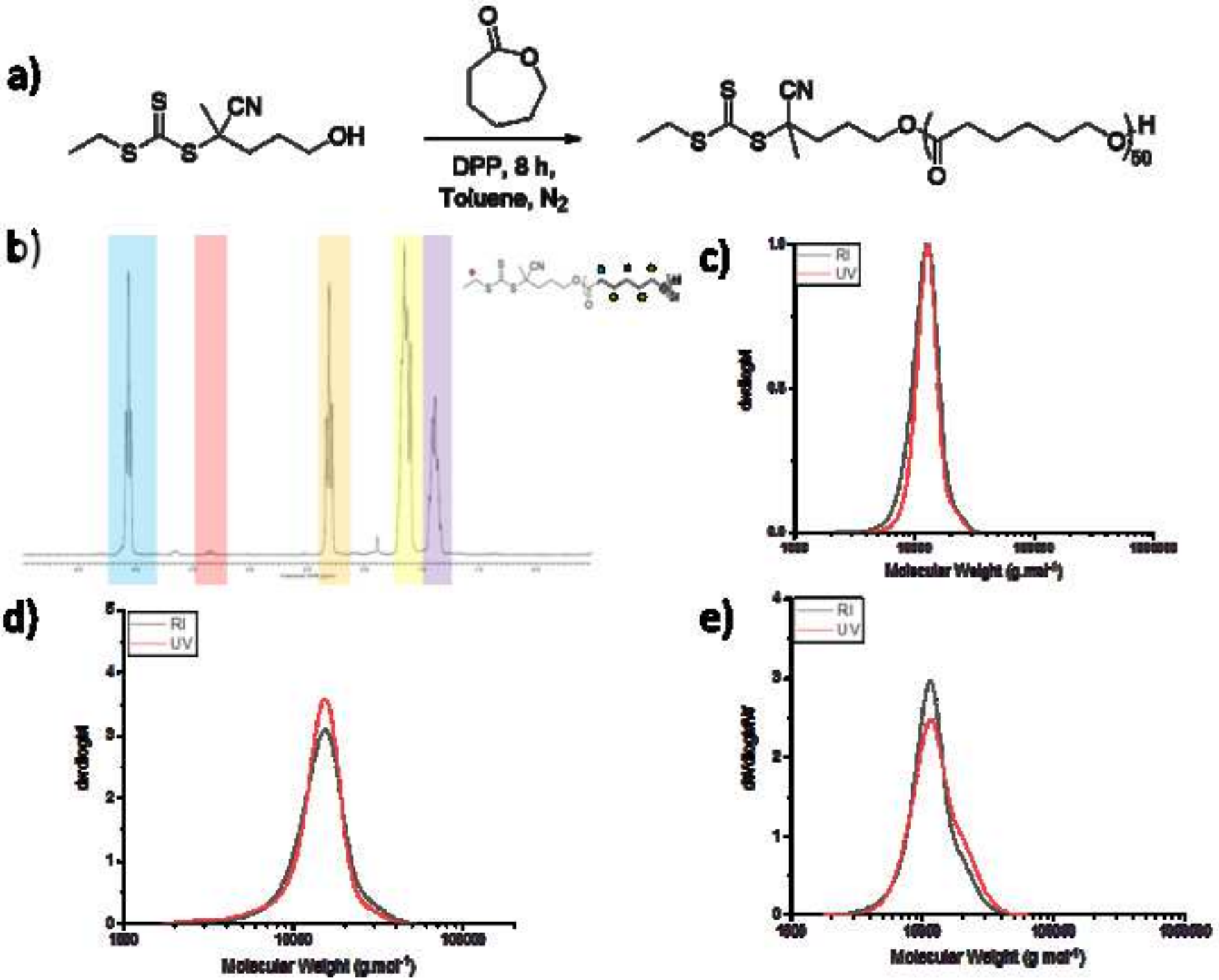


Figure 2

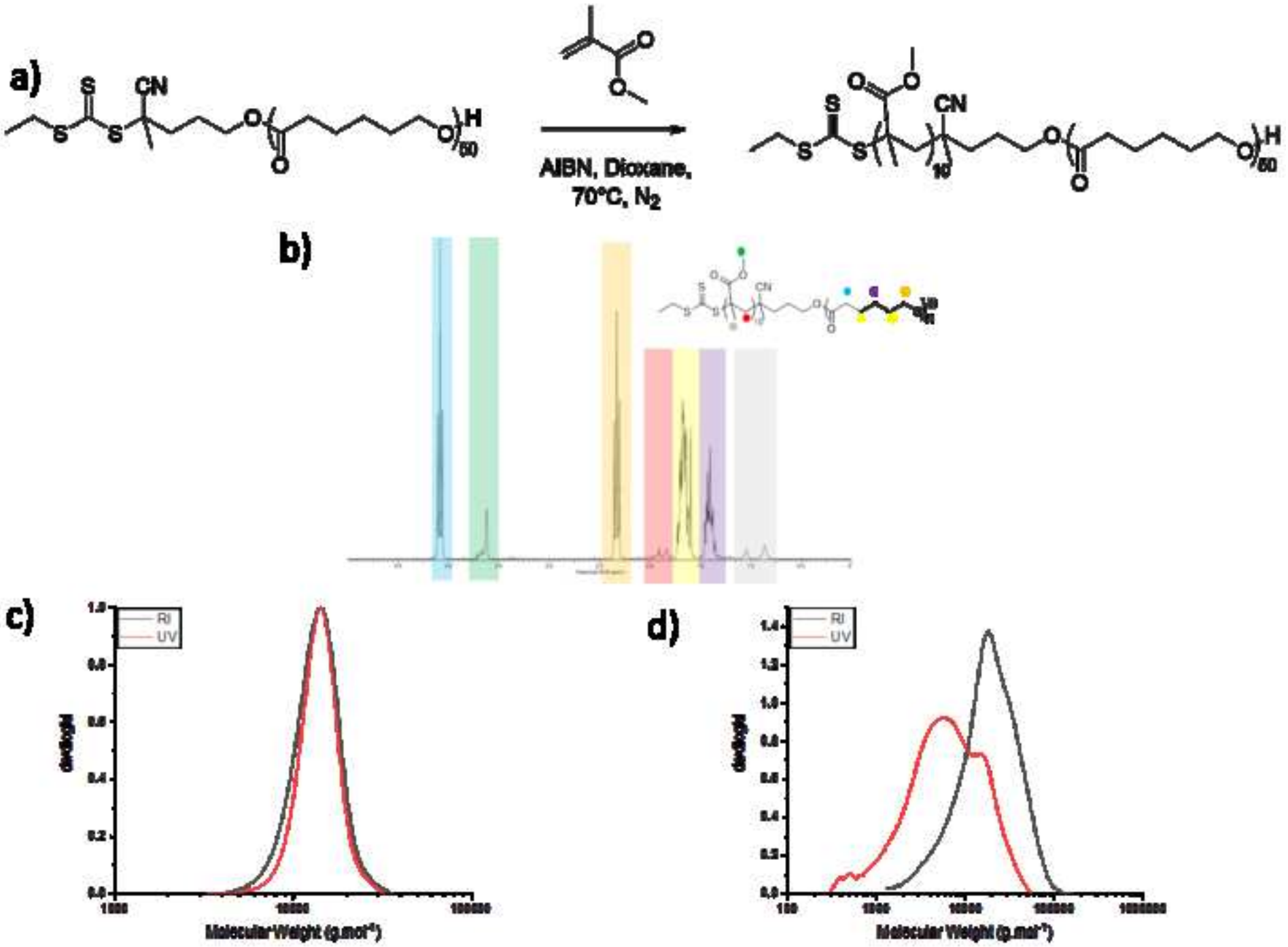


Figure 3

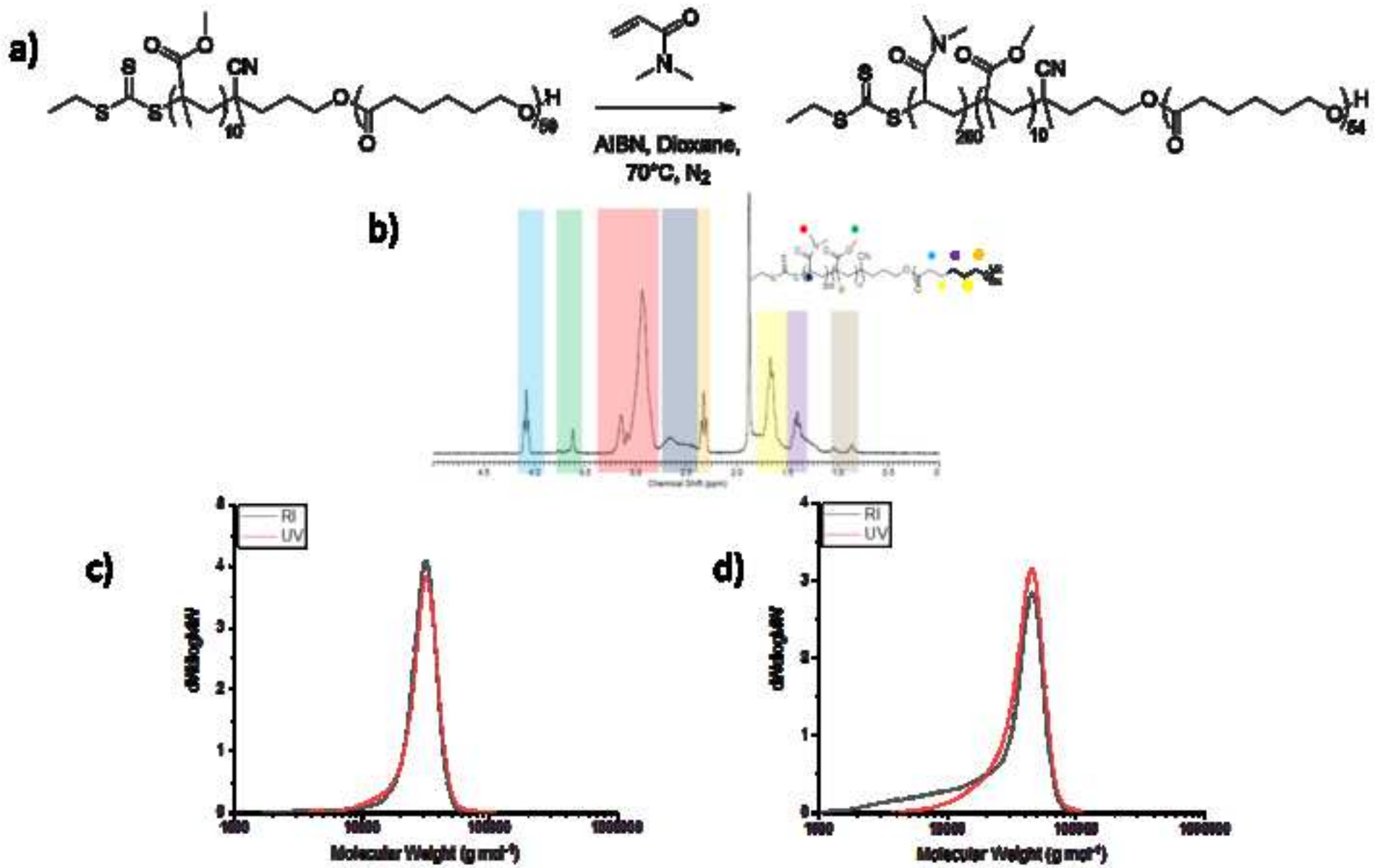


Figure 4

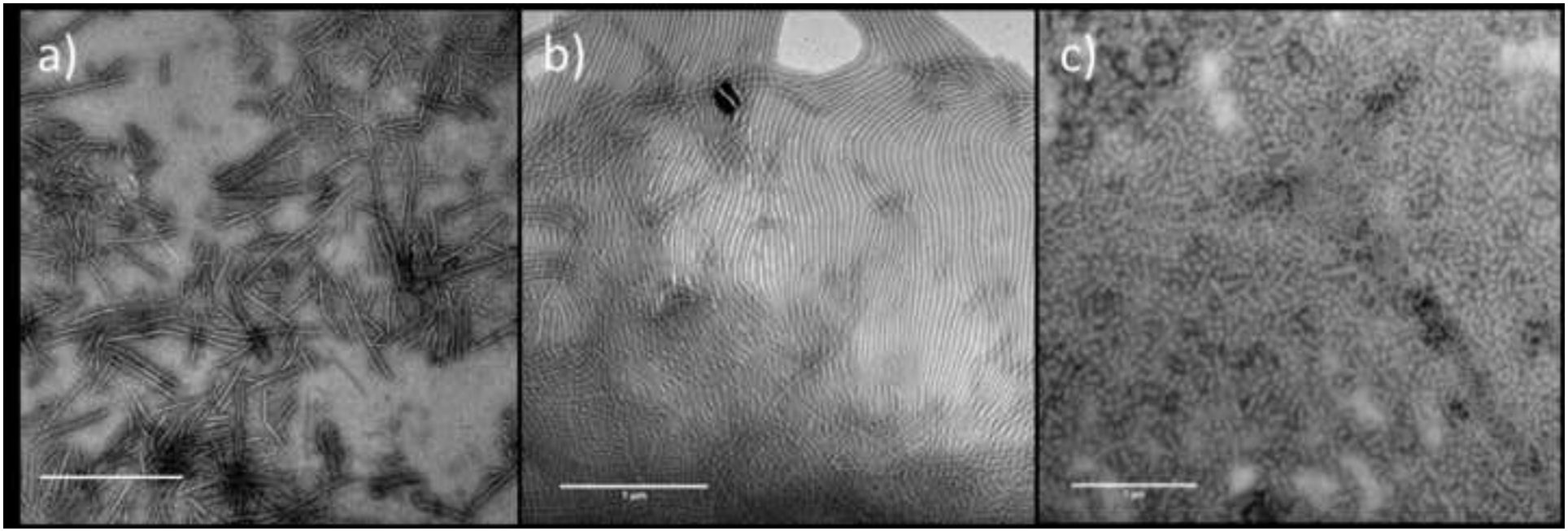
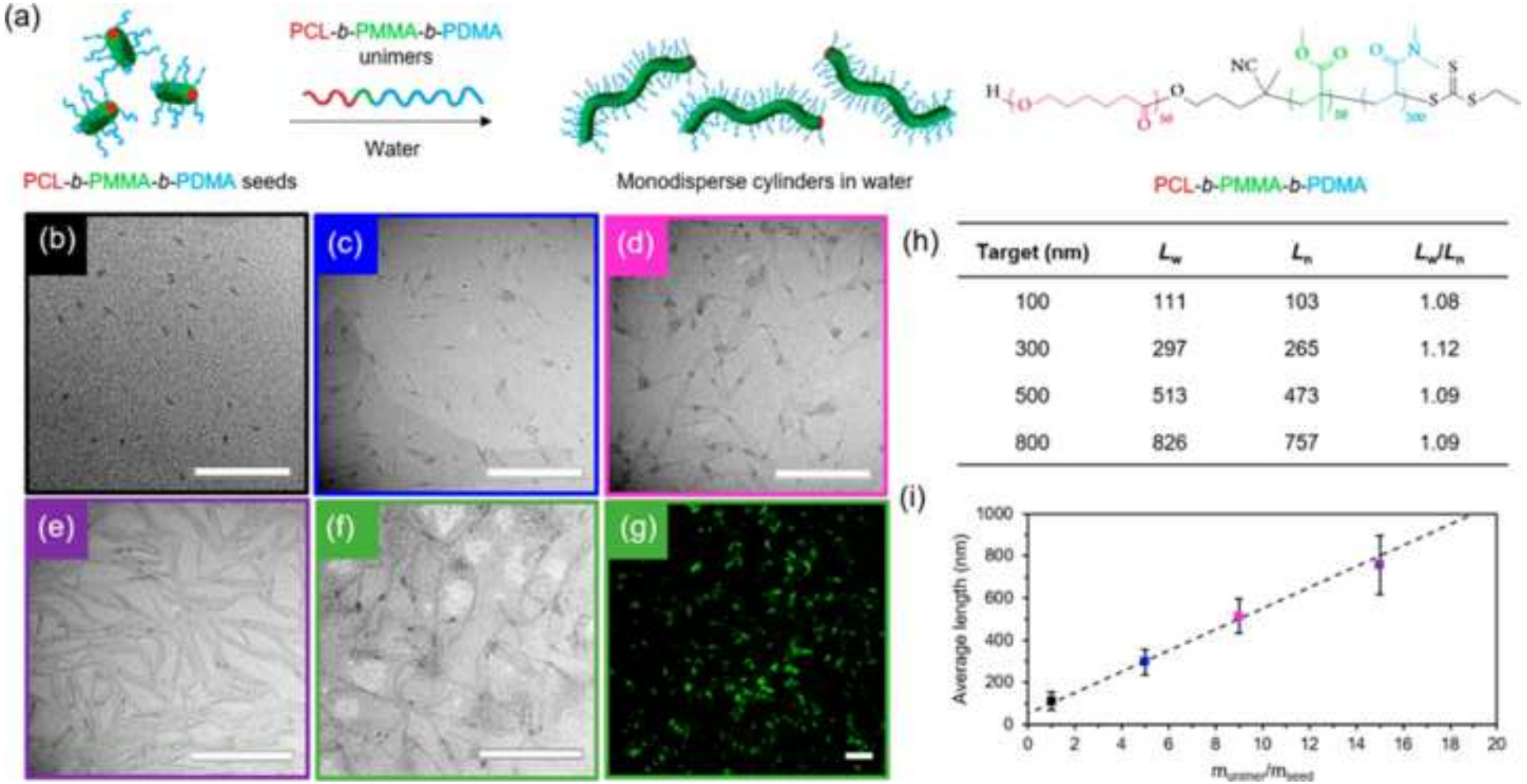


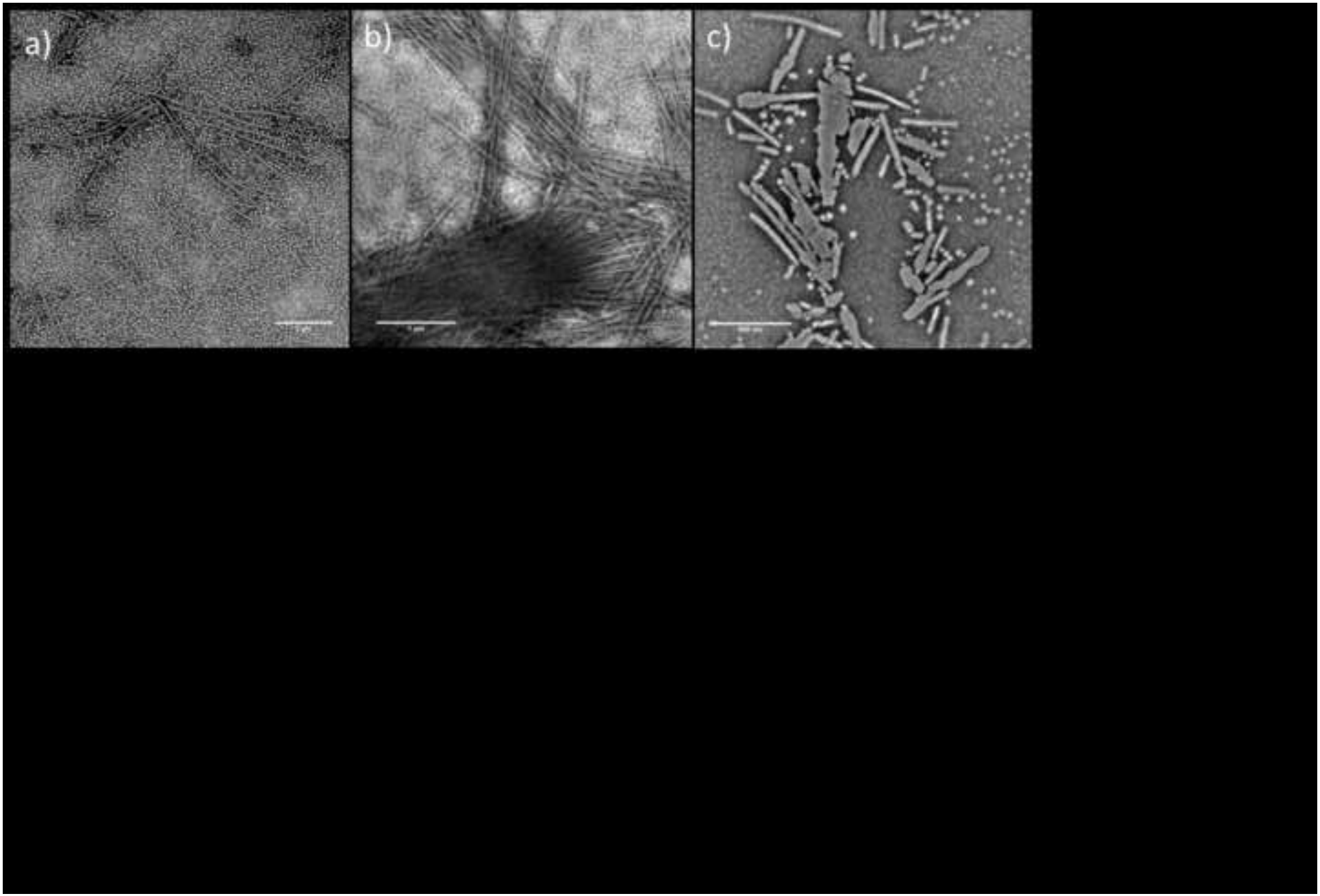
Figure 5



Target (nm)	L_w	L_n	L_w/L_n
100	111	103	1.08
300	297	265	1.12
500	513	473	1.09
800	826	757	1.09

Figure 6

Click here to access/download;Figure;Figure 6.tif



Name of Material/ Equipment	Company	Catalog Number	Comments/Description
2,2'-azobisisobutyrnitrile	Sigma Aldrich		
250 mL ampoule			
250 mL two neck RBF			
Ampoule (25 mL)			
B19 tap			
B24 stopper			
Basic Alumina	Fluka		
Buchner Flask			
Buchner Funnel			
Caclium Hydride			
Cannulae			
caprolactone	Arcos Organics		
Chain Transfer Agent	Made in House		
Conical Flask (multiple sizes)			
Dessicator			
Diethyl Ether	Merck		
Dioxane	Fisher		
diphenylphosphate	Sigma Aldrich		
Distillation Condenser			
Ethanol	Fisher		
Filter Paper (multiple sizes)			
Gel Permeation Chrmoatography Instrument	Agilent Technologies Infinity 1260 II		Running DMF at 50 °C

Glovebox	Mbraun, Unilab	
Hotplate	IKA, RCT basic	
Mercury Thermometer		
Methyl Methacrylate	Sigma Aldrich	
Molecular seives	Fisher	MS/1030/53
N,N -dimethyl acrylamide	Sigma Aldrich	
NMR spectrometer	Bruker 400 MHz	
Phosphorus pentoxide	Sigma Aldrich	
RBF (multiple sizes)		
Schlenk Cap (B24)		
Schlenk Flask (250 mL)		
Schlenk Line		
Sonication Probe	Bandelin Sonoplus	
Suba Seal (multiple sizes)		
TEM grids	EmResolutions, Formvar/carbon film 300 mesh copper	
THF	Merck	
three neck adaptor		
Toluene	Fisher	
Transmission Electron Microscope	Jeol 2100	

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Author(s):	Zachary Coe, Andrew P Dove, Rachel K O'Reilly.

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Date:	31/01/2019.

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   - Addressed

2. Please ensure that open access is not a requirement with your UK funding. You have currently only selected standard access.
   - Open Access is not a requirement and the authors will publish under standard access.

3. Please sort the Table of Materials alphabetically.
   - This has been addressed.

4. Figure 1: Please use the SI abbreviation for time: h instead of hr
   - This has been addressed.

5. Figure 2: The ordering of the panels is not intuitive: d is before c? Please revise.
   - This has been addressed.

6. Please provide email addresses for all authors.
   - Z.Coe@pgr.bham.ac.uk
   - A.C.Weems@bham.ac.uk
   - R.OReilly@bham.ac.uk
   - A.Dove@bham.ac.uk

7. Please avoid personal pronouns in the protocol.
   - These issues have been addressed.

8. Please specify any temperature requirements for the reactions.
   - ‘at room temperature’ has been specified in the ROP protocol.

9. Please provide reprint permissions for any figures that have been previously published.
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10. If there are no acknowledgments, please write as such.
    - This has been addressed.

11. Please do not abbreviate journal titles.
    - This has been addressed

Reviewers' comments:

Please note that novelty is not a requirement for publication and reviewer comments questioning the novelty of the article can be disregarded.

Reviewer #1:

1. Page 8, line 271-273: A high molecular weight shoulder was observed after polymerization of ε-CL for 12 h with proper drying (Figure 1e) and the authors attributed it to transesterification between polymer chains. Was it due to the longer polymerization time? How should we do if we would like to prepare block copolymers with high molecular weight PCL block and still a low polydispersity?

- Obviously we have not explained this point in enough detail and more clarity is needed. It is less of a polymerization time issue and more of a conversion issue. At degrees of polymerization of 50, polymerization times over 8 hours have reached very high conversions and thus transesterification starts to occur, impacting dispersity. Higher molecular weight polymers can indeed be achieved with a lower dispersity, but conversion must be kept below 95% (monitored with ^{1}H NMR spectroscopy). This point has been clarified in the main text also.

2. Why was PCL-b-PMMA-b-PDMA triblock terpolymer, instead of PCL-b-PDMA diblock copolymer, prepared? This point should be explained.

- This was overlooked and has now been addressed in the main text.

3. The references are not complete. I think that following references are highly related to this work.
For crystalline cylindrical micelles of PCL-containing BCPs:
(1) He WN, Zhou B, Xu JT, Du BY, Fan ZQ. Two growth modes of semicrystalline cylindrical poly(ε-caprolactone)-b-poly(ethylene oxide) micelles. Macromolecules, 2012, 45, 9768-9778.
(2) Rizis G, van de Ven TGM, Eisenberg A. Crystallinity-driven morphological ripening processes for poly(ethylene oxide)-block-polycaprolactone micelles in water. Soft Matter, 2014, 10, 2825-2835.
For crystalline cylindrical micelles of PE-containing BCPs:
(1) Fan B, Liu L, Li JH, Ke XX, Xu JT, Du BY, Fan ZQ. Crystallization-driven one-dimensional self-assembly of polyethylene-b-poly(tert-butylacrylate) diblock copolymers in DMF: Effects of crystallization temperature and the corona-forming block. Soft Matter, 2016, 12, 67-76.
Growth kinetics of crystalline cylindrical micelles of block copolymers:
(1) Boott CE, Leitao EM, Hayward DW, Laine RF, Mahou P, Guerin G, Winnik MA, Richardson RM, Kaminski CF, Whittell GR, Manners I. Probing the growth kinetics for the formation of uniform 1D block copolymer nanoparticles by living crystallization-driven self-assembly. ACS Nano, 2018, 12, 8920-8933.
(2) Zhang T. Y., Xu J. T. One-dimensional growth kinetics for formation of cylindrical crystalline micelles of block copolymers. Polymer Crystallization. 2018;1-12. https://doi.org/10.1002/pcr2.10047.

- The authors agree and the references have been included in appropriate places in the main text.

Reviewer #2:
Manuscript Summary:
In this manuscript, the authors described that the synthesis of a PCL-b-PMMA-b-PDMA triblock copolymer. The purpose is to examine a crystallization-induced self-assembly behavior of this triblock copolymer. In the past year, there have been a lot

of reports in this regards. This work deals with a behavior of triblock copolymer. This reviewer has the following major concerns:

Major Concerns:
The authors did not clearly tell that purpose to synthesize the ABC triblock copolymer copolymer. What are the roles of blocks, PMMA and PDMA? In the section of Introduction, the authors should add to discuss the effect of block copolymer architectures such as AB, ABA and ABC.

- The authors agree that the choice of a triblock terpolymer over a diblock copolymer was not clearly outlined and a significant amount of text describing the reasons why (the diblock cylinders fracture when transferred to water due to the large PCL surface tension, whereas the PMMA block acts as a buffer region, preventing this) has been provided.

- The authors disagree with the inclusion of block copolymer architectures due to several reasons. Firstly, this level of detail is not relevant for what we are trying to achieve with this manuscript – which is to highlight and address the synthetic issues related to CDSA. Thus a portion of text describing the physics behind CDSA is not relevant. Secondly, there are very few papers that deal with block copolymer architecture on the outcome of CDSA.

Editors Comments #2:

The authors have adjusted all points in the document except point 4. All of these points have been coherently addressed throughout the text and adjustment to the discussion would require adjustment to the whole protocol (in lieu of repeating ourselves).