Journal Name

ARTICLE

Functional Multisite Copolymer by One-Pot Sequential RAFT Copolymerization of Styrene and Maleic Anhydride

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Guillaume Moriceau,[a] Guillaume Gody,[a] Matthias Hartlieb,[a] Joby Winn,[b] HyungSoo Kim,[c] Antonio Mastrangelo,[b] Timothy Smith,[B] and Sébastien Perrier*[a,d,e]

A Multisite copolymer with functionalizable units inserted at precise locations was synthesised by one-pot Reversible Addition-Fragmentation Chain-Transfer (RAFT) polymerization and sequential Single Monomer Unit Insertion (SMUI) and Chain Extension (ChainExt) using Styrene (Sty) and Maleic Anhydride (MAnh) as comonomers. The multisite copolymer was based on a polystyrene (PSty) backbone (*c.a.* 5,700 g mol^{-1}) with MAnh units inserted locally at four positions in the backbone. First, a well-defined macroCTA (1,400 g mol^{-1} – $Đ$ = 1.07) was synthesised by optimized RAFT polymerization (high conversion, high livingness and low dispersity) of styrene (DP = 10) using industrial grade butyl-2-methyl-2-[(dodecylsulfanylthiocarbonyl)sulfanyl] propionate as chain transfer agent (CTA-Ester – 80% pure). Subsequently, the polystyrene macroCTA was used for one-pot SMUI using a small excess of MAnh monomer (DP_{target} = 1.5). The copolymer was chain extended by styrene leading to a polystyrene backbone with MAnh units (1.5 in average) located in the middle of the chain. By repeating SMUI and ChainExt, several units of MAnh were inserted locally along the polystyrene backbone (every 10 units on average) to give a functionalizable multisite copolymer ($Đ$ = 1.35). Long alkyl chains (stearyl) were added by esterification of maleic anhydride moieties to obtain branched architecture.

Introduction

With the emergence of living polymerization processes, such as controlled radical polymerization (CRP) methods, many advances have been achieved enabling the design of polymeric architectures with controlled microstructures and well-defined properties.[1-8] Nowadays, various structures such as alternating copolymers[9], multiblock copolymers[7, 10-15], graft copolymers, star-type[16], macro-cycles and macromolecular brushes[17], are accessible *via* a variety of polymerization techniques.[18-20]
Among controlled radical polymerization techniques, Reversible Addition-Fragmentation Chain-Transfer (RAFT) polymerization process appears to be the most versatile in terms of monomers choice, polymeric architectures and reaction conditions.[21-26] RAFT polymerization is a free-radical polymerization mediated by a chain transfer agent (CTA) allowing reversible activation/deactivation of the propagating radical and providing a "living" character. In this context, sequential copolymerization of two or more monomers can allow the formation of block and multiblock copolymers.[27]

Simultaneous copolymerization involving two monomers can also lead to block-like structure but generally lead to statistical, random, or alternating microstructures depending on monomers reactivity, polarity and steric hindrance. The radical copolymerization of electron donor monomers (styrene) with electron acceptor such as unsaturated cyclic anhydrides (maleic anhydride or *N*-substituted maleimides) is particularly interesting. Due to their suitable properties (low k_p compared to their high rate coefficient of cross-propagation k_{cp}) and *via* exploitation of radical addition kinetics, conventional or controlled-radical polymerizations such as RAFT polymerization of these comonomer pairs typically lead to almost perfect alternating copolymers.[28-32] This exceptional feature has been used to achieve sequence-controlled alternating copolymers,[33-36] polymer end-chain functionalization[37-39] and single monomer unit insertion.[40-46]
Lutz and co-workers[40, 41, 47, 48] have used the donor/acceptor copolymerization strategy to synthesize sequence-controlled macromolecules by NMP[4], ARGET ATRP[49] and SET-LRP.[50] They demonstrated incorporation of a large library of *N*-substituted maleimides into polystyrene (PSty) growing chains using one-pot sequential addition of various functional *N*-substituted maleimides at different times during the controlled-radical polymerization process. This approach is very promising as it is rapid and versatile. However, it is limited in terms of sequence-control because of the statistical nature of chain-growth copolymerization. One main drawback is the formation of chain-to-chain sequence defects. Also, statistical insertion of monomers results in the presence of chains without insertion,

[a] Department of Chemistry, The University of Warwick, CV4 7AL, UK
[b] Lubrizol Limited. The Knowle, Nether Lane, Hazelwood, Derbyshire DE56 4AN. UK
[c] The Lubrizol Corporation. 29400 Lakeland Blvd. Wickliffe, OH 44092, USA
[d] Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, VIC 3052, Australia.
[e] Warwick Medical School, The University of Warwick, Coventry CV4 7AL, UK
* E-mail: s.perrier@warwick.ac.uk
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

as well as chains with short alternating and/or statistical blocks.[51, 52]

To allow strict single monomer unit insertion (SMUI), an excess of acceptor monomer is required for the extension of the donor chains. However, this process implies tedious purification steps. Kallitsis *et al.* first described polystyrene chain end functionalization with an excess of maleic anhydride (MAnh) using ATRP[37] followed by Harth *et al.* by NMP.[38] McLeary *et al.* were the first to observed SMUI during alternating copolymerization of styrene (Sty) and maleic anhydride using RAFT.[53, 54] Feng and coworkers described the synthesis of miktoarm ABC star copolymers using SMUI of MAnh after a polystyrene block followed by chain extension (ChainExt) with methyl acrylate and acrylamide monomers.[42] In another approach, Stayton *et al.* described SMUI of *N*-substituted maleimide using methacrylate and acrylamide polymers as macroCTA and showed their possible extension with styrene.[44] In their study, they also mentioned the possibility to obtain multiple bioconjugation sites at defined intervals along a polymer backbone using sequential block copolymerization, opening the way to uniquely defined architectures. This new copolymer structure has been mentioned in recent publications under the name of multisite copolymer.[52, 55, 56] Recently, Dong Fu *et al.* have used this concept to produce well-defined amphiphilic polymer co-network by RAFT.[45, 57] A linear polystyrene backbone with controlled numbers of functional groups (2, 4, and 6 maleimides), precisely inserted at certain positions, was achieved. Although a synthesis of a well-defined copolymer with a high level of sequence-control was shown, the process involved many undesired purification steps.

Recently, a RAFT-based one-pot sequential addition method has been reported by our group yielding well-defined multiblock copolymers containing complex sequences.[10] By tuning RAFT parameters, full conversions were achieved and a high fraction of "living" chains was retained after each block extension, thus greatly simplifying the preparation of multiblock copolymers with number of very short blocks as high as 20 and relatively low molar mass distributions ($Đ$ < 1.4).[58, 59]

In this study, we use optimized RAFT copolymerization to produce a multisite copolymer in a one-pot process by sequential SMUI and ChainExt using styrene and maleic anhydride as comonomers (Scheme 1). RAFT polymerization was optimized (high monomer conversion, minimum initiator concentration) to synthesize a well-defined polystyrene macroCTA. Maleic anhydride was then inserted by chain extension using a small excess of monomer (1.5 equiv.).

The copolymer was chain extended by styrene leading to a polystyrene backbone with MAnh units in the middle. By repeating SMUI and ChainExt, several units of MAnh were inserted locally in polystyrene backbone (every 10 units on average) to give a functionalizable multisite copolymer. The sequential SMUI/ChainExt process combined with high conversion of each block allows for good control on the microstructure (precise insertion) while the one-pot process decreases the number of purification steps usually needed to achieve such complex architectures. However the process differ slightly from strict SMUI since 1.5 equiv. of MAnh is used, in a one-pot process, which leads to more than one unit inserted locally. Finally, esterification of each maleic anhydride unit with aliphatic alcohols was performed in order to prepare well-defined graft architectures with a controlled location and density of side chains.

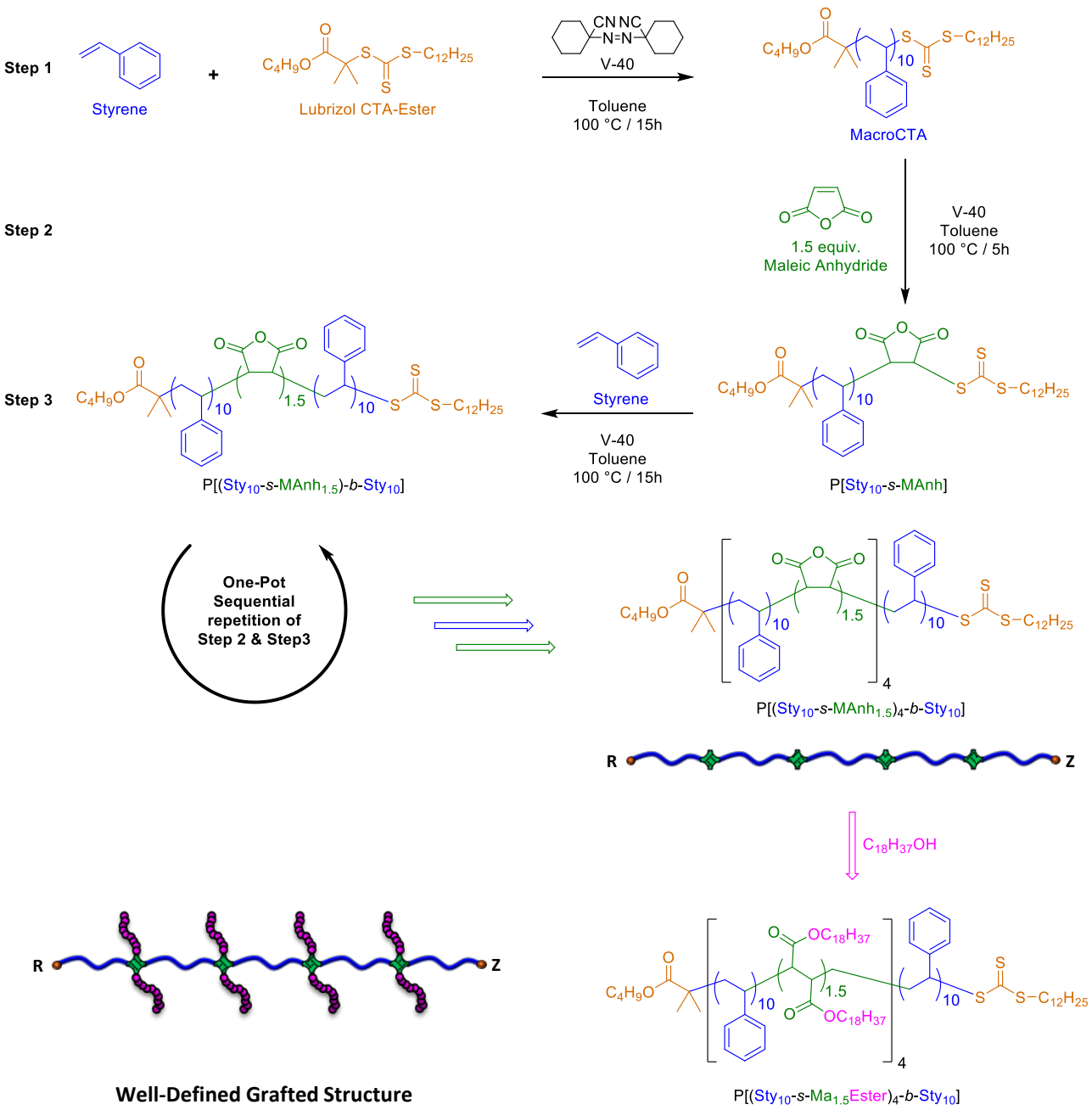
Results and discussion

Synthesis of Polystyrene MacroCTA

The industrial production of well-defined polymeric architectures such as blocks, multiblocks, or star copolymers by RAFT polymerization is a challenge and despite common monomers are commercially available most of RAFT agents are not. Butyl-2-methyl-2-[(dodecylsulfanylthiocarbonyl)sulfanyl] propionate (CTA-Ester) is one of the rare RAFT agents produced in industrial scale.[60, 61] Prior to synthesizing a multisite copolymer by one-pot sequential SMUI and ChainExt process (Scheme 1) using industrial grade CTA-Ester (80% pure), the performances of the CTA with styrene were probed by measuring the apparent chain transfer constant (C_{tr}^{app}) and by studying the kinetic of the polymerization (Supporting Information – SI-1).[62-68] The value of the C_{tr}^{app} was relatively high (C_{tr}^{app} = 28 – Figure S1) and consistent with kinetic investigation as pseudo-first order kinetic (Figure S2 - A) and a linear evolution of Mn with conversion at low dispersities (Figure S2 - B) were observed under typical experimental conditions (Table S1). These results were particularly interesting as the CTA-Ester was used without purification.

As mentioned before, careful optimization of RAFT polymerization conditions are required to achieve multiblock structures by one-pot chain extension while maintaining control over the microstructure.[10, 58, 59] Ideally, each block should be achieved with near-quantitative conversion (conv. ≥ 98%) to avoid further purification and with high degree of livingness to allow sequential chain extension (L ≥ 98%). To synthesize the first polystyrene block (macroCTA) high monomer concentration ($[Sty]_0$ = 5 M) at 100°C in toluene (Table S1) was used, a temperature and solvent allowing good rate of propagation for styrene (*i.e.* high k_p), while maintaining a low rate of monomer self-initiation compared to azo-initiator initiation.[69] Working at high monomer concentration has the advantage to allow high polymerization rate, however, the viscosity of resulting polymer can be problematic for following steps. 1,1'-Azobis(cyclohexane-1-carbonitrile) (V-40) was used as radical initiator because of its suitable decomposition rate at 100°C (10 hours half-life decomposition temperature of 88°C – 2 h at 100°C). The preliminary kinetic study (Figure S2 - A) showed that a ratio $[CTA]_0/[Initiator]_0$ = 11 leads to high conversion (conv. > 90%) within 12 h.. One key factor to achieve high polymerization rates is the generation of large amounts of radicals, by using a high initiator concentration. However, this factor also limits the livingness as shown in Eq (4)[10]. The polystyrene macroCTA was achieved with reasonable conversion (kept around 90% due to the low k_p of styrene), low

dispersity and high livingness using optimized conditions (Table 1).



Scheme 1 Schematic representation of the one-pot synthesis of a multisite copolymer by sequential single monomer unit insertion and chain extension by RAFT and functionalization of maleic anhydride units by post-polymerization esterification. As no official nomenclature exist for multisite-like structure, the letter "*-s-*" (site) was chosen here in the same way "*-b-*" is used for block copolymers.

Maleic Anhydride Single Monomer Unit Insertion by One-Pot RAFT Chain Extension

The polystyrene macroCTA was used to perform SMUI of MAnh in a one-pot fashion (Scheme 1). Preliminary attempts reaction of the macroCTA with MAnh, a small excess of MAnh was therefore used (1.5 unit per chain). While this strategy introduces defects in the microstructure (chains with slightly more than one unit), it should ensure the presence of grafting point locally inserted along the polymer backbone. Furthermore, the small excess of MAnh units introduced (0.5 (not shown) with 1 equivalent led to almost complete SMUI but residual PSty chains were observed. To ensure the complete

for a 10 DP of Sty) leads to negligible structural defects. Toluene was used to dilute the reaction mixture and to maintain a processable viscosity, whereas a small amount of dioxane was used to solubilize the MAnh prior to addition. The initiator was mixed with the

Table 1. Feature summary for polymer after each step

Entry	Monomer	DP_{targ}	$M_{n,targ}$ g mol^{-1}	% conv.$_{sty}$ [a]	$M_{n,theo}$ [a] g mol^{-1}	$M_{n,SEC}$ [b] g mol^{-1}	$Đ$ [b]	% $L_{cumul.}$ [c]
$P[Sty]_{10}$	Styrene	10	1500	93	1400	1400	1.07	96
$P[Sty_{10}\text{-}s\text{-}MAnh_{1.5}]_1$	MAnh	1.5	1600	-	-	1400	1.09	92
$P[(Sty_{10}\text{-}s\text{-}MAnh_{1.5})_1\text{-}b\text{-}PSty_{10}]$	Styrene	10	2700	97	2500	2300	1.15	89
$P[Sty_{10}\text{-}s\text{-}MAnh_{1.5}]_2$	MAnh	1.5	2800	-	-	2300	1.15	86
$P[(Sty_{10}\text{-}s\text{-}MAnh_{1.5})_2\text{-}b\text{-}PSty_{10}]$	Styrene	10	3800	92	3500	3200	1.17	82
$P[Sty_{10}\text{-}s\text{-}MAnh_{1.5}]_3$	MAnh	1.5	4000	-	-	3200	1.22	80
$P[(Sty_{10}\text{-}s\text{-}MAnh_{1.5})_3\text{-}b\text{-}PSty_{10}]$	Styrene	10	5000	99	4700	4100	1.24	75
$P[Sty_{10}\text{-}s\text{-}MAnh_{1.5}]_4$	MAnh	1.5	5200	-	-	4000	1.28	73
$P[(Sty_{10}\text{-}s\text{-}MAnh_{1.5})_4\text{-}b\text{-}PSty_{10}]$	Styrene	10	6200	89	5700	4700	1.35	68

[a] Calculated by ^{1}H NMR,[b] determined using SEC-THF with polystyrene standards,[c] cumulative livingness using eq.(4). MAnh conversion was not measurable by ^{1}H NMR as peak overlap with styrene.

monomer and added at the same time to the reaction vessel, which was heated at 100°C under a nitrogen atmosphere. The ^{1}H NMR spectrum of the macroCTA (Figure 1, A and C) shows the typical styrene backbone peak between 1.3-2.5 ppm and aromatic protons between 6.4-7.3 ppm. The peak between 4.6-5.0 ppm was due to methine proton of the styrene unit located next to the trithiocarbonate and is typical for polystyrene made by RAFT. The peaks at 0.88, 1.26, 1.65, 3.25, and between 3.4-3.6 ppm are characteristic for CTA-Ester chain end. The peak from residual styrene monomer (5.25 and 5.75 ppm) and impurities from industrial grade CTA-Ester (4.10, 3.30 and 2.65 ppm) were also observed. After SMUI (Figure 1, B and D) the signal of the methine proton of the styrene unit next to the trithiocarbonate disappeared and a new peak between 4.0-4.5 ppm appeared, corresponding to the methine proton of the maleic anhydride unit, inserted next to trithiocarbonate Feng *et al.* observed a similar peak shift after functionalizing the polystyrene chain end using MAnh and dithiobenzoate RAFT agent.[42] Thus, it was possible to monitor the SMUI yield by following the appearance/disappearance of peaks between 4.0-5.0 ppm. Figure 1D shows that after 5 h, the styrene end group was fully replaced by maleic anhydride. Unfortunately, the conversion of MAnh was not accessible by ^{1}H NMR as the vinyl peak of the cyclic anhydride (7.0 ppm) overlapped with the aromatic protons of styrene (6.4-7.3 ppm). It was also worth noticing that after SMUI the peaks corresponding to unreacted styrene (5.25 and 5.75 ppm) disappeared indicating that short alternating and / or random blocks were formed due to the presence of styrene from previous step and excess of MAnh. Figure S3 shows the SEC-RI traces of macroCTA before and after SMUI, as well as the CTA-Ester impurities. After SMUI, a small shift towards higher molar mass was observed while no change of the molecular weight distribution was detected (Table 1). The residual peak at low molecular weight was attributed to unreactive CTA-Ester impurities. The diversity of populations obtained after SMUI was also investigated by MALDI-TOF mass spectrometry. Figure S4 shows spectra of polystyrene macroCTA before (blue) and after maleic anhydride insertion (green). The spectrum obtained for polystyrene macroCTA shows one narrow main population with m/z matching with the theoretical molar mass (1,390 g mol^{-1}). The proposed structure contains the R group from the CTA-Ester, a styrene block and a double bond at ω-chain end (Figure S4 – A – structure A). The comparison between measured and calculated isotopic distribution (Figure S4 - C) was consistent with the proposed structures. Fragmentation of the Z group into a stable conjugated double bond is commonly observed for polystyrene made by RAFT and is mainly attributed to the presence of silver salt which can act as a catalyst to cleave the weak C-S bond between polymer and chain transfer agent in the mass spectrometer.[70, 71] Minor populations with similar structures but with different end-group fragmentation types were observed and are shown in supporting information (Table S2). After SMUI and ChainExt (Figure 2), a relatively clean spectrum at higher molar mass was obtained confirming successful chain extension. The spectrum showed the expected copolymer structures with one and two MAnh units (Figure 2 – structures A-D) while a signal for homopolystyrene was almost not noticeable (Figure 2 – structures E). The very weak intensity of chain extended homopolystyrene shows that SMUI were achieved with high yield. The presence of structures with two maleic anhydride units (Figure 2 – structures B) was expected and is a result of the use of 1.5 equiv. of maleic anhydride. A MALDI spectrum just after SMUI was also recorded (Figure S4 – B-D) but higher laser intensity was required to obtain similar signal strength as compared to styrene terminated fragments (*ca.* 5% higher). The addition of maleic anhydride at the end of polystyrene chains seems to make the polymer more difficult to ionize under these conditions and a more complex spectrum was obtained. Structures corresponding to the copolymer with one

and two ring-opened anhydride units as well as starting macroCTA were observed (Table S2). Structures with opened MAnh ring result of the reaction of cyclic anhydride with silver salt either during sample preparation or ionization process. The structure obtained with two MAnh is due the incomplete conversion of the first block (93%).The high intensity of the peak

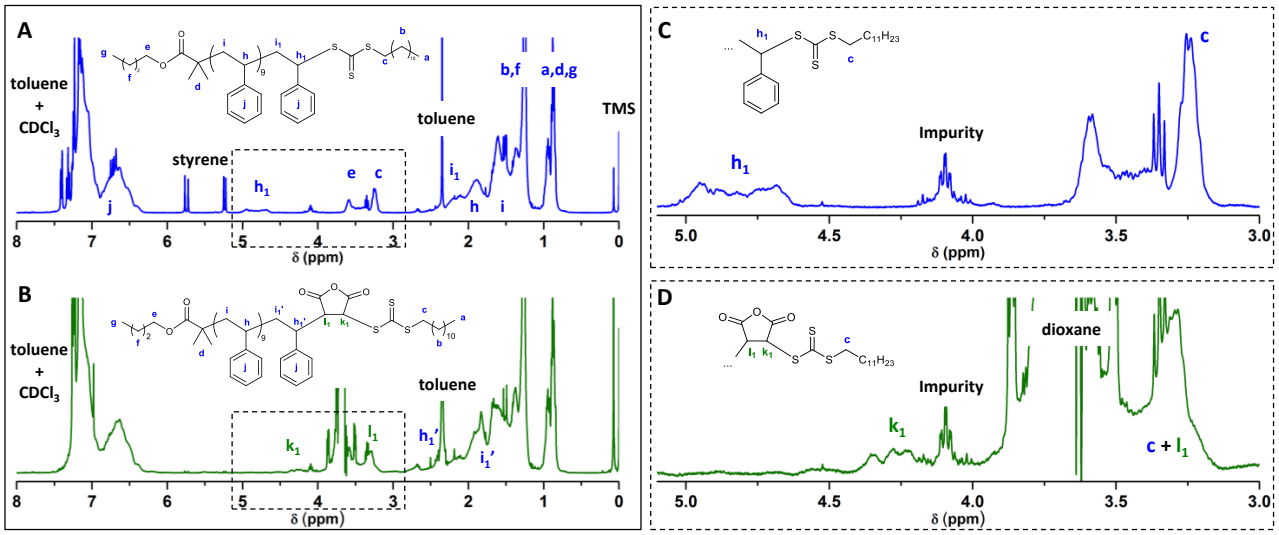


Figure 1 A and B are full ^{1}H NMR spectra ($CDCl_3$) for polystyrene macroCTA ($P[Sty]_{10}$) after 15h reaction and copolymer ($P[Sty_{10}$-*s*-$MAnh_{1.5}]_1$) after 5h of SMUI reaction respectively. C and D are zoomed spectra for A and B respectively showing the position of methine proton next to trithiocarbonate for each monomer.

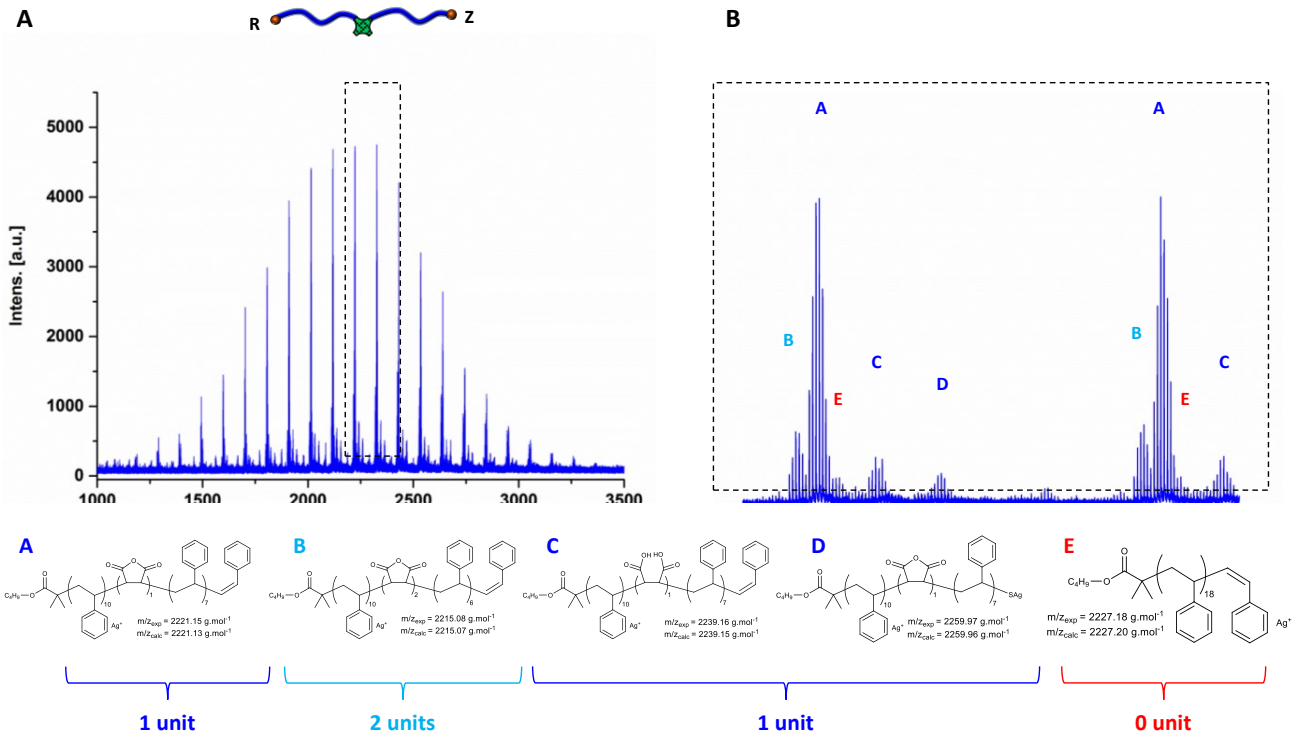


Figure 2 A) MALDI-TOF mass spectrum for poly(Sty_{10}-s-$MAnh_{1.5}$)-$PSty_{10}$. B) zoom corresponding to the region in the dashed square in A).

corresponding to residual macroCTA can be explain by the differences in ionisation process which appear to be more difficult for polymer with MAnh as terminal unit. Note that since MALDI is not a quantitative method, a residual amount of macroCTA can appear to be the main population due to it favourable ionisation process.

Multisite Copolymer Synthesis by One-Pot Sequential SMUI and ChainExt by RAFT

The copolymer poly[Sty_{10}-*s*-$MAnh_{1.5}$] was used as a macroCTA and chain extended with styrene before performing sequential SMUI and ChainExt as describe in Scheme 1. Four cycles were performed to achieve a multisite copolymer composed of a polystyrene backbone with MAnh monomers located every 10 units in average. Figure 3 shows the ^{1}H NMR spectrum of initial polystyrene macroCTA and a magnified area of spectra after each SMUI and chain extension showing the alternation of styrene block and MAnh unit. The process can be followed by the appearance/disappearance of typical peak due to the methine proton of the last styrene unit (4.6-5.1 ppm). A similar behaviour was also observed for last MAnh unit (4.0-4.5 ppm) however, it was less clear due to the overlap with CTA impurities peak (4.1 ppm). The conversion of styrene obtained after each chain extension was relatively high (> 89%) and was controlled by maintaining relatively low ratio between CTA and initiator concentrations after each step (Table S1). Dilution after each chain extension was necessary to maintain low viscosity. As a result the monomer and initiator concentration after each step was restricted, leading to limitations in conversion and livingness. Table 1 shows that after four cycles of SMUI and ChainExt the cumulative livingness had dropped to 68%. Thus, it became difficult to control the polymerization after further chain extension as only two third of chain left were still living. This is the consequence of slow propagation monomers such as styrene. The large amount of initiator required to reach high conversion by RAFT inherently increases the number of terminated chains. According to styrene conversion values, the theoretical molar mass of the final material was estimated at 5,700 g mol^{-1}. The polymer was purified by precipitation in hexane and 20 g of material were recovered (Figure S5). SEC analysis showed monomodal distributions and a clear shifts to higher molar masses after each chain extension confirming the synthesis of multiblock-like structure (Figure 4). However, a low molar mass tailing as well as high molar mass shoulders appeared after performing several cycles. The low molar mass tailing was mainly associated to the accumulation of dead chains (30% after 9 cycles) and initiator derived chains, as relatively high initiator concentrations were used in this system. The high molar mass shoulder was ascribed to bimolecular termination. The final material was recovered without CTA-Ester impurities (low molar mass peak) and with relatively narrow distribution for such complex architecture ($Đ$ = 1.35). Also, the molar mass obtained by SEC ($Mn_{,SEC}$ = 4,700 g mol^{-1}) was consistent with theoretical value calculated from NMR results. A small deviation from the theoretical value was observed and can be explain by the increase of low molar mass chains after the third insertion which results in an underestimation of the overall molar mass of final material ($Mp_{,SEC}$ = 6,000 g mol^{-1}). Also, a deviation was not surprising as the nature of the polystyrene backbone was affected by the presence of MAnh and as homopolystyrene standards were used for SEC calibration. The presence of MAnh in final material was also confirmed by FT-IR as the peaks at 1850 cm^{-1} and 1770 cm^{-1} characteristic for carbonyl stretching bands from cyclic anhydride were observed (Figure S6). An ester peak corresponding to CTA-Ester (1730 cm^{-1}) was also observed. Figure 5 shows MALDI-TOF mass spectroscopy results after each polymerization step. An increase in molar mass after each chain

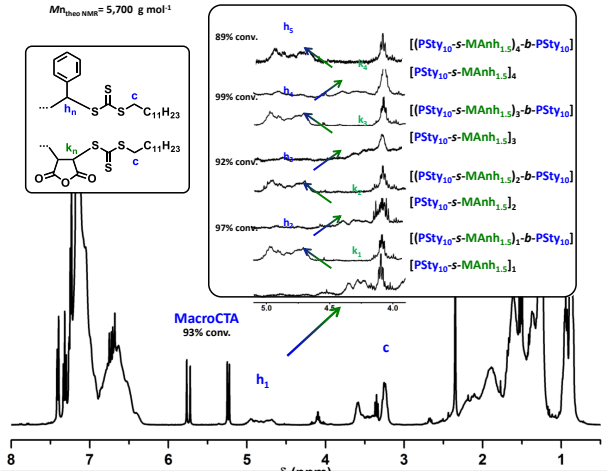


Figure 3 Sequential SMUI / ChainExt monitored by ^{1}H NMR.

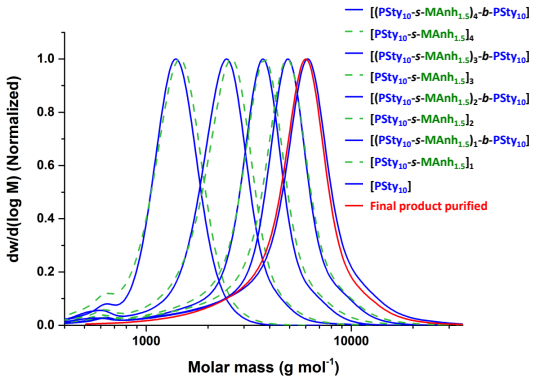


Figure 4 SEC spectrum for polystyrene macroCTA following by each step of SMUI (dashed green) and chain extension with styrene (blue). The final product after purification is shown in red. SEC in THF with polystyrene standards.

extension was observed and the final spectrum shows clearly a population between 4,000-7,000 g mol^{-1} confirming the results previously obtained by NMR and SEC. The different m/z values observed were assigned after each steps (Figure S7-S12) and structures with 3, 4, 5 and 6 MAnh units were observed for the final material. As a small excess of MAnh (1.5 equiv.) was used for each SMUI, an average of 6 units was expected after 4 cycles of SMUI and ChainExt. Thus, it was not surprising to observe structures with 4, 5 and 6 units inserted. The structure with 3 units was the results of an incomplete SMUI. However, as mention previously these defects seems marginal indicating that the SMUI were successful in majority of cases. After three cycles, strong signal corresponding to dead chains and initiator derived chains were observed at low molar mass increasing complexity of the MALDI-TOF spectra.

The high intensity for these chains compare to the final material can be explained by the difference of length. These low molar mass chains were clearly over-represented by MALDI-TOF as just a low molar mass tail was observed by SEC (Figure 4). In addition, quantitative ^{13}C NMR was attempted on the final purified copolymer and revealed the presence of approximately 6 MAnh units (Figure S14 – peak at 171.7 ppm) incorporated as short alternating blocks or SMUI (Figure S14 – zoom 135-150 ppm) [72, 73] confirming the synthesis of a multisite copolymer with the proposed structure.

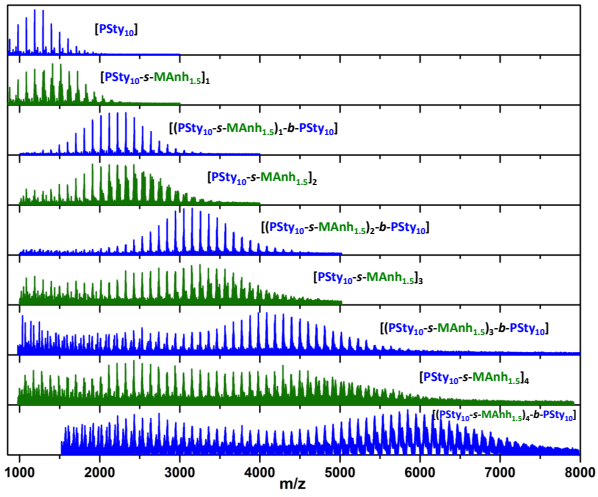


Figure 5 MALDI-TOF spectrum measured after each polymerization steps from the polystyrene macroCTA at the top to multisite copolymers after four SMUI at the bottom. The spectrum were recorded using DTCB as a matrix and AgTFA as ionization agent.

Functionalization of the Multisite Copolymer by Esterification of Maleic Anhydride Moeties

To demonstrate the potential of the described well-defined functionalizable multisite copolymer, esterification of maleic anhydride units with a long alkyl alcohol (stearyl – $C_{18}H_{37}OH$) were attempted. The incorporation of long alkyl chains might change the physical properties of the copolymer such as increasing its hydrophobicity and allowing it solubilisation in non-polar organic solvents. The esterification was performed using Dean-Stark apparatus under reflux of toluene and using methane sulfonic acid as a catalyst (MSA). The reaction of cyclic anhydride with the alcohol is fast and irreversible for the mono-esterification process, whereas esterification of the free acid is relatively slow and requires a catalyst and the elimination of water. Figure 6 shows ^{1}H NMR spectrum of the copolymer before (top) and after (bottom) esterification. A peak corresponding to methylene protons next to ester bond (3.3-4.2 ppm) clearly appeared after esterification showing that functionalization occurred. The number of alkyl chain inserted was determined by comparing the integral before and after esterification for the regions containing protons from alkyl chain (0.5-3.15ppm = 35H) and using aromatic protons from styrene as a reference. The calculated number of alkyl chains (12 per polymer chain) was consistent with a complete esterification, as an average of six maleic anhydride units were inserted in the multisite copolymer. The functionalization of all maleic anhydride moieties was also confirmed by FT-IR (Figure 7) as the peaks corresponding to cyclic anhydride disappeared (1770 and 1850 cm^{-1}) and a strong ester peak appeared (1730 cm^{-1}). SEC study showed an increase in molar mass after esterification while no change was observed for molar mass

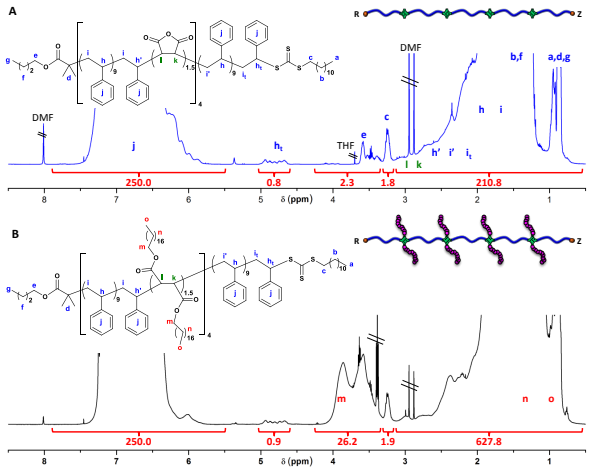


Figure 6 ^{1}H NMR spectra ($CDCl_3$) for multisite copolymer before (A) and after (B) esterification

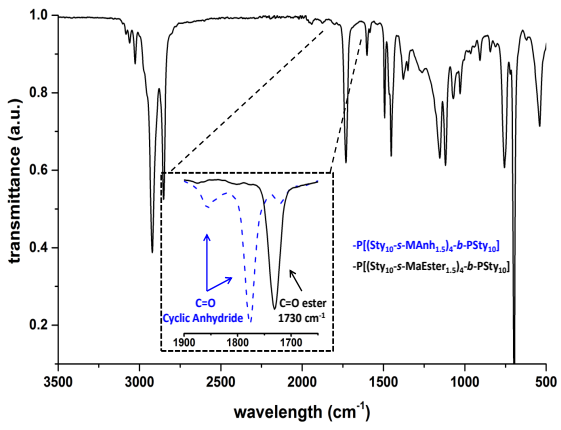


Figure 7 IR spectrum for esterified multisite copolymer.

distribution (Figure S13). MALDI-TOF mass spectroscopy was attempted but not results were obtained using previous conditions. A change in polymer properties was observed as well, as it became soluble in hexane.

Conclusion

A multisite copolymer with precisely inserted pendent alkyl chains was synthesized by one-pot RAFT polymerization process and post-polymerization functionalization. The preparation of the functionalizable multisite copolymer was achieved *via* sequential single monomer unit insertion and chain extension using styrene and maleic anhydride as comonomer and industrial grade CTA-Ester. After four cycles of SMUI and ChainExt, a polystyrene backbone with four precisely located functionalizable areas was achieved. An average of six MAnh units were inserted along the polystyrene backbone as either one local monomeric unit or as short alternating / random blocks with an overall dispersity of 1.35. The quantitative functionalization of each MAnh moieties was achieved by esterification with stearyl alcohol leading to a well-defined graft polymeric structure. By controlling the position and number of side chains, it is therefore possible to produce graft copolymers with a controllable location and density of side chains.

Experimental

Materials & Instrumentation

Styrene monomer (Sty, ≥99%) was obtained from Sigma-Aldrich and passed through neutral alumina prior to use to remove inhibitor. Maleic anhydride (MAnh, ≥99%), 1,1'-Azobis(cyclohexane-1-carbonitrile) (V-40, $T_{1/2\text{-}10h}$ = 88°C, 98%) and methane sulfonic acid (MSA, 70wt% in H_2O) were obtained from Sigma-Aldrich and used as received. Industrial grade CTA-Ester was provided by Lubrizol and used without purification (80% pure).[60, 61] Tetrahydrofuran (THF), diethyl ether and chloroform were obtained from Sigma-Aldrich and used as received. 1, 4-dioxane and toluene were obtained from Fisher Scientific and used as received. Hexane (99%) was obtained from VWR chemical. Chloroform-d (99.9% D atom) and acetone-d_6 (99.9% D atom) obtained from Sigma-Aldrich were used for ^{1}H NMR analysis.

^{1}H NMR spectra were recorded on either a Bruker DPX-300 or DPX-400 spectrometer at 27°C using deuterated solvents obtained from Sigma-Aldrich. For in-situ chain transfer constant determination, kinetics of reaction were recorded on a 600 MHz Bruker Advance instrument by real-time NMR at desired reaction temperature (90°C) and using a delay time (d1) of 25 s and a number of scans of 4. Esterification yield was determined using a Bruker AVHD500 instrument at 25°C using a delay time of 5s and a number of scans of 128. TMS contained in the solvent was used as internal standard and chemical shift values (δ) are reported in ppm.

SEC was carried out using Agilent 390-LC MDS instrument equipped with differential refractive index (DRI) and dual wavelength UV detectors. The system was equipped with 2 x PLgel mixed D columns (300 x 7.5 mm) and a PLgel 5 µm guard column and autosampler. The eluent was THF with 2 % TEA (triethylamine) and 0.01 % BHT (butylated hydroxytoluene) additives. Samples were run at 1 ml min^{-1} at 30˚C. Polystyrene standards (Agilent EasyVials) were used for calibration. Ethanol was added as a flow rate marker. Analyte samples were filtered through a PVDF membrane with 0.22 µm pore size before injection. Respectively, experimental molar mass (Mn,SEC) and dispersity (Đ) values of synthesized polymers were determined by conventional calibration using Agilent GPC/SEC software.

Matrix-Assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF-MS) was performed on a Bruker Daltonics Ultraflex in the positive ion and reflection mode using external calibration (PEG1,500 and PEG5,000). Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), were used as matrix (300 mg mL^{-1} in THF) without further purification (Sigma-Adrich). AgTFA salt was used as ionization agents (10 mg mL^{-1} in THF). Matrix, salts and polymer solution (10 mg mL^{-1} in THF) were mixed in a 1:1:1 ratio and then, 2 µL of the mixture was deposited onto the MALDI target before insertion into the ion source chamber.

Synthesis of Polystyrene MacroCTA

Chain transfer agent (2.6 g, 5 mmol), monomer (5.3 g, 50 mmol), initiator (0.11 g, 0.45 mmol) and solvent (1.2 mL) were introduced into a two-neck round bottom flash equipped with a magnetic stirrer bar, a condenser on one neck and a rubber septum on the other neck (Table S1). The solution was degassed using argon (or nitrogen) for *ca.*15 min before being placed in a thermostated oil bath set at 100°C. After overnight reaction (*i.e* 15 h) a sample was taken from polymerization medium for characterization.

Typical Synthesis for Sequential Single Monomer Unit Insertion and Chain Extension

The reactor vessel was flooded with a nitrogen stream and solvent was added for dilution. Monomer and initiator were added to polymerization medium *via* syringe (Table S1, Supporting Information, for the quantity of reagents). The reaction mixture was degassed for *ca.*15 min and allowed to polymerize at 100°C for desired reaction time. A sample was taken from polymerization medium before and after each chain extension for characterization. As MAnh is a solid hardly soluble in toluene, it was dissolved in a small amount of dioxane before addition. Importantly, the amount initiator remaining after each cycle was taken in account for following polymerization step. The final material was dissolved in minimum amount of chloroform and precipitated in hexane prior characterization and functionalization.

Functionalization of the Multisite Copolymer by Esterification of MAnh Moieties

The dried multisite copolymer (3 g, 0.5 mmol), stearyl alcohol (5 g, 18 mmol) and toluene (*ca.*17 mL) were added in a two-neck round bottom flask equipped with a dean stark apparatus. The flask was placed in a thermostated oil bath set at 130°C and keep under nitrogen flux for 3h. Then, the temperature was set at 90°C and the methane sulfonic acid catalyst (0.25 g, 1 wt%) was added by a syringe through the septum. The temperature was set at 130°C and the reaction was kept under nitrogen flux overnight. After evaporating the toluene, the mixture was dissolved using minimum amount of diethyl ether and precipitated three time in ethanol at room temperature. The precipitate was dry in oven and recovered as yellow solid.

Determination of monomer conversion.

After each extension with styrene, the conversion was estimated using styrene vinyl protons (5.74 ppm) and by comparing integrals obtained after reaction with integrals at time zero. The peak corresponding to methyl protons from CTA-Ester end-chain (0.88 ppm) was use as internal reference. Due to the overlapping between vinyl peak from the cyclic anhydride (7.0 ppm) and aromatic of styrene (6.4-7.3 ppm) the conversion of MAnh was not estimated.

Calculation of $M_{n,th}$

For the polystyrene macroCTA (homopolystyrene), the theoretical number-average molar mass ($M_{n,th}$) was calculated using eq(1):

$$M_{\mathrm{n,th}} = \mathrm{DP_{targ}} \times \mathrm{conv_{mono}} \times \mathrm{M_{mono}} + \mathrm{M_{CTA}} \quad (1)$$

where DP_{targ} is the targeted degree of polymerization, $conv_{mono}$ is the monomer conversion, M_{mono} and M_{CTA} are molar mass of monomer and CTA respectively. After SMUI, Eq(1) cannot be used (conversion not accessible by ^{1}H NMR) but as only one unit of MAnh is supposed to be inserted, the $M_{n,th}$ was evaluated using Eq(2):

$$M_{\mathrm{n,th,SMUI}} = M_{\mathrm{n,th,macroCTA}} + \mathrm{M_{MAnh}} \quad (2)$$

where $M_{n,th,SMUI}$ is the theoretical number-average molar mass after SMUI, $M_{n,th,macroCTA}$ is the theoretical number-average molar mass of polystyrene macroCTA and M_{MAnh} the molar mass of maleic anhydride. To determine the $M_{n,th}$ after further chain extension with styrene Eq(3) was used:

$$M_{\mathrm{n,th}} = \mathrm{DP_{targ}} \times \mathrm{conv_{Sty}} \times \mathrm{M_{Sty}} + M_{\mathrm{n,th,SMUI}} \quad (3)$$

Calculation of the cumulative Livingness (L_{cumul})

The fraction of living chain generated at each step was calculated using Eq(4)[10].

$$L\,(\%) = \frac{[\mathrm{CTA}]_0}{[\mathrm{CTA}]_0 + 2.f.[\mathrm{I}]_0.(1 - e^{-k_d.t}).(1 - \frac{f_c}{2})} \quad (4)$$

L is the number fraction of living chains, $[CTA]_0$ and $[I]_0$ are the initial concentration of chain transfer agent and initiator respectively. The term '2' is included as one molecule of azo-initiator generates two radicals with a certain efficiency *f* (taken as 0.5 in this study). k_d is the decomposition rate coefficient of the initiator. The term 1-*fc*/2 represents the number of chains produced by bimolecular terminations with a coupling factor *fc* (*fc* describes the preference for termination by either combination or disproportionation). As styrene terminate by combination we use *fc* = 1 whereas 0 was used for MAnh as no information about MAnh termination are reported.

The cumulative livingness at specific step was calculated by combining the fraction of living chain generated after at each step.

Acknowledgements

We gratefully acknowledge the University of Warwick and the company Lubrizol for supporting this research. We thank the company Lubrizol for providing CTA-Ester and for sponsoring the project. MH gratefully acknowledges the German Research Foundation (DFG, GZ: HA 7725/1-1) for funding.

References

1 Matyjaszewski, K. *Prog. Polym. Sci.* 2005, **30**, 858-875.
2 Matyjaszewski, K. *Science* 2011, **333**, 1104-1105.
3 Szwarc, M. *J. Polym. Sci., Part A: Polym. Chem.* 1998, **36**, 9-15.
4 Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* 2001, **101**, 3661-3688.
5 Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* 2001, **101**, 3689-3746.
6 Moad, G.; Rizzardo, E.; Thang, S. H. *Polymer* 2008, **49**, 1079-1131.
7 Bates, F. S.; Hillmyer, M. A.; Lodge, T. P.; Bates, C. M.; Delaney, K. T.; Fredrickson, G. H. *Science* 2012, **336**, 434-440.
8 Lutz, J.-F.; Ouchi, M.; Liu, D. R.; Sawamoto, M. *Science* 2013, **341**.
9 Cowie, J. M. G., Springer Science & Business Media: 2013.
10 Gody, G.; Maschmeyer, T.; Zetterlund, P. B.; Perrier, S. *Nat Commun* 2013, **4**.
11 Martin, L.; Gody, G.; Perrier, S. *Polym. Chem.* 2015, **6**, 4875-4886.
12 Zhang, J.; Gody, G.; Hartlieb, M.; Catrouillet, S.; Moffat, J.; Perrier, S. *Macromolecules* 2016.
13 Engelis, N. G.; Anastasaki, A.; Nurumbetov, G.; Truong, N. P.; Nikolaou, V.; Shegiwal, A.; Whittaker, M. R.; Davis, T. P.; Haddleton, D. M. *Nat Chem* 2017, **9**, 171-178.
14 Anastasaki, A.; Nikolaou, V.; Pappas, G. S.; Zhang, Q.; Wan, C.; Wilson, P.; Davis, T. P.; Whittaker, M. R.; Haddleton, D. M. *Chemical Science* 2014, **5**, 3536-3542.
15 Chuang, Y.-M.; Ethirajan, A.; Junkers, T. *ACS Macro Letters* 2014, **3**, 732-737.
16 Ren, J. M.; McKenzie, T. G.; Fu, Q.; Wong, E. H. H.; Xu, J.; An, Z.; Shanmugam, S.; Davis, T. P.; Boyer, C.; Qiao, G. G. *Chem. Rev.* 2016, **116**, 6743-6836.
17 Sheiko, S. S.; Sumerlin, B. S.; Matyjaszewski, K. *Prog. Polym. Sci.* 2008, **33**, 759-785.
18 Davis, K. A.; Matyjaszewski, K., Springer Heidelberg: Berlin, 2002; 1-13.
19 Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Mays, J. *Prog. Polym. Sci.* 2006, **31**, 1068-1132.
20 Gregory, A.; Stenzel, M. H. *Prog. Polym. Sci.* 2012, **37**, 38-105.

21 Goto, A.; Fukuda, T. *Prog. Polym. Sci.* 2004, **29**, 329-385.
22 Braunecker, W. A.; Matyjaszewski, K. *Prog. Polym. Sci.* 2007, **32**, 93-146.
23 Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* 1998, **31**, 5559-5562.
24 Le, T.; Moad, G.; Rizzardo, E.; Thang, S., Chem. Abstr, 1998; 1998.
25 Perrier, S.; Takolpuckdee, P. *J. Polym. Sci., Part A: Polym. Chem.* 2005, **43**, 5347-5393.
26 Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* 2009, **62**, 1402-1472.
27 Gody, G.; Barbey, R.; Danial, M.; Perrier, S. *Polym. Chem.* 2015, **6**, 1502-1511.
28 Alfrey, T.; Lavin, E. *J. Am. Chem. Soc.* 1945, **67**, 2044-2045.
29 Tsuchida, E.; Tomono, T. *Makro. Ch.* 1971, **141**, 265-298.
30 Jenkins, A. D. *Brit. Polym. J.* 1987, **19**, 91-91.
31 Matsumoto, A.; Kubota, T.; Otsu, T. *Macromolecules* 1990, **23**, 4508-4513.
32 Rzaev, Z. M. O. *Prog. Polym. Sci.* 2000, **25**, 163-217.
33 De Brouwer, H.; Schellekens, M. A. J.; Klumperman, B.; Monteiro, M. J.; German, A. L. *J. Polym. Sci., Part A: Polym. Chem.* 2000, **38**, 3596-3603.
34 Chen, G.-Q.; Wu, Z.-Q.; Wu, J.-R.; Li, Z.-C.; Li, F.-M. *Macromolecules* 1999, **33**, 232-234.
35 Lutz, J.-F.; Kirci, B.; Matyjaszewski, K. *Macromolecules* 2003, **36**, 3136-3145.
36 Benoit, D.; Hawker, C. J.; Huang, E. E.; Lin, Z.; Russell, T. P. *Macromolecules* 2000, **33**, 1505-1507.
37 Koulouri, E. G.; Kallitsis, J. K.; Hadziioannou, G. *Macromolecules* 1999, **32**, 6242-6248.
38 Harth, E.; Hawker, C. J.; Fan, W.; Waymouth, R. M. *Macromolecules* 2001, **34**, 3856-3862.
39 Sasso, B.; Dobinson, M.; Hodge, P.; Wear, T. *Macromolecules* 2010, **43**, 7453-7464.
40 Pfeifer, S.; Lutz, J.-F. *Chem. Eur. J.* 2008, **14**, 10949-10957.
41 Pfeifer, S.; Lutz, J.-F. *J. Am. Chem. Soc.* 2007, **129**, 9542-9543.
42 Feng, X.-S.; Pan, C.-Y. *Macromolecules* 2002, **35**, 4888-4893.
43 Shi, G.-Y.; Tang, X.-Z.; Pan, C.-Y. *J. Polym. Sci., Part A: Polym. Chem.* 2008, **46**, 2390-2401.
44 Henry, S. M.; Convertine, A. J.; Benoit, D. S. W.; Hoffman, A. S.; Stayton, P. S. *Bioconjugate Chem.* 2009, **20**, 1122-1128.
45 Zhou, C.; Qian, S.; Zhang, A.; Xu, L.; Zhu, J.; Cheng, Z.; Kang, E.-T.; Yao, F.; Fu, G. D. *RSC Advances* 2014, **4**, 8144-8156.
46 Xu, J.; Fu, C.; Shanmugam, S.; Hawker, C. J.; Moad, G.; Boyer, C. *Angew. Chem.* 2016, n/a-n/a.
47 Zamfir, M.; Lutz, J.-F. *Nat Commun* 2012, 1138.
48 Lutz, J.-F.; Schmidt, B. V. K. J.; Pfeifer, S. *Macromol. Rapid Commun.* 2011, **32**, 127-135.
49 Jakubowski, W.; Min, K.; Matyjaszewski, K. *Macromolecules* 2006, **39**, 39-45.
50 Anastasaki, A.; Nikolaou, V.; Nurumbetov, G.; Wilson, P.; Kempe, K.; Quinn, J. F.; Davis, T. P.; Whittaker, M. R.; Haddleton, D. M. *Chem. Rev.* 2016, **116**, 835-877.
51 Lutz, J.-F. *Acc. Chem. Res.* 2013, **46**, 2696-2705.
52 Gody, G.; Zetterlund, P. B.; Perrier, S.; Harrisson, S. *Nat Commun* 2016, **7**.
53 McLeary, J. B.; Calitz, F. M.; McKenzie, J. M.; Tonge, M. P.; Sanderson, R. D.; Klumperman, B. *Macromolecules* 2004, **37**, 2383-2394.
54 van den Dungen, E. T. A.; Rinquest, J.; Pretorius, N. O.; McKenzie, J. M.; McLeary, J. B.; Sanderson, R. D.; Klumperman, B. *Aust. J. Chem.* 2006, **59**, 742-748.
55 Lutz, J.-F. *ACS Macro Letters* 2014, **3**, 1020-1023.
56 Rowan, S. J.; Barner-Kowollik, C.; Klumperman, B.; Gaspard, P.; Grubbs, R. B.; Hillmyer, M. A.; Hutchings, L. R.; Mahanthappa, M. K.; Moatsou, D.; O'Reilly, R. K.; Ouchi, M.; Sawamoto, M.; Lodge, T. P. *ACS Macro Letters* 2016, **5**, 1-3.
57 Zhou, C.; Deng, L.; Yao, F.; Xu, L.; Zhou, J.; Fu, G. D. *Ind. Eng. Chem. Res.* 2014, **53**, 19239-19248.
58 Gody, G.; Maschmeyer, T.; Zetterlund, P. B.; Perrier, S. *Macromolecules* 2014, **47**, 3451-3460.
59 Gody, G.; Maschmeyer, T.; Zetterlund, P. B.; Perrier, S. *Macromolecules* 2014, **47**, 639-649.
60 Brzytwa, A. J.; Johnson, J. *Polymer Preprints* 2011, **52**, 533.
61 Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* 2002, **35**, 6754-6756.
62 Moad, G.; Chiefari, J.; Mayadunne, R. T.; Moad, C. L.; Postma, A.; Rizzardo, E.; Thang, S. H., Macromol. Symp., 2002; Wiley-Blackwell, USA: 2002.
63 Chong, Y. K.; Krstina, J.; Le, T. P. T.; Moad, G.; Postma, A.; Rizzardo, E.; Thang, S. H. *Macromolecules* 2003, **36**, 2256-2272.
64 Chiefari, J.; Mayadunne, R. T. A.; Moad, C. L.; Moad, G.; Rizzardo, E.; Postma, A.; Thang, S. H. *Macromolecules* 2003, **36**, 2273-2283.
65 Moad, G.; Chiefari, J.; Chong, Y. K.; Krstina, J.; Mayadunne, R. T. A.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polym. Int.* 2000, **49**, 993-1001.
66 Walling, C. *J. Am. Chem. Soc.* 1948, **70**, 2561-2564.
67 Destarac, M. *Polymer Reviews* 2011, **51**, 163-187.
68 Adamy, M.; van Herk, A. M.; Destarac, M.; Monteiro, M. J. *Macromolecules* 2003, **36**, 2293-2301.
69 Khuong, K. S.; Jones, W. H.; Pryor, W. A.; Houk, K. N. *J. Am. Chem. Soc.* 2005, **127**, 1265-1277.
70 Ladavière, C.; Lacroix-Desmazes, P.; Delolme, F. *Macromolecules* 2009, **42**, 70-84.
71 Gruendling, T.; Hart-Smith, G.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *Macromolecules* 2008, **41**, 1966-1971.
72 Ha, N. T. H. *Polymer* 1999, **40**, 1081-1086.
Barron, P. F.; Hill, D. J. T.; O'Donnell, J. H.; O'Sullivan, P. W. *Macromolecules* 1984, **17**, 1967-1972.