Dual stimuli triggerable degradation of graft copolymers

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Dual stimuli triggerable degradation of graft copolymers

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Here we report one-pot stimuli-responsive tandem degradation of a graft copolymer with alternating backbone functionality. A well-defined polymeric species is formed by selectively cleaving one of the structural repeat units in the mainchain, which can be further 'cut' in half by cleaving the second structural unit. The high selectivity of these two orthogonal steps allows nondescrimation of the order of the stimuli employed.

Over the last century the use of synthetic polymers has risen in a variety of applications, from commodity plastics to drug delivery, owing to the tuneable properties of these polymers with synthetic design. One of the increasing issues is the non-degradable feature that is common to most synthetic polymers, leading to plastic pollution and health-related concerns. For this reason, research in designing degradable polymers has gained much attraction.[1] Moreover, employing on-demand degradable functionality that is selective towards a specific stimulus, such as light,[2] pH[3], chemical,[4] and temperature,[5] has led to a new generation of environmentally responsive polymers.[6, 7]

In the literature, degradable polymers synthesized by controlled radical polymerization (CRP) techniques, such as nitroxide-mediated polymerization (NMP),[8] atom-transfer radical polymerization (ATRP),[9] and reversible addition fragmentation chain transfer (RAFT),[10] have only achieved limited incorporation of degradable units throughout the polymer backbone,[11, 12] typically via creative design of initiators and/or specific backbones. For example, disulfide containing difunctional initiators have been implemented to synthesize block copolymers via ATRP that can subsequently be degraded

by cleavage of the disulfide bond, resulting in polymers having half the molecular weight of the original species.[13] In another example, Luo et al. prepared multiblock polymers with enzyme degradable units between the blocks, by clicking together diazide peptide and telechelic alkyne functional RAFT polymer.[14]

More recently, we reported RAFT step-growth polymerisation [15] that combines the user-friendly nature and high functional group tolerance of RAFT polymerisation with versatility in polymer backbone functionality of step-growth, thus providing access to highly functional polymer backbone in a facile manner.[15-20] RAFT step-growth polymerisation is typically carried out by using stoichiometrically equivalent bifunctional monomer and chain transfer agent (CTA) reagents that can undergo efficient Single Unit Monomer Insertion (SUMI) process. Additionally, the *in situ* generated pendant RAFT agents along the backbone can be directly used to graft polymeric side chains via RAFT chain-growth polymerization, permitting the synthesis of graft copolymers with unique step-growth backbone functionality.[15-18] A key advantage to $A_2 + B_2$ RAFT step-growth polymers is the integration of degradable linkers into the backbone by either the monomer or CTA units.[15, 17, 18] As a result, degradation of the graft-copolymer can be triggered throughout the polymer backbone, revealing a unimolecular species with a narrow molecular weight distribution (**Scheme 1A**).[15, 17, 18]

Materials that can respond to more than one stimulus are becoming increasingly desirable for certain applications such as drug delivery where drug release kinetics can be optimized towards targeted sites,[21-23] yet there are relatively few reported examples of polymers where two or more orthogonally degradable functionalities are incorporated into the polymer backbone. In one such example, Chang et al. demonstrated dual stimuli responsive sequential degradation of a terpolymer containing both disulfide and phosphoester moieties prepared via ring opening metathesis polymerization (ROMP).[24]

Since $A_2 + B_2$ step-growth polymerization can allow alternating sequence of functionalities to be embedded along

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Scheme 1: Dual stimuli triggerable graft copolymer in this work.

A) Previous work: stimuli-triggered backbone cleavage of graft copolymers
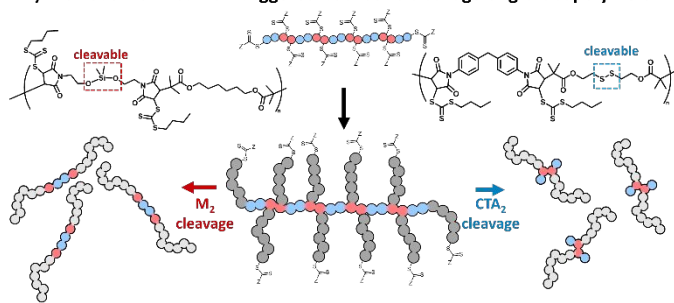
B) This work: dual stimuli-triggered backbone cleavage of graft copolymers
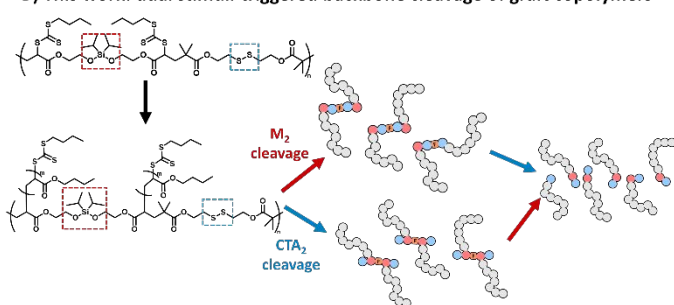
the polymer backbone, we hypothesized that $A_2 + B_2$ RAFT step-growth could employ two orthogonally degradable functionalities, individually embedded in the bifunctional monomer and CTA reagents, to achieve orthogonal degradation. Furthermore, by grafting from this backbone we could prepare dual stimuli responsive graft copolymer that can selectively degrade with the desired linker, allowing for tuneability and tandem degradation with high level of precision (**Scheme 1B**).

To demonstrate this concept, we chose to employ silyl ether and disulfides as orthogonally degradable functionalities (**Scheme 1B**). Following closely to our previous report, the disulfide functionality was incorporated into the bifunctional CTA (CTA_{2SS}) and silyl ether into the bifunctional monomer (M_2). In contrast to our previous report, we selected acrylate as the monomer unit, as it can be readily synthesized compared to bis-maleimides. Additionally, a more sterically substituted diisopropyl silyl ether was selected to prevent inadvertent hydrolysis that was observed in our previous report with less sterically substituted dimethyl silyl ether.[25]

We first investigated the synthesis of the backbone using thermal-initiated RAFT step-growth between the diisopropyl silyl ether tethered diacrylate monomer (M_2) and the disulfide tethered RAFT agent (CTA_{2SS}) (**Figure 1**). We employed stoichiometrically balanced reaction conditions for diacrylates in dioxane and using AIBN as the initiator at 70 °C as previously reported ([CTA_{2SS}]$_0$:[M_2]$_0$:[AIBN]$_0$ = 1.0 M : 1.0 M : 0.05 M).[17] ^{1}H-NMR was used to determine monomer conversion and SEC analysis to determine the molecular weights relative to polystyrene standards in THF.[17] The polymerization proceeded nicely reaching modest molecular weight and conversion after 4.0 hours (p = 93 %, M_w = 12.2k) (**Table S1**, **Figure S2-S3**, and **Figure 1B**). Furthermore, the polymerization followed the

expected step growth molecular weight evolution (M_n, M_w, and M_z) with conversion as predicted by Flory's equations (**Figure 1A**).[26]

We next employed our backbone with the embedded silyl ether and disulfide moieties (P(CTA_{2SS}-*alt*-M_2)) to graft poly(butyl acrylate) (PBA) via RAFT polymerization with a monomer to CTA functionality ratio of 40 ([BA]$_0$/[CTA]$_0$ = 40) in dioxane ([BA]$_0$ = 3.0 M). Using AIBN as the thermal initiator ([CTA]$_0$/[AIBN]$_0$ = 20) at 65 °C, the reaction proceeded to 84 % monomer conversion after 4.0 hours, yielding a theoretical M_n of about 4.8k per PBA sidechain (**Figure S4-S5**). A visible shift in the SEC trace of the graft copolymer towards lower retention time from the precursor backbone was observed, consistent with the formation of the graft copolymer (**Figure 2B** and **Figure S6**). Moreover, the absolute M_n from the light scattering detector was consistent with the theoretical M_n calculated from the number of structural repeat units approximated from the absolute M_n of the precursor backbone, and the theoretical M_n per PBA sidechain (4.8k). Additionally, the Mark-Houwink plot by triple detection SEC (dRI, LS, VS) analysis (**Figure S6**) of both the isolated backbone and graft copolymer reveals α values of 0.634 and 0.479, respectively (**Figure 2A**). An α value of 0.634 is consistent with the molecular weight distribution for linear polymers (where typical α values range from 0.5 to 0.8), and a significant decrease in alpha value for the graft copolymer (α = 0.479) confirms the shift to a branched conformation (**Figure 2A**).[27]

We next screened various conditions to target selective cleavage of the silyl ether and disulfide groups embedded in the mainchain of the graft copolymer backbone, using conventional SEC analysis to characterize the degraded polymers. Indeed, selectively cleaving only one of the structural units along an alternating step-growth backbone will result in uniform species that consists of two polymeric side chains. We anticipated that the efficiency of each cleavage may strongly depend on the solvent; therefore we investigated two different solvents for
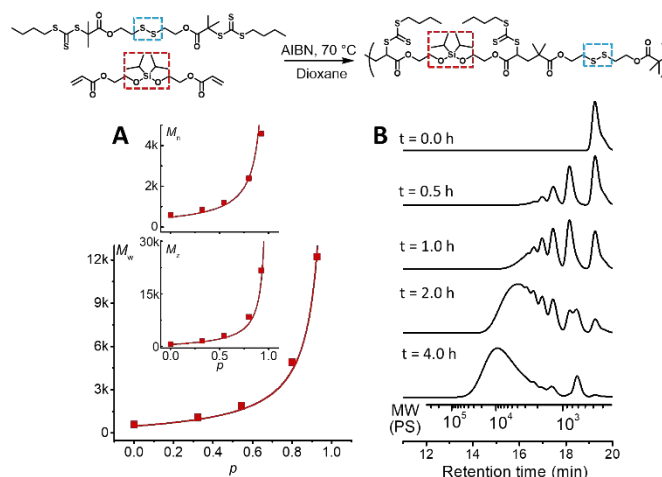
Figure 1: $A_2 + B_2$ RAFT step-growth polymerization. (A) Evolution of the molecular weight averages (M_w, M_n, and M_z) from SEC analysis using polystyrene calibration, plotted with monomer conversion (p) determined by ^{1}H-NMR spectroscopy. These are plotted with the theoretical line for step-growth molecular weight evolution that assumes no cyclization.[26] (B) THF-SEC (normalized dRI) analysis of RAFT step-growth polymerization. The molecular weight is relative to polystyrene calibration.
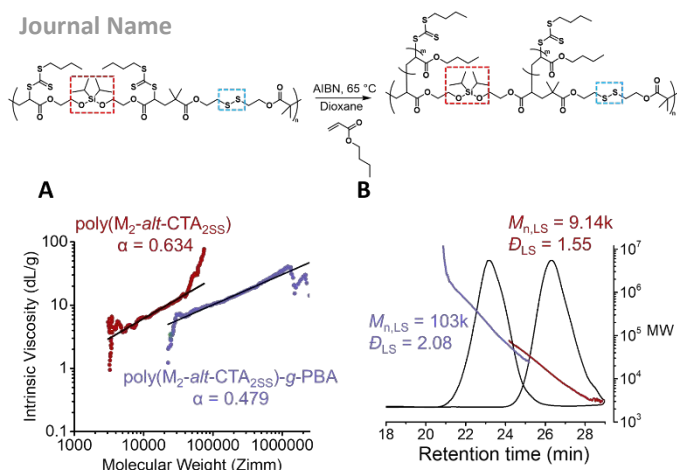
Figure 2: Graft polymerization of RAFT step-growth backbone via RAFT. (A) Mark-Houwink plot for degradable RAFT step-growth backbone and graft copolymer, the slope is determined from linear regression across the same range of data points used in molecular weight analysis (red-line in Figure S6). (B) LS-SEC of P(M$_2$-*alt*-CTA$_{2SS}$) and P(M$_2$-*alt*-CTA$_{2SS}$)-*g*-PBA.

each reaction, butanol as a protic solvent and THF as an aprotic solvent.

We first examined the selective cleavage of the silyl ether groups using a fluoride source.[28] Experimentally, we employed 20 equivalence of tetra-*n*-butylammonium fluoride (TBAF) and acetic acid (AcOH) relative to the silyl ether units.[15] We observed successful selective cleavage of the graft copolymer backbone – resulting from selective cleavage of the silyl ether – after 2.0 hours in THF (M_n = 14k, Đ = 1.09). By contrast, partial cleavage of the graft copolymer backbone was observed after 3.0 hours in butanol (M_n = 36k, Đ = 1.44, **Figure S7**). We attributed this solvent-dependent behaviour to the reaction mechanism which likely proceeds through the SN$_2$ pathway, and favours aprotic solvents.[29]

We next screened the appropriate conditions to selectively degrade the disulfide bond using redox chemistry. Here, we used 20 equivalence tributyl phosphine (PBu$_3$) with respect to the disulfide bond.[17] Though mechanistically the reduction of the disulfide with phosphines has been reported to proceed through the SN$_2$ pathway, a proton source is inherently required for the reduction.[30] Typically the presence of water is necessary to drive the reaction, though previously we found the reduction could proceed to completion in alcoholic solvents without the addition of water.[15] Here we found selective cleavage of the disulfide in butanol to reach completion in under 1.5 hours (M_n = 14k, Đ = 1.13). It is important to emphasize the silyl ether did not undergo metathesis with this solvent (butanol) and remained intact in the polymer backbone during this process. Interestingly, reduction of the disulfide was also observed in THF, albeit to a lesser extent (M_n = 16k, Đ = 1.39 after 2.0 hours, **Figure S8**) despite the lack of proton source. We believe this is likely due to the residual methanol present from the purification process as methanol was used to precipitate the PBA graft copolymer. Nevertheless, employing protic solvent was necessary for complete cleavage of the disulfide bonds.

Using these conditions we identified above, we investigated one-pot, two step tandem degradation of the graft copolymer by first employing 20 equivalence TBAF/AcOH in THF, giving a unimodal molecular weight distribution with a narrow dispersity corresponding to two PBA side chains tethered by a disulfide bond (M_n = 14.9k, Đ = 1.11, Figure 3A). As protic solvent was necessary for the second degradation step, THF was removed under reduced pressure and replaced with butanol prior to the addition of PBu$_3$ (20 eq). Pleasingly, this resulted in a noticeable shift in the SEC chromatogram corresponding to one PBA side chain (M_n = 7.6k, Đ = 1.07, **Figure 3A**).

To further demonstrate the versatility of our dual stimuli triggerable degradation of the graft copolymer backbone, we carried out the tandem degradation in the reverse order to above. Specifically, cleaving the disulfide bonds with PBu$_3$ (20 eq) in butanol (M_n = 12.7k, Đ = 1.18, **Figure 3B**) followed by cleaving the silyl ether units with TBAF/AcOH (20 eq) in THF (M_n = 6.3k, Đ = 1.18, **Figure 3B**). It is worth noting that, in practice removing butanol is more challenging than THF due to its higher boiling point; nonetheless it can be removed by blowing compressed gas on the surface of the solution. Here we used argon to prevent inadvertent oxidation of the liberated thiols during the solvent removal. In summary, we successfully demonstrated that tandem degradation of the alternating cleavable functionality embedded in the mainchain of the graft copolymer backbone can be performed sequentially independent of the order.

Furthermore, to improve atom efficiency we examined the selective degradation conditions with reduced equivalency of the reagents. We found 10 equivalences of TBAF/AcOH in THF was sufficient to fully cleave the silyl ether units within 2 hours (M_n = 14.9k, Đ = 1.12), whilst further lowering the equivalence to 5 equivalence required longer reaction time of 48 hours (M_n = 14.6k, Đ = 1.07, **Figure S9**). Additionally, we found 10 equivalences of PBu$_3$ in butanol was also sufficient to fully cleave the disulfide units within 2.0 hours (Mn = 14.1k, Đ = 1.13, **Figure S10**).

Finally, combining these two new conditions, we examined one-pot tandem degradation with reduced equivalences. Pleasingly, 10 equivalence TBAF/AcOH in THF followed by 10 equivalence PBu$_3$ in butanol revealed unimolecular species with narrow dispersity corresponding to two PBA side chains (M_n = 14.9k, Đ = 1.12, **Figure S11**) and one PBA side chain (M_n = 7.6k, Đ = 1.07, **Figure S11**), respectively.
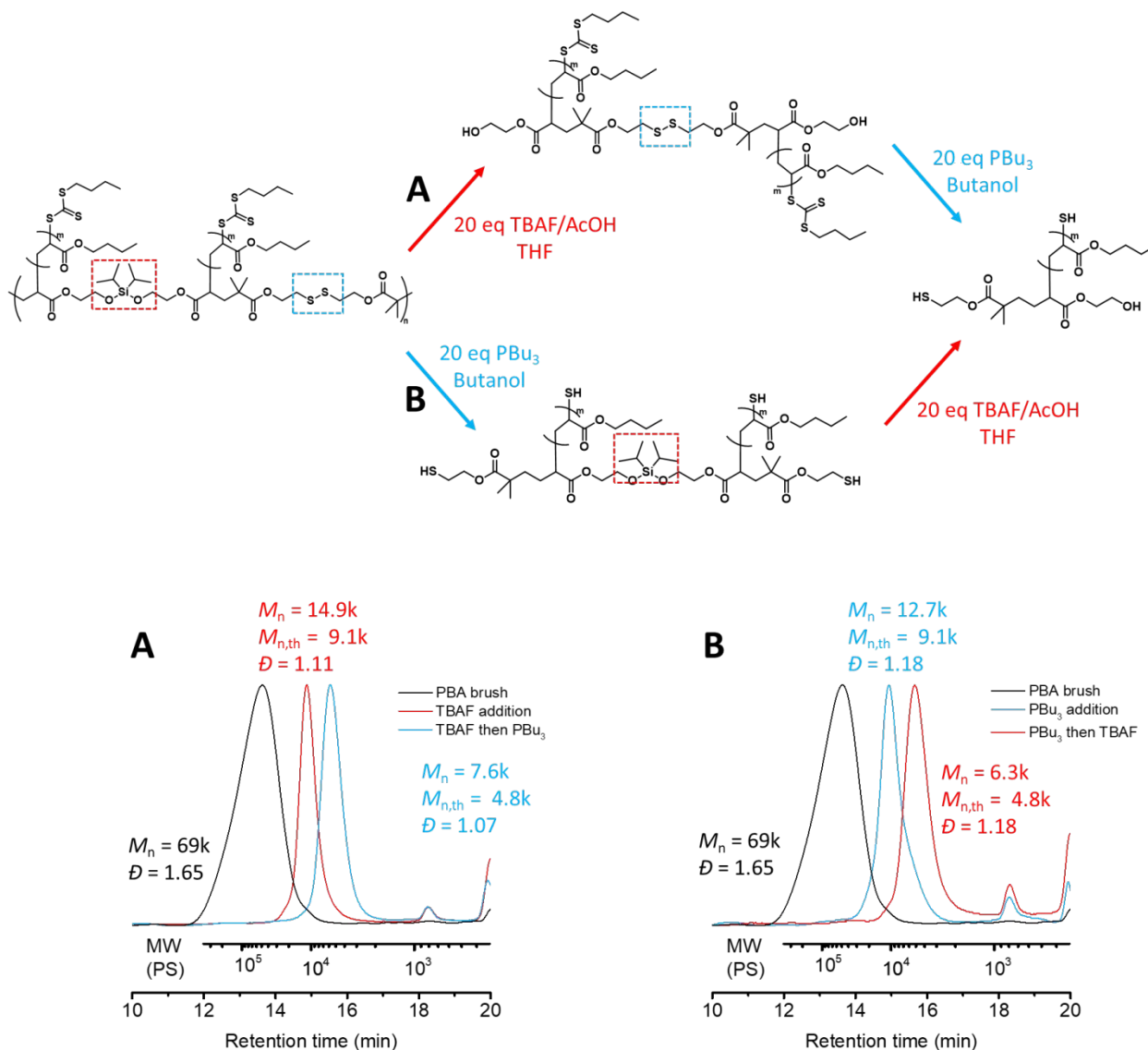
Figure 3: Orthogonal degradation of graft copolymer backbone (A) Dual stimuli degradation of silyl ether group (red), then the disulfide bond (blue). (B) THF-SEC (normalized dRI) chromatograms of P(CTA$_{2SS}$-alt-M$_2$)-g-PBA (black curve, M_n = 69k, $Đ$ = 1.65) dual stimuli degradation of the disulfide bond (blue), then the silyl ether group (red).

To summarize, an alternating functional backbone was easily prepared by A$_2$ + B$_2$ RAFT step-growth polymerization using functional bifunctional monomer and CTA reagents, which was directly grafted via RAFT controlled chain-growth polymerization. Here, a graft copolymer with alternating cleavable functional groups of silyl ether and disulfide bonds embedded in the structural repeat units of the mainchain backbone was prepared. The orthogonal nature of these two stimulus-triggerable functionalities allowed us to accomplish dual-stimuli selective tandem degradation of the graft copolymer backbone into well-defined polymeric species, consisting of two polymeric grafts after the first stimuli, which can be further precisely 'cut' in half after the second stimuli. This was carried out by employing TBAF/AcOH and PBu$_3$ to selectively cleave the silyl ether and disulfide bond units respectively, which can be done independently of the order. By judiciously selecting different (orthogonal) stimulus-triggerable units, one can expand our methodology to a wide range of multiple stimuli triggerable degradation of copolymers.

Author Contributions

The manuscript was written through contributions of all authors.

Conflicts of interest

The authors declare the following competing financial interest(s): J.T. and W.Y. are named inventors on a patent application owned by UNC-Chapel Hill (PCT/US2022/042087) which laid the foundation for this work. Dr. You is also a co-founder of Delgen Biosciences, a startup company that has licensed this UNC patent application.

Acknowledgements

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