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Single UV or Near IR Triggering Event Leads to Polymer Degradation into Small Molecules

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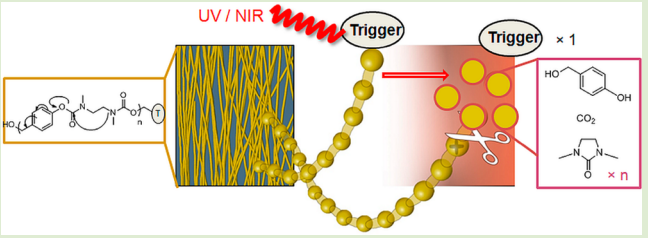
Supporting Information

ABSTRACT: We report two polymers with UV- and NIR-removable end-caps that respond to a single light activated event by complete cleavage of the polymer backbone via a self-immolative mechanism. Two photocleavable protecting groups were used to cap the polymers; *o*-nitrobenzyl alcohol (ONB) and bromo-coumarin (Bhc). GPC and ^{1}H NMR confirmed complete degradation of the ONB-containing polymer in response to UV. The polymers were formulated into nanoparticles; fluorescence measurements of encapsulated Nile red confirmed release upon photolysis of the end-caps. Contrary to previous work using a similar backbone structure that degrades upon hydrolysis, here, the disassembly process and burst release of the payload are only activated on demand, illustrating the powerful capacity of light to trigger release from polymeric nanoparticles. Our design allows the signal to be amplified in a domino effect to fully degrade the polymer into small molecules. Thus, polymers and nanoparticles can reach maximal degradation without having to use intense or long periods of irradiation.



Smart polymeric biomaterials promise the development of efficient drug and diagnostic delivery systems that target diseased tissue, increasing drug efficiency at needed sites and minimizing side effects in healthy tissues.[1−6] Controlled release from these polymeric carriers can be activated in response to different triggering events: biological conditions (pH, reactive oxygen species) or external stimuli (thermal, magnetic, electrical).[7] However, these mechanisms allow only limited control over the time and location of delivery. This can be overcome when the drug is attached to or protected in light sensitive carriers (microcapsules, polymeric nanoparticles, and hydrogels), as light can be remotely applied with high spatial and temporal precision and modulated (wavelength, intensity, duration of the irradiation).[8] Thus, for materials that are optically responsive, disassembly can be remotely activated in response to a particular wavelength to deliver the payload at a chosen concentration, time and location. Light as a trigger for capsule release has the additional benefit of being less likely to affect other parts of the molecules than other stimuli.[9] Near infrared (NIR), via two-photon absorption, is more relevant to biological applications than UV light due to its deeper penetration into tissue and lower risk of damage.[10] However, because of its low energy, photocleavage is often less efficient.

Several polymeric systems able to release their payload in response to light have been reported, most of which convert light to a structural change via photothermal conversion,[11−19] photochemical switch,[20−24] hydrophobicity switch,[25−29] or photo cross-linking[30] and de-cross-linking,[31,32] leading to either disruption or alteration of the carrier's permeability. These mechanisms of release by light suffer from the drawback that most of the system remains intact; the remaining macromolecular carrier complexes may not be easily cleared by the body. However, a few self-immolative polymeric-based nanoparticle materials[33−35] have been investigated so far that allow triggered release.

The UV- and NIR-degradable polymers so far developed require long periods of irradiation to degrade, meaning that the brief periods of irradiation likely to be useful for biological applications yield mostly oligomer strands rather than small molecules.[34,35] To create a system that is more sensitive to brief irradiation, here, we combine the backbone cascade degradation published elsewhere[36] and UV- or NIR-sensitive chemistries our group has previously employed. The backbone design employs a well-established quinone-methide self-immolative disassembly mechanism.[37−39] This overall concept is similar to that of polymers reported recently, incorporating Boc and Fmoc triggering groups, which also translate a single triggering event into complete degradation.[40] For this study, we used *o*-nitrobenzyl (ONB) and 4-bromo-7-hydroxycoumarin (Bhc) as end-cap moieties, both known to undergo photocleavage upon UV and NIR[41,42] light through one- or two-photon excitation, respectively. Disassembly of these linear self-immolative polymers is triggered when the terminal polymer headgroup of the backbone absorbs light; the backbone amplifies this

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signal in a domino effect to fully degrade the polymer into small molecules (Figure 1a). This is an alternative to the previous amplification system reported by our group[34] whose degradation is proportional to the amount of irradiation (Figure 1b).

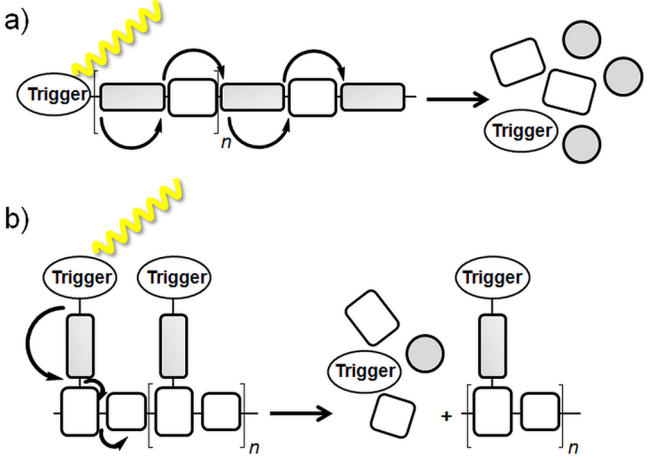

Figure 1. Illustration of the disassembly process. (a) Degradation of light sensitive polymers **2** and **3′** upon irradiation: light is amplified in a domino effect to fully degrade the polymer into small molecules. (b) Previous degradation system[34,35] in which degradation is proportional to the amount of irradiation so that more light is required to completely degrade the polymer.

We synthesized both polymers starting from monomer **1** (Figure 2). Monomer **1** and light-sensitive end-cap compounds were synthesized according to previously published procedure.[36,37,43,44] Synthetic routes and ^{1}H NMR are provided in the Supporting Information (SI). Following deprotection of the Boc group with TFA in DCM to reveal the free amine moiety and allow polycondensation, 5% of either end-cap **a** or **b** was added in the presence of Et_3N and 4-dimethylaminopyridine (DMAP) in toluene (Figure 2a). Weight-average molecular weights (MW) were determined by GPC to be 38000 Da (PDI = 1.23), 35000 Da (PDI = 1.27), and 62000 Da (PDI = 1.29) for **2**, **3**, and **3′**, respectively, relative to polystyrene standards. These PDI values were measured post removal of low molecular weight oligomers by gel filtration or precipitation. The molecular weight of polymer **3′** was almost twice as high as those of **2** and **3** because **3′** was obtained starting from a reaction mixture twice as concentrated in monomer.

To assess how long polymers must be irradiated at 350 nm to allow complete cleavage of the protecting group, we measured changes in the UV/visible absorbance spectrum of polymer **2** in acetonitrile/H_2O (9/1). This method does not allow analysis of photocleavage in **3** or **3′** because the absorbance of its cleaved product overlaps that of the polymer. UV irradiation of **2** induces cleavage of the ONB group and the release of 4,5-dimethoxy-2-nitrosobenzaldehyde (Figure 3a), which appears as a decrease in intensity of the peak corresponding to the 4,5-dimethoxy-2-nitrobenzyl carbamate photolabile end-cap (at 346 nm) and the formation of a new peak at 400 nm. However, no change was observed after 14 min of irradiation, indicating that cleavage had reached maximum conversion. Different mixtures of solvent were investigated to optimize both the photocleavage yield and the degradation speed. When the polymer is dissolved in a buffered system (acetonitrile/phosphate buffer (pH = 7.4), 4/3), optimal and complete deprotection occurred within less than 10 min. Better photoisomerization efficiency of the ONB

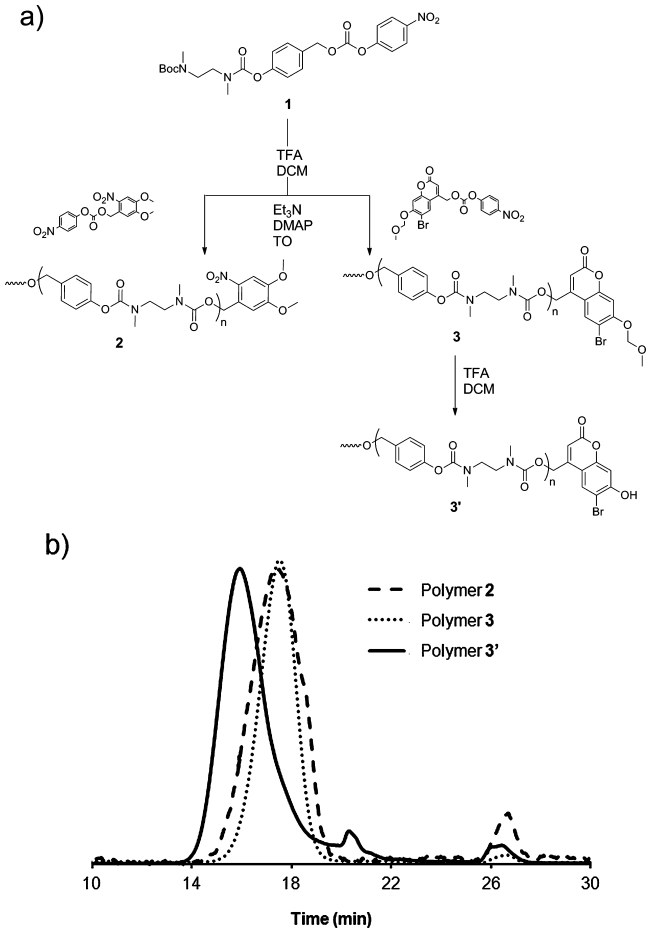

Figure 2. (a) Synthesis scheme of linear self-immolative light-responsive polymers **2**, **3**, and **3′**. (b) GPC chromatograms of **2**, **3**, and **3′** (UV detection, 254 nm). Peaks observed at 26.5 min result from toluene added as reference.

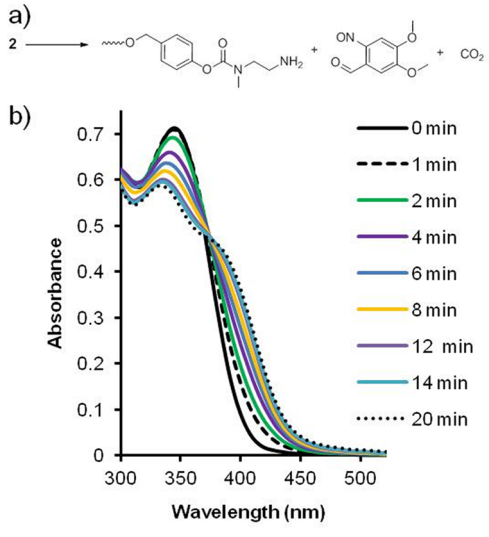

Figure 3. Complete removal of ONB from polymer **2** within 14 min of UV irradiation. (a) Molecular structures obtained upon photocleavage of end-cap in **2**. (b) Change of the UV−vis spectra of **2** (0.5 mg/mL) in acetonitrile/H_2O (9/1) with varying irradiation times at λ = 350 nm.

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derivative into *o*-nitrosobenzaldehyde in buffer solution at pH 7.4 is expected per its mechanism.[9] Moreover, this buffered system is known to facilitate the diamine cyclization process and thereby enhance the degradation speed of our polymer.

Because we confirmed that photocleavage was efficient, we next investigated the degradation of these materials by GPC (in acetonitrile/phosphate buffer) and ^{1}H NMR (in acetone-d_6/deuterium phosphate buffer; Figure 4). In the irradiation regime of complete cleavage, the polymer solutions were incubated at 37 °C and both loss of molecular weight and appearance of new small degradation molecules were monitored after various periods of time.

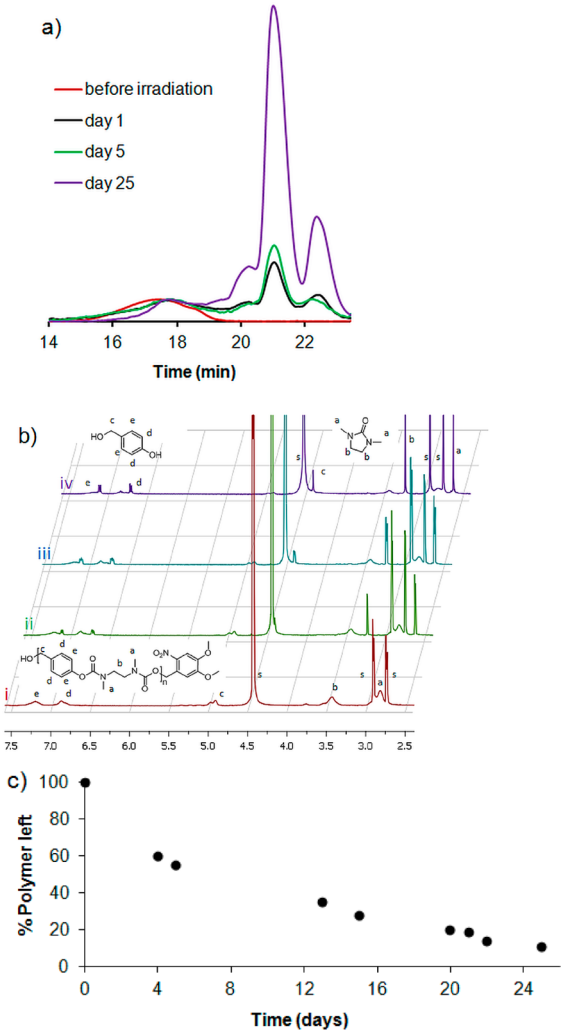

Figure 4. Complete polymer **2** degradation over 25 days. (a) Normalized GPC traces of **2** before irradiation (red line) and after removal of the protecting group and incubation at 37 °C during 1, 5, and 25 days (UV detection, 254 nm). (b) ^{1}H NMR spectra of polymer **2** in $(CD_3)_2CO$/0.1 M phosphate buffered D_2O pH 7.4 (4:3): (i) before irradiation, after irradiation at 350 nm, and incubated at 37 °C for (ii) 4 days, (iii) 15 days, and (iv) 25 days; s refers to DMF and D_2O. (c) Degradation kinetics for polymer **2** monitored by ^{1}H NMR, determined by integration of the peak corresponding to the intact polymer.

First, using GPC, we examined how much time was required for complete degradation. The shift toward small molecules by GPC indicated that degradation was almost complete around day 25 (Figure 4a). Sectional analysis of the GPC data (Figure S2 in the SI) indicated that only a faint peak of polymers (<5%, MW = 26000 Da) remained. A small percentage of intact polymer that remains may indicate that not all of the polymer molecules bear ONB end-caps. The relative inefficiency of degradation can be explained by the solvent mixture used, as cyclizations are faster in polar environments. However, considering the hydrophobicity of our polymers, a mixture of 0.1 M phosphate buffer (pH 7.4) and acetonitrile (3:4) was the most polar system that both allowed full dissolution of the material and maintained a stable nonacidic pH necessary for cyclization. The lack of shift in retention time indicated the relative absence of oligomers during the disassembly process. In parallel, the appearance of new low molecular weight species peaks (at around 21 and 23 min) indicated that, once disassembly started, only small molecules were formed; no oligomer strands were detected. A significant shift in retention time, leading to a mixture of polymer, oligomers, and polymer building blocks, is usually observed for systems containing multiple light-sensitive triggering groups per polymer chain.[34,35]

^{1}H NMR spectroscopy was used to verify the identity of the degradation products and the time course of degradation as measured by GPC. Assignment of the NMR peaks confirmed the expected degradation products of the sequential cascade of diamine cyclizations and 1,6-eliminations (Figure 4b). Sharper peaks characteristic of 4-hydroxybenzyl alcohol (peaks labeled c−e, Figure 4b,iv) and *N*,*N*′-dimethylimidazolidinone (peaks labeled a and b, Figure 4b,iv), first adjacent to polymer peaks, indicate that the degradation products consist only of these two small molecules. Over 25 days, almost all polymer or oligomer strands disappeared. Degradation with UV light of **3** and **3**′ is not presented because we would expect the same result upon complete photocleavage of both polymers.

This linear polymeric structure has been designed to reach maximal degradation without having to use long or intense radiation (Figure 5). To test this hypothesis, and to determine whether the Bhc-containing polymer (**3**′) could degrade in response to NIR, we evaluated the degree of degradation after 4 days of incubation after two-photon irradiation with NIR (Figure 5). NIR light is more desirable for many biological applications due to its deeper penetration into tissue and lower risk of cellular damage. This polymer was deliberately chosen

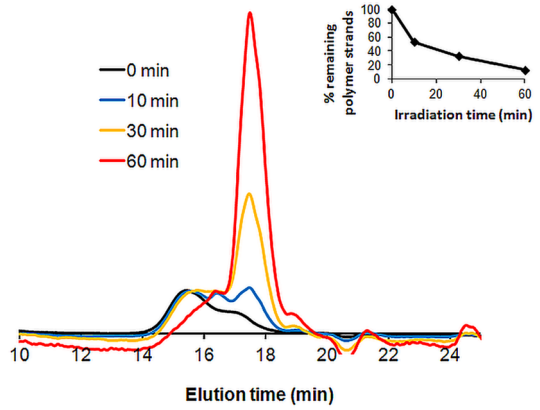

Figure 5. **3**′ degrades completely in response to brief two-photon NIR irradiation. Normalized GPC traces of **3**′ after exposure to NIR (2.35W) for 0, 10, 30, or 60 min and incubation for 4 days at 37 °C (UV dection, 350 nm). The inset, obtained by sectional analysis of the GPC, correlates the yield of activation or depolymerization with the irradiation time.

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over **2** because of the higher two-photon uncaging cross-section of Bhc compared to ONB. Thus, solutions of **3′** (0.1 mg/mL) in a mixture of 0.1 M phosphate buffer (pH 7.4) and acetonitrile (3:4) were irradiated for 10, 30, or 60 min (740 nm, 2.35 W) and incubated at 37 °C for 4 days. When the material was irradiated for 10 min, low molecular weight fragments (50 or 70% lower than intact polymer) were already in coexistence with the intact polymer, validating the polymer's NIR sensitivity and indicating that the polymer degrades completely in response to brief irradiation. Significant fragmentation (70% lower than intact polymer) was achieved after longer irradiation periods (30 and 60 min). As Bhc cleavage is known to be more efficient at higher pH,[44] we also examined degradation at pH 7.6, which allowed complete degradation in 30 min, half the time required at pH 7.4.

We investigated whether nanoparticles consisting of these polymers release contents in response to light. We encapsulated the hydrophobic dye Nile red (NR) using single emulsion to allow measurement of release via the dye's fluorescence. NR fluorescence is quenched when the dye encounters aqueous solution, so a decrease indicates release. By measuring the NR fluorescence intensity, a burst release is observed upon irradiation with 350 nm light. Nanoparticles made from **2** and **3′** respond with a 65% and 40% drop in fluorescence (Figure 6a,b), respectively, upon 1 and 5 min irradiation.

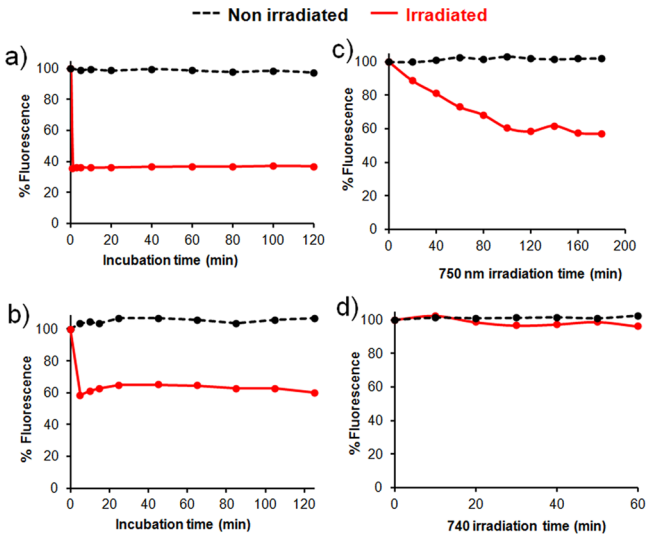

Figure 6. Burst release of encapsulated Nile red from polymeric nanoparticles. (a,b) Release from **2** upon 1 min (a) and from **3′** upon 5 min (b) irradiation with 350 nm light and incubation at 37 °C. (c,d) NR release from nanoparticles (c, **2**; d, **3′**) in response to irradiation with 750 or 740 nm light for 20 min intervals followed by 10 min of incubation at 37 °C (red lines) or incubation at 37 °C (dotted lines).

Prolonged irradiation of **2** resulted in a further decrease in fluorescence (80% after 5 min; Figure S4 in SI). We speculate that the reason **3′** needs longer irradiation to release NR is that its degradation is slightly slower, as Bhc requires interaction with water to be cleaved. Unlike previous micelles built on this backbone but incorporating ethylene glycol in place of photosensitive groups,[36] nanoparticles **2** and **3′** show excellent stability in buffer without irradiation, as no change in fluorescence intensities was measured after several days (**2** over a week and **3′** over 3 days; Figure S5 in SI). Moreover, although the nanoparticles started to fall apart at only one activated site, after the removal of the backbone end-cap, the burst release of NR upon UV irradiation is similar to our previous design, including multiple triggering sites throughout the backbone.[34] This suggests that the amount of photocleavage obtained during 1 min could translate into enough particle shell degradation to expose NR to the hydrophilic aqueous media. This result suggests that only partial polymer degradation within each nanoparticle is required to cause release.

We also studied the response of nanoparticles **2** and **3′** to two-photon absorption upon irradiation with a Ti:Sapphire laser tuned to 750 and 740 nm light, respectively. A 40% gradual decrease in intensity was observed over 2 h of irradiation (Figure 6c,d). This is not surprising given that the NIR two-photon process is less efficient than the photocleavage obtained with UV irradiation. No bulk response or NR release were observed for **3′** upon NIR irradiation, likely because the hydrophobic environment created around this photosensitive moiety strongly affects the absorption properties and quantum yields of this triggering group. Thus, although the Bhc-capped polymer is sensitive to NIR when in solution (Figure 5), it does not respond in bulk.

As these polymers could prove useful as tools for biological research, we examined their effect on cell metabolism. An MTT assay revealed that our polymers and their degradation products are equally as well-tolerated as poly(lactic-*co*-glycolic acid), which is FDA-approved and widely used in biological research (Figures S6 and S7 in SI).

In conclusion, our new light-responsive polymeric nanoparticles degrade completely into small molecules and release their payload upon irradiation with UV or NIR light. These nanoparticles release the small hydrophobic dye NR, used as a drug model, with similar kinetics to those of our previous light-degradable polymers and are similarly stable in the absence of irradiation. This new system is fully degradable on demand, yielding only small molecules that should be more easily cleared by the body than intact polymer strands. Despite incorporating only one light-absorbing group per polymer strand, both polymers are NIR-degradable; nanoparticles from **2** release cargo in response to NIR and **3′** degrades completely. This combination of degradation into small molecules, NIR degradability, and minimal cytotoxicity suggests that these polymers could be developed into tools for drug or protein delivery.

■ ASSOCIATED CONTENT

Supporting Information

Characterization and experimental details for the synthesis of monomer **1**, polymers **2**, **3**, and **3′**, one-photon photolysis of **2**, ^{1}H NMR and GPC degradation studies, formulation of the nanoparticles, DLS and SEM data, fluorescence analyses of NR release, and nanoparticle cytotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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