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Design and Implementation of Multi-Responsive Azobenzene Triggers in Self-Immolative and Degradable Polymers

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Self-immolative polymers are a recently developed class of degradable polymers capable of undergoing end-to-end depolymerization following the reaction of their endcaps with appropriate stimuli. Self-immolative materials originated in the field of prodrug chemistry, and evolved into self-immolative oligomers, dendrimers, and most recently, linear polymers. Many stimuli-responsive endcaps have been developed, but typically can only respond to one stimulus. Azobenzenes are a well-known class of stimuli-responsive molecules most commonly used as photoswitches, due to their facile trans-cis isomerization. In addition to their photochemistry, azobenzenes have recently been found to be selectively reduction-sensitive, and are therefore of interest as endcaps in self-immolative polymers. The two fields of azobenzenes and self-immolative polymers have not previously been combined, and it is the work described herein that is the first to do so. This thesis demonstrates that azobenzenes can be useful as multistimuli-responsive units in self-immolative polymers.

First, the reduction-sensitivity of azobenzene was demonstrated in the context of two self-immolative polymer backbones. The synthesis and depolymerization of these materials showed that azobenzene endcaps could be successfully incorporated into – and used to trigger – self-immolative polymers. In the next study, a library of reduction-sensitive azobenzenes was prepared to determine which azobenzene compounds were most suited for use as reduction-sensitive endcaps. A 2-Cl azobenzene derivative was reduced most quickly, and this compound was incorporated as pendant units in an amphiphilic chain-shattering graft copolymer based on a poly(ester amide) backbone. It was found that these azobenzenes imparted both photo- and reduction-sensitivity to aqueous polymer assemblies, and could respond synergistically to both stimuli. In the final study, two distinct linear self-immolative polymer backbones, a polycarbamate and a polyglyoxylate, were synthesized with an azobenzene linker, and conjugated to poly(ethylene oxide) using click chemistry. The synthesized amphiphilic block copolymers were reduction-sensitive, and their aqueous assemblies were shown to encapsulate and release a hydrophobic cargo under reducing conditions. The multifaceted applicability of azobenzene was highlighted in these studies, first as a reduction-sensitive endcap, then as a dual-responsive trigger for chain-shattering poly(ester amide)s, and finally as a reduction-sensitive linker in diblock copolymers.
Keywords

Azobenzene, self-immolative, polymer, stimuli-responsive, reduction, reduction-sensitive, isomerization, photoisomerization, chain-shattering polymer, block copolymer, amphiphilic, nanoassembly, degradation, glyoxylate, carbamate.
Co-Authorship Statement

The work described in this thesis contains contributions from the author as well as coworkers Thomas M. Güngör, Alexander L. Prinzen, Bo Fan, and supervisor Dr. Elizabeth Gillies. The contributions of each are described below.

Chapter 1 was written by the author and edited by Dr. Elizabeth Gillies.

Chapter 2 describes a project proposed by the author, supervised by Dr. Elizabeth Gillies, and for which the experimental work was carried out in part by the author, and in part by the Masters student Thomas M. Güngör. The synthesis and degradation of small molecules was carried out by the author. The design, synthesis, and degradation of the elimination-based polymer and its controls was completed by the author, while the synthesis and degradation of the alternating cyclization and elimination-based polymer was completed by Thomas M. Güngör. Data analysis was carried out by the author, and the manuscript was prepared as a collaboration between the author and Dr. Elizabeth Gillies.

Chapter 3 describes a project conceived of and designed by the author and Dr. Elizabeth Gillies, and its experimental component was shared between the author and Alexander L. Prinzen, a fourth-year undergraduate student supervised directly by the author. The majority of azobenzene compounds were synthesized and tested by Alexander L. Prinzen, using a synthetic route and methodology developed by the author. Design of the polymers for use in the study was a collaboration between all parties, while synthesis of the polymers was completed by Alexander L. Prinzen. Testing and degradation of the polymers and their controls was shared between the author and Alexander L. Prinzen. Analysis of relevant data was carried out by the author, and assisted by Alexander L. Prinzen. The manuscript was prepared by the author and Dr. Elizabeth Gillies.

Chapter 4 details a project designed by the author and Dr. Elizabeth Gillies, and for which the experimental component was completed in part by the author, and in part by the Ph.D. candidate Bo Fan. The azobenzene endcap was prepared by the author, in addition to the polycarbamates. Modification of the azobenzene endcap to a chloroformate, and subsequent synthesis of polyglyoxylates, their controls, and their amphiphilic block copolymers was
completed by Bo Fan. All degradation experiments and data analysis were completed by the author. The manuscript was drafted by the author and Dr. Elizabeth Gillies.
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<tr>
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<th>Definition</th>
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<tbody>
<tr>
<td>A</td>
<td>absorbance</td>
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<tr>
<td>A₀</td>
<td>initial absorbance</td>
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<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
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<td>Asp</td>
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<td>Boc</td>
<td>tert-butyloxy carbonyl</td>
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<td>br</td>
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<td>°C</td>
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<td>CDI</td>
<td>carbonyldiimidazole</td>
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<td>cm</td>
<td>centimeter</td>
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<tr>
<td>CSP</td>
<td>chain-shattering polymer</td>
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<td>Δ</td>
<td>heat/change</td>
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<td>dispersity</td>
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<td>EDC·HCl</td>
<td>1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloric acid salt</td>
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<tr>
<td>kₐₗbs</td>
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<td>--------</td>
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</tr>
<tr>
<td>$\lambda$</td>
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<tr>
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<td>NEt$_3$</td>
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<tr>
<td>$\nu_{\text{max}}$</td>
<td>wavenumber of maximum absorbance/minimum transmission (cm$^{-1}$)</td>
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</tr>
<tr>
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<td>poly(ethylene oxide)</td>
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Chapter 1

1 Azobenzenes and Self-Immolative Polymers: Two Worlds Collide

1.1 Azobenzene

1.1.1 Overview

Azobenzene is a molecule defined by one or more nitrogen-nitrogen double bonds (diazene bonds) that separate aryl rings. Primary azobenzenes are thus defined as those compounds containing a single diazene bond, while secondary azobenzenes contain two diazene bonds. Azobenzene and its derivatives have an extensive history in synthetic chemistry, and have seen use in a myriad of applications. Originally conceived as dyes (Figure 1.1), azobenzenes have also been used in molecular photoswitches, molecular machines, linkers in biological macromolecules, guests in inclusion complexes, surfactants, ligands, polymers, liquid crystals, and most recently, as multiresponsive triggers in degradable polymers.

Figure 1.1 – Examples of a primary (Methyl Red) and secondary (Congo Red) azobenzene used as dyes.

1.1.2 Applications of Azobenzene

The main focus of azobenzene research has been its photochemistry. The excitation of these molecules by ultraviolet (UV) light allows extremely rapid and efficient isomerization from the trans to cis conformation (Figure 1.2). The isomerization is typically completed with almost negligible side-reactions, and can be tuned to a wide range
of wavelengths via the incorporation of various functional groups. Furthermore, the properties of cis azobenzene are usually significantly different from the trans isomer, which has led to the exploitation of this class of molecules. Properties that undergo changes include polarity, molecular volume, and as a result of the modification in the degree of conjugation, a change in absorption of light. Stilbenes are structurally similar to, and isoelectronic with azobenzenes; however, these compounds are plagued by instability and cannot be isomerized reliably without side reactions. Azobenzene is generally regarded as a stable compound, even upon irradiation, and as such, azobenzene is often used in photochemistry due to its limited capacity for side reactions. However, it has been demonstrated that azobenzene is selectively reduction-sensitive. This capacity for reduction has opened new pathways for the use of azobenzenes, specifically as a selective triggering mechanism.

![Image of azobenzene isomerization](image)

**Figure 1.2 – Isomerization of azobenzene from trans to cis under the action of light.**
The forward reaction is typically generated using UV light, while the reverse uses visible light or heat.

### 1.1.3 Synthesis of Azobenzene Derivatives

Many methods of synthesizing azobenzenes have been reported, and thus only a select few will be reviewed here. Some synthetic approaches are most useful for the synthesis of symmetrical azobenzenes, while others are capable of producing asymmetric variants. Different substrates can require alternate pathways, depending on their reactivity and substitution. One of the simplest methods is the oxidation of the analogous diarylhydrazine, but this method is requires a highly specific starting material, and thus cannot often be applied.
1.1.3.1 Electrophilic Aromatic Substitution

One of the most common methods of azobenzene synthesis involves the formation of a diazonium salt on an aryl ring by diazotization, and this reactive intermediate can subsequently react with a second, electron-rich ring via an electrophilic aromatic substitution reaction (Figure 1.3). This reaction forms the basis of many organic dye synthesis pathways, and is effective at coupling complicated ring systems. This pathway requires that one starting material is an aniline that can be diazotized, usually by acidic sodium nitrite, and that the second ring will possess selectivity for the requisite electrophilic aromatic substitution at a specific position. Multiple nucleophiles have been used, including phenols, anilines, and organometallics. This method is incompatible with oxidation-sensitive and acid-sensitive groups. In addition the intermediate diazonium cation is sensitive and can be lost as dinitrogen gas in some cases. The most notable advantages of this method are its use of low-cost reagents and the one-pot synthesis.

![Diagram of two-step synthesis of an unsymmetrical azobenzene via a diazonium salt. Symmetrical azobenzenes may also be synthesized using this method, where R1 = R2.](image)

1.1.3.2 Reductive Coupling of Nitrobenzenes

Nitrobenzene derivatives are ubiquitous, and therefore it is no surprise that many methods have been developed for their reductive coupling to yield azobenzenes (Figure 1.4). Many of these processes utilize metal catalysts, including platinum nanowires and a variety of gold species to name only a few. There have been many advancements in this field in recent years, and nearly quantitative yields have been reported. However, one of the main drawbacks of this method is that it can only be used to produce symmetrical azobenzenes, and in cases where two nitrobenzenes are added, a statistical mixture of products is generated. This can be problematic in the design of functional materials, which are most
often unsymmetrical. Furthermore, without fine-tuning of the reaction conditions, the process may produce over-reduced anilines, or under-reduced azoxybenzenes, which must then be separated. A more selective system has been reported, involving a trivalent indium salt and a hydrosilane reducing agent\textsuperscript{28} with very few side products observed using the optimized conditions. However, in the production of asymmetric azobenzenes, a large excess of one reagent is required, and homodimers are still formed. Therefore, this method is best suited for the production of symmetrical materials.

![Figure 1.4 – Reductive synthesis of an azobenzene using a nitrobenzene starting material. This reaction is typically metal-catalyzed.](image)

### 1.1.3.3 Oxidative Coupling of Anilines

Analogous to the reductive coupling of nitrobenzenes is the oxidative coupling of anilines (Figure 1.5), which aims to incompletely oxidize the functional groups such that a coupling may take place before the groups reach the nitro oxidation level. Oxidative aniline couplings have many of the same problems as reductive methods, namely their inefficient syntheses of asymmetrical compounds. Various metal catalysts have been used for the coupling of anilines, including transition metals such as yttrium\textsuperscript{29} or gold,\textsuperscript{30-31} and various lanthanides.\textsuperscript{32} Copper is a common metal for the transformation, either as a salt\textsuperscript{33} or as a metal.\textsuperscript{34} A manganese-porphyrin complex has also been used in conjunction with tetrabutylammonium peroxymonosulfate to provide selective catalytic oxidation.\textsuperscript{35} However, the reaction required a freshly-prepared oxidant and highly specific porphyrins.

One of the newest methods for the synthesis of azobenzenes is the use of tert-butyl hypoiodite, which is generated \textit{in situ} using tert-butyl hypochlorite and sodium iodide. The reagent is simple to prepare, metal free, and tolerant of most functional groups. Asymmetric azobenzenes are inefficiently synthesized using this method, but some selectivity for the heterodimer has been achieved.\textsuperscript{36-37} Most of the oxidative coupling
methods accomplish the formation of a single N-N bond, which can then be oxidized in the presence of air to the product azobenzene.

\[
\begin{align*}
2 & \begin{array}{c}
\text{R}_1 \text{NH}_2 \\
\text{C}_6 \text{H}_5 \\
\end{array} \xrightarrow{\text{[O]}} \text{cat.} & \begin{array}{c}
\text{R}_1 \text{N} = \text{N} \\
\text{C}_6 \text{H}_5 \\
\text{R}_1 \\
\end{array}
\end{align*}
\]

**Figure 1.5** – Oxidative coupling of anilines can produce azobenzenes under the correct conditions. Examples which selectively produce asymmetric azobenzenes are rare.

### 1.1.3.4 Nitrosobenzene-Aniline Condensation (Mills Reaction)

The Mills reaction is a condensation reaction between nitrosobenzenes and anilines, and is highly selective. This reaction can be catalyzed by either acid or base, and is a simple and high-yielding reaction analogous to imine formation between an amine and aldehyde. The reaction has been utilized for many years as a method of accessing azobenzene derivatives. Furthermore, this method is ideal for the synthesis of asymmetrical azobenzenes, due to the lack of self-reactivity of the reacting species under the mild conditions, and the ability to produce beforehand the reactive nitrosobenzene (Figure 1.6).

\[
\begin{align*}
\begin{array}{c}
\text{R}_1 \text{NH}_2 \\
\text{C}_6 \text{H}_5 \\
\end{array} \xrightarrow{\text{KHSO}_5} & \begin{array}{c}
\text{R}_1 \text{N}_2 \\
\text{C}_6 \text{H}_5 \\
\end{array} & \begin{array}{c}
\text{R}_1 \text{NH} \\
\text{C}_6 \text{H}_5 \\
\text{R}_2 \\
\end{array} \xrightarrow{\text{H}_2 \text{N} \text{R}_2} & \begin{array}{c}
\text{N}_2 \text{N} \\
\text{C}_6 \text{H}_5 \\
\text{R}_1 \text{R}_2 \\
\end{array}
\end{align*}
\]

**Figure 1.6** – Synthesis of an asymmetric azobenzene via the Mills reaction. The partial oxidation of one aniline to the nitrosobenzene allows for greater selectivity in producing asymmetric azobenzene derivatives. Condensation with a second aniline in a subsequent step yields the product with elimination of water.

This method is most effective with electron-poor nitrosobenzenes because electron-rich rings promote over-oxidation. Preparation of the nitrosoarene is typically straightforward starting from an aniline. A suitably mild oxidant such as oxone is used in
a biphasic oxidation, and a good yield of nitrosoarene is usually isolated with minimal workup.\textsuperscript{39-40} The use of Oxone (potassium peroxymonosulfate, KHSO\textsubscript{5}) rarely leads to over-oxidation, and as such is a preferred reagent. A wide range of asymmetrical azobenzenes can be synthesized this way, excluding those that are intolerant to mildly oxidizing or acidic conditions.

\subsection{Azobenzenes in Stimuli-Responsive Materials}

\subsubsection{Azobenzene Photoswitches for Nanoscale Control}

Azobenzenes are ubiquitous photoswitches, known for their reversible \textit{trans}-\textit{cis} isomerization upon exposure to UV and/or visible light.\textsuperscript{1} The field of photoswitching is broad, but azobenzene has maintained a strong foothold since the initial description of its photoresponsiveness.

Two conformations of azobenzene are typical; the \textit{trans} (E) form is commonly favored, while the \textit{cis} (Z) form is typically less stable. In the \textit{trans} state, azobenzene is fully conjugated through the diazene bond, while isomerization to the \textit{cis} state disrupts this conjugation because it leads to strain that causes the aromatic rings to rotate out of planarity. In the \textit{cis} conformation, the nitrogen lone pairs are forced to lie in the same plane, and in combination with the steric interactions of the ring substituents, this causes the \textit{cis} form to be on the order of 12 kcal mol\textsuperscript{-1} less stable than the \textit{trans},\textsuperscript{41} with an energy barrier of approximately 23 kcal mol\textsuperscript{-1}. There are exceptions to this rule, and there are examples for which the \textit{cis} isomer is the thermodynamically favored conformation. Connection of the aryl substituents, for example, can force an azobenzene to lie the \textit{cis} state until isomerized to the \textit{trans} by light.\textsuperscript{42}

The absorption of azo derivatives in the UV and visible regions is well-known, and the main $\pi-\pi^*$ band has been tuned to absorb from the UVB region (290-320 nm) well into the blue and green ranges (450-570 nm)\textsuperscript{3} of the visible spectrum. The spectral properties of azobenzene can be further exploited by utilizing the $n-\pi^*$ transition, which can be tuned to absorb red light (~600 nm).\textsuperscript{43} Two-photon processes have been reported with azobenzene derivatives using near-infrared light (850 nm),\textsuperscript{44-45} and this highly tunable range allows for application-specific selection of azobenzenes.
While many wavelengths are potentially available for the isomerization of azobenzenes, by far the most commonly observed transitions are found at ca. 320-350 nm for the π-π* band (S_0-S_2) and ca. 400-450 nm for the n-π* band (S_0-S_1). In the trans isomer, the molar extinction coefficient (ε) of the π-π* band is much larger than that of the n-π* band (≈2-3 × 10^4 M^-1 cm^-1, ≈4 × 10^2 M^-1 cm^-1), as the latter transition is symmetry-forbidden, but weakly allowed by vibronic coupling. The π-π* band of the cis isomer is generally stronger than its n-π* transition, which is stronger than the n-π* band of the trans isomer. In the cis isomer this transition is formally allowed.

Complete isomerization of azobenzene is typically challenging due to the overlap in absorbance between the two isomers, which leads to the generation of a photostationary state, a point at any given irradiation wavelength when the rates of the forward and reverse isomerization are equal, and thus further irradiation can produce no more of either product.

In azobenzene derivatives with poorly separated absorption bands, thermal relaxation to the preferred isomer is often the only means by which complete reversion can be accomplished. As a result, the synthesis of azobenzene derivatives with tunable rates of thermal relaxation is of interest. Applications such as data storage require a nearly indefinite reversion time so as not to lose information, while in biological systems a rapidly relaxing azobenzene can be advantageous in that a second light source is not required to return the molecule to its original conformation.

Despite the differences in molar absorptivity for the π-π* and n-π* transitions, isomerization of azobenzene can be accomplished via irradiation into either band. The mechanism for isomerization is thought to vary depending on the excitation chosen. Irradiation into the π-π* band is thought to lead to rotation, likely due to the cleavage of the π component of the diazene bond, while excitation of the n-π* band is thought to produce inversion at one of the nitrogen atoms. As both methods involve excitation of an electron into the π* LUMO, it is not unexpected that the calculated LUMO orbital is antibonding across the N-N bond, and thus excitation into it allows previously restricted motions.
One of the most noted aspects of the photoisomerization of azobenzene is the concomitant changes in the physical properties of the materials in which they are incorporated. Specifically, the isomerization alters the molecular shape from a planar rod-like configuration to a bent and twisted structure. The rings can no longer lie coplanar, and due to repulsive interactions of the $\pi$-clouds, one ring is likely to orient itself perpendicular to the other such that a proton points towards the face of the other ring. Furthermore, during this transformation the end-to-end length of the molecule (C4-C4') decreases from ~9 Å to ~5.5 Å, and the molecule essentially becomes three-dimensional, gaining an appreciable thickness. The significant change in molecular shape and volume has been exploited previously to effect a physical change via the conversion of light energy into a mechanical force.\textsuperscript{41}

Importantly, a significant change in polarity is also observed; \textit{trans} azobenzene has a dipole moment of ~0, while \textit{cis} azobenzene has a moment of ca. 2-3 Debye. \textsuperscript{4} Substituted azobenzenes generally experience the largest change in polarity, while 2- and 3-substituted derivatives possess a more polar \textit{trans} isomer to begin with. The main change, however, arises from a loss of conjugation between the rings, and the reorientation of the diazene lone pairs towards the same direction.\textsuperscript{48}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{azobenzene_isomerization.png}
\caption{A bridged azobenzene which demonstrates a preference for the \textit{cis} isomer. The isomerization kinetics of this type of molecule is inverted compared to standard azobenzenes.}
\end{figure}

The structure of azobenzene can be modified such that the \textit{cis} isomer becomes the thermodynamically favored isomer. By bridging the two aryl substituents, it is possible to synthesize what might be thought of as a reverse azobenzene, whose ground state is the excited state for the majority of its analogues (Figure 1.7). Tethering the rings together also
has the effect of decreasing their rotational freedom, which greatly affects the wavelengths of light needed to produce isomerization.\textsuperscript{42}

1.2.1.1 Substitution of Azobenzenes Affects their Isomerization

The ring-substitution of azobenzenes can have a significant impact on their properties, including the absorbance spectrum, thermal relaxation half-life, and chemical reactivity. The tuning of the thermal relaxation time has applications at short or very long timescales, depending on the intended use of the azo switch.

Examples of tetra-ortho-substituted azobenzene derivatives have been reported for a number of applications (Figure 1.8). Tetrafluoro azobenzenes have been reported to extend the lifetime of the \textit{cis} isomer significantly, while simultaneously separating the n-\(\pi^*\) bands of both isomers for more efficient switching using visible light.\textsuperscript{49} Without such a significant separation of bands, the \(\pi-\pi^*\) band would need to be irradiated to effect the trans-\textit{cis} transformation. These compounds may thus prove useful in the storage of information, as each azobenzene could be seen as a single binary unit with two stable and selectively switchable states.

![Figure 1.8 – Examples of tetra-ortho-substituted azobenzene derivatives used in photoswitching applications. Various effects can be achieved by altering substituents near the diazene bond.](image-url)
Similar to the tetrafluoro azobenzenes, tetrachloro and tetrabromo ortho-substituted azobenzenes have been synthesized, and their photochemical properties examined. These compounds similarly demonstrated a redshifted n-π* absorbance band, and to such an extent that red light could be used for photoswitching. The use of red light is advantageous over UV or even green visible light due to its safety and penetration depth in oxygenated tissues. These derivatives also had relatively long-lived cis states, relaxing thermally with half-lives of approximately 6 hours, which allowed for the formation of a significant portion of the cis isomer.

The installation of four methoxy substituents ortho to the azo bond led to a similar effect as the halogenated azobenzenes, but ostensibly via a different mechanism. Again, the n-π* bands of both isomers were separated such that visible light could be used for photoswitching, but in addition to acting as σ-withdrawing groups, methoxy groups are largely electron donating via resonance. In this case, it seems likely that the high concentration of electron density around the azo bond makes the ground state less favourable than in the cis form, where the nitrogen lone pairs are fully exposed to solvent interactions. X-ray crystallography of methoxy azobenzene derivatives demonstrated the highly twisted conformation of the aryl rings in the ground state, which may further explain the red-shifting and longevity of the cis isomer.

This structural motif was thus expanded to an analogous chalcogen-containing azobenzene in a later work. Four thioethers were installed and found to produce a similar red-shift, and also greatly increased the absorbance above 450 nm. However, this effect was most strongly observed in an azobenzene with dual para amine substituents, and thus these rings were highly electron-rich. The thermal relaxation of these thioether azobenzenes was found to be ~20 minutes in aqueous media, which could prove advantageous where fast relaxation is needed, or only one of the wavelengths for photoisomerization can be used.

Azobenzene has been used as a reversible on/off switch for chemical reactivity. Hecht and coworkers reported the synthesis of an azobenzene-containing amine base, which could be activated with UV light to expose the reactive lone pair and allow a base-
catalyzed reaction. Systems such as this are critical as they demonstrate the variety of utility available via rational design using ubiquitous photoswitches such as azobenzenes. In this system, a bulky azobenzene was positioned such that its trans isomer provided a steric block for an N-tert-butyl piperidine functionality. The piperidine was functionalized with a bulky substituent in order to ensure the positioning of its lone pair in a position obscured by the azobenzene. Upon irradiation, the piperidine was revealed, and was able to deprotonate nitroethane to catalyze a Henry condensation with 4-nitrobenzaldehyde, whereas a non-irradiated sample was unable to perform the same task (Figure 1.9). This on/off behaviour allowed for the direct control of reactivity within a flask, which could potentially be applied to those areas highly dependent on reaction time, and to perhaps limit unwanted side reactions.

![Mechanism for the photo-selective Henry condensation of 4-nitrobenzaldehyde and nitroethane in the presence of a hindered base. Isomerization exposes the lone pair and allows the reaction to proceed.](image)

**Figure 1.9** – Mechanism for the photo-selective Henry condensation of 4-nitrobenzaldehyde and nitroethane in the presence of a hindered base. Isomerization exposes the lone pair and allows the reaction to proceed.
1.2.1.2 Control of Polymeric Systems and Assemblies

An important application of azobenzene photoswitches is in the control of nanostructures in both temporal and spatial dimensions. A high degree of selectivity can be attained through the use of light as a stimulus due to its nearly instantaneous action, lack of dependence on diffusion, and the degree of control over its intensity and wavelength. These aspects have allowed for the control of intricate systems with relative ease. While useful in the lab, this type of control is most highly valued in the context of biological systems, where more constraints exist regarding appropriate stimuli.3-4,50,52

Drug release is an application in which temporal and spatial control are important, due to the potential for negative side effects from premature drug release. An example of azobenzenes used as gatekeepers and molecular paddles was recently reported, wherein a porous ceramic matrix was loaded with drug, and its release was accelerated by irradiation.53 Such behavior could be explained by many of the important transformations experienced by azobenzenes during isomerization, but most importantly in this case, their change in polarity, and their molecular motion during the transition.

Azobenzene-containing polymers have been explored in a wide range of applications, including liquid crystals,9,54-57 and thermoplastic elastomers,58-59 but most relevant to this work is the exploration of azobenzene block copolymer assemblies.60 Azo polymer assemblies have many uses, such as switchable aggregation,61 or reversible dissociation, which could be useful in the design of drug delivery vehicles.

Drug release has been reported using azobenzene-containing block copolymers. A diblock glycopolymer was synthesized such that assemblies of the material could be irradiated and triggered to disassemble and release a drug upon reaching target cells.62 An azobenzene photoswitch was incorporated into an amphiphilic diblock copolymer with a relatively small (7-15 units) hydrophobic azobenzene block, and a comparatively large (150-250 units) hydrophilic glycopolymer block based on galactose. The small hydrophobic component allowed for the disintegration of the micelles by photoisomerization, due to the large shift in polarity, from the virtually nonpolar trans form to the more polar cis form. This system was able to deliver a hydrophobic drug model to
A375 human carcinoma cells due to the specific interaction between the multivalent galactose block with galectin-3 receptors native to the cell membranes.

Block copolymer vesicles or micelles loaded with some cargo can be slow to release such payloads, but if the polymer itself can be reversibly altered such that the assembly entirely disintegrates, then highly selective burst release can be attained. Furthermore, the reverse process can be used to reform the nanostructures, such that they may be used for *in situ* encapsulation. Poly(acrylic acid) (PAA), \(^8\) N-isopropyl acrylamide (NIPAM), and poly(ethylene oxide) (PEO) blocks \(^{64}\) have been used as the hydrophilic component, but in principle these systems are highly modular and can be designed to meet many specific requirements. For example, a system in which irradiation served only to soften the hydrophobic azobenzene layer of a block copolymer vesicle has been reported. \(^{65}\) These polymers incorporated a lower fraction of azobenzene monomers into the hydrophilic block, which allowed for the tuning of photoresponsiveness. In this way, irradiation did not lead to disassembly, rather it increased the permeability of the vesicle membrane (Figure 1.10).

![Figure 1.10 – Irradiation of an azobenzene-containing vesicle can lead to an increase in membrane permeability by changing the polarity of the azobenzene units.](image)

A wide array of block copolymers with high azo content have been synthesized, and have potential as stimuli-responsive materials or in biological applications. \(^{66}\) Typically the inclusion of more azobenzene units increased the impact of photoisomerization due to the large mass percentage of the polymer corresponding to azobenzene, and this effect was
exploited by careful design. Most of these polymers have been synthesized using controlled radical polymerization, a method that can provide high molar masses and low dispersity, but generate non-degradable carbon-carbon bonds. Many systems were designed specifically to alter their morphology upon irradiation, either to allow for the release of some cargo, or to affect a secondary property such as solution viscosity.

A PEO-Azo block copolymer was synthesized from an ATRP initiator-terminated PEO chain using an azo-containing methacrylate monomer, was found to self-assemble with the azobenzene pendants in the trans state, and upon isomerization with visible light, was found to lose its self-assembled nanostructure, implying that it may be useful in drug delivery (Figure 1.11). Furthermore, the azobenzene was attached with a flexible linker, allowing the core of the micelle to form a highly organized and fluorescent structure via an aggregation-induced emission mechanism.\(^{67}\)

![Diagram: A diblock copolymer system with a high azobenzene content in the hydrophobic block may be used to reversibly form assemblies in solution. These structures respond to light such that they can potentially release encapsulated cargo.](image)

_A similar copolymer of N-isopropyl acrylamide and an azobenzene monomer was found to form vesicular structures, which underwent reversible fission when irradiated with UV or visible light. The method of fission was also studied, and found to be a result of both the change in azobenzene structure, and a photo-thermal heating effect on the polymer backbone.\(^{68}\) The change in structure observed here was similar to the growth of wormlike micelles from vesicles upon irradiation of a mixture of surfactants, where the change in the hydrophilic mass fraction of the materials resulted in the reformation of a more stable nanostructure.\(^{69}\)_
A reversible change in structure was also accomplished using a combination of azo-containing block copolymer, and the supramolecular chemistry discussed previously. In one report, a hydrophobic-hydrophobic polystyrene-azobenzene diblock copolymer was synthesized, and the pendant azobenzenes were free to interact with cyclodextrin in solution. These micellar structures were sensitive to light, and isomerization-induced decomplexation led to aggregation, despite the presence of the more polar cis azobenzene. Despite aggregation, the process was reversible.

Multiresponsive materials, prepared using combinations of different stimuli-responsive groups offer the possibility to provide additional levels of function. One such dual-responsive polymer system incorporated a visible light-absorbing azobenzene monomer with acrylic acid to form a random copolymer enabling the preparation of photo- and pH-responsive polymer assemblies. Another example used a zwitterionic surfactant monomer instead of acrylic acid, but was still found to be sensitive to pH and light. The use of more than one stimulus can provide a great deal of control over properties such as drug release from nanoaggregates, as different effects can be observed for the various combinations of applied stimuli. In another example, a triple-responsive azobenzene-containing polymer was reported which responded to changes in temperature, pH, and irradiation, as well as subsets of those stimuli. The incorporation of N,N-(dimethylamino)ethyl methacrylate allowed for control via temperature change due to its lower critical solution temperature (LCST), as well as by pH change due to the presence of tertiary amines. Furthermore, the glycopolymer component allowed for both recognition events and increased solubility.

1.2.2 Supramolecular Chemistry of Azobenzene-containing Materials

Azobenzenes lend themselves well to supramolecular chemistry, where their photoresponsiveness can be exploited to elicit macroscopic changes. The change in these cases is most often due to the increase in polarity of cis azobenzenes compared to trans, but can also make use of the change in physical dimensions.
1.2.2.1 Small Molecule Azobenzene Interactions

The interactions of azobenzenes are often strong enough that small molecules such as surfactants based on the azobenzene motif are able to self-assemble into stimuli-responsive nanomaterials. Photosensitive surfactants are of interest because depending on the isomer present, different species may be solubilized, or the type of assembly may be altered. The inclusion of one or more stimuli-responsive groups in assemblies is an area that has been explored to prepare vesicles that can reversibly be reformed into wormlike micelles, or wormlike micelles that can shrink and grow by irradiation with the proper wavelength. Other systems in which multiple small molecule azobenzenes are held together by ionic interactions to form a polymer have been reported, and shown to disassemble or reassemble via irradiation. The same principle has been applied to azobenzene-containing surfactant systems, ionic liquids, and phase-transfer systems.

1.2.2.2 Host-Guest Azobenzene Complexes

The interaction between azobenzene and supramolecular hosts such as cyclodextrin or cucurbit[8]uril has been described in the literature. The inner core of such macrocycles has limited space, and is hydrophobic compared to the periphery. The combination of these factors allows for an associative interaction with planar, hydrophobic molecules such as azobenzene. In addition, the photoisomerization of the azo guest is known to cause dissociation, due to both the increase in polarity and width of the molecule, such that it can no longer fit within the host cavity. Therefore, with respect to azobenzene-containing materials and self-assembly, the reversible interaction between these host-guest systems is of interest in the design of stimuli-responsive supramolecular assemblies (Figure 1.12). Such photoresponsive host-guest complexes have been designed for use in drug delivery, modification of bulk properties such as viscosity, as well as in the non-covalent formation of pseudo-block copolymers.
1.2.3 Chemistry of the Diazene Bond

Selective reduction of the diazene bond could potentially be exploited for new stimuli-responsive materials (Figure 1.13). While many typical hydride reduction conditions such as sodium borohydride yield no reaction, the enzyme azoreductase is able to fully cleave the azobenzene into its aniline components (Figure 1.13, c).

Partial reduction to the hydrazobenzene is also possible (Figure 1.13, a), although traditionally these reductions can only be carried out using metal-catalyzed conditions. However, non-metal catalyzed methods have recently been reported, involving chemical reductants such as hydrazine or glutathione. Glutathione is particularly interesting as a stimulus due to its significant concentration in human tissues. This section will review the...
various reactive pathways azobenzenes follow, and the potential for molecular design based on such reactions.

1.2.3.1 Enzymatic Reduction of Azobenzenes

Drug delivery via enzymatic cleavage of the azo bond has been explored. The colon-specific drug 5-aminosalicylic acid was delivered by a multivalent dendrimer, and the drug was attached directly by the diazene bond, such that when it was cleaved by azoreductase in the intestine, the drug was released in the appropriate location. It is therefore of interest for the treatment of colon-specific illnesses such as Crohn’s disease, IBS, and colitis. The bacterial method of cleavage results in full reduction to the corresponding anilines, and does not stop at the hydrazine intermediate. The robust nature of the azo bond could also potentially allow for oral administration of the drug. Azobenzenes as triggering mechanisms for drug release have potential in vivo, as the azobenzene bond is tolerant to changes in pH, which is critical for oral drug delivery. More recent examples of azoreductase as a stimulus for degradation in polymeric systems include the work of Khan and coworkers, who were able to take advantage of enzymatic azo cleavage to either destroy diblock copolymer micelles or selectively form them using self-immolative chemistry.

1.2.3.2 Reductions Involving Metal Centers

Reductions of azobenzenes involving metal catalysts and metal-containing reagents are well-known. Azobenzenes are known to coordinate to metal centers via the non-bonding electron pairs on the azo nitrogens. This interaction activates the bond for reaction and facilitates its reduction to hydrazobenzene or aniline derivatives.

Many metals and their salts have been examined for their potential to reduce diazene bonds, including nickel, magnesium, zinc, palladium, iron, copper, and tin. Many examples have also been reported involving titanium salts and photocatalytic titanium oxide which is able to produce reactive oxygen radical species to react with the azobenzene. More recently, the use of gold nanoparticles has been explored in combination with irradiation with light. In most cases, the metal acts in a catalytic fashion, activating the azobenzene for reduction by a sacrificial reducing
agent such as formic acid,\textsuperscript{105, 107} isopropanol,\textsuperscript{120} hydrazine,\textsuperscript{102-104, 113} ammonia-borane,\textsuperscript{121} or hydrogen.\textsuperscript{109} Tributyltin hydride has also been demonstrated to react with azobenzene, although the reaction products are better described as cyclized than reduced.\textsuperscript{122} Diisobutylaluminum hydride reduced azobenzene where NaBH$_4$ failed due to coordination with the diazene nitrogens.\textsuperscript{123}

### 1.2.3.3 Metal-free Reductions of Azobenzene

Various agents have been reported for the reduction of azobenzene derivatives, and fall into the two broad categories of small-molecule reductants and catalytic transfer agents. Both typically involve hydride transfer via activation of the diazene bond. Borane in THF has been shown to produce the hydrazobenzene, made possible by the coordination of BH$_3$ by azobenzene.\textsuperscript{14} This mechanism differs from reductions using NaBH$_4$ or LiAlH$_4$ due to the availability of the empty p-orbital for coordination at the beginning of the reaction. An ammonia/borane system has been reported in combination with a ligated phosphorus center for concerted transfer hydrogenolysis, which was also shown to reduce azobenzene.\textsuperscript{124-125} The redox cycle in this example was centered at phosphorus, which was highly reversible between P(III) and P(V), which is similar to many metal-catalyzed reactions. It was also found that electron-poor azobenzenes were reduced more quickly, but electron-rich substrates were reduced completely to anilines.

Sodium dithionite is another reducing agent known to reduce azobenzenes to anilines, either by direct attack on the diazene bond,\textsuperscript{5, 126} or by use of an electron transporter such as dioctylviologen, which was shown to prevent over-reduction to aniline.\textsuperscript{15} The reactivity of dithionite alone also provides a useful model for the enzymatic reduction of azobenzene, which similarly produces anilines, and this reaction has been used in cellular environments in the study of membrane organization.\textsuperscript{127} Optimization studies have been conducted on azobenzene derivatives for specific use with sodium dithionite, as a means of using azo derivatives as cleavable linkers in peptide synthesis.\textsuperscript{128}

A fluoride-sensitive intramolecular reduction has also been reported.\textsuperscript{129-130} The mechanism of reduction is surprising in the sense that fluoride did participate directly, and simply activated a pendant hydrosilane which could then perform an intramolecular
hydrosilylation to reduce the diazene. In addition, these structures provided a potentially tunable colorimetric change, which may be useful in a fluoride sensor system.

The ability of diazene gas (N₂H₂) to act as a reducing agent is largely due to the formation of dinitrogen gas providing a strong driving force for the delivery of an equivalent of hydrogen. Reductions with diazene are thought to undergo a concerted hydrogen transfer with a six-membered ring-like transition state. While diazene is an excellent reducing agent, administering it to a reaction can be difficult due to its volatility, and therefore some reagents have been designed to produce diazene in situ, which then reduce the azo bond (Figure 1.14, top). ¹³¹

![Figure 1.14 – Mechanism for the reduction of azobenzene by diazene (top), and a possible mechanism for the direct reduction of azobenzene by hydrazine (bottom).](image)

Reduction of azo compounds with hydrazine alone was first reported in 1972, ¹³² although these reductions involved complex heteroaromatic substituents adjacent to the diazene, and concurrent side reactions. Recently, this reaction has been shown to be possible for a much wider array of compounds, including azobenzene derivatives. ¹⁶, ¹³³-¹³⁵

The volatility and toxicity of hydrazine make this method unsuitable for biological applications, although the lack of a metal component indicates that the reduction of azobenzenes may be more accessible than previously thought. The exact mechanism of reduction via hydrazine is not well-understood. A proposed mechanism ¹³⁵ involves nucleophilic attack by hydrazine on the diazene bond followed by several proton transfers. The intermediate compound thus contains a linear chain of four nitrogen atoms, and
decomposes via elimination to form one equivalent of diazene, and the reduced hydrazobenzene product (Figure 1.14, bottom). An earlier proposal suggested that it was diazene formed by the oxidation of hydrazine by air that was the true reducing agent, but successful reductions in the absence of oxygen do not support this mechanism.

Despite the removal of a metal catalyst, most reductions of azobenzene derivatives are performed under reflux in alcoholic solvents such as ethanol. Ideally, these reductions would be performed in an aqueous environment under ambient conditions. It was found by Smith and coworkers\(^\text{16}\) that the reduction of diazenes with electron-poor substituents was facile and could be completed in the absence of oxygen, while more electron-rich substrates were only reduced with oxygen present. These results may suggest that two mechanisms of reduction by hydrazine are possible, depending on the electronics near the azo bond.

### 1.2.3.4 Reduction by thiols

The reduction of electron-poor diazenes such diethyl azodicarboxylate (DEAD) by thiol nucleophiles has been known for many years.\(^\text{136-137}\) Indeed, DEAD has been used in the preparation of asymmetric disulfides due to the isolable nature of the intermediate (Figure 1.15).\(^\text{138}\) In these cases, the diazene is directly conjugated to electron withdrawing esters that activate the bond to nucleophilic attack, similar in mechanism to a conjugate addition.

![Figure 1.15 – Mechanism for the reduction of diethyl azodicarboxylate (DEAD) by thiols. DEAD has thus been used in the preparation of asymmetric disulfides, as an oxidizing agent.](image)

Reactivity of the azo bond \textit{in vivo} was first noted in the context of photobleaching of photoswitches, as it was observed that the thermal relaxation from \textit{cis} to \textit{trans} may have been accelerated in some cases by the reversible addition of a thiol.\(^\text{139}\) Elimination of the
thiol produced the trans isomer, while further oxidation by a second equivalent of thiol produced a disulfide and a hydrazobenzene, which was no longer useful as a photoswitch.41

Azobenzenes have been studied extensively in the field of peptide photoswitching, as a way to change their conformation and function, and the issue of photobleaching is prominent in the design of new photoswitches for this application. In one system, an azobenzene was shown to resist reduction in up to 10 mM glutathione, likely due to its electron-rich nature.4

However, in an example where four ortho-methoxy groups were added to redshift absorbance into the visible region, reduction was found to be an issue, despite the highly electron-rich nature of the rings.50 Halogenated azobenzenes were surprisingly less susceptible to reduction despite their electron-poor character, but were not absorbent enough for the photoswitching application. The discrepancy was explained mainly by the strong ability of the ortho methoxy groups to promote protonation of the azo bond and favor nucleophilic attack by thiols.3 When thioethers were used in the place of ethers at these positions, a sensitivity to reduction was not observed, reportedly due to a decrease in hydrogen bonding while maintaining high electron density.43

In these studies it was shown that reduction-sensitivity of azobenzene photoswitches was attenuated by the introduction of electron-rich substituents, when they were not able to form 6-membered rings via hydrogen bonding interactions. In addition, it was demonstrated that the tetra-ortho substitution pattern may slow reductive processes by the proximity of high electron density zones near the reactive site.

The reduction of photoswitching azo compounds by glutathione (GSH) is seen as a negative side reaction in the field of photoswitching, and much effort has been devoted to designing photoswitches that can withstand the intracellular conditions. However, reduction of azobenzenes by thiols could potentially increase their utility in the fields of biodegradable polymers and targeted drug delivery, as reduction-sensitive materials.
1.3 Self-Immolative Polymers

1.3.1 Overview

Many types of degradable polymers have been prepared with the aim of providing a temporary macromolecular structure, material, or device that can later be broken down into molecular byproducts. The design of such materials with tunable rates and methods of degradation has been important. One of the most industrially relevant classes of degradable polymers is the polyesters, and these products have become ubiquitous due to their cheap components and simple syntheses. However, they possess several shortcomings that can make them ill-suited for some applications. Their degradation is dependent on one chain scission reaction for every monomer unit, and the process is random; the cleavage of any ester linkage is as likely as any other. The problem in this case is two-fold, as one reaction can either lead to a drastic decrease in polymer molecular weight, or have virtually no impact at all.

The concept of a self-immolative polymer descends from work in prodrug chemistry, and the reactions of traceless linkers which served to conjugate targeting groups to drug molecules. This concept was later expanded to oligomeric chains to extend the physical separation between the triggering site and the drug molecule, and then to dendritic systems, where they were useful in signal amplification. Finally, the chemistry of self-immolative spacers was applied to linear polymers, which will be the main focus of this section.

1.3.2 Origin of self-immolative polymers

1.3.2.1 Pro-drug chemistry and self-immolative spacers

The delivery of drugs to targeted areas in the body is an area of great importance, as many active agents are quite toxic when administered directly, or in some cases unable to traverse obstacles such as the blood-brain barrier. Many prodrugs have been developed to address the targeting aspect, and to allow for triggered release of the active compound at the appropriate site.
Prodrugs that consist of a targeting group conjugated directly to the active are known as bipartite, as they have two components. It was hypothesized that the efficacy of these compounds could be increased by adding a spacer between the targeting group and the active, and these tripartite prodrugs formed the chemical basis for self-immolative materials (Figure 1.16). Self-immolative spacers can be broadly categorized by their mechanism of action, which allows them to release their drug cargo or other payload. Elimination spacers are quite common, and rely on the unmasking of an electron-rich heteroatom to facilitate the expulsion of an appropriate leaving group. Often, a quinone methide or analogue is produced, which can then be trapped by a nucleophile, thus restoring aromaticity. Cyclization spacers function similarly in that a nucleophilic heteroatom is exposed during the triggering reaction, which is then able to undergo an intramolecular cyclization to produce a stable (typically 5-membered) ring, while releasing the payload from the opposite side. These structural motifs can be tuned in many ways, including via the modification of their substituents and composition, while maintaining a self-immolative functionality. Furthermore, they can be combined to produce systems that degrade via both elimination and cyclization reactions.
1.3.2.1.1 Elimination-based Self-Immolate Spacers

The initial self-immolative or “traceless” spacers were based on the 1,6-elimination of 4-aminobenzyl alcohol.\textsuperscript{144} Liberation of the aniline allows for elimination from the benzylic position to yield an azaquinone methide, which is then trapped by water or another nucleophile to regenerate the aniline. Spacers based on elimination react quickly due to the lack of steric restraints on the electronic rearrangement. Many examples have been reported of elimination spacers in prodrugs,\textsuperscript{150} and while most use the original 4-aminobenzyl alcohol,\textsuperscript{151-158} 4-hydroxybenzyl alcohol and its derivatives are also effective (Figure 1.17).\textsuperscript{159-161}

![Mechanism of action of an elimination-based prodrug](image_url)

**Figure 1.17** – Mechanism of action of an elimination-based prodrug. Cleavage of the sugar at the anomeric position by β-glucuronidase reveals a phenol capable of 1,6-elimination, and this process releases 10-hydroxycamptothecin, an analogue of camptothecin.
In some cases the elimination reaction can be incorporated within the structure of the drug itself.\textsuperscript{162} Thiobenzyl alcohol has been reported to carry out the same reaction, which can be exploited such that the linker is attached to the triggering group via a disulfide bond.\textsuperscript{163} The critical aspect of these linkers is a free lone pair of electrons on the heteroatom conjugated to the ring system; if it is not available, the elimination cannot take place.

In addition to the original 1,6-elimination reaction, this type of spacer has been expanded to include 1,4-,\textsuperscript{157,164-165} and 1,8-elimination spacers,\textsuperscript{146} and factors such as ring substitution\textsuperscript{166} have also been investigated. An elimination based system has also been reported using a substituted pyridine instead of benzene to the same effect.\textsuperscript{167} When 1,4- and 1,6-elimination reactions are in direct competition, the 1,6-elimination proceeds slightly faster, and an intermediate species is observed prior to elimination.\textsuperscript{164} Eliminations of other species are also possible, as demonstrated by the elimination from a hemiaminal to release an amine drug.\textsuperscript{168} This example also illustrates the capability in prodrugs to conjugate several self-immolative spacers together in a chain. One of the main disadvantages and criticisms of elimination spacers based on aminobenzyl alcohol or hydroxybenzyl alcohol is the ability of their respective (aza)quinone methides to alkylate biological species. Therefore, the toxicity of such intermediates has been studied to some degree,\textsuperscript{169-170} although further studies would be necessary to determine at what level the specific self-immolative intermediates become cytotoxic.

\textbf{1.3.2.1.2 Cyclization-based Self-Immolative Spacers}

Cyclization spacers have been less frequently reported in the literature, but they are advantageous in their lack of reactive intermediates compared to the quinone methide-producing elimination spacers. Furthermore, their rates of cyclization can be tuned more extensively than elimination spacers, by changing the nucleophile, electrophile, and substituents along the cyclizing chain (Figure 1.18).\textsuperscript{171}
Figure 1.18 – Various mechanisms of action of cyclization spacers used in prodrug chemistry. A) The cyclization of an amine on a carbamate to form a urea; B) The cyclization of a phenol on an amide to form a dihydrocoumarin; C) A lactam cyclization promoted by proximity.

The earliest reported mention of a self-immolative cyclization spacer involved the intramolecular reaction of an amine on a phenyl carbamate to release the phenol and produce a cyclic urea. This structural motif has been expanded to include cyclizations of thiols on esters to form thiolactones or on carbamates to produce thiocarbonates or thiiranes. With the exception of thirane, most cyclization products are not immediately harmful via alkylation, but do suffer from kinetic constraints based on the stability of the electrophile, nucleophilic character of the cyclizing heteroatom, and steric effects.

Progress has been made towards the rate-tuning of cyclization spacers. Aminobutyric acid ester derivatives functionalized at the alpha position have been studied in the context of self-immolative spacers, and it was shown that bulky substituents and inductively withdrawing groups both accelerated the cyclization of an amine on the ester. Spacers with structurally rigid substitution were shown to decrease the half-life
significantly, from 39 seconds for an unsubstituted derivative, to 2.0 seconds for a
cyclopentyl variant, as a result of the Thorpe-Ingold and reactive rotamer effects.\textsuperscript{177}

Dihydrocoumarin derivatives have also been investigated as cyclization spacers
due to the stability of their bicyclic lactone species, which is so favorable that a typically
weak nucleophile such as phenol is able to directly attack a stable amide.\textsuperscript{179-180} A similar
cyclization has also been demonstrated between a phenol and an adjacent carbamate.\textsuperscript{181}
Comparable cyclization reactions have been demonstrated for use in solid-supported
synthesis procedures, as a release mechanism for the finished product.\textsuperscript{182}

\subsection*{1.3.2.1.3 Combinations of Self-Immolative Spacers}

Combined self-immolative linker strategies made the first steps towards self-immolative
polymers. Spacers used in series serve to further extend the distance between the targeting
group of prodrugs and the active compound, thereby increasing the relative rates of release
by making the trigger more available.\textsuperscript{183} This strategy requires that the self-immolative
reactions are much faster than the initial cleavage, or the elongated spacer becomes
inefficient for release in a target area. Drugs conjugated with cyclization spacers were also
found to be more stable to random cleavage yet more rapidly cleaved under triggered
conditions, and thus added a degree of stability.

A duocarmycin prodrug was prepared to employ both elimination and cyclization
spacers, and subsequently undergo a Winstein cyclization in a similar fashion to a self-
immolative 1,6-elimination, forming the active cyclopropane drug (Figure 1.19). One other
advantage of using a two-part spacer is that phenolic or alcohol-containing drugs can be
attached via carbamate instead of a carbonate, and thus are more stable to random
hydrolysis.

A further advantage to an increase in the number of self-immolative linkers is the
possibility for inclusion of self-immolative reporter molecules, such that release of the
prodrug cargo can be monitored by spectroscopy.\textsuperscript{184}
Figure 1.19 – A prodrug of a duocarmycin analogue utilizing a two-part combination self-immolative linker. Cleavage of the sugar leads to a 1,6-elimination and decarboxylation reaction, which reveals an amine which can cyclize to release the compound and form a cyclic urea. The released seco-drug undergoes a Winstein cyclization to form the active compound \textit{in situ}.

1.3.2.1.4 From Prodrugs to Sensors

The idea of using self-reporting self-immolative materials is particularly attractive in the field of chemical sensors. The strategy is even more effective in cases where the stimulus of interest leads to signal amplification by releasing more of the stimulus as its payload. Fluoride sensors which release two additional equivalents of fluoride are able to amplify the stimulus of very low concentrations of fluoride in a geometric fashion,\textsuperscript{185-186} and similar systems for the detection of piperidine have also been prepared.\textsuperscript{187} While these materials
lead to rapid degradation of all nearby sensors, they provided only a single output in the form of an absorbent molecule. Other non-geometric systems have been demonstrated to release one or two different materials as well as a reporter, such that a single stimulus has multiple effects.\(^{188}\)

![Chemical structures](image)

**Figure 1.20** – A sensor based on self-immolative chemistry. Interaction with Penicillin G Amidase reveals a hemi-thioaminal, which eliminates a thiol that can undergo cyclization either to a cyclic thiocarbonate (solid arrows), or thiirane and carbon dioxide (dashed arrows). This process releases attached coumarin 1.52 which becomes fluorescent.

Sensors have been developed for stimuli such as including thiols,\(^{189}\) and several new self-immolative spacers have been developed for use in sensors, including a hemithioaminal linkage which undergoes 1,2-elimination to release a thiol, and later a fluorescent dye (Figure 1.20).\(^{190-191}\)

A wide range of self-immolative linkers has been developed, and while not all species have been incorporated into polymeric materials, a vast potential still exists for new development in this area.
1.3.2.2 Self-immolative dendrimers

The first self-immolative dendrimers were developed simultaneously by three separate groups, who focused on either carbamate-based elimination and cyclization systems or benzyl ethers. 

![Diagram of dendrons and a self-immolative G3 dendrimer](image)

**Figure 1.21** – Schematic diagram demonstrating the generational structure of dendrons, and an example of a self-immolative G3 dendrimer capable of undergoing complete degradation to release 8 equivalents of a reporter molecule at the periphery.

These materials were based directly on prodrug chemistry, and focused on multiple generations of the self-immolative spacers frequently used in that field. Several articles review the field of self-immolative dendrimers and their uses, specifically as sensors with an amplified stimulus response. These materials possessed the ability to release multiple groups as the result of a single reaction, but suffered from a tedious synthesis.
Their branched generational structure, however, allows for the incorporation and release of many groups from the periphery in response to a single reaction (Figure 1.21).

1.3.3 Linear Self-Immolative Polymers

Linear self-immolative polymers are advantageous over dendritic systems due to their relative ease of synthesis. While dendrimers are grown stepwise to produce a single monodisperse macromolecule, linear polymers are generally prepared in a single step. At the expense of producing a mixture of chain lengths, the synthetic burden can be greatly decreased by using a one-step polymerization.

1.3.3.1 Polycarbamates and their Derivatives

Of the growing number of linear self-immolative polymers reported in the literature, those structures based on polycarbamates (polyurethanes) have the longest history. The carbamate linkage is simultaneously stable and synthetically accessible, while providing the means for incorporation of a wide variety of amines and alcohols. Such versatility has led to a range of self-immolative materials for a broad array of applications.

1.3.3.1.1 Poly(benzyl carbamate)s

The progenitor of all linear self-immolative polymers was based on initial work with the carbamates of 4-aminobenzyl alcohol in prodrug chemistry. This traceless linker was extended into oligomeric systems, and eventually polymerized via thermal rearrangement of a phenyl carbamate. During the polymerization phenol was liberated to yield an intermediate isocyanate, which reacted with a benzyl alcohol in the presence of a tin catalyst to generate a stable poly(benzyl carbamate), endcapped by an alcohol that could later be cleaved in a β-elimination reaction by bovine serum albumin (BSA). Removal of the tert-butyl ester with trifluoroacetic acid (TFA) permitted the use of the polymer in an aqueous environment.
Figure 1.22 – An example of the synthesis of an enzyme-triggered self-immolative poly(benzyl carbamate). An isocyanate is generated *in situ* via the thermal rearrangement of a phenyl carbamate, and polymerization occurs between the benzyl alcohol in the same monomer in an AB fashion. Removal of the endcap releases several equivalents of a fluorescent monomer via alternating 1,6-elimination and decarboxylation reactions.

The aminobenzyl alcohol used in 1,6-elimination chemistry is highly functionalizable, as the self-immolative chemistry is compatible with ring-substitution. The released aniline monomer in this case was also fluorescent due to the incorporation of an acrylate moiety, providing a convenient method to monitor the release by fluorescence (Figure 1.22). A similar material was later used for the labeling of enzymes via the intermediate azaquinone methide once the enzyme of interest had cleaved the triggering group.\(^{198}\) The concept of signal amplification was expanded to include the release of non-monomer components from pendant groups, thus accomplishing the release of many molecules with a single trigger.\(^{199-200}\)

Recent work with this polymer backbone has demonstrated the usefulness of the material as a readout for highly sensitive assays of peroxide,\(^{201}\) enzymes,\(^{202}\) and metal ions,\(^{203}\) by making use of the increased permeability of the material to water upon triggered depolymerization.
Poly(benzyl carbamate)s have also been applied to larger-scale vehicles for triggered release. The aforementioned examples were mainly concerned with the release of monomer units or covalently linked payloads, but assemblies and polymer capsules have also been developed.\textsuperscript{204} A microcapsule system which could be triggered under various conditions, depending on the endcap, was shown to release its cargo upon treatment with either acid or base.\textsuperscript{205} This system was also prepared from a pendant-based self-immolative monomer, but in this case the side-chains were used to crosslink the polymer shell to better contain its cargo and prevent premature de-encapsulation. A non-crosslinked block copolymer system was also reported, where the hydrophilic poly(\(N, N\)-dimethyl acrylamide) block allowed for assembly into vesicular structures in aqueous solution. Once again, a range of stimuli could be used to trigger depolymerization, including UV light, visible light, or thiol reduction.\textsuperscript{206} The same SIP-PDMA block copolymer system has also been prepared by ATRP, and a thermo-sensitive trigger incorporated (Figure 1.23).\textsuperscript{207} Block copolymers of self-immolative materials are perhaps more easily prepared than crosslinked microcapsules, because they do not rely on inclusion of a crosslinkable group within the self-immolative monomer.

![Figure 1.23 - Thermally triggered retro-Diels Alder depolymerization of a self-immolative block copolymer.](image)

As a parallel to the trend in prodrug chemistry, cyclization spacers have been successfully incorporated into self-immolative polymers. The first reported example of such a polymer contained alternating phenyl and benzyl carbamates, and was prepared from 4-hydroxybenzyl alcohol and \(N, N\)’-dimethylethylene diamine spacers.\textsuperscript{208} This
polymer could be prepared without a heavy metal catalyst by using an amine nucleophile with an activated carbonate electrophile. This methodology lengthened the monomer synthesis, but offered increased chain flexibility, and less toxic degradation products, with similar stability due to its carbamate linkages. Various endcaps have been used, including those responsive to UV or visible light,\textsuperscript{209} or strong acid or ester hydrolysis (Figure 1.24).\textsuperscript{208}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Structure of an alternating polycarbamate with various endcaps. Removal of the endcap triggers end-to-end depolymerization via cyclization, decarboxylation, and 1,6-elimination reactions.}
\end{figure}

1.3.3.1.2 Kinetics of Polycarbamate Depolymerization

The kinetics of the self-immolation of carbamate-based polymers has been studied at some length to determine the relevant parameters and limitations to depolymerization. The structure and substitution of elimination spacers has been investigated,\textsuperscript{166} and it was found that electron-rich substituents accelerated the rate of elimination, as well as extended conjugation to a lesser extent. These systems are more amenable to the delocalization of electrons from an exposed phenol or aniline, and as such more rapidly eliminate a leaving group. The kinetics of depolymerization of linear alternating benzyl phenyl carbamates was also investigated in detail.\textsuperscript{210} In this work a series of monodisperse oligomers up to the octamer were prepared and their depolymerization monitored. The assumption was made that cyclization would be the rate determining step in the depolymerization of each monomer repeat unit, and that the elimination and decarboxylation reactions would be comparatively fast. Using these constraints it was demonstrated that self-immolative
materials exhibit a pseudo-zero order degradation profile, with an initial linear section in which the concentration of degrading polymer is constant (Figure 1.25). Once the degradation reaches the final monomer repeat, the rate becomes first order. The effects of dispersity and chain length were also probed, and it was shown that a highly disperse polymer sample tended to degrade in a pseudo-first order fashion, where the initial linear section is overshadowed, and that increased chain length correlated with longer degradation times.

![Figure 1.25 – Kinetics of degradation of a) monodisperse oligomers of length 1 (●), 2 (■), 4 (▲), and 8 (♦) units; b) polymers of low (▲) and high (■) molecular weight. Reproduced with permission from reference (210). Copyright 2013 American Chemical Society.](image)

1.3.3.1.3 Poly(carbonate/thiocarbonate)s and poly(carbonate/carbamate)s

Self-immolative polymers can be tuned via modification of the backbone itself, as well as the chain length. Examples of cyclization-based linear self-immolative polymers have been reported in which the original \(N,N'\)-dimethylethylenediamine spacer was used with a 2-mercaptoethanol unit to yield a poly(carbamate/thiocarbamate) which degraded by alternating cyclizations only.\(^{211}\) This material did not rely on the original 1,6-elimination reaction of the hydroxybenzyl alcohol unit, and the byproducts of depolymerization could not alkylate heteroatoms. The incorporation of a thiol within the polymer also enabled the researchers to endcap the polymer by a disulfide linkage, making this polymer reduction sensitive.
The removal of the elimination spacer from the backbone slowed the rate of depolymerization due to increased steric demands, but the involvement of heteroatoms other than nitrogen can have a significant effect on the rates of cyclization. The cyclizing nucleophile (X) and the electrophile (Y) on which the cyclization occurs are important in the depolymerization reactions. In the case of the alternating cyclization and elimination polymer, an amine cyclizes on a phenyl carbamate. In the alternating cyclization polymer, a thiolate cyclizes on an alkyl carbamate, and the released amine cyclizes on a thiocarbamate.

![Diagram of polymer structures](image)

**Figure 1.26 – Design of self-immolative polymers based on a similar structural motif, using variation only at heteroatom positions X and Y. Polymers synthesized including the initial polycarbamate 1.64, a poly(carbonate/thiocarbonate) 1.66, and a poly(carbonate/carbamate) 1.67.**

The concept of heteroatom tuning was later applied to new self-immolative backbones (Figure 1.26). These new backbones utilized the alternating cyclization and elimination structural motif, but replaced the diamine spacer with either 2-mercaptoethanol or 2-(methylamino)ethanol. This alteration changed the linkages from carbamates to a mixture of thiocarbonates and carbonates, and increased their electrophilicity. Furthermore, the thiolate is more able to attack a carbonate than a carbamate, increasing its rate of depolymerization. The second example utilizing 2-(methylamino)ethanol follows a similar principle. The amine is more able to attack a carbonate than a carbamate, and thus the rate of depolymerization was increased.
1.3.3.2 Poly(benzyl ether)s

Just as carbamate-based dendrons led to linear polycarbamates, benzyl ether dendrimers led to linear benzyl ether polymers. These materials are similar to poly(benzyl carbamate)s, but omit the decarboxylation step during depolymerization, in favour of the direct elimination of a phenol moiety. Few examples of self-immolative poly(benzyl ether)s are found in the literature, but in these examples, various aspects of the class of polymers have been explored, including substitution patterns of the benzyl system, as well as a range of stimuli-responsive triggers for depolymerization (Figure 1.27). Critically, the polymer was stable without an endcap (Endcap = H) under ambient conditions.

![Diagram](image)

**Figure 1.27** – Base-catalyzed polymerization of a substituted quinone methide to generate a poly(benzyl ether) endcapped by one of several triggering groups. Recovered monomers can be reused following depolymerization.

In another example, a polymer was synthesized in which each monomer unit bore a pendant trigger, thus greatly increasing the sensitivity of the material to degradation, and allowing for the depolymerization to begin at any point along the chain. This mechanism of degradation differs from most self-immolative polymers in that it is not truly end-to-end depolymerization, rather middle-to-end, where “unzipping” from the terminus is as likely as degrading from any individual monomer. The degradation of these polymers was also found to produce a directly re-polymerizable monomer, unlike polycarbamates, because they irreversibly lose carbon dioxide upon depolymerization.
1.3.3.3 Polycarbonates

Polycarbonates have been explored recently as degradable polymers for the purpose of recycling efficiency, but they have also been shown to depolymerize from end to end in response to basic conditions and the generation of a terminal alkoxide. These materials thus have some potential as self-immolative polymers, although a more specific mechanism would be ideal, given the limited stability of carbonate functionalities to basic and nucleophilic degradation. Several polycarbonates have been reported to exhibit a linear depolymerization mechanism under various stimuli, but to date have not been used as self-immolative materials.

Figure 1.28 – Synthesis and depolymerization of a recyclable polycarbonate which can undergo end-to-end depolymerization upon exposure of an alkoxide or carbonate. Depolymerization in the absence of CO$_2$ can lead to the formation of an epoxide monomer, while excess CO$_2$ can produce a cyclic carbonate.
The non-nucleophilic base sodium bis(trimethylsilyl)amide, or a metal catalyst have typically been used for the initiation of the polymerization, and resulted in linear depolymerization instead of random backbone cleavage. Different degradation pathways have also been reported depending on the concentration of carbon dioxide (Figure 1.28). These materials are one of the few examples of a depolymerizable material that can be prepared on a large scale and degraded in the absence of water.

Given the broad range of applications for polycarbonate plastics, investigation of self-immolative polycarbonates may be an interesting avenue for future research. To this end, more specific triggers, such as a photocleavable linker, could be incorporated to generate the alkoxide without base or metal complexes.

1.3.4 Chain-Shattering Polymers

Chain-shattering polymers (CSPs) make use of self-immolative chemistry to enhance their degradation, but cannot be classified as true self-immolative polymers, as a single reaction cannot lead to complete depolymerization. However, these materials are an interesting class of materials, because they incorporate rapidly-degrading pendants into the structures of materials which otherwise either degrade slowly or non-specifically, such as polyesters. Furthermore, they can provide to traditionally non-stimuli responsive materials a sensitivity to many different triggers (Figure 1.29).

Polyesters have been prepared as CSPs in several cases, taking advantage of self-immolative cyclization,\textsuperscript{221-222} elimination,\textsuperscript{223-225} or both\textsuperscript{226-227} to effect rapid and trigger-responsive chain scission. This brand of chemistry has also been useful in poly(ester amide)s, which present an alternative biodegradable polymer that incorporates additional functionality via amino acids in the polymer backbone.\textsuperscript{228} A number of studies report the incorporation of self-immolative spacers as pendants in these materials, and their effect on the rate of polymer degradation.\textsuperscript{229} Chain shattering polymers have also been prepared from components which are themselves stimulus-responsive,\textsuperscript{230} but these materials as a whole do not classify as self-immolative, as the triggering reaction causes chain cleavage but not depolymerization.
Figure 1.29 – Degradation of a chain-shattering polymer via self-immolative chemistry. Removal of a triggering unit and decarboxylation reveals an amine that can cyclize to form a cyclic urea. The polymer backbone is then broken via a 1,4-elimination reaction. The addition of water and further self-immolative reactions leads to complete degradation to the small molecules shown.

CSPs are similar to linear SIPs in that the backbone of the polymer is not immediately relevant in the rate of polymer degradation, and that the same polymer backbone can be used with variable triggers. In comparison to SIPs, CSPs have many more stimulus-responsive groups, which increases their likelihood of reaction to stimuli, but does not amplify their response, since the backbone is not completely self-immolative. Therefore the synthesis of one class of degradable material over another will depend on the intended application.
1.3.5 Self-Immolative Polymers Based on the Polymerization of Aldehydes by Reversible Addition

1.3.5.1 Overview

Self-immolative polymers based on a reversible addition reaction are advantageous because their polymerization and depolymerization do not involve irreversible reactions. This property makes the monomer reusable, and allows for control over the reaction equilibrium using temperature. Polymers of this class generally make use of a property known as the ceiling temperature ($T_c$), which occurs at equilibrium ($\Delta G_p = 0$) between enthalpic ($\Delta H_p$) and entropic ($\Delta S_p$) effects in a polymerization (Equation 1.1).

$$\Delta G_p = \Delta H_p - T_c \Delta S_p$$

The enthalpic term encompasses the changes in the strengths of the bonds in the polymer relative to those in the monomer, while the entropic term depends on the number of species present, both monomeric and polymeric. In a low $T_c$ polymer, $\Delta H_p$ is typically small as the strengths of the bonds in the polymer are only slightly greater than those in the monomer. Above $T_c$ the material will begin to depolymerize, as the entropic term dominates over the enthalpy term. Conversely, below $T_c$, the enthalpic gains from bond formation will dominate over the entropic losses. These changes are often dependent on the environment, and thus the ceiling temperature during polymerization is not equivalent to the ceiling temperature in a different environment.

The most common type of reversible addition SIP is the polyacetal. The aldehyde carbonyl of the monomer is polymerized into a polyacetal under acidic or basic conditions, and below the ceiling temperature this material can be maintained as a polymer. The stability of the material is thus dictated by the unstable hemi-acetal at each end of the growing chain, so it follows that reacting these hydroxyls in an irreversible fashion can produce a stable polyacetal. Polymers of this type have been made from many aldehydes, including the engineering plastic poly(oxymethylene) (POM, polyformaldehyde). POM was initially prepared by DuPont without stabilization, leaving an unstable hemiacetal at either end of the polymer. Despite its ability to reach high molecular weight, the plastic
could not be commercialized until it was discovered that reaction of the hemiacetals with acetic anhydride would produce a thermally stable polymer for use in injection-molding.

One of the most important considerations for the polymerization of aldehydes is the electrophilicity of the carbonyl carbon. Highly electrophilic compounds may be more rapid to polymerize, but also slower to depolymerize due to their ceiling temperature. Furthermore, they may be more difficult to purify because of their high affinity for hydration. Conversely, less electrophilic aldehydes are harder to polymerize, but can undergo very rapid depolymerization later. The preparation of glyoxylates,\textsuperscript{233-240} and phthalaldehydes\textsuperscript{241-250} has been more completely explored in recent years, and these materials exhibit similar but contrary properties. While glyoxylates are more readily polymerized, their rate of degradation is not as rapid as phthalaldehydes. Aldehydes in glyoxylates are particularly electron poor, which pushes their ceiling temperature higher than other aldehydes, because they prefer to stay in either a hydrated or acetal-like state.

1.3.5.2 Polyglyoxylates

Polyglyoxylates are a class of aldehyde addition polymers that consist of a polyacetal backbone with pendant alkyl esters. These materials are unstable in the absence of an endcapping agent, and depolymerize readily above their ceiling temperature. The first glyoxylate polymers were prepared from methyl glyoxylate, endcapped with phenyl isocyanate, and then hydrolyzed to the polyacid to form a biodegradable polyanionic surfactant.\textsuperscript{235-236} Poly(methyl glyoxylate) (PMG) was also investigated as a system for drug delivery,\textsuperscript{237-239} although the significant release of methanol from these materials upon ester hydrolysis limits their applicability \textit{in vivo}. Poly(ethyl glyoxylate) (PEtG) was thus developed as an alternative material, with similar properties but reduced toxicity.\textsuperscript{234, 240} These initial materials were degradable by ester hydrolysis followed by acid-mediated cleavage of the acetal backbone, catalyzed by the carboxylic acid pendants. Thus, its degradation was not controllable or triggerable, and it was not further developed for many years.
Figure 1.30 – General synthesis of polyglyoxylates. Polymers have typically been stabilized with phenyl isocyanate (1.86), but recently it has been shown that carbonates (1.87) are similarly able to prevent premature depolymerization. A photosensitive carbonate (1.88) is also shown, and has been demonstrated to work as a self-immolative polymer.

Typical stabilizing groups for glyoxylate polymers included isocyanates, anhydrides, and ethyl-vinyl ether, but none of these were considered stimuli-responsive to conditions which would not directly affect the glyoxylate backbone. However, these materials have been recently reinvestigated as self-immolative polymers, as it was possible to effectively endcap the polymers with carbonates (Figure 1.30). These carbonates, while less stable than ethers or carbamates, provided the necessary structure for the incorporation of stimuli-responsive moieties. Novel glyoxylate polymers have since been synthesized containing variable side-chain esters, granting a means of tuning the bulk polymer properties, and they have been shown to degrade rapidly when triggered with an appropriate stimulus. Ethyl glyoxylate has also been copolymerized with phthalaldehyde, which opens many new avenues for the synthesis of polyaldehydes with highly tunable properties, and stimuli-responsive degradation pathways.
1.3.5.3 Poly(phthalaldehyde)s

Poly(phthalaldehyde) (PPA) is an addition-based aldehyde polymer which forms via alternating inter- and intra-molecular reactions. These polymers are typically synthesized under forcing conditions due to their low ceiling temperature, which can promote rapid depolymerization once a single acetal is converted to a hemiacetal. In comparison with other aldehyde polymers, a significant increase in rigidity is observed due to the bicyclic repeat unit generated during polymerization. This aspect of PPA makes it of potential use as a structural material. PPA has been studied in part as a resist for dry lithography.\textsuperscript{252-254} As a lithographic resist, the spontaneous depolymerization of PPA is highly advantageous, as it provides an amplified response to the etching agent.

Phthalaldehydes have been explored in the field of metastable electronic devices, which are devices that serve a purpose for a specified time before being destroyed. Self-immolative materials such as PPA allow for more general stability and resistance to typical conditions, but heightened sensitivity to the specific stimulus required for degradation.\textsuperscript{246} PPA can similarly be used to pattern the deposition of other materials such as gold nanorods,\textsuperscript{255} and removed easily afterwards with minimal effort.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1_31.png}
\caption{Typical conditions for the anionic polymerization of poly(phthalaldehyde). These polymers have been endcapped with various stimuli-responsive units, including silyl and allyloxy carbonyl groups, as well as non-stimuli-responsive groups including carbamates, esters, and ethers.}
\end{figure}

The mechanisms of polymerization and depolymerization of PPA is of interest, specifically in the context of self-immolative materials. These polymers should be simple to synthesize, and yet also depolymerize from end to end in response to a triggering event.
The synthesis of PPA has been demonstrated in many instances, but new systems for its preparation are still being reported. The synthesis of SIPs on a large scale is also a concern, but recent work has demonstrated that scale-up from a laboratory setting is possible. Finally, the complete endcapping of stimulus-responsive PPAs is paramount to their use, and as such the characterization of many end groups has been studied (Figure 1.31).

Several mechanisms for depolymerization of PPA have been observed. The polyacetal is well known to degrade in the presence of acid, which directly attacks the acetal backbone. In the absence of an appropriate endcap and either anionic or cationic polymerization conditions, 1,2-phthalaldehyde is known to homopolymerize and form cyclic polyacetals, whose ring size is kinetically variable depending on the monomer available. However, despite lacking a specific triggering moiety, these rings can still be triggered to depolymerize by the application of mechanical force such as sonication. Surprising results have even been demonstrated in linear endcapped PPA, where the addition of sub-stoichiometric fluoride to a silyl-ether capped polymer still resulted in complete depolymerization. This effect was not expected, due to the strong silicon-fluorine bond formed during the reaction, but led to the conclusion that water was acting to liberate fluoride for subsequent reactions.

The use of PPA in block, alternating, or random copolymers is a recent development which has mainly aimed to tune the properties of the polyaldehydes for application in a more diverse range of applications. For example, 1,2-phthalaldehyde has been copolymerized with benzaldehyde derivatives to produce polymers with functional handles incorporated, or more recently it has been prepared with ethyl glyoxylate to produce polymers with highly tunable thermal properties. Block copolymers are also of interest, as they afford the capability to pattern surfaces using phase separation, as in a recent example where a polystyrene-PPA block copolymer was synthesized and used to template nano-channels.

PPA has been used in the preparation of macro- and micro-scale devices such as patterned devices or diffusion-controlled pumps, as well as in potential drug
carriers. The self-immolative and physical characteristics of PPA also make these polymers suitable for stimulus-specific patterning of films, where the main chain polymer of both patterning agents is the same. Using the same backbone avoids problems with polymer mixing and incompatibility, and could allow for greater control over polymer degradation.

1.4 Scope of This Thesis

The overarching theme of this thesis is the introduction of azobenzene as a multistimuli-responsive trigger for self-immolative and degradable polymers. At the outset of this work, to the best of our knowledge, azobenzene had never been used as a reduction-sensitive moiety within the framework of degradable polymers, or stimuli-responsive materials. Their main usage reported in the literature most frequently revolved around their photoresponsiveness, and thus the goal at the outset was to demonstrate that azobenzene could provide a unique functionality capable of orthogonal and potentially synergistic effects in response to chemical reduction and photoisomerization. In addition, focus was placed on the investigation of azobenzene-derivatives in amphiphilic polymer assemblies designed for the encapsulation, transport, and release of drugs.

Chapter two describes the initial development of a reduction-sensitive azobenzene endcap for self-immolative polymers. It will be shown that following the synthesis and degradation of small molecule models, that it is possible to utilize azobenzene as a stimulus-specific trigger for the degradation of various self-immolative polycarbamate backbones.

Chapter three expands on the efforts of chapter two by first striving to optimize and enhance the reduction-sensitivity of azobenzene endcaps by the synthesis and analysis of a library of electron-poor azobenzenes. To further the applicability of azobenzenes as multiresponsive units in degradable materials, the optimal azobenzene will be shown to be incorporated at a high loading as pendant groups in a poly(ester amide)-based chain-shattering polymer. The aqueous assemblies of this material will be shown to successfully encapsulate a model compound, and to respond to both reduction and light.
Chapter four further details an expansion of work from chapter two, by the synthesis of amphiphilic block copolymers using an azobenzene-based linker between blocks. This chapter will address limitations of polymer insolubility and the choice of reducing agent, in the aim of producing a system more capable of use in drug delivery applications. Furthermore, it will be demonstrated that the reduction-sensitive azobenzene motif can be applied in the context of polyglyoxylates, a rapidly-developing class of self-immolative polymers. Similar to chapter three, aqueous polymer assemblies will be studied and shown to be able to encapsulate and release a model compound.

Chapter five will discuss the overall conclusions of the thesis, and expand on the potential avenues of investigation opened by this work.

1.5 References


Chapter 2

2 A Multiresponsive Azobenzene Endcap for Self-Immollative Polymers

2.1 Introduction

Self-immollative linear polymers are a class of polymers that undergo end-to-end depolymerization in response to the cleavage of an endcap from the polymer terminus. Their unique features, including a predictable degradation time dependent on polymer length\(^1\) and the ability to change the stimulus to which they respond by simply changing the endcap, have made them attractive materials for a wide range of applications including sensors,\(^2,3\) controlled release systems,\(^4-7\) shape-changing plastics,\(^8\) and self-powered microscale pumps.\(^9\) Thus far, a variety of polymer backbones including polycarbamates,\(^2-3, 10-11\) poly(carbonate/thiocarbonate)s,\(^12\) polyphthalaldehydes,\(^13-16\) poly(benzyl ether)s,\(^17\) and most recently polyglyoxyxlates\(^18\) have been developed. In addition, various endcap cleavage triggers have been explored, including light,\(^4, 7, 18\) pH,\(^10\) reduction,\(^12\) oxidation,\(^2\) and enzymes.\(^3, 19-20\)

Here we show that an azobenzene moiety can be used as a multi-responsive endcap for self-immollative polymers. Azobenzenes are well known chromophores and photoresponsive units that undergo \textit{trans-cis} isomerization in response to irradiation with UV light.\(^21\) A recent report describing the use of azobenzene to prepare substituted hydrazines in the presence of sacrificial hydrazine as a reductant\(^22\) suggests that azobenzenes also have the potential to serve as endcaps for self-immollative polymers because the hydrazobenzene derivative resulting from the reduction process contains an anilinic nitrogen, which can trigger the depolymerization of self-immollative polymers via a 1,6-elimination reaction (Figure 2.1). Unlike nearly all other examples of endcaps, complete destruction of the endcap is not required to initiate the depolymerization, as the reduction process is reversible following cleavage. Furthermore, conversion of the azobenzene to the resulting hydrazobenzene results in a visual colour change, providing an optical read-out of the triggering event.
2.2 Results and Discussion

2.2.1 Synthesis of a Reduction-sensitive Azobenzene Derivative

The first step in demonstrating the utility of azobenzene as an endcap, was to demonstrate the proposed chemistry on a small molecule model compound that could be easily studied by UV-visible and NMR spectroscopic methods. As shown in Figure 2.2, ethyl-4-aminobenzoate was oxidized by oxone to produce ethyl-4-nitrosobenzoate 2.1. The ethyl ester was incorporated to ultimately provide an electron-withdrawing group in the final endcap to enhance the rate of reduction. Reaction of 2.1 with 4-aminobenzyl alcohol provided the azobenzene derivative 2.2, which was acetylated to provide the target model compound 2.3.
2.2.2 Effect of Hydrazine Addition on Model Azobenzene

The reduction-sensitivity of compound 2.3 was evaluated by UV-visible and $^1$H NMR spectroscopy. During the reduction process, the hydrazobenzene 2.4 (Figure 2.2) and acetate should be produced, giving two methods of detection. As shown in Figure 2.3, upon addition of excess hydrazine to a solution of 2.3 in methanol, a hypsochromic shift was observed as the $\lambda_{\text{max}}$ at 330 nm was replaced by a new $\lambda_{\text{max}}$ at 300 nm. This can be attributed to the loss of conjugation between the aryl rings. An isosbestic point was observed at 309 nm, suggesting clean conversion of 2.3 to a single absorbing species over a period of 18 h.
Figure 2.3 – UV-visible spectra showing the clean conversion of 2.3 to 2.4 after the addition of 10-fold excess hydrazine hydrate.

Figure 2.4 shows NMR spectra which provided confirmation of the conversion of 2.3 to 2.4 along with acetate. Spectra of the products showed significant upfield shifts in the peaks corresponding to the benzylic and aromatic protons. The identity of 2.4 was confirmed through independent synthesis under similar conditions (Figure 2.2), followed by full characterization.
To confirm that the observed acetate came from the proposed 1,6-elimination reaction, control reactions were performed on both benzyl acetate and a benzyl carbamate derivative (as a model of the proposed carbamate endcap from Figure 2.1). At the same hydrazine concentration used for the study of 2.3, acetate generation was much slower for benzyl acetate, and no dimethylamine formation was observed for the carbamate, as determined by relative integrations in $^1$H NMR (Figure 2.5). While some hydrolysis of benzyl acetate occurred over 48 hours, the rate of acetate release via azobenzene reduction was much faster, suggesting that this mechanism outcompetes the hydrolytic one. In a less electrophilic carbamate model, no hydrolysis is observed at all, which indicates that the self-immolative polycarbamate might be relatively stable towards non-specific degradation. Combined, these results demonstrate that the proposed reductive/self-immolative mechanism is strongly dominant in comparison with possible nucleophilic or hydrolytic mechanisms.
2.2.3 Reduction of Azobenzene Endcaps with Thiols

While hydrazine is a specific chemical stimulus that may be useful in some applications, it was also of interest to demonstrate that the reduction of 2.3 is possible under biologically relevant conditions, as this would significantly expand the utility of the endcap. Electrophilic diazenes such as diethyl azodicarboxylate (DEAD) are known to undergo reduction in the presence of free thiols. More recently, acceleration of the thermal cis-trans isomerization of azobenzenes by thiols has been reported. In addition, the reduction of azobenzenes by thiols has been observed as an undesirable side reaction in the application of azobenzenes in biological systems. In our case, the reduction is desirable for initiation of depolymerization, so the reduction of 2.3 in the presence of dithiothreitol (DTT) was investigated. It was demonstrated by UV-visible (Figure 2.6) and $^1$H NMR spectroscopy (Figure 2.7) that it was indeed possible to cleanly reduce compound 2.3 to compound 2.4 using DTT providing an additional triggering stimulus that can potentially be applicable in vivo with a water soluble system.

Figure 2.5 - Comparison of the effect of hydrazine on compound 2.3 (●), benzyl acetate (○), and a benzyl carbamate (♦).
Figure 2.6 – UV-visible spectra showing the conversion of 2.3 to 2.4 after the addition of excess DTT at 65 °C in 1:1 methanol/phosphate buffer (100 mM, pH = 7.4).

The $^1$H NMR study showing the reaction of DTT with compound 2.3 is notable due to the apparent presence of only the small amount of cis isomer. This isomer is much more soluble than the trans isomer due to its polarity, and in the methanol/phosphate buffer mixture used for the NMR study, it is the only visible compound. Its reduction appears to be relatively fast, contrary to the UV-visible study which shows a much slower decrease in absorbance of the trans isomer, compared to hydrazine reduction.
Figure 2.7 – Conversion of the cis isomer of compound 2.3 to 2.4 by DTT as shown by $^1$H NMR. The reaction appears to be complete within 1 hour.

2.2.4 Synthesis of an Azobenzene-endcapped Polycarbamate

Having demonstrated the feasibility of cleanly reducing the model endcap 2.3, the next step was to introduce this moiety to the termini of self-immolative polymers. In one example, the azobenzene was incorporated as the endcap for our previously reported self-immolative polycarbamate based on 1,2-dimethylethylene diamine and 4-hydroxybenzyl alcohol.\(^\text{10}\) As shown in Figure 2.8, this was accomplished by the conversion of 2.2 into an activated carbonate by reaction with 4-nitrophenyl chloroformate to provide endcap 2.5. Polymerization using 2.5 with monomer 2.6\(^\text{10}\) in the presence of NEt$_3$ and 4-dimethylaminopyridine (DMAP) provided polycarbamate 2.7. The polymer was analyzed by size exclusion chromatography (SEC) relative to poly(methyl methacrylate) (PMMA).
standards in DMF, which have been shown to have the most accurate correlation with this polymer backbone, and the polymer had an M_w of 9 200 g mol\(^{-1}\) and \(D = 1.6\). UV-visible spectroscopy confirmed the presence of a strong endcap absorbance at 330 nm in addition to the absorbance of the hydroxybenzyl alcohol-based polymer backbone at 270 nm.

![Synthesis of activated azobenzene carbonate 2.5 and condensation polymerization with monomer 2.6 to form the linear self-immolative polymer 2.7.](image)

**Figure 2.8** – Synthesis of activated azobenzene carbonate 2.5 and condensation polymerization with monomer 2.6 to form the linear self-immolative polymer 2.7.

### 2.2.5 Self-immolative Polymer Degradation

The degradation of polymer 2.7 was studied in methanol. As shown in Figure 2.9, upon the addition of hydrazine hydrate the azobenzene endcap was reduced to the corresponding hydrazobenzene (\(\lambda_{\text{max}} = 300 \text{ nm}\)) and the polymer solution changed from orange to colourless. The \(\lambda_{\text{max}}\) shifted to 278 nm, the absorption maximum of 4-hydroxybenzyl alcohol (Figure 2.10), and became sharper and more intense, as the polymer degraded to 4-hydroxybenzyl alcohol and \(N,N'\)-dimethylimidazolidinone via a series of alternating 1,6-elimination-decarboxylation and cyclization reactions. The azobenzene absorbance is lost in the reaction, and replaced with that of the hydrazobenzene compound.
Figure 2.9 – UV-vis spectra in MeOH demonstrating the decrease in azobenzene absorbance (330 nm), and increase in the absorbance of hydrazobenzene (300 nm) and released 4-hydroxybenzyl alcohol (278 nm) during depolymerization of polymer 2.7.

Figure 2.10 – Comparative UV-vis spectra in MeOH demonstrating the formation of 4-hydroxybenzyl alcohol (left) and hydrazobenzene 2.4 (middle) by the reduction of the azobenzene endcap (right) during depolymerization of 2.7.
In NMR spectra, peaks corresponding to the expected depolymerization products 4-hydroxybenzyl alcohol and \(N,N^{'}\)-dimethylimidazolidinone were observed to increase in intensity while the corresponding polymer peaks decreased (Figure 2.11). In addition, SEC showed complete polymer degradation with the exception of a small fraction of cyclic oligomers that cannot degrade (Figure 2.12). As shown in Figure 2.14, in the absence of hydrazine, depolymerization was much slower. A \(t\)-butyloxy carbonyl (Boc)-endcapped control polymer was prepared as previously reported (Figure 2.13),\(^1\) and was also studied both in the presence and absence of hydrazine. Its rate of depolymerization was very similar to that of polymer 2.7 in the absence of hydrazine and the presence of hydrazine caused only a very small acceleration in the rate of depolymerization of this Boc-endcapped polymer.

Figure 2.11 – Degradation of polymer 2.7 (~1.8 mM) in the presence of hydrazine (9 mM) as determined by \(^1\)H NMR spectroscopy in MeOD. Sharp small molecule peaks are shown to replace broad polymer peaks during depolymerization.
Figure 2.12 – SEC traces showing polymer 2.7 (—) and its degradation products after treatment with hydrazine (—-).

Overall, these data suggest that hydrazine does selectively reduce the azobenzene on the endcap, leading to triggered depolymerization. Given the unreactive nature of the small molecule benzyl carbamate and the gradual depolymerization of both 2.7 and the Boc-endcapped control, it seems likely that nonspecific cleavage occurred at the phenyl carbamates in the polymer backbones.

Figure 2.13 – Synthesis of a Boc-endcapped control polymer 2.8 using the monomer precursor 2.6b.
2.2.6 Azobenzene-endcapped Poly(benzyl carbamate)

To eliminate the less stable phenyl carbamate linkages, and demonstrate the versatility of the azobenzene endcap, the azobenzene trigger was also incorporated into a polycarbamate based on 4-aminobenzyl alcohol, developed by Shabat and coworkers. As shown in Figure 2.15, the previously reported monomer 2.9 was polymerized in the presence of compound 2.2 as an endcap using catalytic dibutyltin dilaurate (DBTL) in dry dioxane to provide polymer 2.10.

Figure 2.14 – Degradation profiles of polymer 2.7 (●,○) and its Boc-endcapped control 2.8 (▲,Δ), with exposure to hydrazine (filled) and without (empty).

Figure 2.15 – Synthesis of a poly(benzyl carbamate) 2.10 based on 4-aminobenzyl alcohol, which degrades via alternating 1,6-eliminations and decarboxylations.
Based on $^1$H NMR spectroscopy, the average chain length was ~6 units. The resulting material also appeared oligomeric by SEC, with an $M_W$ of 3 700 g mol$^{-1}$ relative to PMMA, and $D = 1.5$. These results are consistent with the tendency of this polymerization to provide relatively short polymers. In the current work, this provides the advantage of allowing the chemistry of the endcap to be followed readily by spectroscopic methods. A further advantage is that monomer 2.9 can be prepared from commercial products in a single step.

2.2.7 Poly(benzyl carbamate) Degradation

The degradation of polymer 2.10 was studied in DMF due to its insolubility in polar solvents such as water or methanol. Piperidine was added to trap azaquinone methide species released on depolymerization. Upon the addition of hydrazine the solution changed from orange to clear, providing a visual indication that the endcap had been removed, and thus polymer degradation had been initiated.

![Figure 2.16 – Degradation of polymer 2.10 by hydrazine as monitored by UV-vis spectroscopy in DMF. The solution was heated to 50 °C for 45 hours to encourage complete degradation.](image-url)
This reduction was confirmed using UV-vis spectroscopy (Figure 2.16), which showed the same decrease in azobenzene absorbance that was previously observed for polymer 2.7. Following this initial study, the depolymerization reaction was followed by $^1$H NMR spectroscopy, with 17% degradation observed over a period of 9 days at room temperature.

Figure 2.17 – $^1$H NMR spectra overlaid to illustrate the degradation of polymer 2.10 upon the addition of hydrazine and piperidine. Endcap reduction appears complete by 6 hours (disappearance of b), while 17% polymer degradation was reached by 9 days (appearance of peaks d-h).

This slow degradation rate was expected as the depolymerization of this particular class of self-immolative polymers is known to be slow under non-aqueous conditions.\textsuperscript{11}
The difference in rate between azobenzene reduction and depolymerization is more obviously highlighted in Figure 2.18, where it is clear that reduction is complete within 2 days, but polymer degradation proceeds very slowly in a linear process. In the absence of hydrazine, but with piperidine present, no degradation of polymer 2.10 was observed (Figure 2.19). This result may indicate that random hydrolysis is not present in the poly(benzyl carbamate), and also confirms that azobenzene reduction is the cause of polymer degradation.

Figure 2.18 – Comparison in rate of degradation for the endcap (●) and backbone (○) of polymer 2.10 when exposed to hydrazine and piperidine in DMF for 9 days.
Figure 2.19 – No appreciable degradation of polymer 2.10 was observed by $^1$H NMR spectroscopy after 6 days in DMF-$d_7$ solution with piperidine.

A control polymer with an $n$-butyl carbamate endcap was synthesized by the same method as in Figure 2.15, but using $n$-butanol as an endcapping agent. This polymer was designed not to respond to stimuli, and its response to hydrazine was compared with that of polymer 2.10. No degradation of polymer 2.11 was observed even in the presence of hydrazine, again reinforcing the role of azobenzene reduction in the depolymerization of these self-immolative polymers (Figure 2.20).
Figure 2.20 – $^1$H NMR spectra of control poly(benzyl carbamate) 2.11 before and after 2 weeks of exposure to hydrazine and piperidine. No degradation was apparent.

2.2.8 Evaluation of azobenzene endcap photoresponsiveness

In addition to triggering the depolymerization of self-immolative polymers, azobenzenes are well known to undergo trans-cis isomerization in response to irradiation with UV light. This process is of particular interest in the context of the reduction-sensitive moiety because recent studies have suggested an increased rate of reduction of the cis azobenzene relative to the trans isomer, thus providing further control over the onset of degradation. Furthermore, the isomerization of azo-containing monomers in block copolymers has been studied, wherein the polarity and morphology of the polymers were modified via irradiation. The single azobenzene hinge at the interface of two blocks may have the potential to provide a similar morphological disruption in an assembly, either via a change in the conformation, or by modification of the polarity at the polymer interface.
To confirm that the endcaps can also undergo this isomerization process orthogonally to their reduction, both model compound 2.3 and azobenzene endcapped polymer 2.10 were irradiated with UV light (λ = 365 nm) and monitored by $^1$H NMR spectrometry (Figure 2.21, Figure 2.22), and UV-visible spectroscopy (Figure 2.23). After 30 min irradiation, more than half of the azobenzene moieties in each of these materials had converted from the trans conformation to the less stable cis conformation. After 24 h, they had both almost completely converted back to the trans conformation via thermal relaxation.

![Diagram](image.png)

Figure 2.21 – $^1$H NMR spectra showing the photoisomerization of compound 2.3 with UV light (λ = 365 nm) for 30 minutes, and the reverse thermal isomerization. Peaks are shown to move significantly after conversion to the cis form.
Figure 2.22 - $^1$H NMR spectra showing the photoisomerization of polymer 2.10 with UV light ($\lambda = 365$ nm) for 30 minutes, and the reverse thermal isomerization. Endcap peaks move significantly after isomerization, while polymer peaks remain stationary.

Figure 2.23 – UV-vis spectra showing the change in absorbance following irradiation with UV light for 30 minutes ($\lambda = 365$ nm).
As these data show, the azobenzene endcaps can respond in different ways to different stimuli, with reductive conditions leading to depolymerization and UV light leading to isomerization.

2.3 Conclusions

A novel azobenzene endcap responsive to both reducing conditions and light was developed. This highly absorbant dye can act as a trigger for polymer degradation, and as a reporter molecule due to its visual colour change upon reduction. In addition, the azobenzene can undergo trans-cis isomerization in response to UV light without triggering other degradation processes. This isomerization, when incorporated for example between two blocks of copolymer could effect changes in polymer assemblies or in the solid state, and may also modulate the rate of reduction. Thus, this new multiresponsive endcap opens new prospects for the application of self-immolative polymers in a wide range of stimuli-responsive materials.

2.4 Experimental

2.4.1 General Materials and Methods

Ethyl 4-aminobenzoate, 4-aminobenzyl alcohol, oxone, phenyl chloroformate, N,N'-dimethylethlenediamine, 4-nitrophenyl chloroformate, 4-hydroxybenzyl alcohol, 4-dimethylaminopyridine (DMAP), and n-butanol were purchased from Alfa Aesar. Dibutyltin dilaurate (DBTL) was obtained from Sigma-Aldrich. Di-tert-butyl-dicarbonate was purchased from AK Scientific. Dimethyl sulfoxide (DMSO) was distilled from barium oxide before use. NEt₃ and CH₂Cl₂ were distilled from calcium hydride immediately before use. Solvents of reagent grade, trifluoroacetic acid, and methanol and N,N-dimethylformamide (DMF) of distilled-in-glass grade, were purchased from Caledon. Unless noted, all reagents and solvents were used as received. ¹H-NMR spectra were obtained at either 400 or 600 MHz on Varian Inova spectrometers and calibrated according to the residual solvent signal. ¹³C NMR spectra were obtained on the same instruments at either 100 or 150 MHz. High resolution mass spectrometry (HRMS) was performed on a Finnigan MAT 8400 mass spectrometer using electron impact (EI). Size exclusion chromatography (SEC) was carried out with a Waters 515 HPLC pump using two PLgel
mixed-D columns (5 μm pore size) in series, with a Wyatt Optilab rEX refractive index detector. DMF with 1% NEt₃ and 10 mM LiBr was used as the eluent, the temperature was 85 °C, and the flow rate was 1.0 mL min⁻¹. Molecular weight was determined relative to PMMA standards. FT-IR spectra were recorded on a Bruker Vector 33 instrument in transmission mode. UV-visible spectra were obtained on a Varian Cary 300 Bio UV-visible spectrophotometer from 200-800 nm at a scanning rate of 600 nm min⁻¹.

2.4.2 Synthesis of Ethyl-4-nitrosobenzoate (Compound 2.1)

Ethyl-4-aminobenzoate (1.00 g, 6.05 mmol) was suspended in water (50.0 mL). A solution of oxone (7.44 g, 12.1 mmol) in water (50.0 mL) was added and the mixture was stirred for 2 hours at room temperature to generate a yellow precipitate. The suspension was then poured into ethyl acetate (150 mL) to give a bright green organic layer. The organic phase was washed with 1M HCl (2 × 50 mL), saturated NaHCO₃ (2 × 50 mL) and saturated NaCl (2 × 50 mL), then dried on MgSO₄ and the solvent removed in vacuo, to yield 2.1 as a yellow solid (1.06 g, 98%). This compound was used immediately without further purification. ¹H NMR (600 MHz, CDCl₃): δ = 8.30-8.32 (2H, m), 7.92-7.95 (2H, m), 4.44 (2H, q, J = 8.0 Hz), 1.44 (3H, t, J = 8.0 Hz).

2.4.3 Synthesis of Ethyl-4-[4'-((hydroxymethylphenyl)diazenyl]benzoate (Compound 2.2)

Ethyl-4-nitrosobenzoate 2.1 (1.06 g, 5.92 mmol) was dissolved in CH₂Cl₂ (50 mL), and AcOH (5 mL) was added. 4-Aminobenzyl alcohol (364 mg, 2.96 mmol) was added and the mixture was stirred at room temperature for 16 hours. The solvent was removed in vacuo and the residue dissolved in EtOAc (150 mL), then washed with saturated NaHCO₃ (2 × 100 mL), and saturated NaCl (4 × 100 mL). The organic phase was dried on MgSO₄, then filtered through a silica plug with excess EtOAc. The solvent was removed in vacuo and the collected solid was purified by column chromatography (EtOAc/Hex 40:60) to afford compound 2.2 as an orange solid (605 mg, 72%). ¹H NMR (400 MHz, CDCl₃): δ = 8.18-8.23 (2H, m), 7.93-7.98 (4H, m), 7.53-7.57 (2H, m), 4.82 (2H, d, J = 8.0 Hz), 4.43 (2H, q, J = 8.0 Hz), 1.84 (1H, t, J = 8.0 Hz), 1.44 (3H, t, J = 8.0 Hz). ¹³C NMR (100 MHz, CDCl₃): δ = 166.1, 155.1, 152.0, 144.6, 132.1, 130.6, 127.4, 123.4, 122.6, 64.8, 61.3, 14.3. FT-IR
(KBr, thin film, $v_{\text{max}}$/cm$^{-1}$): 1718 (C=O), 1271 (N=N), 1106 (C–O, ester), 1095 (C–O, alcohol). HRMS (EI): calc. for $[\text{C}_{16}\text{H}_{16}\text{N}_{2}\text{O}_{3}]^+$ [M]$^+$: 284.1161, found: 284.1157. m.p. = 159-161 °C.

2.4.4 Synthesis of Ethyl-4-[4'-(acetoxymethylphenyl)diazenyl] benzoate (Compound 2.3)

Compound 2 (200 mg, 0.70 mmol) was suspended in CH$_2$Cl$_2$ (5.0 mL), and freshly distilled pyridine (0.11 mL, 1.4 mmol) was added. Acetic anhydride (0.13 mL, 1.4 mmol) was added slowly, followed by catalytic DMAP (8.6 mg, 0.07 mmol). The suspended solid was observed to dissolve over 5 minutes, and the reaction was stirred at room temperature for 1 hour to yield a transparent red solution. The reaction was poured into EtOAc (100 mL) and extracted with saturated NaHCO$_3$ (2 × 100 mL), 1M HCl (2 × 100 mL), and saturated NaCl (3 × 100 mL), then dried over MgSO$_4$, filtered, and the solvent removed in vacuo. The crude product was then dissolved in minimal EtOAc and run through a silica plug (25 mL). The solvent was removed in vacuo to yield compound 3 as a bright orange crystalline solid (200 mg, 88%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 8.19$-$8.21$ (2H, m), 7.93-$7.96$ (4H, m), 7.50-$7.53$ (2H, m), 5.19 (2H, s), 4.42 (2H, q, $J = 6.0$ Hz), 2.14 (3H, s), 1.43 (3H, t, $J = 6.0$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 170.7$, 166.0, 155.0, 152.2, 139.5, 132.2, 130.52, 128.7, 123.3, 122.6, 65.6, 61.2, 20.9, 14.3. FT-IR (KBr, thin film, $v_{\text{max}}$/cm$^{-1}$): 1742 (C=O, ethyl ester), 1711 (C=O, acetate), 1286 (N=N), 1242 (C–O, ethyl ester), 1114 (C–O, acetate). HRMS (EI): calc. for $[\text{C}_{18}\text{H}_{18}\text{N}_{2}\text{O}_{4}]^+$ [M]$^+$: 326.1267, found: 326.1265. m.p. = 115-121 °C.

2.4.5 Synthesis of Ethyl-4-[4'-hydroxymethylphenylhydrazo] benzoate (Compound 2.4)

Compound 2.3 (50 mg, 0.15 mmol) was suspended in methanol (20 mL), and to this mixture was added hydrazine hydrate (0.5 mL, excess). The solution was stirred at room temperature for 48 hours. A colour change from bright orange to pale yellow was observed, and insoluble crystals of compound 2.4 appeared. The reaction was poured into EtOAc (50 mL) and washed with deionized water (2 × 100 mL). The organic layer was then washed with saturated NaCl (2 × 100 mL) and dried on MgSO$_4$ before the solvent was removed in vacuo.
vacuo. The crude compound was purified by column chromatography (EtOAc/Hex 40:60, gradient to 60:40). Compound 2.4 was recovered as a white crystalline solid (41 mg, 93%).

$^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ = 8.38 (1H, s), 7.78 (1H, s), 7.71-7.77 (2H, m), 7.06-7.12 (2H, m), 6.74-6.79 (2H, m), 6.65-6.69 (2H, m), 4.89 (1H, t, $J$ = 4.0 Hz), 4.35 (2H, d, $J$ = 4.0 Hz), 4.21 (2H, q, $J$ = 6.0 Hz), 1.27 (3H, t, $J$ = 6.0 Hz).

$^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ = 165.8, 154.2, 148.1, 132.4, 130.9, 127.9, 118.2, 111.5, 110.4, 63.0, 59.7, 14.3. FT-IR (KBr, thin film, $\nu_{\text{max}}$/cm$^{-1}$): 3467 (NH), 3307 (OH), 1690 (C=O, ethyl ester), 1607 (NH-NH), 1515 (aryl-NH), 1279 (C-O, ester), 1265 (C-O, hydroxyl). HRMS (EI): calc. for [C$_{16}$H$_{18}$N$_2$O$_3$]$^+$ [M]$^+$: 286.1317, found: 286.1315. m.p. = 135-140 °C.

2.4.6 Synthesis of O-(4-nitrophenyl)-O’-(4’-diazenyl (4’’-carboxyphenyl)phenylmethyl)carbonate, ethyl ester (Compound 2.5)

Compound 2.2 (70 mg, 0.39 mmol) was dissolved in distilled CH$_2$Cl$_2$ (50 mL) and distilled pyridine (0.1 mL, 1.2 mmol) was added. 4-Nitrophenyl chloroformate (PNPOCOCl) (83 mg, 0.41 mmol) was added and the mixture was stirred at room temperature for 2 hours under inert atmosphere. The reaction was then diluted with CH$_2$Cl$_2$ (50 mL) and washed with 1M citric acid (1 x 100 mL). The organic layer was dried on MgSO$_4$ and the solvent removed in vacuo. The crude product was purified by column chromatography (CH$_2$Cl$_2$/Hex 80:20, gradient to 90:10) to yield compound 2.5 (93 mg, 69%) as a light orange solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 8.28-8.33 (2H, m), 8.20-8.24 (2H, m), 7.96-8.04 (4H, m), 7.60-7.64 (2H, m), 7.39-7.44 (2H, m), 5.40 (2H, s), 4.44 (2H, q, $J$ = 8.0 Hz), 1.44 (3H, t, $J$ = 8.0 Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 166.0, 155.4, 154.9, 152.7, 152.4, 145.5, 137.5, 132.4, 130.6, 129.2, 125.3, 123.5, 122.7, 121.7, 70.2, 61.3, 14.3. FT-IR (KBr, thin film, $\nu_{\text{max}}$/cm$^{-1}$): 1753 (C=O, carbonate), 1724 (C=O, ester), 1530 (NO$_2$), 1350 (N=N), 1274 (C-O, ester), 1232 (C-O, carbonate). HRMS (EI): calc. for [C$_{23}$H$_{19}$N$_3$O$_7$]$^+$ [M]$^+$: 449.1223, found: 449.1231. m.p. = 139-141 °C.
2.4.7 Synthesis of an Alternating Cyclization and Elimination-based Polycarbamate (Polymer 2.7)

Monomer 2.6 (0.34 g, 0.66 mmol, 1.0 equiv.), synthesized as previously reported,¹ and compound 2.5 (2.8 mg, 6.2 μmol, 0.01 equiv.) were dissolved in dry toluene (4.0 mL). The solution was cooled to 0 °C. Freshly distilled NEt₃ (1.15 mL, 8.24 mmol, 12.5 equiv.) and DMAP (17 mg, 0.14 mmol, 0.22 equiv.) were added, and the reaction was stirred for 24 hours at 0 °C. The solution was diluted with CH₂Cl₂ and washed with 1M HCl, and then twice with 10% aqueous Na₂CO₃. The organic layer was dried over MgSO₄ and the solvent was removed in vacuo to provide crude polymer 7 (0.21 g) as a yellow viscous liquid. The crude polymer was dialyzed in a 6-8 kDa molecular weight cut-off (MWCO) membrane (Spectra/Por 1, Spectrum Laboratories) against DMF for 24 hours (300 mL, two solvent changes), and then against water for 24 hours (300 mL, two solvent changes). The resulting material was then lyophilized to afford the purified polymer 2.7 (0.10 g, 57%) as an orange powder.

¹H NMR (600 MHz, CDCl₃): δ = 8.20 (2H, d), 7.93 (4H, m), 7.84 (2H, d), 7.40-7.25 (147H, m), 7.20-7.00 (149H, m), 5.15-5.05 (170H, m), 4.43 (2H, q, J = 6.0 Hz), 3.65-3.40 (345H, m), 3.15-3.10 (60H, m), 3.06-3.02 (67H, m), 3.01-2.90 (348H, m). SEC (DMF): Mₙ = 5 600 g mol⁻¹, Mₘ = 9 200 g mol⁻¹, D = 1.6.

2.4.8 Synthesis of an Elimination-based Polycarbamate (Polymer 2.10)

Monomer 2.9 (30 mg, 0.12 mmol), synthesized as previously reported,² and compound 2.2 (66 mg, 0.23 mmol) were placed in a flame-dried round-bottom flask and dissolved in dry dioxane (0.5 mL). DBTL (0.1 mL) was injected and the mixture was heated to 90 °C and stirred for 3 hours. A solution of monomer (300 mg, 1.23 mmol) in dioxane (0.4 mL) was injected to the polymerization flask and the mixture was stirred at 90 °C for an additional 21 hours. The solution was then cooled to room temperature, and precipitated into chilled methanol. The polymer was centrifuged and the methanol decanted. The polymer was dissolved in DMF and re-precipitated in cold methanol. The polymer was collected by centrifugation and dried under vacuum to yield polymer 2.10 as a light orange solid (210 mg, 90%). ¹H NMR (400 MHz, DMSO-d₆): δ = 9.93 (1H, s), 9.78 (4H, s), 9.65 (1H, s), 8.15-8.19 (2H, m), 7.96-8.03 (4H, m), 7.65-7.69 (2H, m), 7.19-7.52 (24H, m), 5.28 (2H,
2.4.9 Synthesis of an Elimination-based Polycarbamate control for comparison with 2.10 (Polymer 2.11)

Polymer 2.11 was synthesized via the same procedure as polymer 2.10, replacing compound 2.2 with nBuOH (18 mg, 0.24 mmol) to yield a white solid (148 mg, 69%). 1H NMR (600 MHz, DMSO-d6): δ = 9.66-9.78 (7H, m), 7.47-7.48 (11H, m), 7.39-7.40 (2H, m), 7.33-7.35 (11, m), 7.20-7.21 (2H, m), 5.04-5.07 (13H, m), 4.41 (2H, d, J = 6.0 Hz), 4.07 (2H, t, J = 6.0 Hz), 1.60 (2H, m), 1.38 (2H, m), 0.91 (3H, t, J = 6.0 Hz). SEC (DMF): M_n = 2 600 g mol^{-1}, M_W = 3 500 g mol^{-1}, D = 1.3.

2.4.10 Procedure for the degradation of 2.3 with hydrazine (UV-vis)

Compound 2.3 was dissolved in spectroscopy-grade methanol at a concentration of 0.01 mg mL^{-1} in a quartz cuvette (3 mL total). Spectra were recorded at ambient temperature using the solvent as a baseline correction. Excess hydrazine hydrate (40-50%) (10 μL, ~180 μmol of N₂H₄ and ~220 μmol of H₂O) was added by syringe. The degradation was then monitored by obtaining UV-visible spectra from 250 - 550 nm at regular time intervals.

2.4.11 Degradation of 2.3 with dithiothreitol (UV-vis)

Compound 2.3 was dissolved in a 1:1 methanol (spectroscopic grade)/phosphate buffer (100 mM, pH = 7.4) at a concentration of 0.01 mg mL^{-1} and was deoxygenated by bubbling nitrogen gas through the solution for 15 minutes. DTT was dissolved in the same solvent at a concentration of 50 mg mL^{-1}, and deoxygenated in the same manner. The DTT solution (0.28 mL) was added to a quartz cuvette containing the solution of compound 2.3 (3.0 mL) under a blanket of nitrogen. The degradation was then monitored by obtaining UV-visible spectra from 250 - 550 nm at regular time intervals.

2.4.12 Degradation of 2.7 with hydrazine (UV-vis)

Polymer 2.7 was dissolved in spectroscopy grade methanol at a concentration of 0.5 mg mL^{-1} in a quartz cuvette. Hydrazine hydrate (40-50%) (20 μL, 360 μmol of N₂H₄ and ~440
µmol of H₂O) was added directly to the cuvette to initiate degradation. The degradation was then monitored by obtaining UV-visible spectra from 250 - 550 nm at regular time intervals.

2.4.13 Degradation of 2.10 with hydrazine (UV-vis)

Polymer 2.10 was dissolved in distilled-in-glass grade DMF at a concentration of 0.05 mg mL⁻¹ in a quartz cuvette. Piperidine (1 µL, ~1.5 equiv.) and excess hydrazine hydrate (20 µL, 360 µmol of N₂H₄ and ~440 µmol of H₂O) were added to the cuvette and the degradation was monitored by UV-visible spectroscopy.

2.4.14 Degradation of 2.3 with hydrazine (¹H NMR)

Compound 2.3 (2 mg, 6.1 µmol) was dissolved in CD₃OD (1 mL). Hydrazine hydrate (40-50%) (20 µL, 360 µmol of N₂H₄ and ~440 µmol of H₂O) was added via syringe, and degradation was monitored by obtaining ¹H NMR spectra at regular time intervals over 48 hours. A presaturation delay was used to remove the signals from water and hydrazine.

2.4.15 Degradation of benzyl acetate with hydrazine (¹H NMR)

Benzyl acetate (2 mg, 13.3 µmol) was dissolved in CD₃OD (1 mL). Hydrazine hydrate (40-50%) (20 µL, 360 µmol of N₂H₄ and ~440 µmol of H₂O) was added via syringe, and degradation was monitored by obtaining ¹H NMR spectra at regular time intervals over 48 hours. A presaturation delay was used to remove the signals from water and hydrazine.

2.4.16 Degradation of N,N-dimethyl benzyl carbamate with hydrazine (¹H NMR)

N,N-dimethyl benzyl carbamate (2 mg, 11.1 µmol) was dissolved in CD₃OD (1 mL). Hydrazine hydrate (40-50%) (20 µL, 360 µmol N₂H₄ and ~440 µmol of H₂O) was added via syringe, and degradation was monitored by obtaining ¹H NMR spectra at regular time intervals over 48 hours. A presaturation delay was used to remove the signals from water and hydrazine.
2.4.17 Degradation of 2.3 with dithiothreitol ($^1$H NMR)

Compound 2.3 was suspended in a 1:1 mixture of CD$_3$OD:phosphate buffer (100 mM, pH = 7.4, D$_2$O) (5.0 mg mL$^{-1}$, 1.0 mL). The entire amount of 2.3 did not dissolve. DTT was dissolved in CD$_3$OD at a concentration of 50 mg mL$^{-1}$ and added (50 μL) to the NMR tube via syringe.

2.4.18 Degradation of 2.7 and 2.8

Polymer 2.7 was dissolved in CD$_3$OD at a concentration of 10 mg mL$^{-1}$ and filtered through a 0.22 μm PTFE syringe filter into an NMR tube. Hydrazine hydrate (40-50%) diluted 20x in MeOD (10 μL, ~9 μmol N$_2$H$_4$ and ~11 μmol of H$_2$O) was then added via syringe to initiate depolymerization. The degradation was monitored by obtaining $^1$H NMR spectra at regular time intervals. A presaturation delay was used to remove the signal from water. A Boc-endcapped polymer with the same backbone was previously reported$^1$ and was studied under the same conditions. Both 2.7 and the Boc-endcapped control were also studied using the same procedure but without the addition of hydrazine. Maximum degradation of polymer 2.7 was determined by a long timepoint at which degradation had plateaued, and set as 100%, and any undegraded polymer was assumed to be cyclic and non-triggerable. To determine the maximum degradation level of the Boc polymer, the polymer was subjected to a TFA/CH$_2$Cl$_2$ (1:1) deprotection as previously reported$^1$ then dissolved in MeOH. The deprotected polymer was stirred at room temperature for 24 hours and the ratio of the aromatic peaks of the polymer and degraded product was used to determine the 100% degradation mark.

2.4.19 Degradation of 2.10 and 2.11

Polymer 2.10 was dissolved in DMF-$d_7$ at 5.0 mg mL$^{-1}$ in an NMR tube, and piperidine (4.4 μL, 1.5 equiv.) was added as a trapping agent for the azaquinone methide species. Hydrazine hydrate (40-50%) (20 μL, ~360 μmol of N$_2$H$_4$ and ~440 μmol of H$_2$O) was then added and the tube was sealed. The degradation was monitored by obtaining $^1$H NMR spectra at regular time intervals. A presaturation delay was used to remove the signals from water and hydrazine. The degradation of polymer 2.10 was also studied under identical conditions, but without the addition of hydrazine.
2.4.20  Photoisomerization of 2.3

Compound 2.3 was dissolved in CDCl$_3$ at a concentration of 3.0 mg mL$^{-1}$. A preliminary spectrum ($^1$H NMR 600 MHz, CDCl$_3$) was recorded and then the sample was irradiated ($\lambda = 365$ nm, 16 UVA bulbs, Hitachi FL8BL-B, 8 watts) for 30 min. A $^1$H NMR spectrum of the irradiated sample was obtained and then the NMR tube was placed in the dark for 24 h at room temperature prior to the collection of the final spectrum.

2.4.21  Photoisomerization of 2.10

Polymer 2.10 was dissolved in DMF-$d_7$ at a concentration of 5.0 mg mL$^{-1}$. The same procedure as described above for photoisomerization of compound 2.3 was then followed.

2.5  References


Chapter 3

3 Poly(ester amide)s with Pendant Azobenzenes: Multi-Responsive Self-Immolative Moieties for Modulating Polymer Assemblies

3.1 Introduction

Stimuli-responsive materials possess properties that change upon exposure to one or more stimuli. Stimuli such as light, or changes in redox potential, pH, temperature, or magnetic fields have been successfully employed to induce reversible or irreversible changes in the chemical, biological, mechanical, or electrical properties of materials. These changes can be exploited to provide functions in a wide range of applications. For example, stimuli-responsive drug carriers can release their payloads in response to the stimulus, affording increased selectivity for their target. Responsiveness to multiple stimuli, both separately or simultaneously, can extend the versatility of stimuli-responsive materials, and increase their sensitivity.

Azobenzene derivatives have been widely used as photoswitches both in the context of small molecules and polymers due to their highly efficient and reversible trans-cis isomerization upon exposure to UV or visible light. Isomerization of the diazene bond from the trans to cis conformation results in a significant increase in its polarity which has been exploited in the context of stimuli-responsive materials. For example, polymer micelles and vesicles prepared from block copolymers containing azobenzene groups in their hydrophobic blocks were introduced, using hydrophilic blocks such as poly(acrylic acid), poly(ethylene oxide) (PEO) or even in linear-dendritic systems. Isomerization of the azobenzene moieties from trans to cis in response to irradiation with UV light destabilized the assemblies. Upon irradiation with visible light, the amphiphilic nature of the polymer was restored, and the assemblies were re-formed in situ. These assemblies underwent organizational changes in response to light, but did not degrade as they were composed of stable polymer backbones.
In recent work, we have demonstrated that in addition to their ability to undergo isomerization, appropriately designed azobenzene molecules could also be used as reduction-sensitive endcaps/triggers to initiate the end-to-end depolymerization of self-immolative polymers (SIPs). It was shown that reduction of the azobenzene to hydrazobenzene generated a species capable of a 1,6-elimination reaction, triggering the depolymerization of a linear self-immolative polycarbamate. The reduction was accompanied by the disappearance of the characteristic strong absorbance of the azobenzene, providing a colourless solution and thus a visual cue that the endcap had been activated. However, this system could not be investigated in water due to the hydrophobicity of the polymer backbone. Furthermore, the potential effects of light-mediated trans-cis isomerization on the system were not explored.

Poly(ester amide)s (PEAs) are polymers that contain both ester and amide functional groups within their backbones. PEAs prepared from diols, diacids, and amino acids are a particularly attractive class of PEAs as the combination of monomers can be easily tuned to afford a wide range of properties and functions. They are of interest for biomedical applications ranging from drug delivery to tissue engineering because they are degradable under physiological conditions, and have been found to be non-toxic and to support cell growth.

Previous research in our group has demonstrated that it is possible to prepare PEA-poly(ethylene oxide) (PEO) graft copolymers through derivatization of the pendant groups of amino acids such as aspartic acid or lysine along the PEA backbone. The resulting amphiphilic copolymers could be assembled into micelles that released drugs such as paclitaxel in a sustained and/or stimuli-responsive manner.

The modularity and versatility of the PEA structure also affords the opportunity to incorporate azobenzene moieties in a manner that can allow their responsiveness to light and reduction to be exploited both separately and synergistically. Described here is first the optimization of the azobenzene chemical structure to afford increased reduction rates. Subsequently, the optimized azobenzene is incorporated as a pendant group on a PEA-PEO graft copolymer and this copolymer is self-assembled to form nanoparticles in aqueous solution. As shown in Figure 3.1, reduction of the azobenzenes is designed to lead to
backbone fragmentation via a self-immolative 1,6-then 1,4-elimination reaction. On the other hand, $trans$-$cis$ isomerization is designed to change the polarity of the assembly core. Synergistically, isomerization can potentially increase the rate of breakdown of the polymer by increasing the polarity of the assembly core, providing enhanced penetration by the reducing agent and water. These changes are probed using UV-visible (UV-vis) spectroscopy, dynamic light scattering (DLS), and the fluorescence of nile red as a probe molecule. To the best of our knowledge, this is the first example exploiting both the photo- and reduction-responsiveness of azobenzene. It is shown that the unique capabilities of azobenzene enable access to both reversible and irreversible disruption of polymer assemblies.

![Figure 3.1](image)

**Figure 3.1** – Schematic demonstrating the proposed multi-stimuli responsiveness of the azobenzene-PEA-PEO system. Reduction of the polymer assemblies leads to irreversible polymer degradation via a cascade of self-immolative elimination reactions, while isomerization with UV light leads to reversible changes in the polarity of the core of the assemblies.

### 3.2 Results and Discussion

In our previous study, an electron-withdrawing ester group was incorporated onto the azobenzene with the aim of promoting reduction. However, the azobenzene structure was not optimized with respect to the rate of reduction. As depolymerization of the self-immolative polycarbamate requires days, endcap reduction was not rate-limiting in
the process and the specific azobenzene structure did not play a significant role in the overall rate of polymer degradation. On the other hand, in the current design (Figure 3.1), many azobenzene reductions are required to completely degrade the polymer, so there was motivation for investigating and optimizing the rate of reduction by tuning the chemical structure of the azobenzene. To achieve this, azobenzenes 3.1 through 3.11 (Figure 3.2) were synthesized by the general route shown in Figure 3.3, involving oxidation of the appropriate starting aniline to its corresponding nitrosobenzene, followed by condensation with 4-aminobenzyl alcohol in the presence of acetic acid (AcOH). Nitrosobenzene intermediates were not isolated due to their instability, and were used for the subsequent step immediately following aqueous workup. All of the derivatives except 3.1 were designed to contain electron-withdrawing groups, as this has previously been shown to enhance the rate of reduction.\textsuperscript{55} Azobenzene 2.2\textsuperscript{27} was included for direct comparison with the synthesized library.

![Chemical structures](image)

**Figure 3.2** – Library of reduction-sensitive azobenzene derivatives synthesized and used in this study.
Figure 3.3 – General two-step synthesis of azobenzene derivatives 3.1-3.11, and their reduction by hydrazine hydrate in THF/water (3/5).

3.2.1 Substituent Effects of the Rates of Azobenzene Reduction

To study the reduction kinetics, a 25 μM solution of each azobenzene in 3/5 tetrahydrofuran (THF)/water mixture was prepared and 250 μM hydrazine hydrate was added as a reducing agent (Figure 3.3). The water/THF mixture was used in order to study the reduction in a primarily aqueous environment, while maintaining solubility of the hydrophobic azobenzene molecules using THF. The 10-fold excess of hydrazine was used to provide pseudo-first-order conditions to facilitate the kinetic analysis. The reduction in absorption (A) of each azobenzene derivative at its maximum absorption wavelength ($\lambda_{\text{max}}$) corresponding to the $\pi - \pi^*$ transition (323 – 336 nm) was monitored over 10 minutes using UV-vis spectroscopy. This technique was chosen due to the hypsochromic shift in absorbance between the decreasing azobenzene peak and growing hydrazobenzene peak (Figure 3.4). Plots of $\ln(A/A_0)$ versus time (Figure 3.5) were used to determine the observed rate constant of reduction ($k_{\text{obs}}$) for each derivative and the results are shown in Table 3.1.
Figure 3.4 – The observed decrease in absorbance of compound 3.2 upon reduction by hydrazine hydrate, as measured by UV-vis spectroscopy (25 μM 3.2 and 250 μM N₂H₄ in THF/water 3/5).

The different azobenzene derivatives exhibited observed rate constants (k_{obs}) for reduction ranging from 1.4 × 10⁻² min⁻¹ to 8.0 × 10⁻² min⁻¹, demonstrating that the rate could be tuned over almost one order of magnitude, depending on the substitution on the aromatic rings. Nearly all derivatives were reduced more rapidly than the unfunctionalized azobenzene 3.1. The introduction of a halogen at the 2-position on the aromatic ring was most effective in increasing the reduction rate. Compound 3.2 (2-Cl) was the most reduction-sensitive azobenzene, exhibiting a nearly four-fold increase in rate over the unfunctionalized azobenzene 3.1 and about a two-fold increase over that of our previously studied compound 2.2 (4-CO₂CH₂CH₃). Compound 3.9 (2-Br) was more rapidly reduced than 3.10 (2-F), but more slowly than 3.2 (2-Cl). The difference in reduction rates between
these three compounds was analyzed in terms of electronic and steric factors. Fluorine is the most electronegative (EN) atom \( \text{EN}_F = 4.0, \text{EN}_{\text{Cl}} = 3.0, \text{EN}_{\text{Br}} = 2.8 \), so the inductive withdrawing effect was expected to be much larger than for the other halogens. However, the orbital overlap of fluorine with the aromatic \( \pi \) system is high due to its 2p orbitals, resulting in stronger \( \pi \)-donation than for the larger halogens.\(^{56}\)

**Table 3.1 - \( k_{\text{obs}} \) for the reduction of azobenzene derivatives 3.1-3.11 and 2.2.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>( k_{\text{obs}} \times 10^{-2} \text{ min}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 (H)</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>3.2 (2-Cl)</td>
<td>8.0 ± 1.1</td>
</tr>
<tr>
<td>3.3 (4-Cl)</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>3.4 (3-Cl)</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>3.5 (4-CN)</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>3.6 (2-CF(_3))</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>3.7 (4-CF(_3))</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>3.8 (4-CO(_2)CH(_2)CCH)</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>2.2 (4-CO(_2)CH(_2)CH(_3))</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>3.9 (2-Br)</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>3.10 (2-F)</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>3.11 (F(_5))</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

While Hammett parameters (\( \sigma \)) do not exist for *ortho* substituents due to complicating effects associated with steric hindrance, the competing \( \sigma \)-withdrawing and \( \pi \)-donation effects of the halogens are reflected in the \( \sigma \) values of halogens as para-substituents (\( \sigma_F = 0.06, \sigma_{\text{Cl}} = 0.23, \sigma_{\text{Br}} = 0.23 \)).\(^{57}\) In the current reaction, ring torsion would be expected to accelerate the reduction rate by raising the energy of the starting material relative to that of a completely conjugated azobenzene. Bromine has the largest radius (\( r \)) of the evaluated halogens (\( r_F = 71 \text{ pm}, r_{\text{Cl}} = 99 \text{ pm}, r_{\text{Br}} = 114 \text{ pm} \)) and would be expected to have the highest impact ring torsion. However, 3.9 (2-Br) was reduced more slowly than
3.2 (2-Cl). This indicates that ring torsion does not play a dominant role in accelerating the reduction rate for this series of azobenzenes.

Figure 3.5 – Plot of ln(A/A₀) for compounds 3.1-3.11 and 2.2, where A = absorbance in UV-vis, following the addition of N₂H₄. A more negative slope indicates a faster rate of reduction.

The role of electronics and steric was further explored by comparing the reduction rates of 3.6 (2-CF₃) and 3.2 (2-Cl). The Hammett parameter for the trifluoromethyl group is significantly larger than for chlorine (σ(Cl) = 0.23, σ(CF₃) = 0.54), perhaps due to the contribution of three electronegative fluorine atoms. Furthermore, the CF₃ group is much larger than Cl,⁵⁸ and thus expected to cause greater ring torsion. Despite all of these factors that should favour more rapid reduction of 3.6 (2-CF₃), it was reduced more slowly than 3.2 (2-Cl). 3.7 (4-CF₃) was reduced at a similar rate to 3.6 (2-CF₃). However, 3.3 (4-Cl) and 3.4 (3-Cl) were reduced more slowly than 3.2 (2-Cl), demonstrating that the ortho position is critical for the Cl substituent.
The introduction of various other electron-withdrawing substituents such as esters (3.8 and 2.2) or a cyano (3.4) at the 4-position also afforded intermediate rates of reduction and there was no significant correlation in the reduction rates of the 4-derivatives with the Hammett values for these substituents (Figure 3.6). Surprisingly the perfluorinated derivative 3.11 was reduced more slowly than all other derivatives and even the unfunctionalized azobenzene 3.1, despite its presumed electron deficiency.

![Figure 3.6 – Hammett plot for 3- and 4-substituted azobenzenes demonstrating poor correlation between σ for the substituents and the observed rate of reduction](image)

Considering the data as a whole, there appears to be a unique feature of 3.2 (2-Cl) that results in its more rapid reduction relative to other derivatives with more electron-deficient character and/or greater torsional strain. We propose that the results may be explained by halogen bonding. Recent studies have shown that aryl halogens are highly anisotropic in their electron distribution, leading to a δ+ region ~180° from the σ-bond referred to as the σ-hole. The presence of a halogen (X) at the 2-position may favor the association of hydrazine with the azobenzene via an N(δ-)-X(δ+) dipole-dipole interaction,
effectively pre-organizing the reagent complex for reduction. This behaviour is not often observed with aryl fluorides, and increases with halogen size.\textsuperscript{59} While this mechanism does not account for the decreased rate of the 2-Br derivative 3.9, the contributions of other factors such as electronegativity may also play a partial role, and the rates of reduction for compounds 3.2 and 3.9 were not significantly different within a 95% confidence interval.

### 3.2.2 Synthesis of a Multi-responsive Poly(ester amide)

To demonstrate the potential of azobenzene as a multi-stimuli responsive moiety, the most rapidly-reduced azobenzene 3.2 was incorporated as a pendant group on an amphiphilic PEA-PEO graft copolymer. The synthesis of a diester monomer incorporating an azobenzene was accomplished according to Figure 3.7. First, azobenzene derivative 3.2 was reacted with carbon tetrabromide and triphenylphosphine in an Appel reaction to afford the benzylic bromide 3.12. A phenoxide was then prepared from 3.13,\textsuperscript{60} and was subsequently alkylated with 3.12, affording the dialdehyde 3.14. The aldehydes of 3.14 were then selectively reduced in the presence of the diazene bond to benzylic alcohols using NaBH\textsubscript{4} to give compound 3.15. Two equivalents of N-t-butyloxycarbonyl (Boc)-protected glycine were then coupled to 3.15 using carbonyldiimidazole (CDI) to afford 3.16. The Boc group was removed from compound 3.16 using trifluoroacetic acid (TFA) directly before polymerization to yield the target monomer 3.17.
As shown in Figure 3.8, monomers 3.17, 3.18, and 3.19 (0.9/0.1/1.0), were then reacted via a condensation polymerization in N,N-dimethylacetamide (DMA) to give polymer 3.20. This polymer had a weight-average molar mass (M_w) of 7200 g mol\(^{-1}\) and a dispersity (D) of 2.26 as measured by size exclusion chromatography (SEC) relative to poly(methyl methacrylate) standards (PMMA). The 0.1 equiv. of the aspartic acid-based monomer 3.18 were incorporated to provide attachment sites for PEO chains following cleavage of the \(t\)-butyl ester protecting groups. Thus, the pendant carboxylic acid groups of polymer 3.20 were deprotected using TFA and subsequently coupled to amine-terminated poly(ethylene oxide) monomethyl ether (PEO-NH\(_2\), 2000 g mol\(^{-1}\)) yielding the target amphiphilic graft copolymer, 3.21.

This polymer had an M_w of 5900 g mol\(^{-1}\) and a D of 2.13 as measured by SEC relative to PMMA. It was surprising that 3.21 had a lower molar mass than its precursor 3.20 but this phenomenon has been previously observed by SEC for PEA-PEO graft copolymers and may be related to conformational effects. Using \(^1\)H NMR spectroscopy, based on the relative integrations of the PEO peak at 3.5 ppm and the peak
at 3.9 ppm corresponding to the α-hydrogens of glycine on the azobenzene monomer, the graft copolymer that was ~42 wt% PEO.

Figure 3.8 – Synthesis of a PEA having pendant azobenzenes, and post-polymerization conjugation of PEO to afford an amphiphilic graft copolymer.

A control polymer 3.23 (Figure 3.9) without azobenzene moieties was also prepared in order to demonstrate that any changes in properties arise from triggering of the azobenzene moieties rather than non-specific cleavage of the esters in the polymer backbone. This polymer had a $M_W$ of 18 200 g mol$^{-1}$, $D$ of 1.58, and a PEO content of ~34 wt%. 
3.2.3 Assembly Formation and Degradation

Nanoassemblies were prepared from the stimuli-responsive polymer 3.21 and from the control polymer 3.23 by the rapid addition of a solution of polymer in DMSO to water. The suspensions were then diluted four-fold with water and dialysed against a 1 kg mol\(^{-1}\) molecular weight cut-off (MWCO) membrane in water to remove the organic solvent. The Z-average hydrodynamic diameters of the resulting assemblies were determined by DLS to be ~90 nm for 3.21 (Figure 3.10A) and ~100 nm for 3.23 (Figure 3.11A). The assemblies were also imaged by transmission electron microscopy (TEM). As shown in Figure 3.10B and Figure 3.11B, the assemblies were solid particles, suggesting that they were micelles or compound micelles.

Figure 3.9 – Control PEA 3.23 synthesized for comparison with 3.21.
3.2.3.1 Effect of UV Irradiation on Polymer Assemblies

First, the UV-responsive properties of the micelles were probed. Irradiation with light was expected to result in reversible changes in the micellar structure. Alternating irradiation with UV and visible light was used to convert the pendant azobenzenes between the trans and cis conformations and the effects were measured by fluorescence and UV-vis spectroscopy. As shown in Figure 3.12, 10 min of irradiation with UV light resulted in a
~35% decrease in the absorbance at 330 nm corresponding to the trans conformation. Irradiation for 10 min with visible light resulted in an increase in the absorbance at 330 nm back to its original level. There were no changes in the maximum and minimum absorbance over 8 cycles, confirming that the isomerization was fully reversible.

Figure 3.12 – Reversible increase and decrease in absorbance of the trans-azobenzene and fluorescence of encapsulated nile red in polymer 3.21 assemblies upon irradiation with UV and visible light in alternating 10 minute cycles.

To further probe the effects of the isomerization on the micelles, 2 wt% of nile red as a probe molecule was encapsulated in the micelles by dissolving nile red in the DMSO polymer solution, and then forming the assemblies as described above. The same cycles of irradiation with UV and visible light were repeated. As shown in Figure 3.12, irradiation with UV light for 10 min resulted in a ~15% reduction in the nile red fluorescence. It is well established that the fluorescence intensity of nile red is strongly dependent on its environment, with increased fluorescence observed in more hydrophobic environments and reduced fluorescence in more polar environments due aggregation and consequent
Therefore, the reduction in nile red fluorescence upon trans-cis isomerization likely results from an increase in polarity of the micelle core, increased water penetration into the micelle core resulting from this polarity, some release of nile red from the micelle into the bulk water or some combination of these phenomena. This process was also reversible upon irradiation with visible light, resulting in an increase in nile red fluorescence back to approximately its initial value. It was noted that the fluorescence varied to some extent with each cycle, suggesting that some minor reorganization of the micelles and the encapsulated nile red might have occurred as a result of the isomerization. Nevertheless, the process was clearly reversible on the assemblies.

Figure 3.13 – Changes in the mean count rate measured by DLS for assemblies of polymers 3.21 and 3.23 under various conditions. The control polymer 3.23 was exposed to hydrazine (○) and a combination of hydrazine and constant UV irradiation (●). The azo polymer 3.21 was exposed to no stimulus (■), hydrazine (Δ), UV irradiation (♦), and the combination of UV irradiation and hydrazine (▲).

In another experiment monitored by DLS, the assemblies were irradiated continuously with UV light. As shown in Figure 3.13, after 8 h of irradiation, a ~25%
reduction in the mean count rate was observed. The count rate in light scattering is proportional to the number, size, and density of the aggregates, but no obvious changes in the particle size distribution were observed (Figure A3.36). Therefore, in agreement with the nile red fluorescence results, this suggests that the particles underwent a more subtle reorganization. Micelles of 3.21 without any stimulus did not exhibit any significant changes in the mean count rate.

3.2.3.2 Effect of Hydrazine Reduction on Polymer Assemblies

Unlike trans-cis isomerization, treatment with hydrazine was expected to result in irreversible disruption of the micelles upon bond cleavage initiated by azobenzene reduction followed by 1,6- and 1,4-elimination reactions. Micelles of 3.21 containing nile red were treated with 250 μM hydrazine. As shown in Figure 3.14, this resulted in a ~80% decrease in nile red fluorescence over a period of 9 hours. This large decrease in nile red fluorescence is consistent with breakdown of the micelles and release of the nile red into the aqueous environment. In the absence of hydrazine, <5% decrease in fluorescence was observed. Control micelles prepared from 3.23 were also treated with 250 μM hydrazine. This resulted in a ~30% reduction in nile red fluorescence, which can likely be attributed to a small amount of ester cleavage induced by hydrazine, which is strongly nucleophilic. However, this degradation was much less than that of 3.21, confirming that the observed effects for 3.21 primarily resulted from bond cleavage induced by azobenzene reduction.

The effects of hydrazine on the DLS count rate were also investigated. As shown in Figure 3.13, assemblies of 3.21 exhibited an initial small increase in the mean count rate immediately following hydrazine addition and this was followed by a small gradual decrease. The initial increase can likely be attributed to scattering by hydrazine. Although it is a small molecule that does not strongly scatter light, its concentration is ~10-fold higher than that of the polymer in terms of molarity.
Figure 3.14 – Changes in the observed fluorescence of nile red-containing polymer assemblies of polymers 3.21 and 3.23. The control polymer 3.23 was exposed to hydrazine (○) and a combination of hydrazine and constant UV irradiation (●). The azo polymer 3.21 was exposed to no stimulus (■), hydrazine (Δ), and the combination of UV irradiation and hydrazine (▲).

It was somewhat surprising that the mean count rate did not decrease more substantially over time, given the large changes in nile red fluorescence observed upon reduction. Examination of the assembly size distributions at different time points during the experiment (Figure A3.37) did not reveal any significant changes. This suggests that the degradation products have some susceptibility to aggregation due to the presence of residual hydrophobes following backbone cleavage events, though these assemblies were clearly less effective at encapsulating nile red and/or are much less hydrophobic. The addition of 250 μM hydrazine to assemblies of 3.23 led to an initial ~30% increase in the mean count rate, likely attributable to scattering by hydrazine, and this count rate remained relatively stable over 9 hours (Figure 3.13), again demonstrating the specific effect of hydrazine on the azobenzene-containing assemblies.
3.2.3.3 Effect of Simultaneous UV Irradiation and Hydrazine Reduction on Polymer Assemblies

Finally, the combination of light and hydrazine as stimuli was investigated. As shown in Figure 3.14, when the 3.21 assemblies were treated with hydrazine and irradiated with UV light for 10 minutes, a >90% decrease in fluorescence in of nile red was observed over 9 hours. This decrease was larger than that observed for hydrazine alone, suggesting that the trans-cis isomerization provides a synergistic effect. Isomerization to the cis azobenzene may increase the polarity of the micelle core, enabling better penetration by hydrazine and/or water. Control 3.23 assemblies were also subjected to the same hydrazine and UV treatment and the decrease in nile red fluorescence was only ~20%. By DLS, more than ~80% decrease in the mean count rate was observed for 3.23 assemblies over less than 8 hours, a much larger decrease than was observed for hydrazine alone. This result suggests that trans-cis isomerization may somehow assist in the disruption of the aggregates remaining from hydrazine treatment alone, providing a synergistic effect on the DLS results. In contrast, treatment of control 3.23 micelles with both UV light and hydrazine did not lead to any significant change in the DLS count rate. This result again confirms the specific role of azobenzene in both light- and hydrazine-mediated disruption of the micelles.

3.3 Conclusions

The rate of azobenzene reduction was optimized through the preparation and study of a library of twelve electron-deficient azobenzenes. It was found that the reduction-sensitivity of these compounds was tunable, with a 2-Cl derivative affording the fastest reduction rate of the studied compounds. This result was attributed to a halogen bonding effect that may promote pre-complexation of the azobenzene with the reducing agent. 2-Chloroazobenzenes were then successfully incorporated as pendant groups on an amphiphilic PEA-PEO graft copolymer. These copolymer was assembled into micellar-like structures in aqueous solution. A combination of UV-vis spectroscopy, fluorescence spectroscopy using nile red as a probe, and DLS were used to probe the responses of the micelles to trans-cis isomerization triggered by light, reduction triggered by hydrazine, and a combination of light and reductive stimuli. It was found that light imparted reversible
changes in the micelle core, whereas hydrazine led to an irreversible reduction in nile red fluorescence. A combination of light and reductive stimuli led to more significant changes in nile red fluorescence and in mean DLS count rate than either stimulus alone, suggesting a synergistic effect. In contrast, control samples of PEA micelles without azobenzene and azobenzene micelles without stimuli only underwent small changes in nile red fluorescence and DLS count rate either in the presence or absence of stimuli. Thus, this work provides the first example demonstrating the response of azobenzene to multiple stimuli, both separately and synergistically and demonstrates that this response can be translated into both reversible and irreversible changes in the properties of materials.

3.4 Experimental

3.4.1 General Materials, Methods, and Procedures

Chemicals were obtained from Alfa Aesar, Sigma Aldrich, and AK Scientific, and were used without further purification unless otherwise noted. Solvents were of reagent grade and obtained from Caledon and Fisher. They were used without further purification unless otherwise noted. Spectroscopy-grade THF was obtained from Fisher Scientific. DMA was refluxed over calcium hydride for 12 hours and freshly distilled prior to use. CH₂Cl₂ was freshly distilled over CaH₂. DMSO for nanoassembly preparation was distilled in glass grade and was obtained from Caledon. Deionized (DI) water was obtained using a Barnstead EASYpure® II system. Dialysis was carried out using regenerated cellulose membranes (Spectra/Por® RC) with molecular weight cutoffs (MWCO) of 1 kg mol⁻¹ or 50 kg mol⁻¹. Compounds 3.1, 2.2, 3.13, 3.18, 3.19 were prepared as previously reported. Thin layer chromatography (TLC) was run on Macherney-Nagel Polygram SIL G/UV254 plates and SiliaFlash P60 silica (40-60 μm, 230-400 mesh) was used for column chromatography. ¹H NMR and ¹³C NMR spectra were obtained using 600 or 400 MHz Varian Inova spectrometers. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signals (δ H, C) of CDCl₃ (δ 7.26 ppm, 77.2 ppm), CD₂OD (δ 3.31 ppm, 49.2 ppm) or DMSO-d₆ (δ 2.50 ppm, 39.5 ppm). Coupling constants (J) are expressed in Hertz (Hz). High-resolution mass spectrometry (HRMS) was performed on a Finnigan MAT 8400 mass spectrometer using electron impact ionization (EI). Size exclusion chromatography (SEC) was performed using a Waters 515 HPLC pump, two PLgel mixed-
D columns (5 μm pore size, 300 mm × 7.5 mm) and their corresponding guard column, and a Wyatt Optilab rEX refractive index detector. DMF with 1% NEt$_3$ and 10 mM LiBr was used as the eluent. The column temperature was 85 °C, and the flow rate was 1.0 mL min$^{-1}$, and samples were analyzed at a concentration of 5 mg mL$^{-1}$. Molar mass was determined relative to PMMA standards. FT-IR spectra were recorded on either a Bruker Vector 33 instrument in transmission mode, or a Perkin Elmer Spectrum Two instrument with a diamond universal ATR attachment. UV-vis spectroscopy was performed using a Varian Cary 300 Bio UV-visible spectrophotometer. Fluorescence emission spectroscopy was carried out using a Photon Technology International QM-4 SE spectrofluorometer. The excitation wavelength was 485 nm and the emission spectrum was measured between 520 and 700 nm. The fluorescence was measured at the maximum emission wavelength. Transmission electron microscopy was performed using a Phillips CM10 microscope operating at 80 kV with a 40 μm aperture. Samples were prepared at a concentration of 0.05 mg mL$^{-1}$ and 10 μL of solution was placed on a 400-mesh copper grid with a formvar coating from Electron Microscopy Sciences and allowed to dry overnight prior to imaging. Dynamic light scattering was performed using a Malvern Zetasizer Nano ZS equipped with a 633 nm laser, using a scattering angle of 173°. For all studies aimed at determining the time-dependent effects of stimuli on the mean count rate of the samples, the attenuator value was fixed according to the computer-optimized attenuator value for the initial assemblies. The temperature was set to 25 °C, and the samples were equilibrated at this temperature for at least 30 seconds prior to measurements. Each measurement was the average of more than 10 scans of the same sample, and three separate samples were measured at each time point.

3.4.2 Determination of $k_{\text{obs}}$ for the Reduction of Azobenzenes 3.1 – 3.11 and 2.2.

Solutions of azobenzenes 3.1 – 3.11 and 2.2 at 25 μM in THF/water (3/5) were prepared. To a solution of 5.0 mL of each azobenzene was added 100 μL of hydrazine hydrate (~50% N$_2$H$_4$) to achieve a concentration of ~250 μM. Absorption spectra were obtained initially and then every minute for 10 minutes over a wavelength range of 200-500 nm in a quartz cuvette with a 1 cm path length. $k_{\text{obs}}$ was determined as the negative slope of the plot of
\[ \ln(A/A_0) \] versus time. The experiment was repeated in triplicate for each azobenzene and the error reported on \( k_{\text{obs}} \) corresponds to the standard deviation of the three values.

### 3.4.3 Formation of Aqueous Polymer Assemblies

A solution of 3.21 or 3.23 was prepared in DMSO at a concentration of 8.0 mg mL\(^{-1}\). 0.1 mL of this solution was added rapidly to 0.9 mL of stirring deionized water. The resulting solution was stirred for approximately 30 minutes, and then diluted four-fold by the addition of 3.0 mL of DI water. The resulting solution was then dialyzed in 1 L of DI water to remove the organic solvent, using a 50 kg mol\(^{-1}\) MWCO membrane for at least 16 hours, to yield 4.0 mL of polymer assemblies at a concentration of 0.2 mg mL\(^{-1}\).

### 3.4.4 Encapsulation of Nile Red

A stock solution of nile red in THF (0.16 mg mL\(^{-1}\)) was prepared. To a clean dry vial was added 0.1 mL of the nile red solution, and the THF was evaporated under a stream of air. The resulting nile red residue was then re-dissolved in 0.1 mL of 8.0 mg mL\(^{-1}\) polymer solution in DMSO, and this solution was used in the formation of polymer assemblies as described above. This provided polymer assemblies with 2 wt% encapsulated nile red dye relative to the polymer mass, at a polymer concentration of 0.2 mg mL\(^{-1}\).

### 3.4.5 Procedure for Alternating Irradiation with UV and Visible Light

The UV light source consisted of 16 UVA bulbs, (Hitachi FL8BL-B, 8 watts) with emission centered at 365 nm, set at a distance of 10 cm from the sample. The visible light source was a 1 watt white LED bulb set at a distance of 1 cm from the sample. 1.0 mL of the 0.2 mg mL\(^{-1}\) assemblies prepared with or without encapsulated nile red as described above was alternately irradiated in a quartz cuvette with UV light for 10 minutes, followed by visible light for 10 minutes. The process was repeated four times. The sample without nile red was analyzed by UV-vis spectroscopy after each irradiation whereas the sample containing nile red was analyzed by fluorescence spectroscopy after each irradiation.
3.4.6 Procedure for Constant Irradiation with UV light

1.0 mL of the 0.2 mg mL\(^{-1}\) 3.21 nanoassembly sample with (for fluorescence studies) or without (for DLS studies) encapsulated nile red was placed in a quartz cuvette and irradiated continuously over 9 hours with the UV light source described above. The temperature was maintained at ~22 °C. At various time points, the sample was briefly removed from the light source and the DLS or fluorescence measurement was performed as described above. The control consisted of 3.21 micelles that were not irradiated with light. The experiments were performed in triplicate and error bars represent the standard deviations on three measurements.

3.4.7 Procedure for Hydrazine-Induced Degradation of Assemblies

To 1.0 mL of the 0.2 mg mL\(^{-1}\) 3.21 or 3.23 nanoassembly sample with (for fluorescence studies) or without (for DLS studies) encapsulated nile red was added 20 µL of hydrazine hydrate (~50% N\(_2\)H\(_4\)). The sample was stirred at room temperature (~22 °C) and then analyzed by fluorescence measurement or DLS as described above at various time points. The experiments were performed in triplicate and error bars represent the standard deviations on three measurements.

3.4.8 Procedure for Hydrazine-Induced Degradation of Assemblies, with UV Light Irradiation

To 1.0 mL of the 0.2 mg mL\(^{-1}\) 3.21 or 3.23 nanoassembly sample with (for fluorescence studies) or without (for DLS studies) encapsulated nile red in a quartz cuvette was added 20 µL of hydrazine hydrate. The sample was then irradiated with the UV light source described above. The temperature was maintained at ~22 °C. At various time points, the sample was briefly removed from the light source and the DLS or fluorescence measurement was performed as described above. The experiments were performed in triplicate and error bars represent the standard deviations on three measurements.
3.4.9 Synthesis of (E)-(4-((2-chlorophenyl)diazenyl)phenyl) methanol (Compound 3.2)

2-Chloroaniline (1.00 g, 7.84 mmol) was dissolved in CH₂Cl₂ (20 mL) and H₂O (50 mL) was added to the solution. A solution of oxone (9.64 g, 15.6 mmol) in water (100 mL) was added and the mixture was stirred for 2 hours at room temperature yielding a green solution. The solution was separated and the aqueous layer was extracted with CH₂Cl₂ (100 mL). The organic phase was washed with 1M HCl (100 mL), saturated NaHCO₃ (100 mL) and saturated NaCl (100 mL), then dried on MgSO₄ and filtered. This solution was used immediately without further purification. AcOH (5 mL) was added to the CH₂Cl₂ solution. 4-Aminobenzyl alcohol (1.06 g, 8.62 mmol) was added and the mixture was stirred at room temperature for 16 hours. The organic phase was washed with saturated NaHCO₃ (100 mL), and saturated NaCl (100 mL). The organic phase was then dried on MgSO₄, filtered and concentrated in vacuo. The collected solid was then purified by column chromatography (EtOAc/Hex 40:60) to provide compound 3.2, as an orange solid (1.78 g, 92%). ¹H NMR (400 MHz, CDCl₃): δ = 4.80 (s, 2H), 7.33-7.38 (m, 1H), 7.39-7.43 (m, 1H), 7.52-7.54 (m, 2H), 7.56-7.58 (m, 1H), 7.70-7.72 (m, 1H), 7.97-7.99 (m, 2H). ¹³C NMR (100 MHz): δ = 152.2, 148.7, 144.4, 135.3, 131.6, 130.7, 127.4, 127.3, 123.6, 117.6, 64.8. FT-IR (NaCl, thin film, νmax/cm⁻¹): 3303 (O–H), 1219 (N=N), 1030 (C–O, alcohol). HRMS (EI): calc. for C₁₃H₁₁ClN₂O+ [M]+: 246.0560, found 246.0556.

3.4.10 Synthesis of (E)-(4-((4-chlorophenyl)diazenyl)phenyl) methanol (Compound 3.3)

The same procedure described for the preparation of azobenzene 3.2 was used except that the starting aniline was 4-chloroaniline (1.00 g, 7.84 mmol). The collected solid was then purified by column chromatography (EtOAc/Hex 40:60) to generate compound 3.3, as an orange solid (1.32 g, 68%). ¹H NMR (600 MHz, DMSO-d₆): [cis] δ = [4.44 (d, J = 5.6 Hz, 2H)], 4.61 (d, J = 5.6 Hz, 2H), [5.22 (t, J = 5.6 Hz, 1H)], 5.38 (t, J = 5.6 Hz, 1H), [6.82-6.83 (m, 2H)], [6.87-6.88 (m, 2H)], [7.25-7.27 (m, 2H)], [7.37-7.39 (m, 2H)], 7.53-7.55 (m, 2H), 7.65-7.67 (m, 2H), 7.87-7.88 (m, 2H), 7.89-7.91 (m, 2H). ¹³C NMR (150 MHz): [cis] δ = [152.3], [151.8], 150.7, [150.5], 146.8, [142.1], 135.78, [131.3], 129.6, [128.9], 127.1, [126.7], 124.1, 122.6, 121.7, [120.0], 62.4, [62.2]. FT-IR (NaCl, thin film,
\( v_{\text{max/cm}}^{-1} \): 3398 (O-H), 1261 (N=N), 1080 (C–O, alcohol). HRMS (EI): calc. for 
\([C_{13}H_{11}ClN_2O]^+ [M]^+\): 246.0560, found 246.0558.

3.4.11 Synthesis of \((E)-(4-((3-chlorophenyl)diazenyl)phenyl)\) methanol (Compound 3.4)

The same procedure described for the preparation of azobenzene 3.2 was used except that 
the starting aniline was 3-chloroaniline (2.00 g, 15.6 mmol). The collected solid was then 
purified by column chromatography (EtOAc/Hex 40:60) to generate compound 3.4, as an 
orange solid (3.43 g, 89%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 4.81 \) (s, 2H), 7.44-7.50 (m, 
2H), 7.53-7.55 (m, 2H), 7.83-7.86 (m, 1H), 7.91-7.95 (m, 3H). \(^{13}\)C NMR (100 MHz): \( \delta = 
153.4, 151.8, 144.4, 135.1, 130.7, 127.4, 123.3, 122.4, 121.8, 64.8. \) FT-IR (NaCl, 
thin film, \( v_{\text{max/cm}}^{-1} \)): 3323 (O–H), 1211 (N=N), 1028 (C–O, alcohol). HRMS (EI): calc. for 
\([C_{13}H_{11}ClN_2O]^+ [M]^+\): 246.0560, found 246.0565.

3.4.12 Synthesis of \((E)-4-((4-(hydroxymethyl)phenyl)diazenyl)benzonitrile\) (Compound 3.5)

The same procedure described for the preparation of azobenzene 3.2 was used except that 
the starting aniline was 4-aminobenzonitrile (1.00 g, 8.46 mmol). The collected solid was then 
purified by column chromatography (EtOAc/Hex 40:60) to generate compound 3.5, as an 
orange solid (1.43 g, 71%). \(^1\)H NMR (600 MHz, DMSO-\(d_6\)): \( \delta = 4.62 \) (d, J = 5.1 Hz, 
2H), 5.42 (t, J = 5.1 Hz, 1H), 7.54-7.56 (m, 2H), 7.91-7.93 (m, 2H), 7.99-8.01 (m, 2H), 
8.06-8.08 (m, 2H). \(^{13}\)C NMR (150 MHz): [cis] \( \delta = [157.3, 154.0, [151.6, 150.7, 147.7, 
[142.7), 133.8, [133.3, 127.2, [126.7, 123.1, [122.9, 120.4, [120.3, 118.4, [118.3, 
113.1, [109.2, 62.4, [62.2. \) FT-IR (NaCl, thin film, \( v_{\text{max/cm}}^{-1} \)): 3366 (O-H), 2359 (C≡N), 
1219 (N=N), 1030 (C–O, alcohol). HRMS (EI): calc. for 
\([C_{14}H_{11}N_3O]^+ [M]^+\): 237.0902, found 237.0906.

3.4.13 Synthesis of \((E)-(4-((2-(trifluoromethyl)phenyl)diazenyl)phenyl)methanol\) (Compound 3.6)

The same procedure described for the preparation of azobenzene 3.2 was used except that 
the starting aniline was 2-(trifluoromethyl)aniline (1.00 g, 6.21 mmol). The collected solid 
was then purified by column chromatography (EtOAc/Hex 40:60) to generate compound
3.6, as an orange solid (1.56 g, 90%). $^1$H NMR (600 MHz, DMSO-$d_6$): δ = 4.63 (d, J = 5.9 Hz, 2H), 5.41 (t, J = 5.9 Hz, 1H), 7.57-7.59 (m, 2H), 7.73-7.76 (m, 1H), 7.80-7.81 (m, 1H), 7.83-7.86 (m, 1H), 7.89-7.90 (m, 2H), 7.96-7.97 (m, 1H). $^{13}$C NMR (150 MHz): δ = 151.7, 149.6, 148.4, 134.5, 132.1, 128.0, 127.4, 124.8 (q, $^1$J$_{C-F} = 274$ Hz), 123.7, 122.1, 117.0, 63.2. FT-IR (NaCl, thin film, $\nu_{\text{max}}$/cm$^{-1}$): 3365 (O-H), 1219 (N=N), 1052 (C–O, alcohol). HRMS (EI): calc. for [C$_{14}$H$_{11}$F$_{3}$N$_2$O]$^+$ [M$^+$]: 280.0823, found 280.0817.

3.4.14 Synthesis of (E)-(4-((4-(trifluoromethyl)phenyl)diazenyl)phenyl)methanol (Compound 3.7)

The same procedure described for the preparation of azobenzene 3.2 was used except that the starting aniline was 4-(trifluoromethyl)aniline (1.00 g, 6.21 mmol). The collected solid was then purified by column chromatography (EtOAc/Hex 40:60) to generate compound 3.7, as an orange solid (1.10 g, 63%). $^1$H NMR (600 MHz, DMSO-$d_6$): [cis] δ = [4.44 (d, J = 5.87 Hz, 2H)], 4.63 (d, J = 5.87 Hz, 2H), [5.22 (t, J = 5.87 Hz, 1H)], 5.41 (t, J = 5.87 Hz, 1H), [6.85-6.87 (m, 2H)], [7.05-7.07 (m, 2H)], [7.25-7.27 (m, 2H)], 7.55-7.77 (m, 2H), [7.69-7.70 (m, 2H)], 7.92-7.93 (m, 2H), 7.96-7.97 (m, 2H), 8.04-8.06 (m, 2H). $^{13}$C NMR (150 MHz): [cis] δ = [156.9], [154.1], [151.6], 150.7, 147.5, 142.5, 130.6, 127.1, [127.0], 126.9, [126.2], 124.9, [124.8], 123.0, [122.8], 120.3, [120.2], [109.7], 62.4, [62.2]. FT-IR (NaCl, thin film, $\nu_{\text{max}}$/cm$^{-1}$): 3365 (O-H), 1219 (N=N), 1044 (C–O, alcohol). HRMS (EI): calc. for [C$_{14}$H$_{11}$F$_3$N$_2$O]$^+$ [M$^+$]: 280.0823, found 280.0817.

3.4.15 Synthesis of (E)-prop-2-yn-1-yl 4-((4-(hydroxymethyl)phenyl)diazenyl)benzoate (Compound 3.8)

The same procedure described for the preparation of azobenzene 3.2 was used except that the starting aniline was prop-2-yn-1-yl 4-aminobenzoate (1.00 g, 5.71 mmol). The collected solid was then purified by column chromatography (EtOAc/Hex 40:60) to generate compound 3.8, as an orange solid (1.23 g, 73%). $^1$H NMR (600 MHz, DMSO-$d_6$): δ = 3.65 (t, J = 2.3 Hz, 1H) 4.62 (d, J = 5.3 Hz, 2H) 5.4 (m, 1H) 7.57 (m, 2H) 7.93 (m, 4H) 8.00 (m, 2H) 8.18 (m, 2H). $^{13}$C NMR (150 MHz): δ = 164.4, 154.7, 150.8, 148.4, 130.7, 127.2, 122.8, 122.7, 120.4, 119.7, 78.1, 62.4, 52.8. FT-IR (ATR, $\nu_{\text{max}}$/cm$^{-1}$): 3400-3000 (br, OH), 3266, 2125, 1722 (C=O), 1370, 1262, 1224, 1095, 1034, 1007. HRMS (EI): calc. for [C$_{17}$H$_{14}$N$_2$O$_3$]$^+$ [M$^+$]: 294.1004, found 294.1010.
3.4.16 Synthesis of (E)-(4-((2-bromophenyl)diazene)phenyl) methanol (Compound 3.9)

The same procedure described for the preparation of azobenzene 3.2 was used except that the starting aniline was 2-bromoaniline (2.00 g, 11.63 mmol). The collected solid was then purified by column chromatography (EtOAc/Hex 40:60) to generate compound 3.9, as an orange solid (3.08 g, 91%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 4.81$ (s, 2H), 7.31-7.35 (m, 1H), 7.39-7.43 (m, 1H), 7.53-7.55 (m, 2H), 7.68-7.70 (m, 1H), 7.76-7.78 (m, 1H), 7.98-8.00 (m, 2H). $^{13}$C NMR (100 MHz): $\delta = 152.1$, 149.7, 144.5, 133.7, 131.8, 128.0, 127.4, 125.7, 123.6, 117.8, 64.8. FT-IR (NaCl, thin film, $\nu_{\text{max}}$/cm$^{-1}$): 3304 (O-H), 1219 (N=N), 1029 (C–O, alcohol). HRMS (EI): calc. for $[C_{13}H_{11}BrN_2O]^+$ [M]$^+$: 290.0055, found 290.0044.

3.4.17 Synthesis of (E)-(4-((2-fluorophenyl)diazene)phenyl) methanol (Compound 3.10)

The same procedure described for the preparation of azobenzene 3.2 was used except that the starting aniline was 2-fluoroaniline (1.00 g, 9.00 mmol). The collected solid was then purified by column chromatography (EtOAc/Hex 40:60) to generate compound 3.10, as an orange solid (1.23 g, 62%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 4.80$ (s, 2H), 7.22-7.25 (m, 1H), 7.28-7.30 (m, 1H), 7.44-7.48 (m, 1H), 7.52-7.53 (m, 2H), 7.76-7.78 (m, 1H), 7.95-7.97 (m, 2H). $^{13}$C NMR (100 MHz): $\delta = 152.1$, 149.7, 144.5, 133.7, 131.8, 128.0, 127.4, 125.7, 123.6, 117.8, 64.8. FT-IR (NaCl, thin film, $\nu_{\text{max}}$/cm$^{-1}$): 3304 (O-H), 1219 (N=N), 1029 (C–O, alcohol). HRMS (EI): calc. for $[C_{13}H_{11}FN_2O]^+$ [M]$^+$: 230.0855, found 230.0858.

3.4.18 Synthesis of (E)-(4-((perfluorophenyl)diazene)phenyl) methanol (Compound 3.11)

The same procedure described for the preparation of azobenzene 3.2 was used except that the starting aniline was 2,3,4,5,6-pentafluoroaniline (1.00 g, 5.46 mmol). The collected solid was then purified by column chromatography (EtOAc/Hex 40:60) to generate compound 3.11, as an orange solid (0.66 g, 40%). $^1$H NMR (600 MHz, DMSO-d$_6$): [cis] $\delta = [4.51$ (s, 2H), 4.63 (s, 2H), [7.10-7.11 (m, 2H)], [7.38-7.39 (m, 2H)], 7.57-7.58 (m, 2H), 7.87-7.88 (m, 2H). $^{13}$C NMR (150 MHz): [cis] $\delta = [151.9$, 151.1, 148.7, [145.2], [141.6],
FT-IR (NaCl, thin film, $\nu_{\text{max}}$/cm$^{-1}$): 3325 (O-H), 1219 (N=N), 1020 (C–O, alcohol). HRMS (EI): calc. for [C$_{13}$H$_7$F$_5$N$_2$O]$^+$ [M]$^+$: 302.0479, found 302.0486.

### 3.4.19 Synthesis of (E)-1-(4-(bromomethyl)phenyl)-2-(2-chlorophenyl)diazene (Compound 13)

Compound 3.2 (4.00 g, 16.2 mmol) was dissolved in CH$_2$Cl$_2$ (200 mL). CBr$_4$ (6.45 g, 19.4 mmol) was added and the solution was cooled to 0 °C. PPh$_3$ (6.38 g, 24.3 mmol) was added to the solution. The mixture was allowed to warm to room temperature and stirred for 1 hour. The solution was then concentrated in vacuo, yielding an orange solid. The collected solid was then purified by column chromatography (EtOAc/Hex 5:95) to provide compound 3.12 (4.91 g, 98%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 4.57$ (s, 2H), 7.35–7.37 (m, 1H), 7.40–7.43 (m, 1H), 7.55–7.59 (m, 3H), 7.70–7.71 (m, 1H), 7.95–7.96 (m, 2H). $^{13}$C NMR (100 MHz): [cis] $\delta = 152.4$, [152.0], 148.6, 141.1, [140.7], [138.1], [136.1], 135.5, 131.9, 130.7, 129.9, [129.4], [129.2], 127.3, 123.73, [123.65], [123.4], 117.5, [117.4], [91.3], [45.6], 32.6. FT-IR (ATR, $\nu_{\text{max}}$/cm$^{-1}$): 1580, 1459, 1427, 1225 (N=N), 1197, 1144, 1088, 1057. HRMS (EI): calc. for [C$_{13}$H$_{10}$BrClN$_2$]$^+$ [M]$^+$: 307.9716, found 307.9727.

### 3.4.20 Synthesis of Compound 3.14

In a flame-dried round bottom flask, compound 3.12 (2.48 g, 7.99 mmol) was dissolved in THF (50 mL), and compound 3.13$^{60}$ (1.00 g, 6.66 mmol) was dissolved separately in THF (20 mL). The solution of compound 3.13 was then transferred by cannula into the solution of compound 3.14. The solution was then cooled to 0 °C and DIPEA (2.60 mL, 14.6 mmol) was added. The reaction mixture was stirred for 1 hour and then was heated to 40 °C and stirred 24 hours. The solution was then diluted with H$_2$O (100 mL) and extracted with CH$_2$Cl$_2$ (3 × 100 mL). The organic phase was washed with 1M HCl (200 mL), saturated NaHCO$_3$ (200 mL), and saturated NaCl (200 mL), and then dried on MgSO$_4$, filtered and concentrated in vacuo. The collected solid was then purified by column chromatography (EtOAc/Hex/PhMe 15:50:35) to provide compound 3.14 (1.26 g, 51%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 5.28$ (s, 2H), 7.36–7.38 (m, 1H), 7.41–7.44 (m, 1H), 7.43–7.46 (m, 1H), 7.55–7.56 (m, 2H), 7.58–7.59 (m, 1H), 7.72–7.74 (m, 1H), 8.01–8.02 (m, 2H), 8.14–8.15 (m,
2H), 10.36 (s, 2H). $^{13}$C NMR (150 MHz): $\delta = 188.4, 163.3, 152.9, 148.6, 137.9, 135.6, 135.5, 132.0, 130.8, 130.3, 129.3, 127.3, 125.2, 123.9, 117.5, 80.7. FT-IR (NaCl, thin film, $\nu_{\text{max}}/\text{cm}^{-1}$): 3398 (C-H, H-CO), 1678 (C=O), 1219 (N=N), 1057 (C-O ether). HRMS (EI): calc. for $[C_{21}H_{15}ClN_2O_3]^+$ [M$^+$]: 378.0771, found 378.0767.

3.4.21 Synthesis of Compound 3.15

Compound 3.14 (500 mg, 1.32 mmol) was dissolved in MeOH (50 mL) and cooled to 0°C. NaBH$_4$ (110 mg, 2.90 mmol) was then added to the solution and the reaction mixture was stirred for 1 hour, then warmed to room temperature. The solution was then neutralized to pH 5-6 with NH$_4$Cl and extracted with ethyl acetate (3 × 50 mL), and the collected organic phase was then extracted with saturated NaHCO$_3$ (100 mL), and saturated NaCl (100 mL). The organic phase was then dried on MgSO$_4$, filtered and concentrated in vacuo. The collected solid was then purified by column chromatography (EtOAc/Hex 20:80) to give compound 3.15 (330 mg, 65%). $^1$H NMR (600 MHz, DMSO-d$_6$): [cis] $\delta = [4.51$ (d, $J = 5.3$ Hz, 4H)], 4.59 (d, $J = 5.3$ Hz, 4H), [4.80 (s, 2H)], 5.01 (s, 2H), [5.08 (t, $J = 5.3$ Hz, 2H)], 5.15 (t, $J = 5.3$ Hz, 2H), [6.64-6.66 (m, 1H)], [6.95-6.96 (m, 2H)], [7.12-7.14 (m, 1H)], [7.13-7.15 (m, 1H)], 7.16-7.18 (m, 1H), [7.33-7.34 (m, 2H)], 7.37-7.38 (m, 2H), [7.44-7.45 (m, 2H)], 7.49-7.53 (m, 1H), [7.51-7.73 (m, 1H)], 7.57-7.60 (m, 1H), 7.70-7.71 (m, 1H), [7.73-7.74 (m, 2H)], 7.73-7.74 (m, 2H), 7.97-7.99 (m, 2H). $^{13}$C NMR (150 MHz): [cis] $\delta = 153.2, [153.0], 151.6, [151.1], 147.9, 141.9, [137.6], 135.1, [135.0], 134.0, [132.7], 130.8, [129.9], 128.6, [128.5], 128.3, 128.1, [128.1], [127.8], 127.3, [124.0], [123.9], 123.0, 119.6, [119.6], 117.6, 74.7, [74.6], 58.0, [57.9]. FT-IR (NaCl, thin film, $\nu_{\text{max}}/\text{cm}^{-1}$): 3377 (O-H), 1219 (N=N), 1057 (C=O, ether) 1021 (C-O, alcohol). HRMS (EI): calc. for $[C_{21}H_{15}ClN_2O_3]^+$ [M$^+$]: 382.1084, found 382.1072.

3.4.22 Synthesis of Compound 3.16

Boc-Gly-OH (332 mg, 1.90 mmol) was dissolved in CH$_2$Cl$_2$ (20 mL). Carbonyldiimidazole (350 mg, 2.16 mmol) was then added and the resulting reaction mixture was stirred for 30 min. Compound 3.15 (330 mg, 0.86 mmol) was added and the reaction mixture was stirred for 3 hours. The solution was quenched with 1M HCl (20 mL) and the organic and aqueous phases were separated. The organic phase was washed with saturated NaHCO$_3$ (20 mL),
and saturated NaCl (20 mL), then dried on MgSO\textsubscript{4}, filtered, and concentrated \textit{in vacuo}. The resulting solid was then purified by column chromatography (EtOAc/Hex 30:70) to give compound 3.16 (225 mg, 37\%). \textsuperscript{1}H NMR (600 MHz, DMSO-\textit{d}\textsubscript{6}, 50 °C (note: elevated temperature was used to eliminate the complicating effects of \textit{cis-trans} isomers)): \(\delta = 1.38\) (s, 18H), 3.75-3.81 (m, 4H), 5.07 (s, 2H), 5.25 (s, 4H), 7.14 (br s, 2H), 7.22-7.24 (m, 1H), 7.46-7.47 (m, 2H), 7.47-7.50 (m, 1H), 7.54-7.57 (m, 1H), 7.68-7.70 (m, 2H), 7.74-7.75 (m, 2H), 7.98-8.00 (m, 2H). \textsuperscript{13}C NMR (150 MHz, 50 °C): \(\delta = 170.01, 169.95, 155.7, 155.1, 151.7, 147.9, 140.8, 133.9, 132.3, 130.4, 129.3, 128.6, 127.7, 124.3, 122.9, 117.3, 78.1, 75.7, 61.0, 42.0, 27.9\). FT-IR (ATR, \(\nu_{\text{max}}/\text{cm}^{-1}\)): 3340, 2978, 2930, 1684, 1529, 1390, 1366, 1292, 1249, 1156, 1056, 1031. HRMS (EI): calc. for [C\textsubscript{35}H\textsubscript{41}ClN\textsubscript{4}O\textsubscript{9}]\textsuperscript{+} [M]\textsuperscript{+}: 696.2562, found 696.2594.

### 3.4.23 Synthesis of Compound 3.17

Compound 3.16 (225 mg, 0.323 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (5 mL) at room temperature. TFA (5 mL) was then added and the reaction mixture was stirred for 2 hours. The solution was then concentrated \textit{in vacuo} to give compound 3.17 (160 mg, 98%). This compound was prepared directly before polymerization, removal of the Boc group was confirmed by \textsuperscript{1}H NMR, and the compound was used immediately as the TFA salt without further purification. \textsuperscript{1}H NMR (600 MHz, DMSO-\textit{d}\textsubscript{6}): \(\delta = 3.91-3.92\) (m, 4H), 5.09 (s, 2H), 5.34 (s, 4H), 7.28-7.31 (m, 1H), 7.52-7.54 (m, 3H), 7.60-7.63 (m, 1H), 7.70-7.72 (m, 1H), 7.74-7.77 (m, 3H), 8.00-8.02 (m, 2H), 8.25 (br s, 6H).

### 3.4.24 Synthesis of Polymer 3.20

Compound 3.17 (160 mg, 0.323 mmol) and compound 3.18\textsuperscript{36} (16 mg, 0.036 mmol) were dissolved in distilled DMA (600 μL). Compound 3.19\textsuperscript{37} (129 mg, 0.359 mmol) was then added to this solution. NEt\textsubscript{3} (115 μL, 0.825 mmol) was added and the resulting mixture was stirred for 48 hours. The resulting gel was then dissolved by dilution with DMA (1 mL) and dialysed against DMF and then water using a membrane with a 1 kg mol\textsuperscript{-1} MWCO. Lyophilization provided 3.20 as an orange solid (135 mg, 65\%). \textsuperscript{1}H NMR (600 MHz, DMSO-\textit{d}\textsubscript{6}): \(\delta = 1.24-1.37\) (m, 1.75H), 1.61 (br s, 0.29H), 2.35-2.37 (m, 4H), 2.61-2.72 (m, 0.47H), 3.83-3.87 (m, 3.57H), 4.00-4.05 (br s, 0.35H), 4.82-4.84 (m, 0.52H), 5.02-
5.09 (m, 2.43H), 5.19-5.21 (br s, 2.64H), 6.61-6.63 (m, 0.25H), 6.93-6.95 (m, 0.53H), 7.18-7.24 (m, 1.42H), 7.36-7.57 (m, 3.89), 7.69-7.72 (m, 2.38H), 7.95-7.97 (br s, 1.18H), 8.32-8.35 (m, 1.70H). SEC (DMF): $M_W = 7200$ g mol$^{-1}$, $D = 2.26$.

### 3.4.25 Synthesis of Polymer 3.21

Polymer 3.20 (50 mg) was dissolved in CH$_2$Cl$_2$ (5 mL). TFA (5 mL) was then added and the reaction mixture was stirred for 1.5 hours. The solution was then concentrated in vacuo to give an orange solid. This was dissolved in DMA (1 mL) at room temperature. EDC·HCl (5.02 mg, 26.2 μmol) was then added to this solution, followed by PEO-NH$_2$ (2 000 g mol$^{-1}$, 52.4 mg, 26.2 μmol). Catalytic DIPEA (9 μL, ~50 μmol) and DMAP (0.2 mg, ~2 μmol) were added and the reaction mixture was stirred for 24 hours. The solution was then dialysed using a 50 kg mol$^{-1}$ MWCO dialysis tubing in DMF (50 mL) and then water (50 mL). The polymer solution was lyophilized to give 3.21 as an orange solid (60 mg, 70%).

$^1$H NMR (600 MHz, DMSO-$d_6$): $\delta = 2.35-2.36$ (m, 4.40H), 2.61-2.72 (m, 0.57H), 3.24 (s, 1.02H), 3.4-3.6 (m, 50.47H), 3.82-3.88 (m, 3.60H), 3.95-4.05 (m, 0.35H), 4.81-4.83 (br s, 0.49H), 5.01-5.09 (m, 2.46H), 5.19-5.21 (br s, 2.66H), 6.62-6.64 (m, 0.34H), 6.93-6.96 (br s, 0.51H), 7.17-7.24 (m, 1.49H), 7.36-7.58 (m, 4.34H), 7.69-7.72 (br s, 2.73H), 7.95-7.97 (br s, 1.26H), 8.13-8.15 (br s, 0.17H), 8.32-8.34 (br s, 1.80H). SEC (DMF): $M_W = 5900$ g mol$^{-1}$, $D = 2.13$.

### 3.4.26 Synthesis of Polymer 3.23

Poly(ester amide) 3.22 was prepared as previously reported$^{43}$ ($M_W = 16500$ g mol$^{-1}$, $D = 1.55$). 3.22 (57 mg) was then dissolved in CH$_2$Cl$_2$ (5 mL) and TFA (5 mL) was added. The solution was stirred for 1.5 h, and then the solvents were removed in vacuo. This material was then dissolved in DMF (1 mL) at room temperature. EDC·HCl (5 mg, 25.8 μmol) was added, followed by PEO-NH$_2$ (2 000 g mol$^{-1}$, 51.66 mg, 25.8 μmol) and the reaction mixture was stirred for 10 min. DIPEA (6.7 mg, 52 μL) and DMAP (cat.) were introduced and the solution was stirred for 24 hours. The solution was then dialysed in 50 kg mol$^{-1}$ MWCO dialysis membrane in DMF (50 mL) and then water (50 mL). The precipitated polymer solution was lyophilized to give polymer 3.23 as a white solid (35 mg, 38%).

$^1$H NMR (600 MHz, DMSO-$d_6$): $\delta = 1.07-1.21$ (m, 9.1H), 1.35-1.49 (m, 9.1H), 1.57-1.59 (m,
0.4H), 2.01-2.05 (m, 4.0H), 2.66-2.70 (m, 0.1H), 2.86-2.90 (m, 1.9H), 2.98-3.01 (m, 1.9H), 3.24 (s, 0.2H), 3.40-3.60 (m, 12.6H), 3.94-3.98 (m, 4.0H), 4.43-4.47 (m, 1.9H), 4.57-4.58 (m, 0.1H), 7.17-7.27 (m, 9.1H), 8.22-8.23 (m, 2.0H). SEC (DMF): MW = 18 200 g mol⁻¹, D = 1.58.

3.5 References

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Chapter 4

4 Linear Self-Immolative Block Copolymers with Multiresponsive Azobenzene Linkers

4.1 Introduction

Self-immolative polymers (SIPs) are a recently-developed class of degradable polymers that degrade selectively from end to end in response to a targeted stimulus. Their reliance on a stimulus to initiate degradation means that the endcap or trigger is the critical link in their depolymerization. Therefore a single self-immolative polymer backbone can be used with a wide variety of endcaps, and each endcap can be used with a range of self-immolative polymers now available. Self-immolative chemistry has expanded beyond the scope of fully self-immolative materials, to those materials that can partially degrade in the presence of a stimulus using self-immolative reactions. As a result, new polymer backbones and endcaps are constantly in development, as each new addition to the field has an amplified effect.

Azobenzene has been recently introduced as a novel endcap and trigger for self-immolative polymers. Reduction of the diazene bond by reductants such as hydrazine was shown to lead to depolymerization of a self-immolative polymer backbone. The rate of reduction of such azobenzenes is enhanced by the presence of electron-withdrawing groups on the azobenzene rings. An initial example containing an ethyl ester was shown to depolymerize upon the addition of hydrazine in a methanol solvent. One limitation of this work was its insolubility in aqueous solution, which precluded its use in polymer assemblies. The rate of azobenzene reduction was also demonstrated to be tunable according to substituent effects on the azobenzene ring. A series of twelve azobenzenes was investigated for their potential as reduction-sensitive triggers for SIPs, and it was shown that ortho-halogen derivatives were the most quickly reduced. The presence of an ester at the para-position also increased the rate of reduction relative to an unsubstituted azobenzene. The esters in the series of azobenzene provided a unique capability for further
functionalization that was not present in the other species, and the propargyl ester in particular was attractive for its potential in copper-assisted click chemistry.

The fastest reducing azobenzene of the series was incorporated into a graft copolymer based on a poly(ester amide),\textsuperscript{18} with pendant stimuli-responsive units at regular intervals. The reduction of the azobenzene was used to trigger local backbone cleavage via self-immolative reactions, effectively breaking the chain. This class of materials is also known in literature as chain-shattering polymers (CSPs),\textsuperscript{19-20} as they generally cause random chain scission, but only upon receiving the appropriate stimulus. While providing a high concentration of azobenzenes, which was useful for their photochemical properties, complete degradation of the polymer required a large excess of reducing agent, and a significant amount of time. It was therefore advantageous to utilize the most sensitive and most rapidly reducing azobenzene for the multiple triggers.

In the CSP, the azobenzene triggers were buried within the hydrophobic core due to their planar aromaticity, and the material’s response to light and reductive stimuli suggested that their accessibility was diminished due to this positioning. Isomerization and the corresponding increase in polarity of the azobenzenes seemed to favour depolymerization, and thus it was hypothesized that accessibility was critical to the reactivity of these species. Therefore it would be an advantage to place the azobenzene at the interface between two polymer blocks of different solvophilicity, such that they would remain accessible to chemical agents. Furthermore, a linear self-immolative polymer system endcapped by azobenzene may be able to take advantage of the amplification of the triggering stimulus caused by their end-to-end depolymerization, which the CSP could not provide.

This work attempts to improve the accessibility of azobenzene endcaps on linear SIPs in aqueous environments through the synthesis of amphiphilic block copolymers.
4.2 Results and Discussion

4.2.1 Synthesis of an Azobenzene-containing Polycarbamate-PEO Block Copolymer

A linear self-immolative polymer was synthesized based on the design described in chapter 2, but with an alkyne functionalized azobenzene developed in chapter 3, such that the endcap would allow further functionalization. A similar procedure to that described in chapter 2, for the preparation of the 4-nitrophenyl carbonate-activated endcap 2.5, was utilized in the preparation of an alkyne-ester variant 4.1 (Figure 4.1).

![Chemical Reaction Image]

**Figure 4.1** – Synthesis of an alkyne-containing azobenzene for use as an endcap linker in self-immolative block copolymers.

The self-immolative monomer 2.6 was prepared as previously described in chapter 2, where the tert-butyl carbamate of precursor 2.6b was removed using trifluoroacetic acid immediately prior to polymerization. The monomer was then mixed with the activated azobenzene endcap linker 4.1, and underwent condensation polymerization to yield the reduction-sensitive alkyne-functionalized self-immolative polymer 4.2. In this case toluene was not used as the solvent, as azobenzene 4.1 was insoluble. Endcapping of SIPs is critical to their function, and thus THF was chosen, because it is a good solvent for all components.
Figure 4.2 – Synthesis of an alkyne-containing azobenzene-endcapped self immolative polymer 4.2 by condensation polymerization.

Polymer 4.2 was characterized by SEC in DMF, relative to poly(methyl methacrylate) (PMMA) standards, and found to have a number average molar mass ($M_n$) of 2 900 g mol$^{-1}$, a weight average molar mass ($M_w$) equal to 4 200 g mol$^{-1}$, and a dispersity ($D$) of 1.45. The final purified polymer had a distinct orange colour, which was indicative of the inclusion of the azobenzene endcap linker. Low molar mass polymers are advantageous in this case because their end-groups can be analyzed more easily by analytical techniques such as $^1$H NMR spectroscopy, wherein their peaks are not lost in the baseline. Endcap incorporation was thus confirmed using $^1$H NMR spectroscopy. The methylene peak of the propargyl ester (4.97 ppm) was integrated vs. the backbone benzylic resonance (5.05-5.15 ppm), that indicated an approximate chain length of 9-10 units relative to endcap peaks and therefore an approximate molecular weight of 2 800 g mol$^{-1}$, including the contribution of the azo endcap (Figure 4.3).
Figure 4.3 – NMR overlay demonstrating the shift in peaks from azo endcap linker 4.1, to homopolymer 4.2, and copolymer 4.4. Integration of peaks c and e were used to calculate $M_n$ for polymer 4.2.

Having prepared the self-immolative block with an alkyne functionality, the next step was the coupling of a hydrophilic block using a copper-assisted azide-alkyne cycloaddition to form a triazole linkage. Azide functionalized poly(ethylene oxide) (PEO) monomethyl ether 4.3 with a molecular weight of 2 000 g mol$^{-1}$ was prepared as previously reported$^2$ by mesylation of the terminal alcohol, followed by nucleophilic displacement of the mesylate with sodium azide. The polymers 4.2 and 4.3 were coupled using click chemistry to form diblock 4.4 according to Figure 4.4, using copper sulfate ($\text{CuSO}_4$) and sodium ascorbate (NaAsc) in dimethyl sulfoxide (DMSO) solution.
Figure 4.4 – Synthesis via click chemistry of an amphiphilic self-immolative block copolymer (4.4) with a central azobenzene unit.

The copper and other small molecule byproducts of the coupling were removed by dialysis against an aqueous solution of 1 wt% ethylenediamine tetraacetic acid (EDTA) from a 1 kDa MWCO membrane, and then into deionized water. The pure diblock copolymer was recovered by lyophilization of the dialyzed solution. The synthesized block copolymer 4.4 was found to have $M_n = 4.600$ g mol$^{-1}$, $M_W = 7.700$ g mol$^{-1}$, and $D = 1.68$ by DMF SEC, and $M_n = 4.800$ g mol$^{-1}$ from $^1$H NMR. A small fraction of uncoupled homopolymer 4.2 remains within the sample, as shown in Figure 4.5, either due to incomplete coupling, or the lack of an endcap in cyclic species. However, no free PEO is observed in the final product, as demonstrated by the overlay of a comparably-sized PEO. The trace of 4.4 has shifted significantly compared to polymer 4.2 due to the large hydrodynamic radius of PEO in DMF, and the small initial size of polymer 4.2. Compared to the reference PEO peak, polymer 4.2 is smaller despite its higher molecular weight. Therefore a large increase in observed size is expected.
Figure 4.5 – SEC traces showing the synthesized azo-endcapped polycarbamate before (—) and after (—-) coupling to an azide-functionalized PEO. A trace of PEO (2000 g mol⁻¹ (●)) is overlaid to demonstrate that there is no free PEO in the final product.

Polymer 4.4 had a hydrophobic/hydrophilic mass ratio of approximately 1.5 based on NMR spectroscopy, as determined by a comparison between the integrations of peaks from both PEO and the polycarbamate backbone. It is possible that the ratio is slightly lower due to the presence of excess self-immolative polymer, but because these species participate in the aqueous assembly process, the ratio calculated from NMR remains a good approximation.

4.2.2 Reduction-triggered depolymerization of polymer 4.4

Nanoassemblies were formed in water from the block copolymer 4.4 using an organic into water nanoprecipitation method. A solution of polymer was prepared in DMSO, added rapidly into stirring water, and then later diluted four-fold. The organic solvent was then dialyzed into water to produce the final solutions of polymer assemblies free of organic
solvent. These assemblies were then characterized by DLS and TEM (Figure 4.6). DLS revealed that the assemblies had a hydrodynamic diameter of approximately 35 nm. TEM showed structures of similar diameter to DLS, along with some aggregates that may have been formed during the drying process. The dark cores observed in these structures and their small size suggests that the assemblies assumed a micellar morphology.

Figure 4.6 – (A) DLS trace showing the intensity distribution of assemblies prepared from polymer 4.4. (B) TEM image showing dark micelle cores.

Hydrazine was chosen as a reductant due to its effectiveness in reducing the azobenzene as demonstrated in the previous chapters. The basicity and nucleophilic character of hydrazine were considered as potential drawbacks, but previous studies have shown only a small effect on the rate of random backbone hydrolysis of the polymer, and a preference for the reduction of azobenzenes to nucleophilic attack. The degradation of the assemblies in response to treatment with hydrazine was studied by UV-visible spectroscopy, DLS, and fluorescence spectroscopy. For fluorescence spectroscopy, nile red dye was incorporated into the assemblies as described in chapter 3. Briefly, a stock solution of nile red was dried and re-dissolved using a DMSO solution of polymer 4.4. The assemblies were then formed using the same fast organic to water nanoprecipitation process.
Figure 4.7 – UV-visible spectra before and after treatment of polymer 4.4 assemblies with hydrazine. Data has been corrected for the overall increase in absorbance due to the decrease in solution turbidity.

When monitored by UV-vis spectroscopy, the reduction of the azobenzene linkers of polymer 4.4 appear to reach completion within 24 h (Figure 4.7). Therefore it can be said that the endcap removal is complete within this period, and that polymer degradation will be the rate-limiting step in the degradation of the polymer assemblies.

Upon treatment of a 0.2 mg mL\(^{-1}\) (1 mL) suspension of assemblies of polymer 4.4 with approximately 125 μM hydrazine hydrate (10 μL, 40-50%) a rapid increase in DLS count rate was observed (Figure 4.8). The increase was followed by a lengthy plateau, and the count rate stayed relatively constant for most of the study. It is of note that the effect is highly variable, as demonstrated by the large error. In dynamic light scattering, an increase in the count rate corresponds either to an increase in the number and/or size of scattering molecules or assemblies in solution. While there does not appear to be a rapid mechanism for the increase in number of scattering species, aggregation of the assemblies following the cleavage of the hydrophilic PEO may contribute to an increase in their size.
Figure 4.8 – DLS count rates for assemblies of polymer 4.4 upon treatment with hydrazine. A rapid increase in the count rate is observed.

Unlike in the chain-shattering design described in chapter 3, reduction of azobenzene followed by 1,6-elimination results in the cleavage of the PEO corona from the hydrophobic core (Figure 4.9). This would be expected to destabilize the micelles by removing their solubilizing component, and thus resulting in their aggregation. While an eventual decrease in the count rate of scattered light would be expected following depolymerization of the polycarbamate cores, this was not observed, as the scattered light intensity remained the same after 24 hours. In previous experiments with assemblies of this polycarbamate, degradation often took several days to reach completion, and so this result was not unexpected. In the current system, reduction of an azobenzene causes the PEO corona to be lost, but in the previous study, a simple ester hydrolysis had a similar effect. These materials were monitored over a longer time period, which suggests that assemblies of polymer 4.4 may in fact degrade completely over a longer time scale. One notable difference between the two systems is that it is clear from UV-vis spectroscopy that all azobenzene endcaps have been removed within 24 hours. Ester hydrolysis is a much slower
reaction, which may occur on a similar timescale to the depolymerization, warranting its examination over many days.

Figure 4.9 – A potential mechanism for the aggregation observed following reduction of the azo linker between blocks. Cleavage of the solubilizing PEO leads to an increase in exposure of the hydrophobic core, and causes aggregation.

The depolymerization of polymer 4.4 micelles was further probed through using nile red as a fluorescent probe. Nile red is a hydrophobic dye molecule that fluoresces in hydrophobic environments. In aqueous or highly polar conditions its emission is quenched and thus it is a good reporter for release from a micellar structure, which has a hydrophobic interior and a hydrophilic corona. The assemblies for fluorescence studies were prepared in a similar fashion to those used in DLS studies, but a portion of stock solution of nile red was first dried, to give a known, small quantity of the dye, and then re-dissolved in the polymer solution. A loading of dye of 0.4 wt% relative to the mass of the polymer was used in this study.

125 μM hydrazine was added to a 0.2 mg mL⁻¹ suspension of polymer 4.4 micelles loaded with 0.4 wt% nile red, and the emission of the dye was measured at 620 nm using fluorescence spectroscopy (Figure 4.10). A large initial increase in fluorescence was observed at the onset of the experiment, which provided further evidence for aggregation. An increase in scattering caused by aggregation leads to an increase in the path length of the excitation photons, and consequently the probability of excitation of the nile red dye. This phenomenon explains the initial increase in fluorescence, followed by its gradual
decrease over 24 hours during polymer degradation, and is consistent with the increased scattering intensity observed by DLS.

![Graph showing changes in fluorescence over time](image)

**Figure 4.10 – Changes in the fluorescence of encapsulated nile red upon treatment of polymer 4.4 assemblies with hydrazine.**

While a significant decrease in fluorescence count rate is observed, the assemblies do not appear to degrade completely, possibly due to the inclusion of un-endcapped SIP, which would naturally aggregate in the hydrophobic interior of the micelle, and not degrade in reducing conditions. Cyclic species of the polycarbamate backbone are occasionally produced during the polymerization via backbiting reactions, and cannot be selectively degraded due to their lack of endcap.\(^9\)\(^-\)\(^10\) The presence of cyclic oligomers would effectively maintain a stimuli insensitive core and perhaps aggregate with other assemblies once the stabilizing block copolymers were degraded. The presence of un-endcapped cyclic species may be suggested by the remaining starting SIP after the coupling reaction, as shown by SEC. Given that an excess of PEO was used during the coupling, and the typically high efficiency of the azide-alkyne click coupling, it seems likely that the remaining SIP starting material could not be coupled due to lack of functionality.
Having investigated the effects of azobenzene isomerization by repetitive UV and visible light irradiation in previous work, it was of interest to determine if the assemblies of 4.4 synthesized in this study would behave similarly despite having a single azobenzene at the interface between the hydrophilic and hydrophobic blocks. The azobenzene content of these polymers is low in comparison to the CSP with pendant azobenzenes, and thus a diminished effect was expected. To study the effect of isomerization on the micelles, they were irradiated at a concentration of 0.2 mg mL\(^{-1}\) in 10 minute intervals with UV (\(\lambda = 365\) nm) and visible (white LED) light (Figure 4.11).

While fluctuation in nile red fluorescence between 90-105% of the initial fluorescence were observed over the alternating periods of UV and visible irradiation, there was no significant trend indicating causation. The lack of effect can be rationalized due to the presence of azobenzene only at the interface of the core and corona of the assemblies, which would not affect the core polarity of the micelle. Therefore, as expected, irradiation of the micelles by UV and visible light did not produce the same oscillating effect as in previous work with pendant azobenzenes. With only one azobenzene per chain, the effect of isomerization was not pronounced. However, systems in which the physical motion of azobenzenes has cause the release of a drug from pores in a solid material have been reported, and thus the motion caused by the isomerization of a peripheral azobenzene could plausibly serve a function. Moreover, the reduction of azobenzenes by thiols has been reported to be accelerated by the presence of the \(cis\) form,\(^{23}\) which is consistent with the behaviour observed in chapter 2 during reduction of azobenzenes by DTT.
Figure 4.11 – Effect of repeated UV and visible light isomerizations of the azobenzene linker between blocks of polymer 4.4. No trend was observed over the course of 8 measurements after 4 irradiations each of UV and visible light.

It could potentially be advantageous that the assemblies and their contents are not directly impacted by irradiation by light, instead allowing for a combined effect from multiple simultaneous stimuli, e.g., promoting reduction in areas of low reducing agent concentration by isomerization of the azobenzene units.

The polycarbamate 4.4 was slow to degrade under the experimental conditions, and may warrant future study over longer time periods. The gradual depolymerization can likely be attributed to several factors, including the constrained hydrophobic interior, in which the cyclization reaction may be unfavourable sterically and electronically, aggregation behaviour upon cleavage of the two blocks, or perhaps the released quinone methide is trapped by nearby amines before diffusing away to be trapped by water. Slow depolymerization may be advantageous in cases where a burst release of cargo is not necessary, as the cargo would be less willing to leave the micelle core. In the interest of
pursuing a more rapid release and depolymerization, polycarbamate 4.4 was set aside to test the azobenzene trigger with a different self-immolative system.

4.3 Synthesis of an Azobenzene-containing Polyglyoxylate Block Copolymer

Polyglyoxylates are a recent addition to the field of self-immolative polymers, and depolymerize based on their ceiling temperature \(T_c\) instead of cyclization or elimination-based mechanisms.\(^{24-25}\) The materials themselves have been used previously to produce anionic detergents,\(^{26-27}\) but their self-immolative characteristics have remained unknown until recently.\(^2\),\(^{16}\) These glyoxylate polymers have many advantages. The most common polymer, poly(ethyl glyoxylate) (PEtG) can be prepared directly from commercially available monomers. Non-commercial glyoxylates have also been prepared in as few as two steps from abundant materials,\(^2\) which makes them ideal for larger scale production of self-immolative polymers. Furthermore, with careful purification of the monomer, it is possible to achieve high molecular weights that are not typical in the condensation polymerizations used to produce other self-immolative materials. Ethyl glyoxylate is a particularly attractive glyoxylate monomer, as it meets many criteria simultaneously: it is commercially available, its purification is well-understood, and its degradation process is well studied,\(^{24-25,28-29}\) the products of which are generally regarded as non-toxic to humans. Glyoxylic acid hydrate (GAH) and ethanol both follow well-known metabolic processes, as GAH is a metabolic intermediate of the glyoxylate cycle occurring in plants, bacteria, and other organisms, and also a byproduct of metabolic reactions in mammals.\(^{29}\)

Glyoxylate polymers are most often synthesized in an anionic-like polymerization mediated by base, and initiated by trace water. Therefore, with the meticulous exclusion of water, it is possible to prepare materials of very high molar mass. Due to the initiation procedure, both chain ends of the polymer are hemiacetal functionalities, as compared to the acetal backbone, and it is these functionalities that are responsible for the depolymerization of non-stabilized polyglyoxylates. Both ends of the polymer must be endcapped for these polyglyoxylates to be used as self-immolative material, and thus a block copolymer synthesized from PEtG is necessarily a triblock copolymer.
4.3.1 Synthesis and characterization of poly(ethyl glyoxylate)

The target triblock copolymer was synthesized by first polymerizing ethyl glyoxylate,² and then endcapping with a chloroformate derivative of the azobenzene alkyne 3.8. The hemiacetal end groups of the PEtG homopolymer are less nucleophilic than the chain end of polycarbamate 4.2, and so to achieve a high degree of endcapping it was necessary to prepare a more electrophilic endcap than the activated carbonate 4.1. Therefore chloroformate 4.5 was prepared from compound 3.8 using a solution of phosgene in toluene (Figure 4.12).

Figure 4.12 – Synthesis of a strongly electrophilic chloroformate derivative 4.5 from compound 3.8 using a solution of phosgene.

The endcapping of the poly(ethyl glyoxylate) homopolymer with compound 4.5 produced an azobenzene-endcapped PEtG homopolymer 4.6 (Figure 4.13). Similar to polymer 4.4, this material possessed the alkyne functionalities necessary for click chemistry and thus the formation of a triblock copolymer.

Figure 4.13 – Synthesis of an alkyne-containing azobenzene endcapped poly(ethyl glyoxylate) homopolymer 4.6 via addition polymerization.
A PEO azide monomethyl ether with a molecular weight of 5 000 g mol\(^{-1}\), polymer 4.7, was prepared as previously reported,\(^2\) such that the eventual amphiphilic block copolymer would produce micellar aggregates. These complementary homopolymers were thus conjugated via click chemistry to yield the final triblock copolymer (Figure 4.14). Homopolymer 4.6 and triblock copolymer 4.8 were characterized using \(^1\)H NMR and THF SEC before and after PEO coupling.

![Figure 4.14 – Synthesis by click chemistry of an azobenzene-containing amphiphilic triblock copolymer based on poly(ethyl glyoxylate) and PEO.](image)

![Figure 4.15 – SEC traces of polymers 4.6 and 4.8. Polymer 4.8 has shifted to lower molecular weight after coupling with PEO.](image)
Following coupling to PEO, the PEO-PEtG-PEO triblock copolymer 4.8 was found to shift to a lower apparent molecular weight than its precursor, homopolymer 4.6 (Figure 4.15). This behaviour was not observed with polycarbamate, but was previously noted in chapter 3 in the case of the chain-shattering graft copolymer.\textsuperscript{18} Polymer 4.4 was of low molar mass as shown in Figure 4.5 compared to PEO (2 000 g mol\textsuperscript{-1}). Thus the large increase in molar mass was expected. The glyoxylate polymer 4.6 was of high molar mass initially, and thus the conjugation of two PEO 5K blocks is a less significant change in mass compared to the carbamate. The graft copolymer similarly gained a small relative mass upon conjugation to PEO, and displayed an apparent decrease in molar mass.\textsuperscript{18} In cases where the mass is observed to decrease after coupling, the shift is often small, and may be attributed to differences in polymer conformation in solution.

4.3.2 Preparation of aqueous polymer assemblies from triblock 4.8

A similar method of nanoprecipitation for the formation of aqueous assemblies was used for polymer 4.8 as was used for polymer 4.4, but the assemblies were left undiluted before dialysis. A solution of 4.8 was prepared in DMSO at a concentration of 8.0 mg mL\textsuperscript{-1}. This solution was added rapidly to stirring DI water at a ratio of 0.1 mL polymer solution for every mL of assembly solution desired. The 0.8 mg mL\textsuperscript{-1} solution of assemblies was then dialyzed against a 1 kDa MWCO membrane in water to remove the organic solvent. Upon preparation of the nanoaggregates it was found by DLS that they had an approximate diameter of 90 nm (Figure 4.16, A). When imaged by TEM, the assemblies appeared to be micellar in structure due to their dark cores. It also appeared that the micelles had aggregated during the drying process, to give the appearance of larger aggregates (Figure 4.16, B).
Figure 4.16 - A) DLS trace showing the intensity distribution of assemblies prepared from polymer 4.8. B) TEM image showing dark micelle cores. Drying effects are visible in the background (white).

A previously reported photo-sensitive PEO-PEtG-PEO triblock copolymer 4.9 prepared previously within the group\(^2\) (Figure 4.17) was used as a control polymer for these studies due to its similar micelle-forming characteristics. Furthermore, its photoresponsive 2-nitrobenzyl moieties are not responsive to reduction under the experimental conditions, and therefore should provide almost identical properties without stimulus-responsiveness. This polymer has been observed to form micellar assemblies with a size of approximately 50 nm as determined by DLS and TEM.\(^2\)

Figure 4.17 – The photoresponsive PEO-PEtG-PEO triblock copolymer 4.9 used as a non-reduction-sensitive control in this study.
Instead of hydrazine, dithiothreitol was chosen as the reducing agent. Hydrazine is highly toxic stimulus, and not found in vivo. Therefore, for the further improvement of the azobenzene-triggered depolymerization of SIPs, it would be useful to use biological reducing agents, or their analogues. These reducing agents are typically thiol-based, the main example being glutathione (GSH), which can be found in the intracellular environment at millimolar concentrations. Azobenzenes have been demonstrated to be reduced by thiols such as DTT in previous studies, and thus given the aqueous environment it was of interest to determine how effectively DTT could be used to trigger depolymerization of this system. In addition to its toxicity, hydrazine is both nucleophilic and basic. One of the main degradation pathways of poly(ethyl glyoxylate), in the absence of specific stimuli which remove the endcap, is the hydrolysis of the pendant esters. The resulting carboxylic acids are then able to catalyze the cleavage of neighboring acetals, causing backbone scission. Thiols are much less able to facilitate this process, and this makes them more appropriate for use with glyoxylates.

The concentration of DTT necessary to reduce the azobenzene and initiate depolymerization was probed using DLS. Various concentrations of DTT were added to 0.8 mg mL\(^{-1}\) of polymer assemblies. The assemblies were monitored by DLS over time, and the decrease in count rate was used as a measure of depolymerization. The observed size of the assemblies remained relatively constant throughout the experiment which is consistent with previous studies in the group, and earlier work with the polycarbamate backbone. While a concentration of 1 mM was too low to generate much depolymerization, 10 mM and 100 mM concentrations were high enough to cause a significant decrease in count rate, ~80% and ~50% respectively, over 25 hours (Figure 4.18).
Figure 4.18 – Optimization of the degradation conditions for triblock 4.8 by the addition of either 1 mM (●), 10 mM (▲), or 100 mM (♦) DTT to a solution of polymer assemblies at a concentration of 0.8 mg mL⁻¹.

The preliminary trials showed that the polymer assemblies were able to reach 50% degradation by 25 hours with 100 mM DTT. Previous polyglyoxylates have been known to fully degrade within a similar time period,² and so the decreased rate of depolymerization was of interest. It is possible that several factors hindered the reaction. The polymer solutions used in the optimization were not purged of oxygen, which may have led to oxidation of the DTT and a lower effective concentration of reducing agent, although this effect is not expected to have a significant impact on higher concentrations such as 100 mM. However, upon the initial reduction of a single polymer chain, many equivalents of ethyl glyoxylate monomer are released. These electron-poor aldehydes are typically hydrated by water, but the thiols of DTT would also be capable of reversibly attacking the aldehydes, again lowering the effective concentration. Finally, as the reduction of the azobenzene trigger was optimized for reaction with hydrazine, the endcaps may not be ideal for reaction with thiols, especially in the trans state, considering the previous work
done with DTT in chapter 2. A highly electron-rich azobenzene with four ortho-methoxy substituents was found to be reduced rapidly by GSH\(^{31}\) in another study, even in the trans state. This reaction would not be favourable with hydrazine as the reducing agent, highlighting the potential difference in mechanism between these two different reductions.

In an effort to maximize the effectiveness of the added DTT, degradation experiments were designed to minimize the aforementioned concerns. Argon was bubbled through the solutions of polymer assemblies for 15 minutes prior to sealing them from the atmosphere, and additional reducing agent was added after 24 hours to compensate for potential DTT degradation. A high initial concentration of 100 mM was used to encourage a more rapid initial reduction. The control polymer 4.9 was subjected to the same conditions for comparison in this study. This polymer was useful in testing for non-specific cleavage reactions that could potentially have been caused by DTT, leading to uncontrolled depolymerization.

To evaluate the rate-limiting step in the depolymerization of the synthesized polyglyoxylates, the polymer assemblies were subjected to 100 mM in degassed solutions and monitored by UV-vis spectroscopy, wherein it was possible to monitor more accurately the reduction of the azobenzene endcaps. During these studies it was found that high concentrations of DTT overlap significantly with the azobenzene absorbance of the polymer endcaps (Figure 4.19). Regardless, it was possible to monitor their reduction over a period of 24 hours. A clear decrease in absorbance above 330 nm is observed, and in the final spectra it is clear that the azo peak has decreased in absorbance.
Figure 4.19 – Degradation of polymer 4.8 assemblies (0.2 mg mL\(^{-1}\)) with DTT (100 mM) monitored by UV-vis spectroscopy over 24 h. Left - The azo peak at ~330 nm is shown to decrease significantly over 8 hours. Right - Overlap with an absorption from the added DTT.

In the initial optimization studies it was noted that the degradation seemed to be roughly linear over the time period monitored. Polyglyoxylates do not typically exhibit a linear degradation over a period of days. Thus, it seemed likely that in this case the reduction is rate limiting. When one reduction does occur, the polymer chain should depolymerize entirely, but with a slow initial reduction the overall effect may be slow to appear. Furthermore, as these triblock copolymers can depolymerize from either end, it is likely that the assembly properties would remain similar when one of two PEO chains is severed. If the reduction is slow compared to depolymerization, it would then be expected that the polymer assemblies would degrade at the rate of reduction. However, in this case it appears that the reduction is complete within 24 hours, while depolymerization takes significantly longer.
The assemblies of polymer 4.8 were then monitored by DLS and shown to have degraded to approximately 50% of the initial count rate over the course of 48 hours when exposed to 100 mM DTT in a degassed solution (Figure 4.20). At these long time points, a plateau had not been reached, indicating that while slow, the degradation had not reached its endpoint. In contrast, the control polymer 4.9 had not shown the same level of degradation, maintaining approximately 85% of the initial count rate over the same time period. This result may suggest some portion of the degradation comes from non-specific degradation reactions.

Figure 4.20 – Decrease in DLS count rate for polymer 4.8 (♦) and control polymer 4.9 (▲) after exposure to 100 mM DTT.

The polymer assemblies were also studied by fluorescence spectroscopy with nile red dye, as in the first portion of this chapter. The dye was encapsulated in a similar fashion, by drying a volume of stock nile red solution, and re-dissolving it into the polymer solution prior to nanoprecipitation, to achieve a loading of 0.4 wt% relative to the polymer mass. The dye encapsulation was carried out for both polymers 4.8 and 4.9. Again, 100 mM of DTT was added to degassed polymer solutions at 0.8 mg mL\(^{-1}\) and the emission of nile red was monitored by fluorescence spectroscopy. In this case the fluorescence emission was
observed at 600 nm rather than 620 nm due to the difference between the polarities of the interiors of the polycarbamate and polyglyoxylate micelles.

Fluorescence spectroscopy of the polymer micelles revealed that the release and fluorescence quenching of the dye was occurring at a similar rate to the decrease in scattered light counts (Figure 4.21). In both samples an initial spike in fluorescence was observed upon the addition of the DTT, up to roughly 130% in both cases within 2 hours. This sharp increase was followed by a roughly linear decrease in fluorescence over the remaining time monitored. At 48 hours, the fluorescence from assemblies of 4.8 had decreased to approximately 50%, similar to the result obtained by DLS. At the same time, the fluorescence from assemblies of polymer 4.9 had decreased to approximately 80%, again similar to the results from DLS.

Figure 4.21 – Fluorescence emission of polymer 4.8 (●) and control polymer 4.9 (○) after treatment with 100 mM DTT.

The azobenzene reduction appears to be mostly complete after a period of 24 hours, and its slower reduction may only be partially responsible for the slow decreases in both
count rate and fluorescence observed in those experiments. It is quite puzzling that these materials appear to degrade in a linear fashion, even after the endcap has been removed. Polyglyoxylates with certain other endcaps under study in our group have been shown to plateau prior to reaching 100% degradation, including the photocleavable polymer 4.9 used in this study as a control. Lacking another explanation for the behaviour of these materials when compared with other data, it seems clear that a new effect is being observed. It is therefore hypothesized that the chain ends of the polyglyoxylate are re-encapped via alkylation of the hemiacetal by the residual hydrazaquinone methide generated by azo reduction (Figure 4.22).

Figure 4.22 – Proposed mechanism for the re-alkylation of the chain ends of poly(ethyl glyoxylate) 4.8 after reduction of the azobenzene trigger.
This species is very electrophilic, and is aided by the electron-withdrawing ester at the opposite end of the azobenzene. Similar azaquinone methides have been known to alkylate biological molecules, and it is known that these species would be in close proximity to the chain ends initially. Furthermore, the endcap has been shown by UV-vis to be reduced in fewer than 24 hours, yet the behaviour of the polymer aggregates do not change after this time. Finally, the cleaved hydrazobenzene is connected to the PEO block via a hydrophobic triazole linkage, such that the cleaved portion is still amphiphilic. This property may predispose the cleaved PEO blocks to remain in the periphery of the assembly such that the aromatic head can reside in the core. Ethers are less easily cleaved by the 1,6-elimination than is the carbonate, which liberates an equivalent of carbon dioxide, but the elimination is possible, which may explain why the polymer appears to degrade very slowly, but is not affected by the addition of excess reducing agent. In addition, this behaviour would not produce a significant difference in the UV-vis spectrum, because the characteristic azo absorbance would still be seen to decrease. However, these proposed mechanisms for the slowed depolymerizations are still under investigation, and require more substantive evidence.

4.4 Conclusions

A reduction-sensitive azobenzene linker was synthesized and incorporated into two self-immolative polymers, a polycarbamate, and a polyglyoxylate. The alkyne ester in the azobenzene endcap allowed a hydrophilic PEO block to be couple to the SIP chain ends using click chemistry. Aqueous nanoassemblies of these materials were then formed, and studied using UV-vis spectroscopy, DLS, and fluorescence spectroscopy, with regard to their reduction-sensitivity and ability to encapsulate hydrophobic cargo. Assemblies of both polycarbamate 4.4 and polyglyoxylate 4.8 were found to degrade under the influence of either hydrazine or DTT, respectively, and were able to encapsulate and release nile red.

The polycarbamate 4.4 was shown to form micellar aggregates approximately 35 nm in diameter as determined by DLS. Upon treatment with hydrazine, these assemblies were found to degrade and release their dye cargo, resulting in the quenching of its fluorescence. By DLS, the micellar structures were found to maintain a similar size during the degradation, and the mean count rate increased after the addition of hydrazine. The
reduction of the azobenzene endcap was shown to be complete after 24 hours using UV-vis spectroscopy, and so this result may indicate that cleavage of the hydrophilic PEO after azo reduction may lead to aggregation. This interaction may increase the hydrophobicity of the core, and slow the depolymerization of the self-immolative block and thus impede the destruction of the assemblies. Cyclic isomerization between trans and cis by UV and visible light irradiation was not found to have a significant effect on the assemblies, based on the observed fluorescence of nile red. The synthesized polymers have a relatively low azobenzene content, and thus their isomerization was not enough to cause a change in the micelle interior. However, the azobenzene isomerization may provide additional functionality either by generating molecular motion, or enhancing the reduction-sensitivity of the linker.

The poly(ethyl glyoxylate)-PEO triblock copolymer 4.8 was also found to be amenable to usage with the azobenzene endcaps, and formed assemblies with an approximate diameter of 90 nm as determined by DLS. A more reactive azobenzene endcapping agent was required for use with the polyglyoxylate, due to the decreased activity of the hemiacetal chain ends relative to the secondary amines of the polycarbamate. A chloroformate derivative was prepared using phosgene gas, and this change provided efficient endcapping of the glyoxylate polymer. The dithiol dithiothreitol was used instead of hydrazine due to its lower reactivity with the pendant esters of PEtG, lower toxicity, and similarity to species found in vivo. The polyglyoxylate backbone depolymerized more slowly than expected from previous work, even at high thiol concentrations. A gradual decrease in both the count rate from DLS and fluorescence of nile red was observed, while the hydrodynamic diameter of the assemblies stayed relatively constant. The relatively linear degradation was possibly caused by the back-reaction of the highly reactive hydrazquinone methide to produce an ether linkage. Such a linkage may be in equilibrium with the freed chain end, and explain the slow depolymerization, but further experiments are required to confirm this hypothesis.
4.5 Experimental

4.5.1 General Materials and Methods

Chemicals were obtained from Alfa Aesar, Sigma Aldrich, and AK Scientific, and were used without further purification unless otherwise noted. Solvents were of reagent grade and obtained from Caledon and Fisher unless stated otherwise. They were used without further purification unless otherwise noted. Triethylamine, pyridine, and CH₂Cl₂ were freshly distilled over CaH₂ before each use. DMSO for preparation of nanoassemblies was distilled-in-glass grade and was obtained from Caledon. Deionized (DI) water was obtained using a Barnstead EASYpure® II system. Dialysis was carried out using regenerated cellulose membranes (Spectra/Por® RC) with molecular weight cut-offs (MWCO) between 1 kg mol⁻¹ and 50 kg mol⁻¹ depending on the sample. Thin layer chromatography (TLC) was run on Macherey-Nagel Polygram SIL G/UV254 plates and SiliaFlash P60 silica (40-60 μm, 230-400 mesh) was used for column chromatography. ¹H NMR and ¹³C NMR spectra were obtained using 600 or 400 MHz Varian Inova spectrometers. NMR chemical shifts are reported in ppm and are calibrated against residual solvent signals (δ H, C) of CDCl₃ (δ 7.26 ppm, 77.2 ppm) or DMSO-d₆ (δ 2.50 ppm, 39.5 ppm), and coupling constants (J) are expressed in Hertz (Hz). FT-IR spectra were recorded on either a Bruker Vector 33 instrument in transmission mode, or a Perkin Elmer Spectrum Two instrument with a diamond universal ATR attachment. High-resolution mass spectrometry (HRMS) was performed on a Finnigan MAT 8400 mass spectrometer using electron impact ionization (EI). Size exclusion chromatography (SEC) in DMF was performed using a Waters 515 HPLC pump, two PLgel mixed-D columns (5 μm pore size, 300 mm×7.5 mm) and their corresponding guard column, and a Wyatt Optilab rEX refractive index detector. DMF with 1% NEt₃ and 10 mM LiBr was used as the eluent. The column temperature was 85 °C, and the flow rate was 1.0 mL min⁻¹, and samples were analyzed at a concentration of 5 mg mL⁻¹. Molar mass was determined relative to poly(methyl methacrylate) (PMMA) standards. Size exclusion chromatography in THF was performed using a Viscotek GPC Max VE2001 solvent module equipped with a Viscotek VE3580 RI detector operating at 30 °C, an Agilent Polypore guard column (50 × 7.5mm) and two Agilent Polypore (300 × 7.5 mm) columns connected in series. Molecular weight determination was carried out
using a calibration based on polystyrene standards. UV-visible spectroscopy was performed using a Varian Cary 300 Bio UV-visible spectrophotometer. Dynamic light scattering was performed using a Malvern Zetasizer Nano ZS equipped with a 633 nm laser, using a scattering angle of 173°. For all studies aimed at determining the time-dependent effects of stimuli on the mean count rate of the samples, the attenuator value was fixed according to the computer-optimized attenuator value for the initial assemblies. The temperature was set to 25 °C, and the samples were equilibrated at this temperature for at least 30 seconds prior to measurements. Each measurement was the average of more than 10 scans of the same sample, and three separate samples were measured at each time point. Fluorescence emission spectroscopy was carried out using a Photon Technology International QM-4 SE spectrofluorometer. The excitation wavelength was 485 nm and the emission spectrum was measured between 520 and 700 nm. The fluorescence was measured at the maximum emission wavelength. Transmission electron microscopy (TEM) was performed using a Phillips CM10 microscope operating at 80 kV with a 40 μm aperture. Samples were prepared at a concentration between 0.01-0.02 mg mL\(^{-1}\) and 10 μL of solution was placed on a 400-mesh copper grid with a formvar coating from Electron Microscopy Sciences and allowed to dry overnight prior to imaging.

4.5.2 Typical Self-Assembly Procedure for SIP-b-PEO amphiphilic copolymer 4.6

The copolymer was prepared as a solution in DMSO (8.0 mg mL\(^{-1}\)), and 0.1 mL was added rapidly to stirring water (0.9 mL, MilliQ). The solution was diluted four-fold by the addition of 3.0 mL of water, and then dialyzed (1 kDa MWCO) in water for at least 16 h, and its volume adjusted with additional water to provide a solution of polymer assemblies at 0.2 mg mL\(^{-1}\).

4.5.3 Typical Self-Assembly Procedure for PEO-PETG-PEO amphiphilic copolymers 4.8 and 4.9

The copolymer was prepared as a solution in DMSO (8.0 mg mL\(^{-1}\)) and 0.3 mL was added rapidly to stirring water (2.7 mL, MilliQ). The solution was then dialyzed in water for at least 16 h, then its volume adjusted with additional water to provide a solution of polymer assemblies at 0.8 mg mL\(^{-1}\).
4.5.4 Encapsulation of nile red in assemblies of polymers 4.6, 4.8, and 4.9

A stock solution of nile red in THF (0.16 mg mL\(^{-1}\)) was prepared. To a clean dry vial was added 20 μL of the nile red solution, and the THF was evaporated under a stream of air. The resulting nile red residue was then re-dissolved in 0.1 mL of 8.0 mg mL\(^{-1}\) polymer solution in DMSO, and this solution was used in the formation of polymer assemblies as described above. In the case of the glyoxylate assemblies the initial amount of nile red solution was 60 μL, and the volume of polymer solution was 0.3 mL. This procedure provided polymer assemblies with 0.4 wt% encapsulated nile red dye relative to the polymer mass, at a polymer concentration of 0.2 mg mL\(^{-1}\).

4.5.5 Procedure for alternating irradiation with UV and visible light

The UV light source consisted of 16 UVA bulbs, (Hitachi FL8BL-B, 8 watts) with emission centered at 365 nm, set at a distance of 10 cm from the sample. The visible light source was a 1 watt white LED bulb set at a distance of 1 cm from the sample. 1.0 mL of the 0.2 mg mL\(^{-1}\) assemblies prepared with encapsulated nile red, as described above, was alternately irradiated in a quartz cuvette with UV light for 10 minutes, followed by visible light for 10 minutes. The process was repeated four times. The sample was analyzed by fluorescence spectroscopy after each irradiation.

4.5.6 Procedure for the degradation of polymer 4.6 assemblies with hydrazine

To 1.0 mL of the 0.2 mg mL\(^{-1}\) 4.6 solution of nanoassemblies with (for fluorescence studies) or without (for DLS studies) encapsulated nile red, was added 10 μL of hydrazine hydrate (~50% N\(_2\)H\(_4\)). The sample was maintained at room temperature (~22 °C) and then analyzed by fluorescence measurement or DLS as described above at various time points. The experiments were performed in triplicate and error bars represent the standard deviations on three measurements.
4.5.7 Procedure for the degradation of polymers 4.8 and 4.9 with DTT

Solution of nanoassemblies of 4.8 or 4.9 (1.0 mL, 0.8 mg mL\(^{-1}\)) were bubbled with argon for 15 minutes to remove oxygen. To solutions with (for fluorescence studies) or without (for DLS studies) encapsulate nile red, was added 15.4 mg of DTT for a final concentration of 100 mM. The samples were sealed to keep out air, maintained at room temperature (~22 °C), and analyzed at various time points by fluorescence spectroscopy or DLS as described above. The experiments were performed in triplicate and error bars represent the standard deviations on three measurements.

4.5.8 Synthesis of (E)-Prop-2-yn-1-yl 4-(((4-nitrophenoxycarbonyl)oxy)methyl)phenyl)diazenyl)benzoate (Compound 4.1)

Compound 3.8 (175 mg, 0.595 mmol) was dissolved in CH\(_2\)Cl\(_2\) (50 mL) and pyridine (0.14 mL, 1.78 mmol). 4-Nitrophenyl chloroformate (125 mg, 0.620 mmol) was added, and the reaction was monitored by TLC (80:20 CH\(_2\)Cl\(_2\)/Hex) to completion. The mixture was then diluted with CH\(_2\)Cl\(_2\) (50 mL), and the organic layer was extracted with 1M HCl (100 mL), and brine (100 mL). The solution was dried over MgSO\(_4\), and the solvent removed in vacuo. The crude material was purified by silica flash chromatography (80:20 CH\(_2\)Cl\(_2\)/Hex) to yield compound 4.1 (198 mg, 73%). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta = 8.29-8.31\) (m, 2H), 8.24-8.25 (m, 2H), 7.98-8.01 (m, 4H), 7.62-7.63 (m, 2H), 7.41-7.42 (m, 2H), 5.40 (s, 2H), 4.98 (d, \(J = 2.4\) Hz, 2H), 2.56 (t, \(J = 2.4\) Hz, 1H). \(^13\)C NMR (150 MHz, CDCl\(_3\)): \(\delta = 165.2, 155.4, 155.2, 152.7, 152.4, 145.5, 137.6, 131.3, 130.9, 129.2, 125.3, 123.5, 122.8, 121.7, 117.6, 75.3, 70.2, 52.8\). FT-IR (ATR, \(\nu_{\max }/\text{cm}^{-1}\)): 3282, 1758 (C=O, carbonate), 1733 (C=O, ester), 1733 (C=O, ester), 1521 (NO\(_2\)), 1355 (NO\(_2\)), 1263, 1220, 1088, 862, 772, 709. HRMS (EI) calc. for [C\(_{24}\)H\(_{17}\)N\(_3\)O\(_7\)]\(^+\) [M\(^+\)]: 459.1067; found: 459.1063.

4.5.9 Synthesis of an Azobenzene-functionalized Polycarbamate (Polymer 4.2)

Protected monomer 2.6b (453 mg, 0.9 mmol) was dissolved in dry CH\(_2\)Cl\(_2\) (3.0 mL) in a flame-dried flask. Trifluoroacetic acid (3.0 mL) was added by syringe and the reaction was stirred for 1.5 hrs. The solvents were removed under a stream of air, and additional CH\(_2\)Cl\(_2\)
(2 x 3.0 mL) was added and removed in the same fashion. The monomer 2.6 was then dried completely under high vacuum and dissolved for immediate use in dry THF (5.0 mL). Solutions of endcap 4.1 (41 mg, 0.09 mmol) in THF (2.0 mL), and DMAP (5.5 mg, 0.045 mmol) in THF (0.5 mL) were prepared separately. The endcap in THF was added to the monomer, and the solution stirred, and chilled to 0 °C in an ice bath. Freshly distilled NEt3 (1.44 mL, 11.3 mmol) was added, followed by the solution of DMAP. The mixture was stirred overnight during which time it was allowed to warm to room temperature. The product solution was diluted with CH2Cl2 (50 mL) and washed with 1M HCl (100 mL), then saturated sodium carbonate (3 x 100 mL), and finally with saturated NaCl (100 mL). The organic layer was dried over MgSO4 and the solvent removed in vacuo. The crude product was re-dissolved in DMF (3 mL) and dialysed in a membrane with a MWCO of 1 kg mol-1 against DMF (500 mL) for 16 h, then against water (1 L) for 8 h. The aqueous polymer suspension was then lyophilized to afford the polymer 4.2 (105 mg, 39%) as a light orange solid. 1H NMR (600 MHz, CDCl3): δ = 8.27-8.28 (m, 2H), 8.23-8.24 (m, 2H), 7.88-7.97 (m, 4H), 7.49-7.55 (m, 2H), 7.42-7.45 (m, 2H), 7.32-7.39 (m, 19H), 7.05-7.14 (m, 19H), 5.28 (br s, 2H), 5.20-5.25 (m, 2H), 5.07-5.13 (m, 19H), 4.97 (d, J = 2.4 Hz, 2H), 3.45-3.61 (m, 42H), 2.92-3.14 (m, 61H), 2.56 (t, J = 2.4 Hz, 1H). SEC (DMF, rel. PMMA): M_n = 2 900 g mol-1 M_w = 4 200 g mol-1, D = 1.45.

4.5.10 Synthesis of an azobenzene-containing amphiphilic diblock copolymer (Polymer 4.4)

Polycarbamate 4.2 (20 mg, 0.007 mmol, 1 equiv.), PEO 4.3 (14 mg, 0.007 mmol, 1 equiv.), and sodium ascorbate (2.8 mg, 0.014 mmol, 2 equiv.) were dissolved in DMSO (1.97 mL) and the solution degassed and backfilled with argon to remove oxygen. A solution of CuSO4 (4.6 mg, 0.028 mmol, 4 equiv.) in DMSO (30 μL) was prepared and similarly degassed. The polymer solution was warmed to 30 °C and stirred, then the CuSO4 solution was added, and the mixture stirred for 16 h. The resulting solution was then diluted with DI water (2 mL) and dialyzed against water (1 L) in a membrane with a 1 kg mol-1 MWCO for 16 h. The solution was lyophilized to yield the product polymer 4.4 (27 mg, 79%). 1H NMR (600 MHz, CDCl3): δ = 8.19-8.28 (m, 2.75H), 7.88-7.94 (m, 5.7H), 7.49-7.51 (m, 2.52H), 7.29-7.38 (m, 23H), 7.02-7.09 (m, 22H), 5.51 (s, 2.00H), 5.20-5.27 (m, 3.24H),
5.09-5.12 (m, 23H), 4.55-4.58 (m, 2.16H), 3.89-3.91 (m, 2.01H), 3.62-3.67 (m, 206H), 3.46-3.57 (m, 48H), 3.39 (s, 3.4H), 2.92-3.12 (m, 75H).

SEC (DMF, rel. PMMA): $M_n = 4600$ g mol$^{-1}$, $M_W = 7700$ g mol$^{-1}$, $D = 1.68$.

4.5.11 Synthesis of $(E)$-prop-2-yn-1-yl 4-((4-(((chlorocarbonyl)oxy)methyl)phenyl)diazenyl)benzoate (Compound 4.5)

Caution: Phosgene is a highly toxic gas and must be handled with great care; refer to the MSDS before using. Compound 3.8 (300 mg, 1.02 mmol, 1.0 equiv.) was dissolved in THF (8 mL). The resulting solution was then added dropwise into a phosgene solution (15 wt% in toluene, 2.0 mL, 2.74 mmol, 2.7 equiv.) under an argon atmosphere at room temperature and was stirred for 16 hours. The residual phosgene and solvent was then removed by high vacuum to yield chloroformate 4.5 (354 mg, 95%) as an orange solid. Phosgene collected in the liquid nitrogen-cooled trap was then quenched with methanol (10 mL) and saturated sodium hydroxide solution (10 mL). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 8.24$ (d, $J = 8.8$ Hz, 2H), 7.99 (d, $J = 4.7$ Hz, 1H), 7.98 (d, $J = 4.7$ Hz, 2H), 7.57 (d, $J = 8.8$ Hz, 2H), 5.40 (s, 2H), 4.98 (d, $J = 2.4$ Hz, 2H), 2.56 (t, $J = 2.4$ Hz, 1H). $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta = 165.1, 155.2, 152.8, 150.7, 136.7, 131.3, 130.9, 129.5, 123.5, 122.8, 77.5, 75.2, 72.5, 52.7$. FT-IR (ATR, $\nu_{\text{max}}$/cm$^{-1}$): 3269, 1782 (C=O, chloroformate), 1722 (C=O, ester), 1361, 1269, 1136, 1095, 803, 694. HRMS (EI) calc. for $[C_{18}H_{13}ClIN_2O_4]^+$ [M$^+$]: 356.0564; found: 356.0564.

4.5.12 Azobenzene-endcapped poly(ethyl glyoxylate) (Polymer 4.6)

The poly(ethyl glyoxylate) homopolymer was prepared in a manner similar to that previously reported by our group.$^2$ Ethyl glyoxylate (EtG) in toluene solution (20 mL) was fractionally distilled under vacuum (55 °C, 125 mbar) over P$_2$O$_5$ to remove toluene and trace water in the first, discarded fraction. The residue was then distilled twice successively over P$_2$O$_5$ at atmospheric pressure under argon at 130 °C to obtain the highly pure monomer. The resulting pale yellow liquid (5.0 mL, 50 mmol, 1.0 equiv.) was dissolved in CH$_2$Cl$_2$ (5.0 mL) and NEt$_3$ (3.5 μL, 25 μmol, 0.0005 equiv.). The solution was stirred for 1 h, at −20 °C. Compound 4.5 (0.26 g, 730 μmol, 0.014 equiv.) and NEt$_3$ (100 μL, 730 μmol, 0.014 equiv.) were added at 0 °C to endcap the polymer. The solution was then
warmed to room temperature and stirred for 24 h, then stirred for 16 h at 40 °C. Purification was achieved by precipitation of the crude reaction mixture into methanol. After decanting the excess methanol, the residue was dried *in vacuo* for 48 h to provide polymer 4.6 as a white, sticky material (3.2 g, 70%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 8.23$ (m, 4H), 7.97 (m, 8H), 7.56 (m, 4H), 5.46-5.78 (m, 8H2), 5.31 (m, 4H), 4.98 (m, 4H), 4.10-4.33 (m, 1228H), 1.21-1.44 (m, 1891H). FT-IR (KBr, thin film, $\nu_{\text{max}}$/cm$^{-1}$): 2984, 2942, 1748, 1467, 1447, 1376, 1297, 1214, 1137, 1015, 957, 855, 785, 701. SEC (THF): $M_n = 59$ kg mol$^{-1}$, $M_w = 91$ kg mol$^{-1}$, $D = 1.53$. $T_g = -2 ^\circ$C.

4.5.13 Synthesis of a PEO-PETG-PEO triblock copolymer (Polymer 4.8)

Polymer 4.6 (500 mg, 8.4 µmol, 1 equiv.) and PEO 4.7$^2$ (167 mg, 33 µmol, 4 equiv.) were dissolved into DMF (5 mL). After removing the air and refilling with argon, CuSO$_4$ (10 mg, 62 µmol, 8 equiv.) and sodium ascorbate (10 mg, 50 µmol, 6 equiv.) were added into the solution, and the mixture was stirred at 40 °C for 16 h. It was then transferred into a regenerated cellulose membrane (50 kDa MWCO) and dialyzed against deionized water for 48 h (300 mL, 6 solvent changes). The dialyzed material was then centrifuged and the water decanted, to remove excess PEO. The centrifugation process was repeated several times. The polymer pellet was then dried by desiccation to afford polymer 4.8 as a spongey orange solid (430 mg, 79%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta = 8.20$-8.22 (br, 2.06H), 7.94-7.95 (br, 4.54H), 7.55-7.57 (br, 2.00H), 5.52-5.70 (br, 324H), 5.29-5.30 (br, 2.05H), 4.57-4.61 (br, 1.25H), 4.13-4.28 (br, 666H), 3.91-3.92 (br, 1.56H), 3.53-3.77 (br, 467H), 3.39 (s, 2.28H), 1.18-1.40 (br, 1002H). SEC (THF): $M_n = 47$ kg mol$^{-1}$, $M_w = 72$ kg mol$^{-1}$, $D = 1.54$. $T_g = -5 ^\circ$C.

4.6 References


Chapter 5

5 Conclusions and Future Directions

The work presented in this thesis represents the first instance of the use of multifunctional azobenzene units as reduction- and photo-sensitive components of self-immolative polymers. Azobenzene and azobenzene-containing polymers had not previously been studied in the context of self-immolative materials, and furthermore, the reduction-sensitivity of these well-known dye molecules had not been explored in the context of a non-synthetic application prior to this work.

In the study presented in chapter 2, it was demonstrated conclusively that the reduction of an electron-poor azobenzene by either hydrazine or dithiothreitol was possible, and that the produced hydrazobenzene was capable of a 1,6-elimination reaction. This process was similar to that of anilines commonly seen in self-immolative chemistry, and it provided the basis for the use of these azobenzenes as endcaps in self-immolative polymers. These reduction-sensitive azobenzenes were then incorporated into two different self-immolative polymer backbones based on carbamate linkages, and that degraded either by alternating decarboxylation, 1,6-elimination, and cyclization reactions, or alternating decarboxylation and 1,6-elimination reactions. In the synthesis of these different polymers it was shown that the azobenzene endcap could be incorporated into the backbone as a nucleophile, or prepared as an activated carbonate to be incorporated as an electrophile. This dual reactivity expands the number of potential polymers into which the endcap can be incorporated. Several key features of the azobenzene endcap were exploited in the depolymerization of the polymers. A visible colour change indicated that reduction was complete, and the azobenzene endcapped polymers exhibited a substantial increase in the rate of degradation relative to control polymers. The capability of these azobenzene triggers to undergo trans-cis isomerization in response to UV light was confirmed in both small molecule and polymeric examples, which demonstrated that the polymer backbone did not hinder this ability or lead to premature degradation, thus providing a further avenue for investigation.
In the next study, presented in chapter 3, the applicability of reduction-sensitive azobenzenes was expanded significantly. Firstly, a library of electron-poor azobenzenes was prepared in an effort to allow for the tuning of the rate of reduction. A range of electron-withdrawing substituents was investigated, including esters, halogens, nitriles, and trifluoromethyl groups. A spectrum of reaction rates was observed, and it was found that ortho-halogens, specifically the 2-Cl derivative, were the most rapidly reduced. This result was unexpected, because the halogen-containing compounds were not highly electron-withdrawing, and thus were not predicted to react rapidly. A wide range of factors influencing the reduction were investigated, including electronegativity, atomic radius, and Hammett parameters, but in the end it was concluded that halogen anisotropy was responsible for a preorganization effect that favoured reduction by hydrazine. In an effort to expand the usefulness of azobenzene triggers further, a chain-shattering polymer based on a poly(ester amide) backbone was designed. The polymer was prepared as an amphiphilic graft copolymer with poly(ethylene oxide), and formed micellar structures in aqueous solution. Due to the large number of azobenzene units incorporated, it was critical that they be reduced as rapidly as possible, because a single reduction resulted in a single chain-breakage. The fastest-reducing azobenzene was used in the polymer synthesis. The micellar structures were capable of the encapsulation of the hydrophobic cargo nile red. The degradation of these assemblies was studied using DLS and fluorescence spectroscopy, and it was found that they responded to UV light, reduction, or a combination of the two stimuli, in unique ways. A combination of both irradiation and reduction resulted in the most rapid degradation, while cyclical isomerization of the azobenzene pendants by UV and visible irradiation was found to be a fully reversible process, and changed significantly the polarity of the micelle core.

The final study presented in chapter 4 represents a culmination of the ideas and concepts produced in the first two studies. In the first study, linear self-immolative polymers with azobenzene endcaps were studied, but in organic solution only. In order to move these self-immolative polymers into aqueous media it was necessary to synthesize a block copolymer capable of self-assembly. In the second study, an amphiphilic polymer with multiple azobenzenes was prepared, but as a chain-shattering polymer, the backbone was not fully degradable from end to end after a single stimulus, and therefore lacked the
multiplicative effect common to linear self-immolative polymers. Therefore two novel systems containing azobenzene linkers at the junctions of the blocks of amphiphilic block copolymers were prepared. An alkyne-functionalized azobenzene prepared in chapter 3 was used with the self-immolative polymer backbone of chapter 2, and then functionalized with a hydrophilic poly(ethylene oxide) block using click chemistry. This diblock copolymer was found to form micellar structures in aqueous media, and demonstrated reduction-sensitivity when treated with hydrazine. Similar to chapter 3, it was demonstrated that this material was capable of the encapsulation and release of hydrophobic cargo. As a result of the incorporation of a single azobenzene per polymer chain, the responsiveness of this polymer to UV irradiation was not strongly expressed. The azobenzene linker was next applied to a poly(ethylene oxide)-poly(ethyl glyoxylate)-poly(ethylene oxide) triblock copolymer. These materials were also capable of forming aqueous assemblies and encapsulating hydrophobic cargo, but represent a significant step towards the preparation of self-immolative polymer assemblies that produce non-harmful byproducts upon depolymerization. In an effort to emulate cellular conditions, these assemblies were treated with dithiothreitol instead of hydrazine, and were shown to depolymerize under these reducing conditions. Their degradation was slower and more linear than previous studies on the same material would predict. This behaviour was attributed to the propensity for the reduced hydrazobenzene to be reattached reversible via alkylation to the depolymerizing polyglyoxylate, although further experiments are required to confirm this behaviour.

In any future studies on the topic of azobenzenes in degradable polymers, it would be of interest to better understand the systems reported in this thesis. In degradation studies of polymer assemblies conducted in chapters 3 and 4, it was found that the size and count rate of the micelles stayed relatively constant, despite clear evidence of azobenzene reduction. It is thus of interest to determine what could cause this behaviour in future studies. It was hypothesized that the behaviour was caused by some combination of micelle aggregation. One possible method of interrogation would be to encapsulate in one set of micelles a fluorescent molecule, and in another set a quenching agent. In a mixture of the two, if aggregation of the micelles were to occur upon degradation, the cores of multiple micelles should coalesce, and the fluorescence of the solution may be shown to decrease.
The ratio of hydrophilic and hydrophobic polymer blocks is also of interest in the tuning of polymer assemblies. The poly(ester amide), polycarbamate, and polyglyoxylate used in this study could all be synthesized using different chain lengths of PEO. A small change in the hydrophobic fraction could cause assemblies to form vesicular structures, which would be useful for the loading of both hydrophilic and hydrophobic cargoes.

Chapter 4 involved the incorporation of an alkyne-containing azobenzene, and poly(ethylene oxide) was used as a hydrophilic block. However, the hydrophilic block could potentially be stimuli-responsive as well. The polymer poly(2-dimethylaminoethyl methacrylate), for example, has been shown to be responsive to CO₂, pH, and temperature. Conjugation of this polymer to the azobenzene-containing polymers synthesized here could provide a polymer that responds to five different stimuli: light, reduction, CO₂, pH, and temperature. Many multi-responsive polymers have been prepared, but very few have incorporated self-immolative polymers. The current system has the potential to allow for a large number of stimulus-responses to be packed into a single material that can undergo triggered depolymerization.

It should also be possible through careful design to generate a hydrophilic self-immolative polymer block, such that its degradation could take place in an aqueous environment, and not limited by its chain-aggregated state in the micelle core. The combination of two self-immolative blocks of different solvophilicity would then lead to a fully self-immolative block copolymer. Another strategy to increase the rate of degradation within the core is the preparation of vesicular assemblies by decreasing the length of the hydrophilic block. The presence of an aqueous environment in the vesicle core would result in an increase in degradation rate following endcap removal.

The number of possible azobenzene compounds is essentially limitless, and thus it may be advantageous to further explore this class of compounds for application-specific species. For example, the studies contained in this thesis used either hydrazine or dithiothreitol as reducing agents at relatively high concentrations, thus the design of an azobenzene with increased sensitivity to the reductive stimuli found in vivo would be advantageous. Furthermore, the use of the enzyme azoreductase has not yet been explored
with self-immolative materials, and may be an interesting avenue for colon-specific degradation. The library of electron-poor azobenzenes synthesized in chapter 3 was optimized specifically for reduction by hydrazine, so tuning towards reduction by more biologically relevant stimuli is of interest for the next generation of azobenzene triggers. Similarly, azobenzenes that are water-soluble, or photoswitchable with visible light, could be investigated more thoroughly for biological applications.

In addition to their photosensitivity, brightly coloured appearance, and reduction sensitivity, azobenzenes have been well-known to participate in supramolecular chemistry. Their interaction with macromolecular structures such as cyclodextrin or curcubiturils has been studied extensively, and presents another mode of stimulus-responsiveness to be exploited. A self-immolative block copolymer might then be synthesized without covalently binding the blocks together, instead relying on the host-guest behaviour of azobenzene for this linkage. This interaction has also been shown to be reversible and responsive to the trans-cis isomerization of the azobenzene guest. In the case of the chain-shattering polymer discussed in chapter 3, this concept would allow for multiple interactions with each polymer chain. In the case of a linear self-immolative polymer as described in chapters 2 and 4, this process could potentially be used to generate supramolecular block copolymers.

As there are many possible azobenzenes, it is possible to imagine an endcap capable of functionalization by a number of reactions. For example, an allyl ester in place of the alkyne ester described in chapters 3 and 4 could be useful for either thiol-ene chemistry, or an alkene metathesis reaction. There are very few limitations on the potential azobenzenes that could be prepared and used in a reduction-sensitive capacity.

The fields of self-immolative polymers and azobenzenes as stimulus responsive materials are ever-growing, and there is still a great deal of room for expansion beyond this work.
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Appendix 2: NMR spectra for compounds 2.2-2.5, polymers 2.7, 2.10, and 2.11.
Figure A2.1 – $^1$H NMR spectrum of compound 2.2 (400 MHz, CDCl$_3$).

Figure A2.2 – $^1$H NMR spectrum of compound 2.3 (600 MHz, CDCl$_3$).
Figure A2.3 – $^1$H NMR spectrum of compound 2.4 (400 MHz, DMSO-$d_6$).

Figure A2.4 – $^1$H NMR spectrum of compound 2.5 (400 MHz, CDCl$_3$).
Figure A2.5 – $^1$H NMR spectrum of polymer 2.7 (400 MHz, CDCl$_3$). The polymer has an endcap incorporation >70% as determined from the final degradation point by determining the total percentage of cyclic oligomers remaining after depolymerization (e.g. Figure 2.12).
Figure A2.6 – $^1$H NMR spectrum of polymer 2.10 (400 MHz, DMSO-$d_6$).

Figure A2.7 – $^1$H NMR spectrum of polymer 2.11 (600 MHz, DMSO-$d_6$).
Figure A2.8 – $^{13}$C NMR spectrum of compound 2.2 (100 MHz, CDCl$_3$).

Figure A2.9 – $^{13}$C NMR spectrum of compound 2.3 (100 MHz, CDCl$_3$).
Figure A2.10 – $^{13}$C NMR spectrum of compound 2.4 (100 MHz, DMSO-$d_6$).

Figure A2.11 – $^{13}$C NMR spectrum of compound 2.5 (100 MHz, CDCl$_3$).
Figure A3.1 – $^1$H NMR spectrum of compound 3.2 (400 MHz, CDCl$_3$).

Figure A3.2 – $^1$H NMR spectrum of compound 3.3 (600 MHz, DMSO-$d_6$). Small peaks correspond to the cis isomer.
Figure A3.3 – $^1$H NMR spectrum of compound 3.4 (400 MHz, CDCl$_3$). Small peaks correspond to the cis isomer.

Figure A3.4 – $^1$H NMR spectrum of compound 3.5 (600 MHz, DMSO-$d_6$).
Figure A3.5 – $^1$H NMR spectrum of compound 3.6 (600 MHz, CDCl$_3$).

Figure A3.6 – $^1$H NMR spectrum of compound 3.7 (600 MHz, DMSO-$d_6$). Small peaks correspond to the cis isomer.
Figure A3.7 – $^1$H NMR spectrum of compound 3.8 (600 MHz, DMSO-$d_6$). Small peaks correspond to the cis isomer.

Figure A3.8 – $^1$H NMR spectrum of compound 3.9 (400 MHz, CDCl$_3$). Small peaks correspond to the cis isomer.
Figure A3.9 – $^1$H NMR spectrum of compound 3.10 (400 MHz, CDCl$_3$). Small peaks correspond to the cis isomer.

Figure A3.10 – $^1$H NMR spectrum of compound 3.11 (600 MHz, DMSO-$d_6$). Small peaks correspond to the cis isomer.
Figure A3.11 – $^1$H NMR spectrum of compound 3.12 (600 MHz, CDCl$_3$).

Figure A3.12 – $^1$H NMR spectrum of compound 3.14 (600 MHz, CDCl$_3$).
Figure A3.13 – $^1$H NMR spectrum of compound 3.15 (600 MHz, DMSO-$d_6$). Small peaks correspond to the cis isomer.

Figure A3.14 – $^1$H NMR spectrum of compound 3.16 (600 MHz, DMSO-$d_6$, 50 °C). Elevated temperature was used to reduce the signal intensity of the cis isomer and identify peaks.
Figure A3.15 – $^1$H NMR spectrum of compound 3.17 (600 MHz, DMSO-$d_6$). Small peaks correspond to the cis isomer.
Figure A3.16 – $^1$H NMR spectrum of polymer 3.20 (600 MHz, DMSO-$d_6$). Note a mixture of trans and cis isomers was observed in this NMR solvent. An approximate 9:1 ratio of monomers 3.17/3.18 as per the feed ratios indicated in Figure 3.8 is supported by the relative integrations of the peak at 3.8 ppm corresponding to the $\alpha$-hydrogens on the glycine of monomer 3.17 (labeled a) and the peak at 4.0 corresponding to $\text{-CH}_2\text{-CH}_2\text{O-C(O)-}$ on monomer 3.18 (labeled b).
Figure A3.17 – $^1$H NMR spectrum of polymer 3.21 (600 MHz, DMSO-$d_6$). Note a mixture of trans and cis isomers was observed in this NMR solvent. Approximately quantitative coupling of PEO-NH$_2$ to the carboxylic acids is supported by the relative integrations of the peak at 3.5 ppm corresponding to PEO (labeled b) and the peak at 3.9 ppm corresponding to the $\alpha$-hydrogens on the glycine of monomer 3.17 (labeled a).
Figure A3.18 – $^1$H NMR spectrum of polymer 3.22 (600 MHz, DMSO-$d_6$). This polymer was previously reported (see Figure 3.9) and this spectrum is included for comparison with 3.23.
Figure A3.19 – $^1$H NMR spectrum of polymer 3.23 (600 MHz, DMSO-$d_6$).

Approximately quantitative coupling of PEO-NH$_2$ to the carboxylic acids is supported by the relative integrations of the peak at 3.5 ppm corresponding to PEO (labeled b) and the peak at 2.0 ppm corresponding to the $\alpha$-hydrogens of the sebacic acid component (labeled a).
Figure A3.20 – $^{13}$C NMR spectrum of compound 3.2 (100 MHz, CDCl$_3$).

Figure A3.21 – $^{13}$C NMR spectrum of compound 3.3 (150 MHz, DMSO-$d_6$). Peaks corresponding to the cis isomer are observed.
Figure A3.22 – $^{13}$C NMR spectrum of compound 3.4 (100 MHz, CDCl$_3$).

Figure A3.23 – $^{13}$C NMR spectrum of compound 3.5 (150 MHz, DMSO-$d_6$). Small peaks correspond to the $cis$ isomer.
Figure A3.24 – $^{13}$C NMR spectrum of compound 3.6 (150 MHz, DMSO-$d_6$).

Figure A3.25 – $^{13}$C NMR spectrum of compound 3.7 (150 MHz, DMSO-$d_6$). Small peaks correspond to the cis isomer.
Figure A3.26 – $^{13}$C NMR spectrum of compound 3.8 (150 MHz, DMSO-$d_6$).

Figure A3.27 – $^{13}$C NMR spectrum of compound 3.9 (100 MHz, CDCl$_3$).
Figure A3.28 – $^{13}$C NMR spectrum of compound 3.10 (100 MHz, CDCl$_3$).

Figure A3.29 – $^{13}$C NMR spectrum of compound 3.11 (150 MHz, DMSO-$d_6$). Small peaks correspond to the *cis* isomer.
Figure A3.30 – $^{13}$C NMR spectrum of compound 3.12 (100 MHz, CDCl$_3$). Small peaks belong to the *cis* isomer.

Figure A3.31 – $^{13}$C NMR spectrum of compound 3.14 (100 MHz, CDCl$_3$).
Figure A3.32 – $^{13}$C NMR spectrum of compound 3.15 (150 MHz, DMSO-$d_6$). Small peaks belong to the cis isomer.

Figure A3.33 – $^{13}$C NMR spectrum of compound 3.16 (150 MHz, DMSO-$d_6$, 50 °C).
Figure A3.34 – SEC (DMF) traces of polymers 3.20 and 3.21. The peak at 18.3 min corresponds to the eluent of the system.

Figure A3.35 – SEC (DMF) traces of polymers 3.22 and 3.23. The peak at 18.3 min corresponds to the eluent of the system.
Figure A3.36 – Intensity distribution of polymer 3.21 as determined by DLS after constant UV irradiation.

Figure A3.37 – Intensity distribution of polymer 3.21 assemblies as determined by DLS after treatment with hydrazine.
Appendix 4: NMR spectra for compounds 4.1 and 4.5, and polymers 4.2, 4.4, 4.6, and 4.8.
Figure A4.1 – $^1$H NMR spectrum of compound 4.1 (600 MHz, CDCl$_3$). Small peaks correspond to a small percentage of cis isomer.

Figure A4.2 – $^1$H NMR spectrum of polymer 4.2 (600 MHz, CDCl$_3$).
Figure A4.3 – $^1$H NMR spectrum of polymer 4.4 (600 MHz, CDCl$_3$).

Figure A4.4 – $^1$H NMR spectrum of compound 4.5 (600 MHz, CDCl$_3$).
Figure A4.5 – $^1$H NMR spectrum of polymer 4.6 (600 MHz, CDCl$_3$).
Figure A4.6 – $^1$H NMR spectrum of polymer 4.8 (600 MHz, CDCl$_3$).
Figure A4.7 – $^{13}$C NMR spectrum of compound 4.1 (600 MHz, CDCl$_3$).

Figure A4.8 – $^{13}$C NMR spectrum of compound 4.5 (600 MHz, CDCl$_3$).
Curriculum Vitae for Andrew D. Wong

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NSERC Graduate Scholarship CGS-D (Western) 05/2012 – 04/2015
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Society of Chemical Industry Merit Award (Queen’s) 2010
Highest-performing undergraduate in Engineering Chemistry

Dean’s Scholar (Queen’s) 2007 – 2010
Average >80%

M. Sullivan and Son Scholarship (Queen’s) 2010
Best Thesis in Engineering Chemistry

Lawrence M. Hunter Memorial Award (Queen’s) 2009
Humanitarian Efforts

NSERC USRA (Queen’s) 2009
Butyl Rubber Modification

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2009 – 2010

Queen’s University  
Summer Research Assistant (NSERC USRA)  
2009

Queen’s University/Kingston Process Metallurgy  
Summer Research Assistant (NSERC USRA)  
2008

TEACHING EXPERIENCE

Undergraduate Student Mentor  
for Alexander Prinzen (CHEM 4490)  
09/2014 – 09/2015

Engineering Design Studio Assistant  
(ES 1050, Dr. Shahzad Barghi)  
Winter 2014

Laboratory Teaching Assistant  
(CBE 2207b, Dr. Jose Herrera)  
Winter 2013

Laboratory Teaching Assistant  
(CBE 2206, Dr. Elizabeth Gillies)  
Fall 2012

Undergraduate Student Mentor  
for Thalia Wells (NSERC USRA)  
Summer 2012

Laboratory Teaching Assistant  
(CBE 2217a, Dr. Jose Herrera)  
Winter 2012

Laboratory Teaching Assistant  
(CBE 2206, Dr. Paul Charpentier)  
Fall 2011

Laboratory Teaching Assistant  
(CBE 2207b, Dr. Jose Herrera)  
Winter 2011


**PUBLICATIONS**


Hemery, Gauvin; Garanger, Elisabeth; Lecommandoux, Sebastien; **Wong, Andrew;** Gillies, Elizabeth; Pedrono, Boris; Bayle, Thomas; Jacob, David; Sandre, Olivier. (2015). Thermosensitive polymer-grafted iron oxide nanoparticles studied by in situ dynamic light backscattering under magnetic hyperthermia. *Journal of Physics D: Applied Physics, 48* (49), 494001.


**CONFERENCE PRESENTATIONS**

**Wong, Andrew D.;** Prinzen, Alexander L.; Gillies, Elizabeth R. *Azobenzene as a Multifunctional End-cap and Linker for Self-Immolative Polymers and their Assemblies*, 98th Canadian Society of Chemistry Conference and Exhibition, Ottawa, Ontario (2015), *Poster Presentation*

**Wong, Andrew D.;** Güngör, Thomas, M.; Gillies, Elizabeth R. *Azobenzene as a Multiresponsive Endcap for Self-Immolative Polymers* 36th High Polymer Forum, Gananoque, Ontario (2014), *Oral Presentation*

**Wong, Andrew D.;** Gillies, Elizabeth R. *Cascade Degradable Polycations: Potential Use in Gene Delivery*, 2nd Annual Distinguished Lecturer and Research Day, Center for Advanced Materials and Biomaterials Research (2012), *Poster Presentation*

