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Single chain technology: Toward the controlled synthesis of polymer nanostructures

Christopher Kenneth Lyon

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SINGLE CHAIN TECHNOLOGY: TOWARD THE CONTROLLED SYNTHESIS OF POLYMER НANOSTRUCTURES

BY

CHRISTOPHER LYON

DISSERTATION

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry

May, 2016
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On December 21, 2015

Original approval signatures are on file with the University of New Hampshire Graduate School.
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ABBREVIATIONS

SCNP – Single chain nanoparticles
ADMET polymerization – Acyclic diene metathesis polymerization
RAFT polymerization – Reversible addition fragmentation chain transfer polymerization
SET-LRP – Single-electron transfer living radical polymerization
NMR – Nuclear magnetic resonance
FMOC – Fluorenylmethyloxycarbonyl
AIBN – Azobisisobutyrylnitrile
DMAEMA – Dimethylaminoethyl methacrylate
TFA – Trifluoroacetic acid
UPy – Ureidopyrimidinone
BTA – Benzetrifluorooxycarboline
ROMP – Ring opening metathesis polymerization
SEC – Size exclusion chromatography
MALS – Multi-angle light scattering
BCB – Benzocyclobutene
DLS – Dynamic light scattering
DOSY – Diffusion ordered NMR spectroscopy
AFM – Atomic force microscopy
TEM – Transmission electron microscopy
SANS – Small angle neutron scattering
SAXS – Small angle X-ray scattering
MD – Molecular dynamics
TOF – Turnover frequency
PEG – Poly(ethylene glycol)
SDP – Diphenylphosphino styrene
CRP – Controlled radical polymerization
AuNP – Gold nanoparticle
MRI – Magnetic resonance imaging
MA – Maleic anhydride
ABBREVIATIONS (CONT.)

NEM – N-ethyl maleimide
NPM – N-pyrene maleimide
ICAR ATRP – Initiators for continuous activator regeneration atom transfer radical polymerization
RI – Refractive index
MHS – Mark-Houwink-Sakurada
THF – Tetrahydrofuran
DP_n – Number average degree of polymerization
DA – Diels Alder
DCC – Dicyclohexylcarbodiimide
PEGMA – Poly(ethylene glycol) methacrylate
DMF – N,N-Dimethylformamide
DMSO – Dimethylsulfoxide
TMS – Trimethylsilyl, trimethylsilane
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ABSTRACT

SINGLE CHAIN TECHNOLOGY: TOWARD THE CONTROLLED SYNTHESIS OF POLYMER NANOSTRUCTURES

by

Christopher Lyon

University of New Hampshire, May, 2016

A technique for fabricating advanced polymer nanostructures enjoying recent popularity is the collapse or folding of single polymer chains in highly dilute solution mediated by intramolecular cross-linking. We term the resultant structures single-chain nanoparticles (SCNP). This technique has proven particularly valuable in the synthesis of nanomaterials on the order of 5 – 20 nm. Many different types of covalent and non-covalent chemistries have been used to this end.

This dissertation investigates the use of so-called single-chain technology to synthesize nanoparticles using modular techniques that allow for easy incorporation of functionality or special structural or characteristic features. Specifically, the synthesis of linear polymers functionalized with pendant monomer units and the subsequent intramolecular polymerization of these monomer units is discussed.

In chapter 2, the synthesis of SCNP using alternating radical polymerization is described. Polymers functionalized with pendant styrene and stilbene groups are synthesized via a modular post-polymerization Wittig reaction. These polymers were exposed to radical initiators in the presence (and absence) of maleic anhydride and other electron deficient monomers in order to form intramolecular cross-links. Chapter 3 discusses templated acyclic diene metathesis (ADMET)
polymerization using single-chain technology, starting with the controlled ring-opening polymerization of a glycidyl ether functionalized with an ADMET monomer. This polymer was then exposed to Grubbs’ catalyst to polymerize the ADMET monomer units. The ADMET polymer was hydrolytically cleaved from the template and separated. Upon characterization, it was found that the daughter ADMET polymer had a similar degree of polymerization, but did not retain the low dispersity of the template. Chapter 4 details the synthesis of aldehyde- and diol-functionalized polymers toward the synthesis of SCNP containing dynamic, acid-degradable acetal cross-links. SCNP fabrication with these materials is beyond the scope of this dissertation.
CHAPTER 1 INTRODUCTION

Precisely defined linear polymers folded into functional nanostructures, capable of completing complex tasks, are ubiquitous in nature. In this light, an obvious yet unmet research goal becomes apparent: exploiting our understanding of biomolecules to mimic this behavior in the laboratory using recent advances in controlled polymerization chemistry and the well-known theories of modern polymer physics. Advances in this technology will have applications in catalysis,\textsuperscript{1-5} sensors,\textsuperscript{6} nanoreactors,\textsuperscript{7} nanomedicine,\textsuperscript{8-11} etc.

The utility of biomacromolecules is a result of their well-defined tertiary structure: a specific three-dimensional shape with precise placement of functionality on the surface of the structure or its interior. Tertiary structure is permitted by a pristine primary structure, a quality that is currently inaccessible in synthetic polymers. Recent advances in contemporary controlled polymerization chemistry allow the synthesis of multiblock polymers with narrow molecular weight distributions\textsuperscript{12} or materials with controlled monomer sequences\textsuperscript{13,14} by step-growth and chain-growth methods. These techniques are an enormous step forward, but still result in microstructural heterogeneities or broad molecular weight distributions. In analogy to the globular three-dimensional structures of folded biomolecules, dendrimers have been considered as a means to imitate this behavior owing to their monodispersity and highly regular structures. However, their syntheses are traditionally arduous and often result in prohibitively low yields coupled with the inability to precisely control the placement of chemistry at the interior or specific sites on the surface. Although recent strategies employing “click” chemistry have improved upon traditional
methods,\textsuperscript{15} this situation is markedly different from the precise architectural control observed in nature.\textsuperscript{16}

In order to fabricate functional soft nanomaterials that more closely mimic folded biomolecules in structure and activity, the new paradigm in polymer synthesis involves manipulating single polymer chains.\textsuperscript{17} Among the various techniques employed to these ends, one in particular has garnered increased attention recently: the collapse or folding of linear polymer chains into architecturally defined nanostructures (Figure 1.1). This process is simple in principle.

\textbf{Figure 1.1} Linear polymer chains are decorated with functional groups that will promote intra-chain interactions when triggered in dilute solution. Reproduced with permission from Springer.\textsuperscript{18}

These single-chain nanoparticles (SCNP), while simple in concept, exhibit behaviors far more complex than initially anticipated and are currently the topic of intense focus by a number of research groups globally,\textsuperscript{19-22} including our own.

This introduction will highlight the current state of the art by examining (i) the chemistry and processing conditions used to synthesize SCNPs, (ii) the analytical techniques used to characterize SCNPs, including a discussion of their behaviour and morphology, and (iii) current and potential applications.
1.1 Synthesis of single-chain nanoparticles

A variety of synthetic methodologies have been applied to the formation of SCNPs. In most cases, appropriately functional polymers are synthesized followed by post-polymerization transformation in dilute solution (typically <1 mg mL⁻¹) to promote intra-chain cross-linking. Consequently, the chemical reactions that are used must meet the criteria of any effective post-polymerization functionalization reaction: they must be efficient and produce no side products.²³

Nature takes advantage of many different orthogonal covalent cross-links (e.g. disulfides) and non-covalent interactions (e.g. hydrogen bonding, metal ligation), and dynamic covalent chemistry (e.g. acetal formation) in folded biomacromolecules. Taking this as inspiration, SCNP synthesis follows these same motifs. In this section, the discussion of intra-chain cross-linking chemistry is divided into three major categories: covalent, dynamic covalent, and non-covalent. Table 1.1 highlights these three themes, along with illustrations of the chemical transformation used as well as the structure of the cross-link that is created by this chemistry.

Table 1.1 Covalent chemistries used in SCNP synthesis

<table>
<thead>
<tr>
<th>Before cross-linking</th>
<th>Structure of cross-link</th>
<th>Type of chemistry</th>
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<tr>
<td></td>
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<td>Friedel-Crafts alkylation²⁴</td>
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<td></td>
<td></td>
<td>Thermal [4+4] cycloaddition²⁵-²⁸</td>
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<tr>
<td>Before cross-linking</td>
<td>Structure of cross-link</td>
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<td>Free radical polymerization$^{29,30}$</td>
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<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td>Photoinduced [4+2] cycloaddition$^{31}$</td>
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<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td>Isocyanate amine$^{32}$</td>
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<td><img src="image10.png" alt="Image" /></td>
<td>Olefin metathesis$^{33}$</td>
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<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
<td>Azide-alkyne “click” chemistry$^{10,34-36}$</td>
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<td>Thiol-ene “click” chemistry$^{37}$</td>
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<td>Oxidative polymerization of thiophene$^{38}$</td>
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<td>Sulfonyl nitrene insertion/coupling$^{39}$</td>
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<td>Menschutkin reaction$^{41}$</td>
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<td>Thymine, diaminopyridine hydrogen bonding$^{51,52}$</td>
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<td>2-Ureido-$4[1H]$-pyrimidinone (UPy) hydrogen bonding$^{53-57}$</td>
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<td>Benzene-1,3,5-tricarboxamide (BTA) hydrogen bonding\textsuperscript{1,5,6,17,58}</td>
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<td>Dendritic self-complimentary hydrogen bonding\textsuperscript{59}</td>
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<td>Rhodium coordination\textsuperscript{60}</td>
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<tr>
<td><img src="image" alt="Copper coordination" /></td>
<td><img src="image" alt="Copper coordination" /></td>
<td>Copper coordination\textsuperscript{4}</td>
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1.1.1 Covalent cross-linking reactions

Hawker and coworkers synthesized architecturally defined SCNPs via intramolecular dimerization of the benzocyclobutene group at high temperatures (Figure 1.2). As previously mentioned, SCNP preparation requires low polymer concentrations (ca. 1 mg mL$^{-1}$) to avoid intermolecular coupling. The authors report syntheses of various random copolymers of 4-vinylbenzocyclobutene with various vinyl monomers via nitroxide mediated free radical polymerization. A continuous addition method was employed in which a concentrated polymer solution was added to heated solvent, with an overall polymer concentration of ca. 2.5 mg ml$^{-1}$ (0.05 M). The continuous addition technique proved to be much more efficient than typical ultra dilute conditions, by requiring less solvent while still preventing intermolecular coupling. The formation of nanoparticles using different polymeric backbones displays the versatility of this strategy. As a follow up to this work, Harth and coworkers reported a vinylbenzosulfone monomer which was more synthetically accessible than the previous vinylbenzocyclobutene, while still exhibiting similar crosslinking characteristics. The synthesis of this monomer is simpler, with mild reaction conditions and fewer purification steps than previously reported methods. SCNPs were synthesized from vinylbenzosulfone containing polymers using a continuous addition strategy, similar to the work involving the aforementioned benzocyclobutene polymers.

The high temperature conditions for these benzocyclobutene reactions preclude the incorporation of sensitive functionalities in the polymer architecture. Harth and coworkers remediated this issue by placing an electron donating group on the cyclobutene ring, lowering the isomerization temperature to 150 °C. While the continuous addition strategy decreases the amount of solvent that is used, it is impractical to produce commercially relevant quantities of material by this method, a major challenge that is yet unmet in SCNP research.
High efficiency, high functional group tolerance, and mild reaction conditions make “click” reactions an attractive candidate for synthesizing SCNPs.\textsuperscript{22} To date, copper-mediated azide–alkyne cycloaddition,\textsuperscript{10,34–36} thiol-ene addition,\textsuperscript{37,62} and amine-isocyanate addition\textsuperscript{32} click reactions have been used as cross-linking methods for SCNP fabrication. “Click” reactions involving alkynes and alkenes often involve protection or post-polymerization modification strategies, due to their incompatibility with radical polymerizations. Alternatively, if one reactive
partner is incompatible with polymerization chemistry, it can instead be placed in an external cross-linking agent to avoid interference.

In interesting work by Pomposo and coworkers, direct polymerization of unprotected terminal alkynes was conducted via redox-initiated RAFT polymerization. Subsequent exposure of a dilute polymer solution to copper-catalyzed Glaser–Hay coupling conditions led to SCNP formation.

O'Reilly and coworkers reported the synthesis of SCNPs via the tetrazine–norbornene reaction. While not traditionally considered a “click” reaction, this technique benefits from fast and quantitative conversion at room temperature without the need for catalyst and therefore meets the “click” criteria, at least in this context.

Photochemical reactions are often clean, high yielding, relatively fast, and require no chemical catalysts. A number of photoinduced coupling reactions have been examined for SCNP formation, including the photochemically triggered Diels–Alder reaction between 2,5-dimethylbenzophenone and maleimide, the photo-dimerization of coumarin, the photo-dimerization of anthracene, and the photoinduced nitrile imine mediated tetrazole-ene cycloaddition.

Zhu and coworkers reported photoinduced Bergman Cyclization to form polymeric nanoparticles via intramolecular collapse. The desired reactive motif possessed high photoreactivity with phenyl substituted triple bonds and double bonds locked in a methylbenzoate ring. Various random copolymers containing enediyne monomer and butylacrylate were synthesized via SET-LRP. The resulting linear copolymers were further subjected to Bergman cyclization conditions in toluene under dilute conditions by the continuous addition technique to form corresponding nanoparticles.
The reactive sulfonyl nitrene moiety, formed by thermal extrusion of nitrogen from sulfonyl azide groups, has been used by Pu and coworkers to form SCNPs.\textsuperscript{39} This reaction requires high temperature (190 °C), and the cross-links that are formed are not well-defined, due to the highly reactive nature of sulfonyl nitrenes. Similarly, Li and coworkers synthesized azido-functionalized polystyrene. The azido group forms a nitrene upon exposure to UV radiation and cross-links are formed by nitrene insertion. If the crosslinking is not driven to completion, it is possible to functionalize the remaining azide groups using click chemistry.

Coates and coworkers used olefin metathesis to synthesize polymer nanoparticles from linear polycarbonates containing pendant vinyl groups.\textsuperscript{33} The copolymerization of vinylcyclohexene oxide, cyclohexene oxide, and CO\textsubscript{2} with a BDI-ligated zinc catalyst produced the desired vinyl-functionalized polymer. The degree of cross-linking mediated by Grubbs’ catalyst can be easily monitored spectroscopically. Additionally, the authors determined the percent of cross-linked units by hydrolysis of the polymer and NMR analysis of the small-molecule fragments.

Thayumanavan and coworkers synthesized a copolymer of styrene, FMOC protected aminostyrene, and chloromethylstyrene. The chloromethylstyrene was used as a reactive handle to incorporate pendant styrene groups, which were subsequently polymerized in dilute solution using AIBN as an initiator, forming SCNPs. The FMOC groups were then removed, resulting in amine-functionalized nanoparticles.\textsuperscript{29}

Pomposo and coworkers recently reported the synthesis of SCNPs with catalytic activity.\textsuperscript{1} Various linear polymer precursors with glycidyl functionality were collapsed \textit{via} B(C\textsubscript{6}F\textsubscript{5})\textsubscript{3} catalyzed polymerization. Once the polymerization of the glycidyl units is complete, the catalyst remains in the nanoparticle. The authors found that linear polymers greater
than 100 kDa required a higher degree of dilution in order to avoid intermolecular cross-linking. The catalytic activity of these nanoparticles is discussed further in the application section.

Pyun and coworkers reported the synthesis of a novel propylenedioxy-thiophene functionalized polymer, which was cross-linked to synthesize SCNPs via oxidative polymerization of the thiophene units. The ester linkage connecting the polystyrene backbone to the polythiophene was further cleaved to separate the polymer chains to prove the formation of polymerized propylenedioxy-thiophene cross-links.

Zhao and coworkers reported the synthesis of SCNP shape amphiphiles, in which a hydrophobic polystyrene tail was attached to a hydrophilic poly(2-(dimethylamino)ethyl methacrylate)-based SCNP. The p(DMAEMA) block was collapsed using a quaternization reaction of the tertiary amine with 1,4-diiodobutane. The authors also reported the solution behavior of the shape amphiphiles in polar and non-polar solvents. These shape amphiphiles represent SCNP with multiple functionalities and well controlled three-dimensional structure.

1.1.2 Dynamic covalent chemistry

The synthesis of SCNP via dynamic covalent bonds is an interesting route to adaptable and responsive nanostructures. Under certain conditions, these bonds are reversible in nature and can be kinetically fixed or cleaved in response to change in environmental conditions, such as pH, oxidation, or temperature.

Fulton and coworkers utilized dynamic covalent acylhydrazone bonds to synthesize SCNP with reversible character. In this work, a bis(hydrazone) crosslinker was continuously added to aldehyde functionalized polystyrene, followed by catalytic trifluoroacetic acid (TFA). Before isolation, the TFA was quenched with triethylamine to kinetically trap the hydrazone bonds such that SCNP would remain intact upon isolation. The cross-linking density of the nanoparticles was
controlled by the amount of cross-linker added. Interestingly, no collapse was observed at higher cross-linking densities in these studies. The dynamic nature of the acylhydrazone bond was confirmed by formation of SCNPs via an exchange reaction of bis(hydrazide) crosslinker with copolymers adorned with monohydrazide. In a subsequent publication, Fulton et al. reported a similar system, functionalized with oligo(ethylene glycol) side chains to impart thermoresponsive behavior. At low pH, a solution of nanoparticles is kinetically trapped; however, upon exposure to acid and heat, the thermoresponsive nanoparticles precipitate, followed by hydrogel formation. Upon cooling, this process is reversed.\textsuperscript{44}

Pomposo, Fulton, and coworkers synthesized SCNPs capable of reversibly undergoing a coil to globule transition via enamine bond formation, which is reversible under acidic conditions.\textsuperscript{48} Beta-keto ester functionalized linear polymers were synthesized and condensed with butylamine. SCNPs were synthesized by an enamine exchange reaction with ethylene diamine under dilute conditions. The cross-links were cleaved upon addition of phosphoric acid and reform again with additional ethylene diamine, exemplifying the reversible nature of the process.

Disulfides are a dynamic moiety of interest due to their presence in biological systems and sensitivity to redox chemistry. Work in this area from our laboratory involved the synthesis of anhydride functionalized linear polymers where by intramolecular disulfide linkages were installed by addition of 4-aminophenyl disulfide. The disulfides were reversibly cleaved and reform in dilute solution by treatment with dithiothreitol (reducing) and iron(III) chloride (oxidative), respectively.\textsuperscript{46}
1.1.3 Non-covalent chemistry

Supramolecular interactions, such as H-bonding and π–π interactions, are the dominant intra-chain linkage in folded biopolymers. Many groups have examined similar chemistry in SCNP synthesis. Often, monomers are functionalized with hydrogen bonding units that are protected in some way to prevent the polymers from forming aggregates during their synthesis. This is demonstrated in several publications by Meijer and coworkers involving 2-uriedopyrimidinone (UPy) dimerization (Figure 1.3). Deprotection in dilute solutions allows the formation of intra-chain quadruple hydrogen bonds which in turn facilitates chain folding. The authors refer to these SCNPs as “metastable” because when cast into a film, the polymers remain soluble in chloroform; however, upon heating, the SCNPs uncoil and the UPy moieties form inter-chain linkages resulting in an insoluble supramolecular network.\(^{31}\)

![Figure 1.3 Schematic of SCNP formation via photo-deprotection of UPy groups. Reprinted with permission Copyright 2010 American Chemical Society.\(^{53}\)](image)

UPy dimerization has been used to study the effect of several variables in SCNP formation. The rigidity of the polymer backbone, the placement of additional hydrogen bonding sites in the UPy linker, and the molecular weight of the polymer were shown to have little effect on the ability
of a polymer to form supramolecular SCNPs, while solvent played an important role in disrupting or facilitating H-bond formation.\(^{57}\)

Benzene-1,3,5-tricarboxamide (BTA) has been exploited in the field of supramolecular chemistry for its ability to form helical assemblies via hydrogen bonding;\(^{66}\) consequently, it has also found use in SCNP synthesis. In one instance, a photoprotected BTA containing monomer is used to prevent aggregation during polymer synthesis.\(^{58}\) In subsequent publications, this protection strategy was not necessary; BTA containing monomers were simply polymerized directly in a solvent capable of disrupting H-bonds.\(^{1,2,5,6,67}\) Alternatively, the BTA unit can be attached to a polymer via azide–alkyne “click” chemistry.\(^{56}\)

Cucurbit[n]urils are known to form host–guest complexes with various aromatic molecules without necessitating the use of protection chemistry. In work by Scherman et al., methyl viologen and naphthyl functionalized polymers were combined with cucurbit[n]urils in dilute solution to form ternary host–guest complexes, resulting in SCNPs. In all cases, the polymers had to be studied at very low concentrations; above 0.1 mg mL\(^{-1}\), significant aggregation was observed.\(^{68,69}\)

To date, there are only a few instances of using metal coordination to form intramolecular cross-links in SCNPs. The routes to these SCNPs have been relatively simple; a metal complex is introduced to a ligand-bearing polymer in dilute solution and a ligand exchange reaction occurs; the high local concentration of polymer-bound ligand drives SCNP formation forward. In one example, rhodium was bound by polycyclooctadiene, which contains 1,5-dienes.\(^{60}\) Another example involves acac functionalized polymers containing copper(II) as a bridging metal for catalytic purposes.\(^4\)


1.1.4 Multiple intra-chain interactions

It has been determined computationally\(^\text{70}\) as well as with experiment that using multiple orthogonal intramolecular interactions results in more compact globular SCNPs. Hosono et al. combined UPy and BTA units in a block copolymer to form SCNPs with orthogonal hydrogen bonding dimers (Figure 1.4).\(^\text{56}\) Additionally, Altintas et al. reported a polymer folded by two different orthogonal hydrogen bonding dimers.\(^\text{51}\) Another example by Chao et al. involved ROMP-synthesized polyolefins collapsed via the supramolecular association of pendant tetraaniline units and covalently crosslinked by thiol–ene “click” chemistry involving the olefins in the polymer backbone.\(^\text{62}\)

![Figure 1.4 Design of a triblock copolymer bearing BTA and UPy moieties. (a) Graphic representation of SCNP formation; (b) chemical structure of triblock copolymers; (c) helical self-assembly of chiral BTAs via threefold-symmetric hydrogen bonding; (d) photoinduced dimerization of o-nitrobenzyl protected UPys via quadruple hydrogen bonding. Reprinted with permission Copyright 2012 American Chemical Society.\(^\text{56}\)](image-url)
1.1.5 Outlook

Surveying the literature it becomes clear that SCNP synthesis using a singular covalent chemistry as an intra-chain cross-link, and even the use of dynamic covalent chemistry or various supramolecular interactions is a well developed, proven technique. Moving this field forward will require innovations with relation to the use of multiple intra-chain interactions, as only a few examples are present in the current literature. Exploring more complicated polymer architectures such as block copolymers and branched structures is an open area for innovation. Combining SCNP synthesis techniques with advanced polymer syntheses that control monomer sequence or functional group placement such as work by Perrier, Lutz, and Whittaker, or well-defined step-growth chemistry such as ADMET, provides possibilities that could take the current state of the art one step closer to the structural precision found in natural folded macromolecules.

1.2 Characterization of SCNP

The corroboration of data provided by multiple techniques is often required to characterize SCNP formation. The appearance or disappearance of functional groups involved in the cross-linking chemistry and changes in the size and morphology of polymer structure can be detected using the techniques described in this section. Importantly, it is often necessary to use techniques that are sensitive enough to detect small concentrations of aggregates that may be formed by intermolecular cross-linking to prove the single molecule nature of these nanostructures.

1.2.1 Size exclusion chromatography

Size exclusion chromatography (SEC) has been an invaluable tool in understanding and characterizing SCNP. Early papers began with qualitative SEC measurements based on
standards\textsuperscript{24,27} and have since evolved into more quantitative measurements using multiple in-line detectors such as multi-angle light scattering (MALS) and viscometry.

Standalone SEC measurements are vital to understanding the behavior of SCNPs. While the molecular weight of globular SCNPs cannot be accurately measured using linear polymer standards, SEC provides other valuable data. An in depth study performed by Harth and coworkers provides an excellent example.\textsuperscript{27} In this work random copolymers of styrene and vinylbenzocyclobutane (BCB) were used to create a family of SCNPs. The molecular weight of these linear polymers was measured using SEC with polystyrene standards. Upon collapse, SEC measurements showed that all of their polymers had an increase in retention time and a decrease in apparent molecular weight. Since the BCB cross-linking does not produce any side products, the decrease in apparent molecular weight can be directly attributed to a decrease in hydrodynamic volume, which is principally what is measured by traditional SEC. Additionally, the authors used the change in apparent molecular weight to calculate the decrease in hydrodynamic volume. This data was corroborated by dynamic light scattering (DLS) measurements. \textsuperscript{1}H NMR was also used to confirm the complete disappearance of the BCB moiety, confirming spectroscopically that changes in solution volume can be attributed to this chemistry.

Often, a decrease in polydispersity index (Đ) is observed \textit{via} SEC when a chain transitions from a linear coil to a SCNP. In a computational study,\textsuperscript{72} Pomposo et al. examined SCNP formation assuming theta conditions for all samples so that a SCNP can be treated as a small linear polymer with a comparable hydrodynamic volume. They found this decrease in polydispersity index arises from the standard SEC calibration equation ($M_{\text{app}} = cM^b$), where the apparent molecular weight uses a scaling factor derived from a hydrodynamic radius equation. This research illustrates the merits in studying the complex physics of the intra-chain cross-linking of
polymers via various mathematical and computational methods. Full three-dimensional modeling of these materials is still needed in order to include a wider range of collapsing chemistries and represents an open area of research opportunity. Even though quantitative data cannot be collected directly from standalone SEC, it still provides an important tool in characterizing SCNPs. Specifically, it is used to observe a qualitative decrease in hydrodynamic radius, and also provides insight regarding intramolecular vs. intermolecular coupling.

1.2.2 Light scattering

The principles of light scattering were established by prominent scientists such as Einstein,73 Raman,74 Debye,75 and Zimm76 at the beginning of the 20th century. It has since been the basis of one of the most useful forms of characterization of macromolecular suspensions and solutions. Light scattering is an absolute method; the molar mass of large macromolecules is calculated based on the intensity of scattered light and the incremental refractive index (dn/dc) value of the polymer solution, and consequently does not produce data relative to standards.77 In regard to SCNPs, dynamic light scattering (DLS) and multi-angle light scattering (MALS) are both indispensable characterization techniques. Work from our laboratory has shown that using a MALS detector in-line with an SEC can prove that the molecular weight is consistent from parent polymer to SCNP.45,46 Several groups have also used DLS as a method for confirming this result.31,37,78

1.2.3 Viscometry

Another valuable technique in the characterization of SCNPs is solution viscometry. A particle's intrinsic viscosity is related to its molecular weight by the Mark–Houwink equation (Equation 1.2). Using the intrinsic viscosity measurement gathered by the viscometer and the
molar mass data from MALS, “K” and “a” coefficients can be calculated which relate to polymer conformation and the interaction between polymer and solvent. Viscometry is also useful in calculating hydrodynamic volume ($V_h$) which can further be used to calculate hydrodynamic radius ($R_h$) via the Einstein–Simha Relation (Equation 1.3 and Equation 1.4). When a hydrodynamic radius is calculated using intrinsic viscosity, it is sometimes referred to as a viscometric radius ($R_\eta$), as seen in Chapter 2 of this thesis.

Equation 1.1

$$\eta_i = (\eta - \eta_s)/\eta_s$$

Equation 1.2

$$[\eta] = \lim_{c \to 0} (\eta_i/c) = KM^a$$

Equation 1.3

$$V_h = M[\eta]/(2.5 N_A)$$

Equation 1.4

$$R_h = (3V_h/4\pi)^{1/3}$$

$\eta_i$ is the relative viscosity increment, $\eta$ is the solution viscosity, $\eta_s$ is the solvent viscosity, $c$ is the solution concentration, $\eta_i/c$ is the reduced viscosity, $[\eta]$ is intrinsic viscosity, $M$ is molar mass, $V_h$ is hydrodynamic volume, $N_A$ is the Avogadro constant, and $R_h$ is hydrodynamic radius.

Hawker et al. used viscometric measurements to characterize the formation of SCNPs synthesized using intra-chain isocyanate chemistry (Figure 1.5). The intrinsic viscosity of a polymer decreases as the degree of intramolecular cross-linking increases. In this case the authors used two polymer samples: 100 kDa, 150 kDa, and their SCNP counterparts, which were formed using an external diamine cross-linker. As expected, the higher molecular weight linear polymer had greater intrinsic viscosity than the lower. However, for the SCNPs, despite a 50% increase in
molecular weight compared to the parent polymers, the intrinsic viscosities of both samples decreased, and were similar to one another. This is consistent with the prediction made by Einstein; that the intrinsic viscosity of a constant density sphere is independent of its molecular weight, *i.e.* \( \frac{5}{2} \) divided by the sphere density.

![Figure 1.5 Plot of (a) reduced viscosity versus concentration for control copolymers (■, 150 kDa; ▲ 100 kDa) and (b) their analogous cross-linked nanoparticles (□, 150 kDa; 4, △ 100 kDa) in THF. Reprinted with permission Copyright 2009 American Chemical Society.](image)

1.2.4 Nuclear magnetic resonance spectroscopy (NMR)

The formation of cross-links in SCNPs has been confirmed by monitoring the appearance or disappearance of signals from external or internal cross-linkers in \(^1\text{H}\) NMR. Some laboratories have shown that other NMR techniques can be useful in observing the coil-to-SCNP transition. Zhao and coworkers observed SCNPs formed by the intramolecular photodimerization of coumarin using \(^1\text{H}\) NMR spin–spin relaxation time \((T_2)\).\(^7\) Spin–spin relaxation time is altered by molecular motion. An increase in the degree of dimerization showed an increase in the spin–spin relaxation time, which confirms a dramatic increase in the fraction of chain segments having
reduced mobility upon collapse. Relaxation time measurements were made at varying percentages of photodimerization, which indicated reduced mobility with increased degree of cross-linking. Reduction of polymer proton signals was also observed in the spectra from random coil to globule. Loinaz and coworkers demonstrated the use of DOSY experiments to determine the diffusion coefficient of poly(N-isopropylacrylamide) based thermoresponsive SCNPs in solution, which is inversely proportional to the hydrodynamic volume. The intramolecular collapse lead to an increase in the diffusion coefficient, as further evidence of the formation of collapsed SCNPs.\textsuperscript{34}

1.2.5 Characterizing the morphology of SCNPs

One of the most challenging aspects of the characterization of SCNPs is accurately deciphering their morphology, which is highly dependent on solvent choice and concentration. A similar situation occurred in the characterization of dendrimers. As Meijer and coworkers discussed in a detailed review,\textsuperscript{16} the initial expectation of dendrimer morphology was not exactly what was encountered through detailed characterization studies. As more studies are published, it is becoming apparent that the expected morphology of SCNPs is not always consistent with experimental results. Solution-free microscopy techniques, primarily atomic force microscopy (AFM) and transmission electron microscopy (TEM), have provided insight into the size, shape, and aggregation of SCNPs. Characterization of SCNP solution morphology has also been achieved using small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS). Currently a combination of microscopy and scattering techniques along with molecular simulations can give insight into the true nature of SCNPs. To observe individual SCNPs, low concentration solutions (typically around 0.01 mg mL\textsuperscript{-1}) have been used, while higher concentrations have shown the formation of aggregations. Certain trends are observed across several studies. As expected, an
increase in molecular weight of the parent polymer chains results in an increase in particle size, while an increase in cross-linking decreases the particle size.

Detailed AFM studies were carried out by Meijer and coworkers.\textsuperscript{53,54} Multiple polymer chains decorated with 2-ureido-pyrimidonone (UPy) units protected with a photocleavable $o$-nitrobenzyl group for UV induced quadruple hydrogen bonding intramolecular collapse were synthesized. The authors were able to deduce the possible morphology of SCNPs formed from different polymer chains using AFM. Considering the adsorbed particles to be hemi-ellipsoidal, a calculation for the diameter of the particles was developed. Samples of these SCNPs at dilute concentrations show AFM images of individual SCNPs with a size distribution similar to SEC results (Figure 1.6). At higher concentrations, aggregation occurred, forming a variety of unique arrays based on solvent and concentration choice.

![Figure 1.6](image1.png)

*Figure 1.6* AFM images of single-chain nanoparticles. Panels A and B are on mica, while panels C and D are on graphite. Reprinted with permission Copyright 2009 American Chemical Society.\textsuperscript{54}

In another study, Meijer and coworkers were able to monitor the deprotection of UPy groups to induce chain collapse by AFM.\textsuperscript{53} Sample concentration and choice of surface and solvent strongly affected these data. Furthermore, a difference between aggregates and individual particles was determined to ensure the characterization applied truly to SCNP and not multi-chain
aggregates. In this study this difference was evident in height and phase images in which SCNPs showed a darkened core in contrast to no observed changes in phase for multi-chain aggregates.

AFM sample preparation involves drop casting onto substrates, which can induce particles to concentrate in the center of a droplet during the final stages of evaporation, causing aggregation. Meijer and coworkers studied this particle aggregation mediated by solvent evaporation. To contrast individual SCNPs versus aggregation they conducted AFM experiments designed to intentionally induce particle aggregation. The evaporation rate was altered by changing the vapor pressure and solvent surrounding the substrate surface. Slow evaporation resulted in an increase in aggregation. The authors concluded that the major factors dictating morphologies observed by AFM are nanoparticle mobility before solvent evaporation and the amount of time required for solvent evaporation.

When SCNPs are drop cast onto a level surface, the morphology of the nanoparticle is altered upon drying; this morphology change has been observed in both AFM and TEM studies. AFM allows for the dimensional analysis of SCNPs, but TEM results have had better diameter correlation with DLS data than AFM, most likely due to the error resulting from the broadness of the AFM tip. In a study by Zhao and coworkers, a direct comparison of AFM, TEM, and DLS results for multiple nanoparticles revealed that AFM indicated rather large diameters while TEM results were much closer to the DLS determined hydrodynamic diameter. Similar results were obtained by Pomposo et al. in a recent publication. These studies indicate that TEM provides a more accurate image of the diameter of SCNPs as they behave in solution, although the diameter is still underestimated as they are swollen in solution and more compact once dry. Direct comparison of results from varying techniques may differ not just from instrumental effects but also due to sample preparation. Although correlations can be made, the profound effect of solvent
choice and concentration on SCNP morphology makes comparison of results from different sample preparation across different techniques difficult. It becomes important, in light of this knowledge, to use multiple methods of characterization and benchmark these data to the growing body of literature in this area.

While solvent free microscopy techniques provide valuable information on the morphology of SCNPs, their behavior in the absence of solvent and their interactions with substrates are still not entirely understood. For species that exist in solution, true morphology can only be observed with techniques that allow for characterization in solution. Small angle scattering techniques like small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) are becoming more common for the characterization of SCNPs. Both SAXS and SANS allow SCNPs to be directly characterized in solution. TEM and AFM tend to display compact morphologies while SAXS and SANS results indicate less compact morphologies under good solvent conditions. These small angle scattering techniques have been used by many groups to measure the radius of gyration and observe form factors of SCNPs. Additionally, molecular dynamic (MD) simulations have proven useful in aiding researchers understand the data provided by small angle scattering techniques.

Meijer and coworkers have demonstrated the use of SAXS to provide further evidence of SCNP formation in which a clear reduction in the radius of gyration from coil to globule results. In a recent publication by O'Reilly and coworkers, large radius of gyration values were obtained using SANS while AFM and TEM provided a more compact image with a smaller size predicted. Increased SANS values were attributed to SCNP aggregations at room temperature, corroborated by DLS temperature studies. These results are an indication that data from solvent free techniques can be misrepresentative of how SCNPs behave in solution. Molecular dynamic
simulations have shown that when decreasing the quality of solvent, increasingly compact conformations result, which is consistent with the compact morphologies that are constantly observed in solvent-free techniques.\textsuperscript{11}

In studies by Pomposo \textit{et al.} a comparison of heterofunctionalized nanoparticles and their homofunctional counterparts was made both experimentally and using MD simulations based on generic bead-spring models.\textsuperscript{70} Experimental SEC/MALS traces and SAXS data both showed more compact nanoparticles for heterofunctional species compared to their homofunctionalized counterparts, this was consistent with MD simulations. Slightly larger sizes were observed in SAXS data in comparison to simulations but were attributed to bending and torsional barriers of the actual polymer that were not taken into account in these simulations.

Voets and coworkers recently studied random copolymers functionalized with benzene-1,3,5-tricarboxamides which were synthesized and transformed into SCNPs based on BTA self-assembly into helical aggregates as a result of strong 3-fold hydrogen bonding between the amides of adjacent BTAs.\textsuperscript{67} An in depth analysis of the folding process as a function of the chain length was obtained through the use of SANS, SAXS, and DLS. Experiments showed that there is a lack of cooperativity in the intramolecular folding of the polymer, which is unexpected because the intermolecular BTA self-assembly is typically a cooperative process.

Although studies have shown that most SCNPs mimic an intrinsically disordered globular coil, simulation of the folding transition of a single chain indicate that SCNPs have the potential to mimic the control of many natural processes. Yoshinaga \textit{et al.} have performed Monte Carlo simulations that provide a great comparison to SCNPs. These studies reveal the potential of SCNPs to mimic globular proteins, but the current state of the art is far from this goal.\textsuperscript{80}
A recent survey of data from the literature suggests that SCNPs do not adopt a truly compact globular conformation.\textsuperscript{81} Cross-linking conditions typically involve dissolving the polymer in a good solvent, as poor solvation leads to aggregation. Consequently, cross-links are formed based on short-range interactions within a chain. The aspect ratio for an ellipsoidal structure was recently observed \textit{via} SANS by Meijer \textit{et al.} (Figure 1.7a).\textsuperscript{67} Similarly shaped SCNPs were also visualized \textit{via} TEM in recent work from our laboratory.\textsuperscript{49} Computational work by Pomposo \textit{et al.} (Figure 1.7c) suggests that using multiple different orthogonal cross-linking chemistries will induce a greater degree of collapse and lead to a more compact, globular state.\textsuperscript{70} Experimentally, this has been confirmed in work from our group\textsuperscript{62} and by the Meijer lab\textsuperscript{56} (Figure 1.7c).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(a) Ellipsoidal structure proposed by Meijer \textit{et al.}; (b) visualization of ellipsoidal SCNPs formed by photocrossinking of pendant anthracene groups; (c) SCNP structure obtained from MD simulations. Reprinted with permission Copyright 2013, 2014 American Chemical Society.\textsuperscript{67,70} Reprinted with permission © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.\textsuperscript{49}}
\end{figure}
1.3 Potential applications

The potential applications of SCNPs include catalysis, \(^1\text{-}^5\) sensors, \(^6\) nanoreactors, \(^7\) and nanomedicine. \(^8\text{-}^{11}\) SCNP technology is advantageous in these areas due in large part to the small size of the nanoparticles and the ease in which they can be tailored to specific uses. While the research involving SCNPs has been primarily fundamental in nature, these materials have found some practical uses. By design, the interior of the particle offers a useful chemical environment. The environment can be hydrophobic or hydrophilic, and the size can be controlled by varying the amount of intra-chain cross-linking and the molecular weight of the parent polymer. Additionally, controlled polymerization techniques and post-polymerization modification techniques allow the incorporation of selective sites for desired function. The following section summarizes recent applied research involving SCNPs.

1.3.1 Catalysis

Enzymes are generally more efficient catalysts than their synthetic counterparts. The active site of enzymes are contained in a hydrophobic pocket, but most synthetic models are exposed to an aqueous environment. \(^82\) Modeling enzymes using polymer backbones opens doors for preparing efficient catalysts by controlling properties including polymer solubility, increased accessibility to a larger library of substrates, and increased turnover frequency (TOF). Another challenge in homogenous catalysis is the regeneration of the catalyst. In most circumstances, the product and catalyst have similar solubility and the reactions are generally performed in nonaqueous solvents. This presents a disadvantage because using a homogeneous catalysts for industrial processes produces a large amount of waste during the chemical work up. Using a polymer support for
Catalysis is an attractive route for homogenous catalysis because the polymer and product can be easily separated. SCNs offer possible solutions to these challenges.

Perez-Baena et al. reported a tris(pentafluorophenyl)boron (B(C₆F₆)₃) containing SCNP. The B(C₆F₆)₃ units also serve as catalytic sites to allow the SCNP to mimic the function of reductase and polymerase. However, the enzymatic activity of the reported SCNP is limited to organic solvents such as halogenated solvents, toluene, and benzene. Solvents that form adducts with boron are unsuitable and quench the catalytic activity. Empirical evidence demonstrates the size, composition, quantity, and location of catalytic compartments influences the kinetics and turnover frequency (TOF) of catalysis. The TOF can be improved by either decreasing the hydrodynamic radius or by increasing the molecular weight of the polymer.

Catalysis of organic reactions in water is desirable; however, many catalytic systems are compatible only with organic solvents. To address this using SCNs, Terashima et al. reported the synthesis of a styrene-based copolymer containing PEG, BTA, and diphenylphosphinostyrene (SDP) using ruthenium catalyzed CRP. The copolymers were able to self-assemble and form SCNPs in an aqueous environment. The presence of SDP induced ligand exchange to incorporate the ruthenium catalyst into hydrophobic cavities within the polymer. The authors demonstrated transfer hydrogenation at the catalytic site. They found the self-assembled α-helices could withstand aqueous catalytic conditions (0.4 M HCOONa and substrate at 40 °C). Cyclohexanone was efficiently reduced to cyclohexanol, and even acetonophenone, which has poor water solubility, was 86% reduced after 18 h. The turnover frequency for this SCNP based catalytic system (1–20 h⁻¹) is comparable to other catalysts (1–40 h⁻¹) under similar conditions. In a subsequent publication from the same researchers, it was determined that the folding induced by BTA units was not essential to the catalysis; the collapse of the polymer induced by SDP ligation
to ruthenium created the catalyst-containing hydrophobic sites necessary to perform the catalysis. However, it was maintained that the SCNP-bound catalyst was more effective than the free catalyst.\textsuperscript{1}

The importance of compartmentalizing an active site within a hydrophobic pocket of a polymer was shown by Huerta \textit{et al.}\textsuperscript{2} A water soluble methacrylate copolymer was synthesized, containing self-assembling BTA units to provide a hydrophobic pocket. An L-proline analog, which is the catalytic site found in class I aldolase, was incorporated into the hydrophobic pocket of the SCNP. The catalytic activity of the nanoparticles was determined using cyclohexanone and \textit{p}-nitrobenzaldehyde as substrates. Nearly 100\% conversion to the aldol product was observed after 120 hours with the L-proline content remaining constant. Conversion was higher for polymers with higher molecular weight (\textasciitilde 38 kDa) \textit{versus} the lower molecular weight (\textasciitilde 28 kDa), while polymers without BTA units did not demonstrate catalytic activity. The catalytic nanoparticles were also found to be reuseable. The high catalytic efficiency can be attributed to the hydrophobic environment, which brings the substrate within close proximity of the catalytic site. This leads to an increase in local concentration of the substrate near catalytic site and results in high conversion. In this case, controlling the size of the SCNP is important for tuning catalytic efficiency.

Pomposo \textit{et al.} reported a polymer functionalized with acetoaceotoxy (acac) groups that formed SCNPs when introduced to copper(II) acetate. The nanoparticles were then used as a catalyst for alkyne homo-coupling. Compared to the free copper complex under the same solvent and temperature conditions, the copper(II) functionalized nanoparticle showed catalytic selectivity toward propargyl acetate and, to a lesser extent, propargyl propionate. Also, the catalyst was effective at an overall lower catalyst concentration (0.5 mol\% compared to 3 mol\%).\textsuperscript{4}
He and coworkers reported the synthesis of SCNPs from coumarin-containing random copolymers of poly(N,N-dimethylaminoethyl methacrylate). The SCNPs were synthesized via photo-cross-linking of the coumarin units and used as nanoreactors to synthesize gold nanoparticles (AuNP) from HAuCl$_4$. The authors found that a higher degree of cross-linking led to faster AuNP formation. By stirring a dilute solution of the samples with HAuCl$_4$, the tertiary amine units of the polymers acted as reductants and stabilizers of the gold nanoparticles. The more compact polymer provided a higher local concentration of the amine, thereby increasing the number of AuCl$_4$ ions around the nano-objects, facilitating the faster reduction. The experiment was conducted in both THF and water; AuNP formation was faster in water, since the SCNPs are less solvated and therefore more compact.\(^7\)

### 1.3.2 Nano-medicine

Perez-Baena and coworkers developed SCNPs with multiple gadolinium(III) sites as a potential MRI contrast agent.\(^9\) Azide functionalized acrylic polymers were cross-linked with the dialkyne functionalized Gd(III) diethylenetriaminepentaacetic acid (DTPA). In addition to being water soluble, the conformationally locked Gd(III) centers showed enhanced relaxation time with a 2-fold increase over a Magnevist, a commonly used MRI contrast agent, as well as a 4-fold increase over the Gd-loaded cross-linker by itself. This suggests that the SCNP architecture was advantageous for this application.

Pomposo et al. used small angle neutron scattering measurements to show their RAFT poly(MMA-r-AEMA) behaves like a disordered multidomain protein.\(^11\) The polymer chains achieved this conformation through intra-chain Michael addition using bi- and trifunctional cross-linking units. Vitamin B$_9$ nanocarriers based on these nanoparticles demonstrated controlled release in water at neutral pH. The release of vitamin B$_9$ was monitored by UV-Vis spectroscopy.
They observed a release exponent approximately of 0.5, which suggests the delivery process proceeds through a Fickian diffusion mechanism. The complete delivery of vitamin $B_9$ from the Michael nanoparticles with a drug loading content of 41 wt% took place in 5–6 hours.

Expanding upon their polycarbonate based nanosponges synthesized via the reaction of a diamine cross-linker with epoxide moieties,$^{37}$ Harth et al. developed a targeted drug delivery system for breast cancer.$^9$ The researchers developed a targeting peptide capable of recognizing tumorous cells upon their exposure to ionizing radiation. The peptide was conjugated to the nanosponge via thiol–ene “click” chemistry, and the nanosponge was impregnated with the anti-cancer drug paclitaxel. Mouse studies showed that the targeted nanoparticle based system resulted in much slower tumor growth, and a greater retention of paclitaxel over time compared to systemic paclitaxel. The same researchers developed a similar system based on a lung cancer model, in which paclitaxel was administered followed by camptothecin.$^8$

### 1.3.3 Chemical sensors

Gillissen et al. designed SCNPs that act as compartmentalized sensors for metal ions (Figure 1.8).$^6$ Polynorbornene polymers were cross-linked with 3,3-bis(acylamino)-2,2-bipyridine substituted benzene-1,3,5-tricarboxamides (BiPy-BTAs). The BiPy-BTA cross-linker also served as the metal binding moiety as well as the fluorescing component via aggregate induced emission. The multifunctional cross-linker is well suited for metal sensing, as the SCNP would lose its fluorescence after binding a metal ion.

The advantage of SCNPs in this system is that the particle is inherently ratiometrically fluorescent upon formation without additional functionalization. The luminescence is caused by aggregate induced emission upon polymer folding. Subsequent quenching experiments with a
variety of metals showed this system was especially sensitive to copper, which provided the most quenching.

![Schematic representation of the sensing function of the BiPy-BTA functional polymers. Reproduced with permission from The Royal Society of Chemistry.](image)

### Figure 1.8

A Schematic representation of the sensing function of the BiPy-BTA functional polymers. Reproduced with permission from The Royal Society of Chemistry.⁶

#### 1.3.4 Self-assembly

In addition to the utility that SCNPs present, they may serve as building blocks for more compartmentalized nano-machinery, comparable to complex biomacromolecules. From a primary structure inherent to the parent polymer, to secondary structures resulting through folding, it may be possible to assemble SCNPs into hierarchically ordered materials.

The secondary structures in enzymes are important for describing substructures of the macromolecule, while the tertiary structure is important for blocking or opening the active site for binding substrates. While many authors report protein-like polymers that adopt secondary structures using BTA units, very few report control over the tertiary structure. The Barner-Kowollik group has taken advantage of the orthogonal H-bonding pairs cyanuric acid-Hamilton...
and thymine-diaminopyridine to mimic polymer self-folding as observed in biomolecules. In each case, one H-bonding unit was attached to an ATRP initiator. These initiators were used to synthesize polystyrene with an active bromide end group, which was used to attach the opposing H-bonding unit via “click” chemistry. In a subsequent publication, the two orthogonal pairs were combined in a single polymer; the polymer structure was designed such that each H-bonding unit was separated by a block of polystyrene. Light scattering measurements demonstrated the reduction in hydrodynamic radius at low concentration. Variable temperature $^1$H NMR was used to demonstrate hydrogen bonding of the cyanuric acid, Hamilton Wedge, and thymine moieties by observing changes in the NH chemical shift. This folding can be reversed upon raising the temperature of the solution to shift equilibrium towards a random coil state.

Wen et al. developed self assembling monotethered SCNP shape amphiphiles based on poly(2-(dimethylamino)ethy methacrylate)-block-polystyrene (PDMAEMA-b-PS). The tertiary amine block was cross-linked with 1,4-diiodobutane via the Menschutkin reaction to form “tadpole” shape amphiphiles; similar to work done by Tao and Liu, as well as Kim et al. These shape amphiphiles, bearing a hydrophilic SCNP head and a hydrophobic polystyrene tail, were capable of self assembling into micelles or vesicles based on solvent. The diameters of these micelles were between 30—80 nm.

1.4 Summary and Outlook

The synthesis and application of single-chain nanoparticles remains an area of increasing research focus. Given the small size of the nanostructures produced by these methods and the relative ease with which they can be tailored to specific end use applications it is likely such efforts will intensify in the coming years. So far, simple chemistry has been utilized and high-level characterization and modeling studies have been applied to understand the process by which these
particles form and how they behave, both in the bulk and in solution. In depth structural characterizations with the level of detail now available for proteins remains extremely challenging and is an open ended problem requiring contributions from both experiment and theory. Expansion of this concept from linear chains to more advanced polymer architectures is another area where innovations are needed. With respect to practical applications, the growing body of work shows that SCNPs are promising candidates for a number of critical technological needs. However, the ultra-dilute conditions required by current synthetic methods pose a significant challenge requiring clever chemistry and process engineering to overcome. It is certainly clear, despite some of these obstacles, that SCNPs are a firmly established research topic in modern polymer science. In this review we outlined the various methods that have been explored to synthesize these materials, summarized the methods of characterization that are required to prove their formation and probe their morphology, and introduced a number of potential applications that are being explored currently. While it is impossible to predict where this work will ultimately lead, we hope this “user's guide” will prove useful to the community as single-chain nanoparticles continue to evolve from academic curiosity to functional technology.
CHAPTER 2 ZIPPING POLYMERS INTO NANOPARTICLES VIA INTRA-CHAIN ALTERNATING RADICAL COPOLYMERIZATION

2.1 Introduction

Many natural macromolecular architectures derive their utility from their shape, and the precise placement of functional groups on the surface or within the structure. This shape is often the result of a precise single-polymer folding process, which is dictated by a perfectly controlled monomer sequence. Polymer chemists have made strides toward the synthesis of sequenced polymers using a variety of techniques, but while impressive, these advances are far from comparable to the complexity found in nature.

A technique for fabricating advanced polymer nanostructures enjoying recent popularity is the collapse of single polymer chains in highly dilute solution. We term the resultant structures single-chain nanoparticles (SCNP). This technique has proven particularly valuable in the synthesis of nanomaterials on the order of 5 – 20 nm. Many different types of covalent and non-covalent chemistries have been used to this end; our research group and others have recently written reviews on this topic. \(^{89,90}\) Recent advances in the field include the synthesis of SCNP containing dynamic disulfide bonds capable of encapsulating hydrophobic guest molecules.\(^ {91}\) Hosono et al recently used single-molecule force spectroscopy to characterize the process of SCNP unfolding, and used the data to tease out previously unattainable kinetic parameters related to intra-chain benzene tricarboxamide (BTA) self-assembly.\(^ {92}\) Another notable example is the synthesis of SCNP cross-linked by palladium coordination, which were catalytically active.\(^ {93}\) These structures represent the first step in a simple approach to the synthesis of hierarchical structures.
There have been efforts on multiple fronts to control structural features and characteristics of single-chain systems. Results include the synthesis of hetero-telechelic polymers with additional strategic singly functional sites installed in the backbone for dual-point single-chain folding, as well as the synthesis of single-chain tadpole and dumbbell structures. In SCNP, functionality has been incorporated directly via the cross-linking process using an external cross-linking agent – examples include incorporating catalytic centers into the cross-links as well as gadolinium-containing cross-linking agents for potential MRI contrast agents.

Covalent crosslinking techniques involving radical chemistry have been used to synthesize SCNP. In our laboratory, a radical process involving a poly(norbornene imide) based polymer was studied. There are also instances in which cross-links are formed via the radical polymerization of pendant olefin units. A caprolactone based monomer functionalized with an acrylate group was polymerized directly using a non-radical based ring opening polymerization technique. To furnish a polymer with methacrylate groups, the Mitsonobu reaction between methacrylic acid and a hydroxy-functionalized polymer was employed. Thayumannevan et al reported the synthesis of SCNP via the polymerization of pendant styrene units, which were incorporated by a post-polymerization S_N2 reaction between benzyl chloride moieties and a styryl-functionalized resorcinol derivative. In all of the aforementioned cases, nanoparticles were formed by heating a dilute solution of the polymer in the presence of AIBN.

“Zipping up” polymers via radical polymerization (depicted in Figure 2.1a) is an attractive route to SCNP due to its efficiency and relatively high functional group tolerance. However, it requires the synthesis of a polymer functionalized with polymerizable vinyl groups, which necessitates either the use of a non-radical/metathesis based polymerization technique, or the incorporation of vinyl groups via a post-polymerization modification route, as highlighted in the
Figure 2.1 a) Radical polymerization through pendant monomer units to synthesize SCNP, b) incorporation of electron-deficient comonomer into cross-linked regions

examples discussed previously. Furthermore, while radical polymerization is robust, it has only been used to induce polymer collapse, and not to control the installation of specific structural features in SCNP, or to incorporate additional functionality. To address these issues, we explored an alternating radical copolymerization strategy (Figure 2.1b).

Electron rich “donor” monomers such as styrene or stilbene are known to undergo alternating radical copolymerizations with electron deficient “acceptor” monomers such as maleic anhydride (MA) or maleimide derivatives. Specifically, stilbene derivatives, maleic anhydride, and maleimide derivatives are known to undergo strictly alternating polymerization with little to no homopolymerization occurring.\(^{98}\) This effect can be quantized in the form of reactivity ratios, which arise from the rate constants of the propagation reactions of each possible radical with each monomer. Alternating copolymerizations result in cases where the reactivity ratio for each monomer is approximately equal to zero; that is, \(r_1 \approx r_2 \approx 0\).\(^{99}\) In addition to stilbene, styrene also has a tendency to undergo alternating polymerization with maleic anhydride.\(^{100}\) Notably, styrene is also capable of homopolymerization.

By employing the principles of alternating copolymerization, it is possible to synthesize advanced polymer architectures. For example, when a functionalized N-substituted maleimide is
added to the controlled polymerization of styrene-derived monomers, the maleimide is consumed very quickly, resulting in the addition of a functional block, the location of which can be manipulated based on the timing of the addition.\textsuperscript{101} This strategy was combined with other synthetic techniques, including protection and post-polymerization modification strategies, to form amine-functionalized polymers with additional functional blocks built into the polyamine backbone.\textsuperscript{102} This strategy has also been used to synthesize single-chain dumbbells.\textsuperscript{96}

We sought to take advantage of these alternating radical copolymerizations as a means to form well-defined cross-links in SCNP. The alternating nature of the polymerization introduces a simple level of structural control while simultaneously incorporating functionality; i.e. the anhydride group of maleic anhydride, or a functionalized maleimide derivative.

Herein we describe the introduction of pendant styrene and stilbene units into a linear polymer via a versatile post-polymerization Wittig reaction, followed by polymer collapse via both homopolymerization of pendant styrene units (P2.1), and the copolymerization of pendant stilbene units with maleic anhydride (MA), N-ethyl maleimide (NEM), or N-(1-pyrene) maleimide (NPM) (P2).
2.2 Results and Discussion

2.2.1 Polymer synthesis

![Scheme 2.1 RAFT copolymerization of 4-VBTPPF4 and styrene, followed by post-polymerization Wittig reaction](image)

In order to synthesize olefin-bearing polymers, we chose to pursue a post-polymerization modification route. Linear poly(4-vinylbenzyl(triphenylphosphonium) tetrafluoroborate) was previously synthesized by Borguet et al using ICAR ATRP.\textsuperscript{103} Due to the poor solubility of the ionic polymer in THF, it was converted to linear poly(divinylbenzene) for SEC analysis via the Wittig reaction with formaldehyde. We found this to be an attractive means to incorporate pendant styrene or stilbene units which would otherwise interfere with the controlled radical polymerization. For our synthesis, we used thermally-initiated RAFT polymerization and included styrene as a comonomer (\textbf{Scheme 2.1}). Following this, the functionalization of these polymers with the Wittig reaction proceeds cleanly, evidenced by the complete disappearance of the phosphonium methylene unit by \textsuperscript{1}H NMR (see appendix pages 4, 5, 6). Styrene-containing \textbf{P2.1} was synthesized using formaldehyde, and stilbene-containing \textbf{P2.2} was synthesized using benzaldehyde. It is worth noting that we were not able to characterize the presence of the RAFT trithiocarbonate end group by NMR before or after the post-polymerization Wittig reaction due to the relatively high molecular weight of the polymers. The trithiocarbonate could conceivably be hydrolyzed as a result of the basic conditions required for the Wittig reaction. The resultant thiols
can potentially oxidize to disulfides under ambient conditions or undergo thiol-ene “click” reactions when exposed to a radical source, but evidence of either transformation was not observed. Specifically, when stilbene-containing P2.2 was heated in the presence of AIBN, there was no shift in SEC retention time (Figure 2.3a).

2.2.2 Nanoparticle synthesis

We adopted the following systematic nomenclature for the materials described here: the number after NP designates which polymer precursor was used to synthesize the SCNP. The number after the hyphen designates the number of equivalents of electron deficient monomer relative to stilbene or styrene units in the precursor polymer (0 is 0 equiv, 1 is 1.2 equiv, 2 is 3.6 equiv, 3 is 6.0 equiv, and 4 is 12.0 equiv). The suffix indicates the identity of the electron deficient monomer. For example, NP2.3-3NEM was synthesized from polymer 3 using 3.6 equivalents of N-ethyl maleimide.

The radical polymerization of pendant styrene units in P2.1 under highly dilute conditions proceeds as expected to form SCNP, as seen in Figure 2.2a. From parent polymer to SCNP, the retention time and molecular weight increase while the viscometric radius (R_η) and intrinsic viscosity ([η]) decrease. The decrease in radius and intrinsic viscosity are due to the decrease in volume which is a result of the coil-to-nanoparticle transition – the nanoparticle is expectedly more globular in nature than its precursor. This result is in consonance with data previously collected by ourselves and others. No large aggregates are observed in the MALS trace. From parent polymer to nanoparticle, a broadening of the aliphatic and aromatic backbone protons is observed by ^1H NMR. This broadening of the NMR signal is due to enhanced T2 relaxation time, which arises as a result of an increase in restriction of molecular motion from parent polymer to nanoparticle.
Figure 2.2 SEC UV detector traces for a) reaction of styrene-functionalized P2.1 with AIBN, and b) reaction of P2.1 with AIBN in the presence of various concentrations of maleic anhydride.
Additionally, the integrations corresponding to the vinyl protons decrease, although it is difficult to quantify due to overlapping resonances.

Interestingly, when MA is introduced, the process becomes less controlled (Figure 2.2b). Generally, the SEC results still indicate shifts to longer retention time, but shoulders on the traces suggest multimodal distributions, and multi-chain aggregates can be observed in the UV, RI, and MALS data. This complication can be explicated using the kinetics of alternating copolymerization. Styrene, in the presence of an excess of maleic anhydride, undergoes strictly alternating copolymerization. The styrene-based radical is much more reactive than the maleic anhydride radical in terms of both homo-propagation and cross-propagation reactions. As a result, styrene radicals react very quickly with maleic anhydride to form MA-based radicals, which have longer lifetimes and are more likely to be involved in termination or inter-chain cross-propagation events. Subsequently, inter-chain cross-linking is more likely to occur. When the concentration of maleic anhydride is increased, this effect is exaggerated, as higher concentration leads to even faster generation of MA-based radicals.

The radical copolymerization of pendant stilbene units in P2.2 with maleic anhydride under highly dilute conditions proceeds smoothly (Figure 2.3b). Larger concentrations of MA result in larger shifts to longer retention times, higher molecular weights, and smaller viscometric radii and intrinsic viscosity values. This effect is observed substantially between 1.2 and 3.6 equivalents of MA; however, the difference between 3.6, 6, and 12 equivalents is minimal, especially when viewing the MALS data (see appendix). This trend can be rationalized by considering the high local concentration of stilbene units in the polymer environment compared to the overall low solution concentration of MA. The homopolymerization of stilbene is highly unfavorable, so alternating copolymerization is the only possible propagation event. Higher concentrations of MA
2.2.4 Nanoparticle synthesis from polymer containing pendant stilbene units (P2)

![Chemical structures and SEC traces](image)

**Figure 2.3** SEC UV detector traces for a) reaction of stilbene-functionalized P2.2 with AIBN, and b) reaction of P2.2 with AIBN in the presence of various concentrations of maleic anhydride
lead to more propagation, i.e. cross-link formation, and fewer termination events. This result is consistent with the previously discussed inter-chain cross-linking of P2.1, in that higher MA concentration leads to more cross-linking; however, based on the aforementioned kinetics argument, it is not entirely clear why inter-chain cross-linking is avoided in the case of P2.2. This topic requires further investigation.

Our stilbene functionalized polymer was also subjected to the same conditions in the absence of an external comonomer. The SEC results indicate very little change, suggesting that the observed SCNP formation in the previously discussed example is solely due to cross-linking as a result of alternating copolymerization. Effectively, the presence of an electron-poor acceptor monomer is integral in the formation of these SCNP. Pertinent SEC data for nanoparticle formation is recorded in Table 2.1.

Table 2.1 SEC data

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$</th>
<th>$M_w$</th>
<th>PDF</th>
<th>$[\eta]$</th>
<th>$\bar{R}_g$</th>
<th>MHS $\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2.1 (styrene)</td>
<td>26</td>
<td>31</td>
<td>1.2</td>
<td>19.0</td>
<td>4.2</td>
<td>0.73</td>
</tr>
<tr>
<td>NP2.1-0</td>
<td>30</td>
<td>36</td>
<td>1.2</td>
<td>8.6</td>
<td>3.4</td>
<td>0.65</td>
</tr>
<tr>
<td>P2.2 (stilbene)</td>
<td>24.3</td>
<td>29.5</td>
<td>1.2</td>
<td>12.8</td>
<td>3.6</td>
<td>0.72</td>
</tr>
<tr>
<td>NP2.2-1MA</td>
<td>25.9</td>
<td>31.4</td>
<td>1.2</td>
<td>10.7</td>
<td>3.5</td>
<td>0.79</td>
</tr>
<tr>
<td>NP2.2-2MA</td>
<td>29.0</td>
<td>34.7</td>
<td>1.2</td>
<td>6.5</td>
<td>3.1</td>
<td>0.65</td>
</tr>
<tr>
<td>NP2.2-3MA</td>
<td>28.5</td>
<td>35.5</td>
<td>1.2</td>
<td>5.5</td>
<td>2.9</td>
<td>0.43</td>
</tr>
<tr>
<td>NP2.2-4MA</td>
<td>34.2</td>
<td>40.3</td>
<td>1.2</td>
<td>5.5</td>
<td>3.1</td>
<td>0.29</td>
</tr>
<tr>
<td>NP2.2-1NEM</td>
<td>27.7</td>
<td>32.9</td>
<td>1.2</td>
<td>10.2</td>
<td>3.5</td>
<td>0.63</td>
</tr>
<tr>
<td>NP2.2-2NEM</td>
<td>33.2</td>
<td>40.3</td>
<td>1.2</td>
<td>6.9</td>
<td>3.3</td>
<td>0.72</td>
</tr>
<tr>
<td>NP2.2-3NEM</td>
<td>31.7</td>
<td>38.1</td>
<td>1.2</td>
<td>6.9</td>
<td>3.2</td>
<td>0.46</td>
</tr>
</tbody>
</table>

a) Obtained using triple detection SEC. See experimental section for more information; b) Obtained from viscometric SEC data. See experimental section for more information; c) Mark-Houwink-Sakurada a-value
To corroborate NMR and SEC data, and to confirm the disappearance of the stilbene moiety, P2.2, NP2.2-2MA, and NP2.2-3MA were characterized by UV-vis spectroscopy. As expected, the characteristic stilbene absorbance is present in P2.2, but is much smaller in NP2.2-2MA and NP2.2-3MA (Figure 2.4a).

![UV-vis spectra overlay for maleic anhydride cross-linking experiments and N-(1-pyrene)malemide cross-linking experiments](image)

**Figure 2.4** UV-vis spectra overlay for a) maleic anhydride cross-linking experiments and b) N-(1-pyrene)malemide cross-linking experiments

Interestingly, for the reaction of P2.2 with MA, the Mark-Houwink-Sakurada (MHS) α values continue to decrease from 6 to 12 equivalents of MA even as the radius does not decrease. It is possible that, after the addition of a certain amount of MA, a maximum cross-linking density is reached. Continued addition of MA units to the polymer does not result in a size change, but
does add to the molecular weight, thereby increasing the density of the particle and further lowering the MHS $\alpha$ value. Additionally, the formation of multi-chain aggregates is not observed. The results are analogous when MA is substituted for N-ethyl maleimide, which is also known to undergo alternating radical polymerization with stilbene (Table 2.1, see appendix for SEC traces). This result, while not surprising, is important for continuing this work, as any substituted maleimide compatible with radical polymerization can conceivably be used.

### 2.2.5 Synthesis of fluorescent SCNP

The use of N-(1-pyrene)maleimide (NPM) was investigated to this end. Two experiments were conducted with 3.6 and 6.0 equivalents of NPM, characterized by $^1$H NMR and UV-vis spectroscopy, and compared to the analogous MA experiments. In order to ensure the complete removal of NPM, the SCNP were precipitated into acetone, resulting in a much lower recovery (<50%), but none of the small molecule pyrene appeared to be present in the $^1$H NMR spectrum (see appendix page 10). Furthermore, a very broad peak at about 8 ppm confirms the incorporation of pyrene units into the nanoparticles. A small amount of peak broadening occurs, however it is much less compared to the $^1$H NMR spectra of NP2.2-2MA and NP2.2-3MA.

The incorporation of pyrene was also confirmed by UV-vis spectroscopy. Interestingly, while more equivalents of MA result in a slight decrease in the characteristic stilbene absorbance, there was a slight increase in the pyrene absorption between 3.6 and 6.0 equivalents of NPM (Figure 2.4b). It is possible that the inclusion of the bulky pyrene group introduces a steric limitation that overshadows the effect of concentration, which could potentially be overcome by adding spacer units between the stilbene group and the polymer backbone. We are continuing to investigate this chemistry.
Lastly, functional group incorporation was qualitatively confirmed using the fluorescent nature of the pyrene-containing SCNP by exposing 1 mg/mL THF solutions of the nanoparticles, their precursors, and their MA analogs to UV light (Figure 2.5). In accord with the previously discussed experiments, these results confirm the incorporation of electron deficient monomer units that contain functionality.

![Figure 2.5](image)

**Figure 2.5** From left to right: 1 mg/mL THF solutions of **P2.2, NP2.2-2MA, NP2.2-2NPM,** and **NP2.2-3NEM** under an ultraviolet lamp to demonstrate fluorescence of pyrene-containing SCNP

### 2.3 Conclusions

Single-chain nanoparticles were synthesized by the radical homopolymerization of pendant styrene units in linear poly(styrene-co-divinylbenzene). However, the addition of maleic anhydride to this process resulted in a loss of control and the formation of multi-chain aggregates. When the styryl groups are replaced with stilbene units, the copolymerization with maleic anhydride or N-substituted maleimides proceeds smoothly to form SCNP, the size of which can be tuned based on the concentration of the added monomer. When no additional monomer is present, the stilbene-functionalized polymer did not show signs of SCNP formation. The successful incorporation of
reactive anhydride units and modular maleimide units is promising for future work involving this system.

2.4 Experimental Section

2.4.1 Materials

4-vinylbenzyltriphenylphosphonium tetrafluoroborate (M2.1) was prepared according to literature.\textsuperscript{103} Styrene was filtered through a plug of basic alumina before use. Reagents were obtained from the indicated commercial suppliers and used without further purification unless otherwise stated: dichloromethane (Fisher Scientific), hexanes (Fisher Scientific), tetrahydrofuran (inhibited with BHT, Fisher Scientific), formaldehyde (37 wt%, stabilized with 10-15\% methanol, ACROS organics), N-ethyl maleimide (ACROS organics), N-(1-pyrene)maleimide (Sigma Aldrich), chloroform (ACROS organics), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)-sulfanyl]pentanoic acid (CTA1, Sigma Aldrich), 2,2’-azobisisobutyronitrile (Sigma Aldrich), styrene (Sigma Aldrich), potassium hydroxide (EMD Chemicals), toluene (EMD Chemicals), benzaldehyde (Alfa Aeser), alumina (activated basic, Alfa Aeser), isopropanol (Pharmco Aaper), ethanol (95\%, Pharmco Aaper), maleic anhydride (Fluka), N,N-dimethylformamide (Omnisolv), chloroform-D (Cambridge Isotope Laboratories), dimethylsulfoxide-D6 (Cambridge Isotope Laboratories).

2.4.2 Instrumentation

\textsuperscript{1}H NMR (400 MHz) spectra were recorded on a Varian Associates Mercury 400/500 spectrometer. Solvents (CDCl\textsubscript{3}, D\textsubscript{6}-DMSO) contained 0.03\% v/v TMS as an internal reference.
UV-vis spectra were obtained using a Shimadzu UV-2450 UV-vis spectrophotometer. All spectra were obtained with THF as a solvent at polymer concentrations of 0.05 mg mL\(^{-1}\).

Size exclusion chromatography (SEC) was performed on a Tosoh EcoSEC dual detection (RI and UV) SEC system coupled to an external Wyatt Technologies miniDAWN Treos multiangle light scattering (MALD) detector and a Wyatt Technologies ViscoStarII differential viscometer. Samples were run in THF at 40 °C at a flow rate of 0.35 mL min\(^{-1}\). The column set was two Tosoh TSKgel SuperMultipore HZ-M columns (4.6x150 mm), one Tosoh TSKgel SuperH3000 column (6x150mm) and one Tosoh TSKgel SuperH4000 column (6x150mm). Increment refractive index value (dn/dc) of 0.185 (polystyrene) was used for all samples. Absolute molecular weights and molecular weight distributions were calculated using the Astra 6 software package. Intrinsic viscosity \([\eta]\) and viscometric radii (R\(\eta\)) were calculated from the differential viscometer detector trace and processed using the Astra 6 software.

All polymer solutions characterized by SEC were 1.0 mg mL\(^{-1}\), and were stirred magnetically for at least 4 hours before analysis.

Qualitative fluorescence experiments were carried out by exposing polymer solutions to UV light in a dark environment using a UVGL-25 Mineralight lamp. Photographs were taken using an iPhone 6 camera.
2.4.3 Experimental Details

2.4.3.1 Synthesis of poly(styrene-co-(4-vinylbenzyltriphenylphosphonium tetrafluoroborate)) (P2.0)

Styrene (3.0 mL), M2.1 (2.2 g), and CTA1 (20 mg) were dissolved in DMF (2.75 mL) in a 10 mL Schlenk flask. A magnetic stirbar was added and the solution was sparged with argon while stirring for 20 minutes. The solution was then heated at 110 °C for 12 hours and monitored via $^1$H NMR. The solution was removed from heat, exposed to atmosphere and allowed to cool to room temperature, diluted with acetone (5 mL), precipitated twice into isopropanol, and dried under vacuum to afford a white powder (2.30 g). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.81 (br, 6.45H, Ar-H), 7.59 (br, 16.0H, Ar-H), 7.00 (br, 16.0H, Ar-H), 6.50 (br, 19.8H, Ar-H), 5.29 – 4.68 (br, 6.9H, -CH$_2$-), 2.27 – 0.57 (br, 34.8H, -CH(Ar)-CH$_2$-). See appendix page 4 for full spectrum.

2.4.3.2 Synthesis of linear poly(styrene-co-divinyl benzene) (P2.1)

$P2.0$ (0.5 g) was dissolved in DMF (13 mL) in a 20 mL scintillation vial with a magnetic stirbar. Aqueous formaldehyde (27 wt%, 0.5 mL) was added and the solution was stirred vigorously. Aqueous KOH (6M, 0.5 mL) was added, causing an orange color to appear for a brief time. The addition of KOH caused a precipitate to form nearly instantly. Over the course of an hour, the precipitate coagulated, and the DMF solution was decanted. DCM (10 mL) was added to the solid followed by 5 min of sonication. The
resulting cloudy solution was rinsed with brine (10 mL). The DCM layer was separated and concentrated under reduced pressure. The resulting residue was dissolved in a minimal volume of chloroform (~0.7 mL), precipitated into 95% ethanol, collected by filtration, redissolved and reprecipitated, and dried under vacuum (~150 mTorr) overnight to afford polymer 1 (0.20 g). $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ 7.43 – 6.82 (br, 30.9H, Ar-H), 6.50 (br, 26.4H, Ar-H), 5.63 (br, 3.8H, -CH=CH$_2$), 5.17 (br, 3.8H, -CH=CH$_2$), 1.92 (br, 12.0H, -CH(Ar)-CH$_2$), 1.40 (br, 23.1H, -CH(Ar)-CH$_2$). See appendix page 5 for full spectrum and appendix pages 1 and 25 for full SEC chromatograph.

**2.4.3.3 Synthesis of linear poly(styrene-co-(4-vinyl stilbene)) ($P2.2$, $P2.2'$)**

P2.0 (0.5 g) was dissolved in DMF (13 mL) in a 20 mL scintillation vial with a magnetic stirbar. Benzaldehyde (0.25 mL, respectively) was added and the solution was stirred vigorously. Aqueous KOH (0.5 mL, 6M) was added, causing an orange color to appear for a brief time. The addition of KOH caused a precipitate to form nearly instantly. Over the course of an hour, the precipitate coagulated, and the DMF solution was decanted. DCM (10 mL) was added to the solid followed by 5 min of sonication. The resulting cloudy solution was rinsed with brine (10 mL). The DCM layer was separated and concentrated under reduced pressure. The organic layer was concentrated under reduced pressure. The resulting residue was dissolved in a minimal volume of chloroform (~0.7 mL), precipitated into 95% ethanol, collected by filtration, redissolved and reprecipitated, and dried under vacuum (~150 mTorr) overnight to afford polymer 2 (0.32 g). $^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.50 (br, 2.1H, Ar-H), 7.35 (br, 2.2H,
Ar-H), 7.30 – 6.79 (br, 37.0H, Ar-H), 6.54 (br, 21.2H, Ar-H), 2.46 – 1.66 (br, 11.2H, (-CH(Ar)-CH₂-), 1.66 – 0.97 (br, 26.1H, (-CH(Ar)-CH₂-). See appendix page 6 for full spectrum and appendix pages 2, 25, and 26 for full SEC chromatograph.

2.4.3.4 Preparation of NP2.1-0

\[
\begin{align*}
\text{P2.1} & \quad \text{AIBN} \\
\text{toluene} & \quad 80^\circ \text{C} \\
\text{NP2.1-0} &
\end{align*}
\]

\(\text{P2.1} (50 \text{ mg})\) was dissolved in 50 mL toluene. AIBN (1 mg) was added to the solution along with a magnetic stirbar in a 100 mL round-bottom flask. The flask was fitted with a rubber septum. The solution was degassed via sparging with argon for 20 minutes then heated under argon at 80 °C overnight. The solution was then allowed to cool to ambient temperature, exposed to air, concentrated, taken up in a minimal volume (~0.7 mL) of chloroform, precipitated into hexanes, collected by filtration and dried under vacuum at 50 °C for 2 hours. Recoveries were typically 45-60 mg. \(^1\)H NMR (500 MHz, Chloroform-\(d\)) \(\delta\) 7.24 (br, 35.7H, Ar-H), 6.58 (br, 18.9H, Ar-H), 5.66 (br, 1.5H, -CH=CH₂), 5.40 – 4.85 (br, 1.2H, -CH=CH₂), 3.63 – 1.68 (br, 19.2H, (-CH(Ar)-CH₂-), 1.68 – 0.16 (br, 23.5H, (-CH(Ar)-CH₂-). See appendix page 7 for full spectrum and appendix pages 1 and 26 for full SEC chromatograph.

2.4.3.5 Preparation of NP2.1-1MA and NP2.1-2MA

\[
\begin{align*}
\text{P2.1} & \quad \text{AIBN} \\
\text{toluene} & \quad 80^\circ \text{C} \\
\text{NP2.1-nMA} &
\end{align*}
\]

\(\text{P2.1} (50 \text{ mg})\) was dissolved in 50 mL toluene. Maleic anhydride (MA) (8, 24 mg, respectively) and AIBN (1 mg) were added to the solution along with a magnetic stirbar in a 100 mL round bottom flask. The flask was fitted with a rubber
septum. The solution was degassed via sparging with argon for 20 minutes then heated under argon at 80 °C overnight. The solution was then allowed to cool to ambient temperature, exposed to air, concentrated, taken up in a minimal volume (~0.7 mL) of chloroform, precipitated into hexanes, collected by filtration and dried under vacuum at 50 °C for 2 hours. Recoveries were typically on the order of 45-60 mg. See appendix pages 1 and 27 for full SEC chromatograph.

2.4.3.6 Preparation of NP2.2‘-0

P2.2‘ (50 mg) was dissolved in 50 mL toluene. AIBN (1 mg) was added to the solution along with a magnetic stirbar in a 100 mL round bottom flask. The flask was fitted with a rubber septum. The solution was degassed via sparging with argon for 20 minutes then heated under argon at 80 °C overnight. The solution was then allowed to cool to ambient temperature, exposed to air, concentrated, taken up in a minimal volume (~0.7 mL) of chloroform, precipitated into hexanes, collected by filtration and dried under vacuum at 50 °C for 2 hours. Recoveries were typically 45-60 mg. See appendix pages 2 and 28 for full SEC chromatograph.

2.4.3.7 Preparation of NP2.2-1MA, NP2.2-2MA, NP2.2-3MA, and NP2.2-4MA

P2.2 (50 mg) was dissolved in 50 mL toluene. Maleic anhydride (8, 24, 40, 80 mg, respectively) and AIBN (1 mg) were added to the solution along with a magnetic stirbar in a 100 mL round bottom flask. The flask was fitted with a rubber
septum. The solution was degassed via sparging with argon for 20 minutes then heated under argon at 80 °C overnight. The solution was then allowed to cool to ambient temperature, exposed to air, concentrated, taken up in a minimal volume (~0.7 mL) of chloroform, precipitated into hexanes, collected by filtration and dried under vacuum at 50 °C for 2 hours. Recoveries were typically 45-60 mg. $^1$H NMR (400 MHz, Chloroform-\textit{d}) $\delta$ 7.51 (b, 2.7H, Ar-H), 7.35 (b, 2.9H, Ar-H), 7.15 (b, 34.4H, Ar-H), 6.53 (b, 22.7H, Ar-H), 3.61 – 1.67 (b, 16.2H, -CH(Ar)-CH$_2$-), 1.67 – 0.12 (b, 21.2H, -CH(Ar)-CH$_2$-). See appendix page 8 for full spectrum and appendix pages 2, 28, 29, and 30 for full SEC chromatograph.

2.4.3.8 Preparation of NP2.2-1NEM, NP2.2-2NEM, and NP2.2-3NEM

\begin{center}
\begin{tikzpicture}
  \node (P2.2) at (0,0) {P2.2};
  \node (NP2.2-nNEM) at (2,0) {NP2.2-nNEM};
  \draw[->] (P2.2) -- (NP2.2-nNEM) node[midway, above] {AIBN};
  \draw[->] (P2.2) -- (NP2.2-nNEM) node[midway, right] {toluene 80 °C};
\end{tikzpicture}
\end{center}

\textbf{P2.2} (50 mg) was dissolved in 50 mL toluene. N-ethyl maleimide (10, 30, 50 mg, respectively) and AIBN (1 mg) were added to the solution along with a magnetic stirbar in a 100 mL round bottom flask. The flask was fitted with a rubber septum. The solution was degassed via sparging with argon for 20 minutes then heated under argon at 80 °C overnight. The solution was then allowed to cool to ambient temperature, exposed to air, concentrated, taken up in a minimal volume (~0.7 mL) of chloroform, precipitated into hexanes, collected by filtration and dried under vacuum at 50 °C for 2 hours. Recoveries were typically 45-60 mg. $^1$H NMR (500 MHz, Chloroform-\textit{d}) $\delta$ 7.47 (b, 4.9H, Ar-H), 7.34 (b, 4.4H, Ar-H), 7.29 – 6.74 (32.7, 11H, Ar-H), 6.52 (b, 117H, Ar-H), 4.19 – 2.49 (b, 6.55H, Et), 2.49 – 1.68 (b, 11.9H, -CH(Ar)-CH$_2$-), 1.68 –
0.11 (b, 22.5, -CH(Ar)-CH2-). See appendix page 9 for full spectrum and appendix pages 3, 30, and 31 for full SEC chromatograph.

2.4.3.9 Preparation of NP2.2-2NPM and NP2.2-3NPM

![Diagram](image)

**P2.2** (50 mg) was dissolved in 50 mL toluene. N-(1-pyrene)maleimide (73 or 121 mg, respectively) and AIBN (1 mg) were added to the solution along with a magnetic stir bar in a 100 mL round bottom flask. The flask was fitted with a rubber septum. The solution was degassed via sparging with argon for 20 minutes then heated under argon at 80 °C overnight. The solution was then allowed to cool to ambient temperature, exposed to air, concentrated, taken up in a minimal volume (~0.7 mL) of chloroform, precipitated twice into pentane and once into acetone, and dried under vacuum for 2 hours. Recoveries were typically 20-30 mg. $^1$H NMR (400 MHz, Chloroform-$d$) δ 8.09 (b, 3.5H, pyrene), 7.53 (b, 2.3H, Ar-H), 7.37 (b, 2.5H, Ar-H), 7.05 (b, 34.9H, Ar-H), 6.56 (b, 21.2H, Ar-H), 1.86 (b, 10.8H, -CH(Ar)-CH2-), 1.44 (b, 24.9H, -CH(Ar)-CH2-). See appendix page 10 for full spectrum.
CHAPTER 3 SYNTHESIS OF LOW DISPERSITY ADMET POLYMERS USING A SINGLE-CHAIN POLYMER TEMPLATE

3.1 Introduction

Using a template to control the spatial arrangement of monomers during a polymerization process sometimes results in polymers with properties that are otherwise unattainable in standard bulk or solution polymerizations. Use of a template potentially affects polymerization kinetics, molecular weight, molecular weight distributions, tacticity, and monomer reactivity ratios. In some cases, the template is a polymer that is involved in non-covalent interactions with the monomer units, resulting in reversible self-assembly and polymerization of the monomer along the template (not necessarily in that order). Alternatively, the monomer is covalently bound to the template, which leads to a double stranded polymer. In order to isolate the templated daughter polymer, the covalent connection between it and the template must be labile under certain conditions. This process is depicted in Figure 3.1. These strategies are applicable to both chain-growth and step-growth monomers.

The latter strategy is reminiscent of recent reports involving intramolecular cross-linking of polymers using the polymerization of pendant monomer units – indeed, the two strategies are analogous, with different goals in mind. Reports include the synthesis and subsequent cross-linking of polymers functionalized with vinyl monomers such as acrylates, methacrylates, styrenes, and stilbenes. The vinyl-functionalized polymers are synthesized either by direct polymerization of a vinyl-functionalized monomer using a non-radical polymerization technique, or by using post-polymerization modification strategies to incorporate pendant vinyl units. In all
the aforementioned cases, the vinyl-functionalized polymers are intramolecularly cross-linked using radical polymerization. Additionally, the cationic polymerization of polymer-pendant epoxide units has been effected in a similar strategy. Each of these examples involves the chain-growth polymerization of monomer units that are covalently attached to a polymer chain in dilute solution.

Pyun and coworkers reported the synthesis of 3,4-propylenedioxythiophene-functionalized polystyrene, which was intramolecularly cross-linked via oxidative step-growth polymerization. The ester linkage between the polystyrene backbone and the daughter polythiophene was subsequently reductively degraded, separating the two polymers. While the mixture was analyzed by SEC, the polymers were not preparatively separated and analyzed individually, possibly due to their similar solubilities.

Ke et al. used a similar strategy for the templated synthesis of low dispersity poly(m-phenylene-vinylene) and poly(benzofuranylene-ethylene). The step-growth monomers of interest were attached to a norbornene-based monomer, which was polymerized via ring-opening
metathesis polymerization (ROMP). Due to the sensitivity of the step-growth monomers to Grubbs’ catalyst, a protection strategy was necessary.

Based on these previous reports, we thought it would be interesting to take advantage of this strategy – the templated polymerization of step-growth monomers mediated by the manipulation of single chains in dilute solution – to synthesize low dispersity precision polyolefins using acyclic diene metathesis (ADMET) polymerization.

In the presence of metathesis catalysts, primary olefins are capable of producing dimers in high yield while releasing ethylene as a by-product.\textsuperscript{111,112} In ADMET polymerization, this principle is applied to acyclic dienes with sufficient space between each olefin to prevent the formation of cyclic products.\textsuperscript{113} ADMET polymerization has proven to be a useful tool for the synthesis of sequence defined polymers with no microstructural defects; specifically, for the synthesis of regularly substituted polyethylene analogs. This regular functional group placement results in unique properties that are not accessible in materials produced by other techniques. ADMET polymers have potential applications ranging from silicon-related surface modification and biological applications, in addition to providing valuable insight into the properties of one of the most widely used commercial polymers.\textsuperscript{114}

In order to drive off ethylene and reach high monomer conversion, ADMET polymerization requires the combination of heat, long reactions times (ca. 72 hours), and use of vacuum. In addition, the statistical nature of step-growth polymerization results in relatively high dispersity with no molecular weight control compared to controlled chain growth polymerizations, based on Carothers equation (Equation 3.1 and Equation 3.2).

\textbf{Equation 3.1}

\[
\bar{M}_n = M_0 \frac{1}{1 - p}
\]
Equation 3.2
\[
\bar{M}_w = M_0 \frac{1 + p}{1 - p}
\]
Where \(M_0\) is the molar mass of a monomer unit and \(p\) is the monomer conversion, defined as

Equation 3.3
\[
p = \frac{N_0 - N}{N_0}
\]
where \(N_0\) is the number of monomers initially present and \(N\) is the number of monomers present at a certain time.

For a step-growth polymerization, expressions for the number and weight average molecular weights are given by Equation 3.1 and Equation 3.2, respectively. Based on these expressions, very high monomer conversion (>99%) is required to achieve high molecular weight polymer. The dispersity is defined as the ratio of these two molecular weights, given in Equation 3.4. From this expression, as conversion grows, the dispersity approaches 2. Since very high conversion is a necessity to polymer formation, the dispersity of a polymer synthesized by a step-growth polymerization technique that obeys Carothers equation is equal to 2. These factors have potential for improvement through the use of template polymerization, making ADMET ideal for the focus of this work.

Equation 3.4
\[
D = \frac{\bar{M}_w}{\bar{M}_n} = 1 + p
\]

Thereby, we set out to design a polymer system where each monomer unit was functionalized with an ADMET monomer. Since ADMET polymerizations involve olefins, the polymerization of a bifunctional ADMET monomer using a controlled radical polymerization technique or ROMP necessitates a protection strategy. Alternatively, a non-interfering controlled
polymerization technique is considered, as seen in Scheme 3.1a, as well as a post-polymerization modification strategy, as seen in Scheme 3.1b. In both cases, a cleavable unit must be present in order to efficiently separate the daughter polymer from the template.

**Scheme 3.1** a) Ring opening polymerization of epoxide, followed by templated ADMET and hydrolysis; b) post-polymerization furan-maleimide Diels-Alder followed by templated ADMET and reverse Diels-Alder.

The selective controlled ring-opening polymerization of glycidyl methacrylate was previously reported.\textsuperscript{115} This technique does not interfere with the vinyl unit of the methacrylate, which is potentially promising for the incorporation of an ADMET monomer unit, or the ester unit, which could be used as a hydrolytically cleaved linker, which has been employed successfully in previous reports.\textsuperscript{110} Additionally, as an acid-containing derivative of polyethylene glycol, the template polymer will likely be water soluble after cleavage of the ester linkage, while the daughter ADMET polymer will not be, resulting in easy separation.
M3.3 was synthesized by the esterification of 1,10-undecadien-6-ol with bromoacetyl bromide, followed by a Williamson ether synthesis with glycidol. The polymerization of M3.3 using the aforementioned epoxide ring-opening polymerization technique proceeds smoothly to form polymer P3.1 with narrow dispersity. Next, P3.1 is exposed to Grubbs’ 1st generation catalyst under dilute conditions, resulting in the intramolecular polymerization of pendant ADMET monomer units in the polymer. Notably, the reaction reached completion within 8 hours in refluxing DCM, with nearly complete disappearance of the terminal olefin peak by $^1$H NMR. This is in pleasant contrast to a typical ADMET procedure.

The $^1$H NMR spectrum of the parent polymer, P3.1, contained sharp peaks, in stark contrast to the corresponding NP3.1, for which the peaks are extremely broad (see page 14 and page 15, respectively). These broad peaks are caused by an enhancement of T2 relaxation time, which is a result of restricted molecular motion caused by cross-linking, as previously mentioned in Chapter 2. In addition, there is an increase in retention time after the templated ADMET polymerization step, corresponding to a decrease in size, as seen in Figure 3.2a. The dispersity of the polymer also appears to increase slightly, changing from a very narrow peak to a slightly more broad one (Figure 3.2b). However, despite this apparent change, the dispersity of NP3.1 is very close to 1 according to SEC results. Certainly, there is a small decrease in molecular weight due to the loss of ethylene, but it is more likely that the apparent change is a result of a change in the size distribution, resulting from varying amounts of ADMET polymerization taking place in each polymer.

Hydrolysis of the ester unit of NP3.1 (depicted in Scheme 3.1a) proceeds efficiently to produce daughter polymer P3.2. After the ester is cleaved, the daughter polymer is easily separated.
from the mixture by precipitation into water. By SEC, the daughter polymer has a retention time that is close to P3.1, and the $^1$H NMR is comparable to literature.\footnote{116} However, the dispersity of P3.2 is 2.04, which is the value expected of a non-templated statistical step-growth polymerization. This suggests that the use of a template did not affect the molecular weight distribution in this case. This was confirmed by the synthesis of structurally similar P3.3 using ADMET polymerization and subsequent SEC analysis, summarized in Figure 3.2a. The distribution of the ADMET polymer P3.3 is close to that of daughter polymer P3.2. In addition, there is a shoulder at longer retention time, possibly corresponding to low molecular weight ADMET polymers. This could be a result of the flexibilities of the template and daughter polymer backbones. These polymer backbones are relatively flexible compared to previously mentioned reports.\footnote{110} More flexibility leads to more conformational freedom, which potentially leads to the reaction of non-adjacent monomer units. In addition, when comparing the lengths of each monomer unit in the parent and daughter polymers (based on number of atoms across each backbone), the daughter polymer is longer, though we did not initially anticipate this issue based on polymer flexibilities.

Although the template did not affect the molecular weight distribution, it did appear to have an affect on the molecular weight. Based on SEC data, the number average molecular weight ($M_n$) of P3.1 is 13.3 kDa, which corresponds to a number average degree of polymerization ($DP_n$) of 47. Thereby, complete conversion of pendant ADMET monomer units corresponds to a theoretical $M_n$ of 7.9 kDa for P3.2. The molecular weight for P3.2 was calculated using both SEC and $^1$H NMR results. These results are summarized in Table 3.1. The molecular weight by SEC is much lower than expected, which may be the result of error in $dn/dc$ calculation. However, by $^1$H NMR, the $M_n$ is 6.5 kDa, which corresponds to a $DP_n$ of 39. This value is quite close to the $DP_n$ of the parent polymer, suggesting that the template is capable of dictating the degree of polymerization
of the daughter polymer. In order to confirm this result, parent polymers of differing molecular weights need to be synthesized and processed similarly.

**Table 3.1** SEC and molecular weight data for templated ADMET polymers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$ (kDa)</th>
<th>$M_w$ (kDa)$^a$</th>
<th>$D^c$</th>
<th>$[\eta]$ (mL/g)$^d$</th>
<th>MHS $\alpha^e$</th>
<th>$dn/dc^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3.1</td>
<td>13.3$^a$</td>
<td>13.6</td>
<td>1.02</td>
<td>14.3</td>
<td>0.96</td>
<td>0.116</td>
</tr>
<tr>
<td>NP3.1</td>
<td>14.9$^a$</td>
<td>15.3</td>
<td>1.02</td>
<td>12.7</td>
<td>1.00</td>
<td>0.171</td>
</tr>
<tr>
<td>P3.2</td>
<td>2.6$^a$, 6.5$^b$</td>
<td>5.31</td>
<td>2.04</td>
<td>7.4</td>
<td>0.35</td>
<td>0.077</td>
</tr>
<tr>
<td>P3.3</td>
<td>13$^b$</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

$^a$ Obtained from triple detection SEC. See experimental section for more details  
$^b$ Obtained from $^1$H NMR  
$^c$ $M_w/M_n$  
$^d$ Obtained from viscometric SEC data. See experimental section for more details  
$^e$ Mark-Houwink Sakurada $\alpha$-value  
$^f$ Calculated using ASTRA software. See experimental section for more details

**Figure 3.2** a) SEC-MALS traces for low-dispersity ADMET-monomer-functionalized polymer P3.1 before and after ADMET polymerization, and after separation of template and daughter polymers, b) comparison of SEC traces for templated and non-templated ADMET polymers
For our post-polymerization modification strategy, we investigated the reversible Diels-Alder reaction between furan and N-substituted maleimides. At lower temperatures (ca. 60 °C) the DA adduct is formed, and at higher temperatures (ca. 120 °C) the cycloreversion is preferred. Low dispersity poly(furfuryl methacrylate) is easy to synthesize from commercially available starting materials using RAFT polymerization. The maleimide-functionalized ADMET monomer M3.5 was synthesized by the DCC coupling reaction between 1,10-undecadien-6-ol and 4-maleimidobenzoic acid. We proposed that this monomer would add to PFMA at low temperature, followed by ADMET polymerization in dilute solution, and cleavage of the daughter polymer from the template by reverse DA at high temperature. While we did not expect these polymers to be separable based on solubility, we thought this initial strategy would be promising as a proof of concept: even if the polymers are not separable in a preparative sense, they may be resolvable by GPC or DOSY. Interestingly, heating PFMA in the presence of M3.5 in THF resulted in gelation. Furan typically only reacts with electron deficient olefins, or in some intramolecular reactions, alkyl-substituted olefins.117 While unexpected, it is possible that the ADMET olefin units were also involved in Diels-Alder additions with unreacted furan units, resulting in a cross-linked network, so it is possible the polymer environment provided by the template enhanced their reactivity. Consequently, this approach may be better suited to a different type of step-growth chemistry.
Scheme 3.2 Possible Diels-Alder reaction between PFMA and terminal olefin to produce cross-linked polymer gel

3.3 Conclusions

Two strategies were attempted toward the templated ADMET polymerization of monomers derived from 1,10-undecadien-6-ol. Both strategies were based on the polymerization of ADMET monomers attached to low-dispersity polymers in dilute solution, synthesized by controlled chain growth polymerization techniques. The first strategy involved the direct polymerization of an epoxide monomer that was attached to an ADMET monomer through a cleavable ester linkage. The successful polymerization of this monomer confirmed the versatile nature of the polymerization technique. The subsequent templated ADMET polymerization was performed successfully, and resulted in a daughter polymer with controlled molecular weight based on the DP of the parent polymer. It is possible that subtle changes to the monomer structure, polymer structure, or the conditions of the templated polymerization may result in control over the molecular weight distribution.

A second strategy was based on the post-polymerization addition of an ADMET monomer to low dispersity poly(furfuryl methacrylate) using the reversible maleimide/furan Diels Alder
cycloaddition. However, exposing the monomer and polymer to Diels Alder conditions resulted in an insoluble gel, possibly due to the undesired cycloaddition of the pendant olefin units to the furan groups.

### 3.4 Experimental

#### 3.4.1 Materials

Reagents were obtained from the indicated commercial suppliers and used without further purification unless otherwise stated: dichloromethane (Fisher Scientific), hexanes (Fisher Scientific), tetrahydrofuran (inhibited with BHT, Fisher Scientific), chloroform (ACROS organics), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)-sulfanyl]pentanoic acid (CTA1, Sigma Aldrich), 2,2’-azobisisobutyrlnitrile (Sigma Aldrich), sodium hydroxide (EMD Chemicals), toluene (EMD Chemical), ethanol (95%, Pharmco Aaper), maleic anhydride (Fluka), bromoacetyl bromide (Alfa Aeser), diethyl ether (Pharmco Aaper), acetic acid (EMD chemicals), tetraoctylammonium bromide (Sigma Aldrich), triisobutyl aluminum (1.1 M in toluene, Acros Organics), glycidol (Acros Organics), 4-dimethylamino pyridine (Sigma Aldrich), sodium hydride (60% dispersion in mineral oil, Sigma Aldrich), pentane (JT Baker), methanol (Pharmco Aaper), Grubbs 1st generation catalyst (Materia), ethyl vinyl ether (Sigma Aldrich), 1-bromo-4-pentene (Matrix Scientific), ethyl formate (Sigma Aldrich), 4-aminobenzoic acid (Sigma Aldrich), magnesium (Sigma Aldrich), chloroform-D (Cambridge Isotope Laboratories). 4-Maleimidobenzoic acid was prepared according to literature.
3.4.2 Instrumentation

$^1$H NMR (400 MHz) spectra were recorded on a Varian Associates Mercury 400/500 spectrometer. Solvents (CDCl$_3$, D$_6$-DMSO) contained 0.03% v/v TMS as an internal reference.

Size exclusion chromatography (SEC) was performed on a Tosoh EcoSEC dual detection (RI and UV) SEC system coupled to an external Wyatt Technologies miniDAWN Treos multiangle light scattering (MALD) detector and a Wyatt Technologies ViscoStarII differential viscometer. Samples were run in THF at 40 ºC at a flow rate of 0.35 mL min$^{-1}$. The column set was two Tosoh TSKgel SuperMultipore HZ-M columns (4.6x150 mm), one Tosoh TSKgel SuperH3000 column (6x150mm) and one Tosoh TSKgel SuperH4000 column (6x150mm). Increment refractive index value (dn/dc) of 0.185 (polystyrene) was used for all samples. Absolute molecular weights and molecular weight distributions were calculated using the Astra 6 software package. Intrinsic viscosity [$\eta$] was calculated from the differential viscometer detector trace and processed using the Astra 6 software.

All polymer solutions characterized by SEC were 1.0 mg mL$^{-1}$, and were stirred magnetically for at least 4 hours before analysis.

3.4.3 Experimental Details

3.4.3.1 Synthesis of acetyl bromide ADMET ester M3.2

Bromoacetyl bromide (5.5 mL) and DCM (180 mL) were cooled and stirred under argon in a 500 mL 3-neck flask using a salt/ice bath. DMAP (0.70 g) was added, followed by dropwise
addition of a solution of M3.1 (10.69 g, 6.3.5 mmol) and triethylamine (9.0 mL) in DCM (100 mL) via addition funnel. The mixture was then allowed to stir for 16 hours at ambient temperature and concentrated under reduced pressure. Ether was added and the insoluble solids were removed by vacuum filtration. The ether layer was concentrated under reduced pressure, and the resulting brown oil was purified by column chromatography with 19:1 hexanes/diethyl ether as eluent to produce M3.2 as a colorless oil (7.57 g, 26.2 mmol, 41%).\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 5.77 (m, 2.06H, =CH-), 4.98 (m, 5.33H, =CH\textsubscript{2}, -CH-O-), 3.81 (s, 2.0H, -CH\textsubscript{2}-Br), 2.06 (m, 4.78H, -CH\textsubscript{2}-), 1.58 (m, 4.67H, -CH\textsubscript{2}-), 1.43 (m, 4.73H, -CH\textsubscript{2}-). See appendix page 11 for full spectrum.

3.4.3.2 Synthesis of glycidol ether ADMET ester M3.3

\[\begin{align*}
\text{M3.2} & \xrightarrow{\text{NaH, THF}} \text{M3.3}
\end{align*}\]

Sodium hydride (60% dispersion in mineral oil, 1.16 g, 28.7 mmol) was added to a solution of glycidol (1.6 mL, 1.86 g, 25.2 mmol) and tetrahydrofuran (30 mL) under argon. The resulting mixture was allowed to stir for 2 hours, then M3.2 (7.57 g) was added and the stirring was continued for an additional 10 hours. Acetic acid (1.7 mL) and diethyl ether (50 mL) were added, and the solution was washed with water (50 mL) followed by saturated sodium bicarbonate (50 mL). The organic layer was dried over sodium sulfate, concentrated under reduced pressure, and filtered through silica with diethyl ether as eluent, concentrated under reduced pressure, and purified by column chromatography with hexanes/ethyl ether (7:3) as eluent to afford M3.3 as a colorless oil (2.8 g, 39%). Alternatively, the compound was purified by fractional vacuum distillation (120 °C, 60 mTorr). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 5.78 (m, 2.0H, =CH-), 4.98 (m, 5.28H, =CH\textsubscript{2}), 4.14 (m, 2.23H, -O-CH\textsubscript{2}-CO\textsubscript{2}-), 3.92 (d, 1.07H, -O-CH\textsubscript{2}-), 3.50 (dd, 1.02H, -O-
CH₂), 3.21 (m, 0.94H, -CH-O-), 2.82 (t, 1.04H, -O-CH₂-), 2.64 (dd, 1.04H, -O-CH₂-), 2.04 (m, 4.52H, -CH₂-), 1.56 (m, 5.38H, -CH₂-), 1.40 (m, 4.82H, -CH₂-). ¹³C NMR (101 MHz, CDCl₃) δ 170.19, 138.49, 115.08, 75.07, 72.27, 68.65, 50.79, 44.26, 33.66, 24.71. See appendix pages 12 and 13 for full spectra.

3.4.3.3 Synthesis of P3.1 - Epoxide ring opening polymerization

\[
\begin{align*}
&\text{M3.3 (1.3 g)} \quad \text{and dry toluene (1.2 mL) were added to a dry 10 mL Schlenk flask under argon and cooled in a bath containing an ethylene glycol/ethanol (70:30) dry ice mixture. A solution of tetraoctylammonium bromide in toluene (0.22 M, 0.22 mL) was added followed by a solution of triisobutyl aluminum in toluene (1.1 M, 0.11 mL). The solution was then allowed to stir at ambient temperature for 2 hours before 2 drops of 95% ethanol were added. The resulting liquid was concentrated under reduced pressure, dissolved in diethyl ether, precipitated into cold methanol, and stored in a freezer for several hours. The methanol was decanted and the remaining residue was transferred to a vial with DCM, concentrated under reduced pressure, and dried under vacuum overnight to afford P3.1 as a highly viscous clear oil (0.81 g). } \\
&\text{¹H NMR (400 MHz, Chloroform-d) } \delta 5.76 \text{ (m, 1H, –CH–), 4.94 (m, 2.59H, –CH₂, -O-CH–), 4.10 (m, 1.07H, -O-CH₂-CO₂–), 3.74-3.57 (m, 2.71H, -CH₂-CH(CH₂-O)-), 2.04 (m, 2.04H, -CH₂–), 1.54 (m, 2.14H, -CH₂–), 1.40 (m, 2.22H, -CH₂–). See appendix page 14 for full spectrum and appendix pages 3 and 32 for full SEC chromatograph.}
\end{align*}
\]
3.4.3.4 Synthesis of NP3.1 - Templated ADMET polymerization

A solution of P3.1 (50 mg) in DCM (100 mL) was sparged with argon for 30 minutes. Grubbs 1st gen. catalyst (14 mg) was added and the solution was heated at 50 °C for 8 hours. Ethyl vinyl ether (0.8 mL) was added and the solution was allowed to cool to ambient temperature. After two hours, the solution was concentrated under reduced pressure, dissolved in a small amount of DCM, and flashed through a plug of silica with additional DCM to ensure complete elution of the polymer. The resulting solution was concentrated under reduced pressure. Hexanes was added and the polymer precipitated upon shaking. The solid was isolated and dried under vacuum overnight to afford NP3.1 (34 mg, 71%). 1H NMR (400 MHz, CDCl3) δ 5.76 (br, 0.03H, =CH-), 5.36 (br, 3.93H, -CH=CH-), 4.97 (br, 2.0H, -O-CH-), 4.11 (br, 4.01H, -O-CH2-CO2-), 3.64 (br, 9.54H, -CH2-CH(CH2-O)-), 1.99 (br, 8.44H, -CH2-), 1.54 (br, 8.80H, -CH2-), 1.35 (br, 11.62H, -CH2-). See appendix page 15 for full spectrum and appendix pages 3 and 32 for full SEC chromatograph.
3.4.3.5 Synthesis of $\textbf{P3.2}$ – separation of template and daughter polymer

NP3.1 (26 mg), THF (1.0 mL), methanol (0.5 mL), and sodium hydroxide (20 mg) were heated in a scintillation vial at 50 °C for 12 hours. Water (15 mL) was added and the precipitate was collected by filtration and solvent transfer with chloroform and concentrated under reduced pressure. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.82 (m, 2.0H, =CH-), 5.40 (br, 79.19H, -CH=CH-), 4.99 (m, 4.88H, =CH$_2$), 3.58 (br, 38.66H, -O-CH-), 2.17-0.96 (br, 813.22H, -CH$_2$-CH$_2$-CH$_2$-). See appendix page 16 for full spectrum and appendix pages 3 and 33 for full SEC chromatograph.

3.4.3.6 Synthesis of $\textbf{P3.3}$

M3.4 (0.5 g) was added to a dry Schlenk flask with a stirbar, exposed to vacuum for 20 minutes, and back-filled with argon. Under a blanket of argon, Grubbs’ 1$^{\text{st}}$ generation catalyst was added (7.3 mg). The solution was then exposed to vacuum for 24 hours, back-filled with argon, and heated at 76 °C for three days. The resulting viscous liquid was precipitated into methanol and dried under vacuum to afford P3.3. $^1$H NMR (400 MHz, CDCl$_3$) δ 5.77 (m, 1.0H, =CH-), 5.36 (m, 128.80H, -CH=CH-), 4.98 (m, 4.18H, =CH$_2$) 4.87 (br, 64.10H, -O-CH-), 2.04-1.96 (m, 448.14H, -C(O)CH$_3$, -CH$_2$-), 1.51 (m, 288.09H, -CH$_2$-), 1.33 (m, 261.74H, -CH$_2$-). See appendix page 17 for full spectrum and appendix pages 3 and 33 for full SEC chromatograph.
3.4.3.7 Synthesis of poly(furfuryl methacrylate) (P3.4)

Furfuryl methacrylate (0.59 g), CTA1 (6.2 mg), AIBN (0.25 mg) and toluene (0.5 mL) were added to a Schlenk flask under argon and sparged for 20 min. The reaction was heated at 80 °C for 9 hours.

The solution was exposed to air and diluted with toluene (2 mL) then precipitated twice into methanol to afford polyfurfuryl methacrylate, P3.4 (303 mg). $^{1}$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.41 (s, 1.0H, furan), 6.34 (m, 2.07H, furan), 4.91 (m, 2.18H, -CH$_2$-), 1.98-1.65 (b, 2.20H, -CH$_3$), 1.52-1.04 (br, 0.57H), 0.87 (br, 1.18H, -CH$_2$-), 0.71 (br, 1.18H, -CH$_2$-). See appendix page 18 for full spectrum.

3.4.3.8 Synthesis of M3.5

1,10-undecadien-6-ol (0.22 g), 4-maleimidobenzoic acid (0.22 g), DMAP (8 mg), and DCM (3 mL) were added to a dry 3 neck flash under argon. DCC (0.25 g) was added and the reaction was stirred for 32 hours. The resultant mixture was concentrated under reduced pressure and purified by column chromatography with 4:1 hexanes/ethyl acetate as eluent. The resulting solid was mixed with cold pentane (0.8 mL) and filtered twice to remove grease, producing M3.5 (90 mg). $^{1}$H NMR
(500 MHz, CDCl$_3$) $\delta$ 8.16 (d, 2.26H, Ar-H), 7.48 (d, 2.21H, Ar-H), 6.89 (s, 2.0H, maleimide), 5.77 (m, 2.14H, =CH-), 5.16 (p, 1.17H, -O-CH-), 4.97 (m, 4.47H, =CH$_2$), 2.05 (m, 5.35H, -CH$_2$-), 1.69 (m, 5.34H, -CH$_2$-), 1.48 (m, 5.06H, -CH$_2$-). See appendix page 19 for full spectrum.

3.4.3.9 Diels-Alder addition of ADMET maleimide to poly(furfuryl methacrylate)

ADMET maleimide (220 mg), poly(furfuryl methacrylate) (100 mg), and THF (3 mL) were heated in a 10 mL Schlenk flask under argon for 12 hours. Over the course of this time, the viscosity gradually increased until gelation occurred. An excess of THF was added, but the gel remained intact.
CHAPTER 4 SYNTHESIS OF ACETAL-CONTAINING SINGLE-CHAIN NANOPARTICLES

4.1 Introduction

Nature’s ability to fabricate advanced functional nanostructures is the result of pristine primary polymer structures, which lead to the formation of secondary, tertiary, and quaternary structures that are capable of undertaking complex tasks. In other words, in the appropriate chemical and physical environment, primary structure allows these polymers to fold in a controlled manner into an exact shape, with specific functionality placed in precise locations, both on the interior and exterior of the structure. The interactions that cause this folding to take place include non-covalent interactions, such as hydrogen bonding and metal coordination, and dynamic covalent interactions, such as disulfide formation.

The synthesis of single-chain nanoparticles involves the manipulation of single polymer chains in highly dilute solution. Taking inspiration from nature, it is advantageous to consider the formation of SCNP using chemistry that is reversible based on chemical environment. In addition, the use of multiple orthogonal cross-linking strategies in a single polymer system is potentially interesting, as discussed previously. While nature’s choice of functional groups is limited to a library of nucleic acids, amino acids, and carbohydrates, synthetic polymer chemists have access to a much larger variety of monomers and functional groups. Therefore, in order to complete the synthesis of hierarchical nanostructures via the manipulation of single polymer chains, it is necessary to first investigate useful and modular synthetic methodologies that can potentially be combined with one another in an orthogonal fashion.
Dynamic covalent chemistry is the study of covalent interactions that are reversible under a certain set of reaction conditions. There are a number of reports involving the synthesis of SCNP using dynamic covalent chemistry, including enamine formation and hydrazone formation. In each of these examples, the cross-links are formed by the addition of a bifunctional cross-linker. These cross-links are stable at high pH, but reversible at low pH. This effect is demonstrated by the addition of a mono-functional unit at both high and low pH. At high pH, when the bonds are not reversible, the mono-functional unit has no effect; however, when added at low pH, there is dynamic exchange between the mono-functional unit and the cross-links in the SCNP, resulting in unfolding to the linear polymer. A recent report also highlights the dynamic covalent nature of disulfide chemistry using similar principles. However, there are no recent examples of SCNP containing acetal cross-links.

In 1962, Kuhn and Balmer reported the intramolecular cross-linking of poly(vinyl alcohol) using terephthaldehyde in the presence of an acid catalyst. A similar report from 1985 uses glutaraldehyde instead. While there are instances of using degradable acetal units in other polymer related work, acetals have remained untouched in SCNP literature since these initial reports. Expanding this work to other polymer backbones that are more soluble and easier to synthesize in a modular fashion would allow for the inclusion of additional functional units and for the combination with other orthogonal cross-linking strategies.

4.2 Results and discussion

In order to synthesize SCNP containing acetal cross-links, we first set out to synthesize aldehyde- and diol-functionalized monomers. These included methacrylate and styrene derivatives.
For initial attempts, diol monomers M4.2 and M4.9 were protected with an acetonide group to avoid polymer solubility issues related to hydrogen bonding during polymerization. The protecting group is subsequently removed by aqueous hydrolysis. The synthesis of copolymers containing both functionalities leads to a polymer that will form acetal cross-links in the presence of an acid catalyst. Alternatively, polymer synthesis involving only one functionality leads to a polymer that will form cross-links with the addition of an external cross-linker. For example, a diol-functionalized polymer is cross-linked in the presence of terephthaldehyde, similar to the previously discussed report. These cross-linking strategies are portrayed in Figure 4.1.

Initially, P4.1 and P4.2 were synthesized by RAFT polymerization and each was subjected to hydrolysis conditions to remove the acetonide group. However, in both cases, the acetonide group showed no sign of removal based on $^1$H NMR analysis. This was likely a result of the hydrophobic nature of these polymers, which were only marginally soluble in the THF/H$_2$O mixture used for hydrolysis.
Moving forward, poly(ethylene glycol) methacrylate (PEGMA) was copolymerized with MMA and M4.2 to afford P4.3 in an attempt to mitigate issues with hydrolysis. The inclusion of a small amount of PEG groups in the polymer improved the hydrophilicity of the polymer without sacrificing its solubility in organic solvents. The hydrolysis of P4.3 proceeded successfully to afford diol-functionalized polymer P4.4.

Additional strategies involving “activated” acetal formation techniques were considered for the synthesis of acetal-containing SCNP, including the synthesis of dimethyl acetal functionalized M4.5 and attempt at copolymerization with unprotected diol-functionalized M4.6. M4.5 was synthesized by the reaction of previously synthesized M4.3 with trimethyl orthoformate in the presence of an acid catalyst. The acetal is formed from these precursors by the exchange of the methoxy groups for the diol – a process that is favorable due to the reactive nature of the acetal. An unprotected version of the diol monomer was used to avoid degradation of the dimethyl acetal during the acetonide deprotection step. Attempts at copolymerizations of these monomers with styrene resulted in gel formation, possibly due to the high temperatures of the polymerization causing unwanted acetal formation. No acid catalyst was intentionally present, though the chain
transfer agent used (CTA2) contained a carboxylic acid functionality. The resulting gel was insoluble in THF, DMF, DMSO, and concentrated sulfuric acid.

The mechanism for acetal formation involves multiple instances of protonation and deprotonation of intermediates using a Bronsted acid catalyst, and the production of one equivalent of water. In some cases, in order to drive equilibrium forward, water must be removed, either by a chemical agent or by distillation. In 1980, Noyori reported an efficient procedure for acetal formation under aprotic conditions using trimethylsilyl triflate as a catalyst. In this case, the starting materials are an aldehyde and a TMS-protected alcohol. This process lends its efficiency to the stability of its by-product: trimethylsilyl ether is produced and remains chemically inert under the reaction conditions, forcing the equilibrium toward acetal formation.

![Scheme 4.2](image)

**Scheme 4.2** a) Standard acetal formation with acid catalyst, b) acetal formation from TMS protected alcohol with trimethylsilyl triflate as catalyst

To take advantage of this chemistry, we synthesized TMS-protected 4-vinylbenzyl alcohol and copolymerized it with M4.7 and styrene according to Scheme 4.2.

### 4.3 Conclusions

Aldehyde- and acetonide-protected diol-functionalized polymers were synthesized using RAFT polymerization. In order to remove the acetonide groups via hydrolysis to expose the diols,
it was necessary to include a solubilizing PEG group. In addition to the straightforward strategy of acid-catalyzed acetal formation from aldehydes and alcohols, activated acetal formation techniques were considered. This includes the synthesis of a dimethyl acetal functionalized monomer and its attempted copolymerization with a diol-functionalized monomer; and the synthesis of a polymer containing TMS-protected alcohols and aldehydes, based on Noyori’s previous work in acetal synthesis. Future work with these projects includes the formation of SCNP via acetal formation using acid catalysts, including identifying good solvents and catalyst concentrations, and analyzing the parent polymers and SCNP using NMR spectroscopy and size-exclusion chromatography.

4.4 Experimental

4.4.1 Instrumentation

$^1$H NMR (400 MHz) spectra were recorded on a Varian Associates Mercury 400/500 spectrometer. Solvents (CDCl$_3$, D$_6$-DMSO) contained 0.03% v/v TMS as an internal reference.

4.4.2 Materials

Styrene and methyl methacrylate were filtered through a plug of basic alumina before use. 4-Hydroxybenzaldehyde was recrystallized from water before use. M4.1, M4.2, M4.4, and M4.6 were synthesized according to literature. Reagents were obtained from the indicated commercial suppliers and used without further purification unless otherwise stated: 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid (CTA$_1$, Sigma Aldrich), 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (CTA$_2$, Sigma Aldrich), dichloromethane (Fisher Scientific), hexanes (Fisher Scientific), tetrahydrofuran (inhibited with BHT, Fisher
4-vinylbenzaldehyde (Sigma Aldrich), 2,2′-azobisisobutylnitrile (Sigma Aldrich), styrene (Sigma Aldrich), alumina (activated basic, Alfa Aesar), ethanol (95%, Pharmco Aaper), 4-hydroxybenzaldehyde (Kodak), hydrochloric acid (EMD chemicals), potassium carbonate (Fisher Scientific), 4-vinylbenzyl chloride (Sigma Aldrich), N,N-dimethylformamide (Omnisolv), trimethyl orthoformate (Sigma Aldrich), p-toluene sulfonic acid monohydrate (Sigma Aldrich), poly(ethylene glycol) methacrylate (M₅ 526, Sigma Aldrich), Methanol (Pharmco Aaper), DL-solketal (Sigma Aldrich), methacryloyl chloride (Sigma Aldrich), methyl methacrylate (Sigma Aldrich), chloroform-D (Cambridge Isotope Laboratories).

### 4.4.3 Experimental methods

#### 4.4.3.1 Synthesis of 4-[(4-vinylphenl)methoxy]-benzaldehyde (M4.3)

4-vinylbenzaldehyde (12.2 g) and potassium carbonate (20.7 g) were added to 100 mL of 95% ethanol in a 250 mL round bottom flask and heated at reflux for 30 minutes. The resultant solution was bright red. 4-vinylbenzyl chloride (17 mL) was added and the mixture was heated at reflux for 17 hours. The reaction mixture was allowed to cool and solidified. Ethyl acetate (100 mL) was added and the organic solution was washed with water (100 mL). The aqueous layer was washed with ethyl acetate (100 mL). The organic layers were combined, dried over sodium sulfate, concentrated under reduced pressure to afford a solid which was recrystallized twice from 95%
ethanol and dried in vacuo at 50 °C overnight to afford 4-[(4-vinylphenl)methoxy]-benzaldehyde as an off-white powder (16.25 g).

4.4.3.2 Synthesis of 4-[(4-Vinylphenl)methoxy]-benzaldehyde dimethyl acetal (M4.5)

M4.3 (0.95 g), trimethylorthofomrate (4.4 mL), and p-toluenesulfonic acid monohydrate (34 mg) were stirred in a scintillation vial under argon at 60 °C for two hours. Solvent was removed under reduced pressure and the resulting solid was purified by column chromatography using the CombiFlash default gradient with ethyl acetate/hexanes as eluent.

\[ \text{M4.3} \xrightarrow{\text{MeO-OMe}} \text{M4.5} \]

\[^1H\text{ NMR (400 MHz, CDCl}_3\] \( \delta \) 7.40 (m, 6.13H, Ar-H), 6.95 (d, 2.03H, Ar-H), 6.71 (dd, 1.00H, vinyl CH), 5.35 (s, 1.09H, acetal CH), 5.27 (d, 1.08H, vinyl CH), 5.06 (s, 2.14H, -CH\_2\-), 3.31 (s, 6.13H, -O-CH\_3).

4.4.3.3 Synthesis of P4.1

MMA (0.55 mL), M4.1 (0.26 g), M4.2 (0.22 g), CTA1 (5.4 mg), AIBN (0.2 mg), and DMF (1 mL) were added to a 10 mL Schlenk flask, sparged with argon for 20 minutes, and stirred at 80 °C
overnight. The mixture was exposed to air, allowed to cool, precipitated twice into methanol, and dried under vacuum to afford P4.1. $^1$H NMR (400 MHz, CDCl$_3$) δ 9.92 (br, 1.0H, -CHO), 7.87 (br, 2.06H, Ar-H), 7.06 (br, 2.12H, Ar-H), 4.33 (br, 5.23H), 4.10 (br, 1.37H), 3.99 (br, 2.10H), 3.77 (br, 1.48H), 3.60 (br, 24.49H, -O-CH$_3$), 2.02-1.58 (br, 21.77H, -CH$_3$), 1.44 (br, 9.62H, C(CH$_3$)$_2$), 1.02-0.83 (br, 21.56H, -CH$_3$). See appendix page 20 for full spectrum.

4.4.3.4 Synthesis of P4.2

\[
\begin{align*}
\text{M4.3} & \quad \text{M4.4} \\
\text{110 °C} &
\end{align*}
\]

Styrene (0.78 mL), M4.3 (0.35 g), M4.4 (0.36 g), and CTA2 (7.3 mg) were added to a 10 mL Schlenk flask, sparged with argon for 20 minutes, and stirred at 100 °C overnight. The mixture was exposed to air, allowed to cool, precipitated twice into methanol and dried under vacuum to afford P4.2. $^1$H NMR (500 MHz, CDCl$_3$) δ 9.87 (br, 1.0H, -CHO), 7.82 (br, 2.05H, Ar-H), 7.55 (Ar-H), 5.00 (br, 2.13H, Ar-CH$_2$-O-), 4.44 (br, 1.82H, Ar-CH$_2$-O-), 4.28 (br, 0.90H, -CH-), 4.03 (br, 0.88H), 3.73 (br, 0.88H), 3.47 (br, 2.63H), 2.10-1.67 (br, 5.71H, -CH(Ar)-CH$_2$-), 1.42 (br, 16.14H, C(CH$_3$)$_2$, -CH(Ar)-CH$_2$-). See appendix page 21 for full spectrum.
4.4.3.5 Synthesis of P4.3

Methyl methacrylate (2.2 mL), M4.2 (0.84 g), poly(ethylene glycol) methacrylate (1.0 g), CTA1 (23 mg), AIBN (1.0 mg), and DMF (30 mL) were added to a Schlenk flask, sparged with argon for 20 minutes and heated at 80 °C overnight. The solution was exposed to air and concentrated under reduced pressure at 45 °C. The resulting sticky solid was dissolved in chloroform, precipitated into hexanes, and dried in vacuo to afford P4.3 (3.24 g) as a powder, which slowly became a sticky, clear solid after sitting overnight. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.32 (br, 0.96H), 4.11 (br, 1.85H), 3.99 (br, 1.74H), 3.65 (br, 26.31H, -O-CH\(_3\), -O-(CH\(_2\))\(_2\)-O-), 1.82 (br, 9.51H, -CH\(_2\)-), 1.59 (br, 12.23H), 1.44 (br, 6.94H, C(CH\(_3\))\(_2\)), 1.03 (br, 5.56H, -CH\(_3\)), 0.86 (br, 9.19H, -CH\(_3\)). See appendix page 22 for full spectrum.

4.4.3.6 Synthesis of P4.4

P4.3 (0.5 g) was dissolved in a mixture of THF (25 mL) and aqueous HCl (2.0 M, 5 mL) and
stirred for 24 hours. The solvent was removed under reduced pressure, and the resulting solid was dissolved in chloroform, precipitated into hexanes, and dried in vacuo to afford P4.4 (0.42 g) as a white powder. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.31 (br, 6.26H), 4.07 (br, 5.0H), 3.65 (br, 39.95H, -O-CH$_3$, -O-(CH$_2$)$_2$-O-), 2.22 (br, 35.90H), 1.82 (br, 12.13H, -CH$_2$-), 1.46-1.26 (br, 4.65H), 1.03 (br, 7.76H, -CH$_3$), 0.83 (br, 12.35H, -CH$_3$). See appendix page 23 for full spectrum.

4.4.3.7 Synthesis of P4.5

Styrene (0.64 mL), M4.3 (0.44 g), M4.7 (0.39 g), and CTA2 (7.3 mg) were added to a 10 mL Schlenk flask and heated at 110 $^\circ$C overnight. The resulting mixture was exposed to air, allowed to cool, diluted with THF, precipitated twice into methanol and dried under vacuum to afford P4.5. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.90 (br, 1.0H, -CHO), 7.52 (br, 2.18H, Ar-H), 7.02 (br, 9.11H, Ar-H), 6.50 (br, 8.50H, Ar-H), 4.58 (br, 1.78H, -CH$_2$-), 2.11-1.20 (br, 13.73H, -CH(Ar)-CH$_2$-), 0.12 (br, 6.89H, -OTMS). See appendix page 23 for full spectrum.
4.4.3.8 Attempted synthesis of P4.6

Styrene (0.77 mL), M4.5 (0.41 g), M4.6 (0.30 g), and CTA2 (7.3 mg) were added to a 10 mL Schlenk flask and heated at 110 °C. The solution formed a gel within a few hours, after which it was exposed to air and allowed to cool. The gel was cut into chunks and exposed to THF, DMF, DMSO, and concentrated sulfuric acid to test solubility, but was not found to be soluble in any of these solvents.
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APPENDIX

Figure A 1 SEC UV & MALS trace overlay from P2.1 radical cross-linking experiment. See pages 51 and 52 for full experimental detail.

Figure A 2 SEC UV & MALS trace overlay from P2.1/maleic anhydride cross-linking experiment. See pages 51 and 53 for full experimental detail.
Figure A 3 SEC UV & MALS trace overlay from P2.2 radical cross-linking experiment. See pages 52 and 54 for full experimental detail.

Figure A 4 SEC UV & MALS trace overlay from P2.2/maleic anhydride cross-linking experiments. See pages 52 and 54 for full experimental detail.
Figure A 5 SEC UV/MALS trace overlay from P2.2/N-ethyl maleimide cross-linking experiments. See page 55 for full experimental detail.

Figure A 6 SEC UV/MALS trace overlay from P3.1, NP3.1, and P3.2. See pages 70, 71, and 72 for full experimental detail.

Figure A 7 SEC UV/MALS trace overlay from P3.2 and P3.3, comparison of templated and non-templated ADMET polymers. See page 72 for full experimental details.
Figure A 8 P2.0 $^1$H NMR (D$_6$-DMSO) see page 51 for experimental
Figure A 9 P2.1 $^1$H NMR (CDCl$_3$) see page 51 for experimental details
Figure A 10 P2.2 $^1$H NMR (CDCl$_3$) see page 52 for experimental details
**Figure A 11** NP2.1-0 $^1$H NMR (CDCl$_3$) see page 53 for experimental details
Figure A 12 NP2.2-3MA $^1$H NMR (CDCl$_3$) see page 54 for experimental details
Figure A 13 NP2.2-3NEM $^1$H NMR (CDCl$_3$) see page 55 for experimental details
Figure A 14 NP2.2-3NPM $^1$H NMR (CDCl$_3$) see page 56 for experimental details
Figure A 15 M3.2 $^1$H NMR (CDCl$_3$) see page 68 for experimental details
Figure A 16 M3.3 $^1$H NMR (CDCl$_3$) see page 69 for experimental details
Figure A 17 M3.3 $^{13}$C NMR (CDCl$_3$) see page 69 for experimental details
Figure A 18 P3.1 $^1$H NMR (CDCl$_3$) see page 70 for experimental details
**Figure A 19** NP3.1 $^1$H NMR (CDCl$_3$) see page 71 for experimental details
Figure A 20 P3.2 $^1$H NMR (CDCl$_3$) see page 72 for experimental details
Figure A 21 P3.3 $^1$H NMR (CDCl$_3$) see page 72 for experimental details
Figure A 22 P3.4 $^1$H NMR (CDCl$_3$) see page 73 for experimental details
Figure A 23 M3.5 $^1$H NMR (CDCl$_3$) see page 73 for experimental details
Figure A 24 P4.1 $^1$H NMR (CDCl$_3$) see page 82 for experimental details
Figure A 25 P4.2 $^1$H NMR (CDCl$_3$) see page 83 for experimental details
Figure A 26 P4.3 $^1$H NMR (CDCl$_3$) see page 84 for experimental details
Figure A 27 P4.4 $^1$H NMR (CDCl$_3$) see page 84 for experimental details
Figure A 28 P4.5 $^1$H NMR (CDCl$_3$) see page 85 for experimental details
Figure A 29 P2.1 SEC trace overlay. See page 51 for full experimental detail.

Figure A 30 P2.2 SEC trace overlay. See page 52 for full experimental detail.
Figure A 31 P2.2’ SEC trace overlay. See page 52 for full experimental detail.

Figure A 32 NP2.1-0 SEC trace overlay. See page 53 for full experimental detail.
Figure A 33 NP2.1-1MA SEC trace overlay. See page 53 for full experimental detail.

Figure A 34 NP2.1-2MA SEC trace overlay. See page 53 for full experimental detail.
Figure A 35 NP2.2’-0 SEC trace overlay. See page 54 for full experimental detail.

Figure A 36 NP2.2-1MA SEC trace overlay. See page 54 for full experimental detail.
Figure A 37 NP2.2-2MA SEC trace overlay. See page 54 for full experimental detail.

Figure A 38 NP2.2-3MA SEC trace overlay. See page 54 for full experimental detail.
Figure A 39 NP2.2-4MA SEC trace overlay. See page 54 for full experimental detail.

Figure A 40 NP2.2-1NEM SEC trace overlay. See page 55 for full experimental detail.
Figure A 41 NP2.2-2NEM SEC trace overlay. See page 55 for full experimental detail.

Figure A 42 NP2.2-3NEM SEC trace overlay. See page 55 for full experimental detail.
Figure A 43 P3.1 SEC trace overlay. See page 70 for full experimental detail.

Figure A 44 NP3.1 SEC trace overlay. See page 71 for full experimental detail.
**Figure A 45** P3.2 SEC trace overlay. See page 72 for full experimental detail.

**Figure A 46** P3.3 SEC trace overlay. See page 72 for full experimental detail.