Macromolecules

Perspective

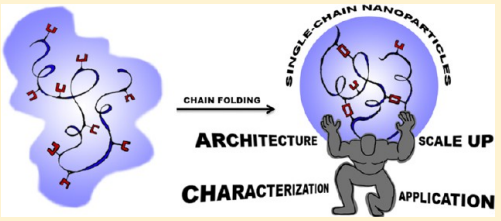
pubs.acs.org/Macromolecules

What Is Next in Single-Chain Nanoparticles?

Ashley M. Hanlon,[†] Christopher K. Lyon,[†] and Erik B. Berda*[,†,‡]

[†]Department of Chemistry and [‡]Materials Science Program, University of New Hampshire, Durham, New Hampshire 03824, United States

ABSTRACT: With the increasing appeal of nanotechnology, there is a demand for development of synthetic techniques for the fabrication of nano-sized objects that allow for precise size control and tailored functionalization. To this end, the collapse or folding of single polymer chains into architecturally defined nanostructures is a rapidly growing research topic in polymer science. Many synthetic approaches have been developed for the formation of single-chain nanoparticles (SCNP), and a variety of characterization methods and computational efforts have been utilized to detail their formation and probe their morphological characteristics. Interest in this field continues to grow partially due to the variety of potential applications of SCNP including catalysis, sensors, nanoreactors, and nanomedicine. While numerous developments have been made, the field is continuing to evolve, and there are still many unanswered questions regarding synthesis and characterization of SCNP. This Perspective serves to identify recent accomplishments in the synthesis and characterization of SCNP while distinguishing areas that are in need of advancement and innovation to move forward. This includes exploring more complex synthetic strategies, obtaining folding control, employing nanoparticle functionalization, developing scalable methods, investigating hierarchical self-assembly of SCNP, and exploiting unique characterization techniques and in-depth simulations.



INTRODUCTION

Nanotechnology presents both the promise and challenge of advancing science in a variety of key areas, including medicine, electronics, and energy. Despite our advances in the fabrication of remarkable soft nanodevices, contemporary synthetic techniques lack the control necessary to produce well-defined structures that operate with the function and precision seen in biological systems. The function in these natural macromolecules is a result of their 3-dimensional shape and the exact arrangement of functional groups on the surface and interior of the molecule, which arises through the folding process dictated by an exact monomer sequence.

Various techniques used to control the structure of synthetic polymers on the nanometer scale include the synthesis of sequence controlled polymers,[1,2] star polymers,[3] dendrimers,[4] and step-growth polymers with evenly spaced functional groups.[5] Each of these has inherent advantages and setbacks, though generally none of them are capable of producing polymers that are completely free of microstructural defects.

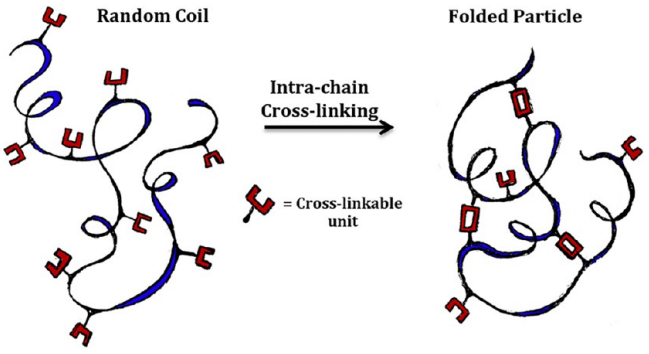


Figure 1. Conceptual illustration of single-chain folding of linear polymer.

Recently, interest in producing macromolecular architectures by the manipulation of single polymer chains is increasing. This area of research is referred to as single-chain technology.[6] In particular, the formation of collapsed single polymer chains in dilute solution has gained much attention. These single-chain nanoparticles (SCNP), while simple in principle, are quite complicated in practice, in terms of both synthetic considerations and characterization.

To our knowledge, the intramolecular cross-linking of linear single polymer chains was first reported in 1962 by Kuhn and Balmer.[7] The topic was revisited in the late 1960s by Rossi et al.[8,9] and again both theoretically and experimentally by various researchers throughout the 1980s[10,11] and 1990s.[12−14] The advent of controlled radical polymerization techniques and the development of efficient postpolymerization modification reactions provided a refreshed interest in the topic at the turn of the century. There are currently several research groups studying what are currently referred to as single-chain nanoparticles (SCNP). An assortment of synthetic methodologies have been used for the formation of SCNP, with the majority of examples utilizing postpolymerization modification reactions in dilute polymer solutions (typically <1 mg mL^{-1}). Postpolymerization cross-linking reactions are typically chosen based on their efficiency; reactions that are low yielding or involve side reactions are undesirable. Intrachain cross-linking

Received: July 2, 2015
Revised: December 8, 2015
Published: December 31, 2015

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

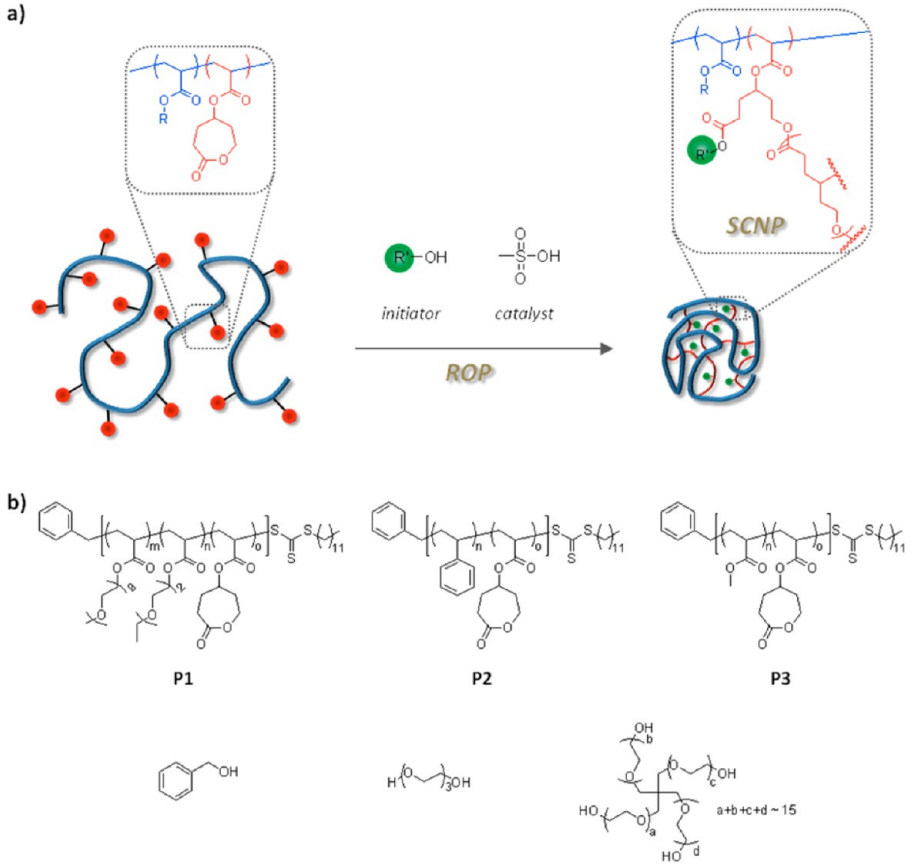


Figure 2. (a) Schematic representation of the formation of nanoparticles from single polymer chains via organo-catalyzed ring-opening polymerization. (b) Linear polymer precursors (**P1**−**P3**) and alcoholic initiators investigated in this study. Reproduced with permission from ref 65.

has been achieved through covalent, dynamic covalent, and noncovalent bonding. These include hydrogen bonding,[15−28] metal ligation,[29−31] photodimerization,[32,33] cycloadditions,[34−39] disulfide formation,[40,41] "click" chemistry,[42−46] and many others,[47−63] as discussed in our previous review on the topic.[64]

THE PRESSING CHALLENGES

Herein, we identify areas that contain unresolved issues or are in need of innovation to distinguish what is important for advancement in this field. Exploration of more complex synthetic strategies, folding control, functionalization, scalable methods, self-assembly, unique characterization techniques, and in-depth simulations will aid in revealing the true potential of SCNP. While the synthetic ability to obtain SCNP is fairly well developed, the capability to impart utility in many of these systems and the ability to fully characterize them remains a challenge

Challenge 1: Complex Synthetic Design of Single-Chain Nanoparticles. SCNP synthesis through intramolecular cross-linking using a singular covalent, dynamic covalent, or noncovalent bonding is well studied.[6,64] Work toward utilizing different types of chemistries to obtain SCNP continues, but more complex designs with utility and synthetic control, rather than simply implementing a new type of cross-linking chemistry, are beginning to emerge. While it is advantageous to expand the library of chemistry used to obtain SCNP, more work toward forming SCNP with unique tunable properties or offer improvement to traditional methods will help to move this research beyond its current proof of concept stage.

Recently, additional instances of previously unexplored cross-linking chemistries have been reported in the literature. While each of these studies introduced a new cross-linking route, they expand their investigation by either examining synthetic factors that influence SCNP formation or developing new applications. Future research will benefit from analyzing the design of the parent polymer and the effects it has on fine-tuning the properties and morphology of the resulting SCNP.

Wong and co-workers developed a system to form SCNP via organo-catalyzed ring-opening polymerization of linear polymer precursors functionalized with pendent polymerizable caprolactone moieties.[65] They studied various linear random copolymer precursors, and results indicated the importance of side-chain brushes in aiding SCNP formation at higher polymer concentrations (Figure 2). It is well-known that a higher molecular weight polymer leads to larger SCNP and an increase in the amount of cross-linking leads to smaller particles. Future research on the effect of the synthetic design of parent polymer or cross-linker species, rather than just size and concentration studies, will aid in understanding SCNP formation.

Another approach in developing new SCNP systems is designing them with built-in utility, showing that SCNP can be used in certain advantageous applications. Recently, Jeong et al. synthesized linear copolymer precursors carrying pendant phthalonitrile groups followed by intramolecular macrocyclization reactions to form Cu-metalated phthalocyanines (CuPc), which simultaneously enabled single-chain nanoparticle formation.[29] The copper phthalocyanine-containing polymeric nanoparticles were soluble in organic solvents, offering a new route to prepare solution-processable CuPc dyes. Willenbacher

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

and co-workers also demonstrated SCNP formation through metal ligation in which polymers decorated with pendant triarylphosphine ligand moieties were cross-linked through the introduction of a palladium species.[66] These Pd-SCNP were characterized using $^{31}P\{^{1}H\}$ NMR spectroscopy and used as catalysts in Sonogashira coupling reactions.

In certain cases, establishing new types of chemistry to use in SCNP synthesis is of interest.[67] However, focus is rightfully shifting to the design of more complex systems and methods of characterization. Work toward designing linear polymers to obtain some control over SCNP size and shape is increasing. Moreno and co-workers determined computationally that using multiple orthogonal intramolecular interactions results in more compact globular SCNP.[68] Currently, there are only a few experimental studies that utilize multiple intrachain interactions, but researchers are beginning to explore the advantages of this strategy. Hosono et al. used a combination of UPy and BTA units in a block copolymer to form SCNP with synergistic supramolecular interactions.[22] Their ABA-type triblock copolymers resulted in compartmentalized SCNP through a stepwise folding process, resulting from orthogonal self-assembly induced noncovalent interactions. Altintas and co-workers also reported using two different orthogonal hydrogen bonding dimers to induce polymer folding.[15] Single polymer chains were functionalized to self-fold based on two orthogonal noncovalent interactions: a cyanuric acid–Hamilton wedge pair and a thymine–diaminopyridine pair. This strategy led to well-defined dual point single chain self-folding, demonstrating that more complex single chain self-assemblies based on synthetic polymers have potential to mimic the folding interactions of natural biomacromolecules on a simplified level. In a different approach, Chao et al. showed that ROMP-synthesized polyolefins collapsed via the supramolecular association of pendant tetraaniline units and covalently cross-linked by thiol–ene "click" chemistry involving the olefins in the polymer backbone.[69] Using sequentially activated noncovalent and covalent intrachain interactions, the polymer was folded from an expanded coil conformation to a more globular conformation with ∼70% reduction in hydrodynamic volume (Figure 3). Each of these examples validates the exploration of multiple intramolecular cross-links, which have demonstrated the synthesis of SCNP with different conformations through a stepwise folding process. Using multiple types of cross-linking in a single polymer chain and embedding the ability to introduce a stepwise folding pattern is bringing synthetic polymers closer to mimicry of biological systems.

Scanning the literature, it is evident that the majority of SCNP systems begin with a simple linear random copolymer. There is merit in exploring more complicated polymer architectures, such as block copolymers and branched structures. Zhang and Zhao recently demonstrated the synthesis of block copolymers of poly(ε-caprolactone) and poly(2-(dimethylamino)ethyl methacrylate) with disulfide bonds at the junction by ring-opening and atom transfer radical polymerization techniques (Figures 4 and 5).[70] These SCNP served as surfactants to prepare surface-tunable polystyrene colloidal particles. The monotethered SCNP significantly reduced the surface tension of the water and could be cleaved from the collodial particles. Cleavage was induced through efficient thiol–disulfide exchange reactions leaving thiols on the surface of the particles for the possibility of further functionalization.

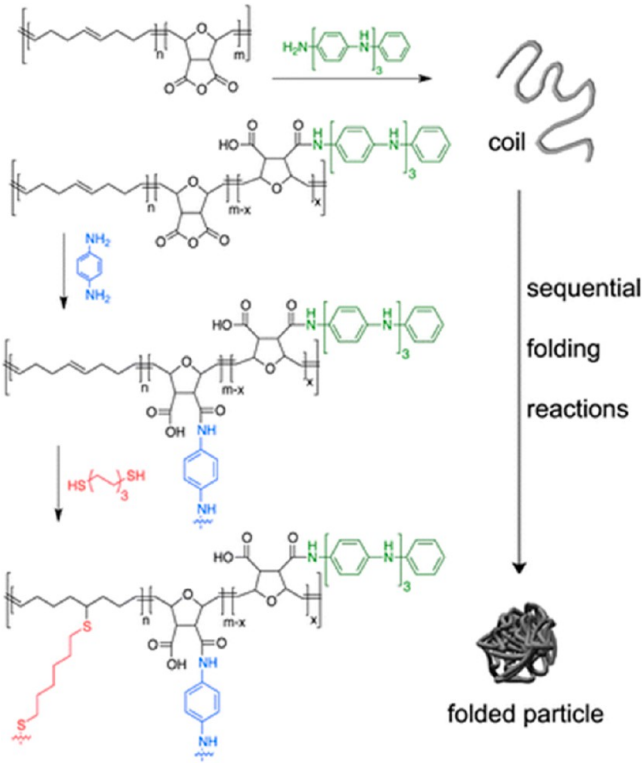


Figure 3. Controlled single-chain folding via sequential intrachain interactions. Reproduced with permission from ref 69. Copyright 2013 the Royal Society of Chemistry.

The value of using block copolymers to synthesize single-chain nano-objects was displayed by Roy and Lutz.[71] They constructed single-chain dumbbells by stepwise intramolecular cross-linking of sequence-controlled polymer precursors (Figure 6). The foldable precursors were synthesized by a copolymerization of styrene with N-substituted maleimides (pentafluorophenyl-4-maleimidobenzoate and TIPS-protected *N*-propargyl maleimide). The two functional N-substituted maleimides allowed for intramolecular cross-linking by both reaction of the activated ester moieties with ethylenediamine and coupling of the deprotected alkynes by the Eglinton reaction. The result was complex single-chain objects containing distinct cross-linked subdomains.

In order to move toward the complexity seen in uniquely folded nanostructures found in nature, it is necessary to focus on complex polymer designs and architectures. The use of block copolymers in SCNP synthesis through sequence-controlled polymerizations could also potentially allow an almost unlimited design of tailored polymer microstructures, in which the amount and positioning of cross-linking sites can be precisely controlled. As polymerization techniques continue to evolve, and synthetic polymers approach precisely controlled monomer placement in the backbone, the design of SCNP parent polymers will continue to enhance the complexity and potentially aid to control the SCNP formation.

Challenge 2: Folding Control. Proteins have the ability to undergo guided folding in solution to complex structures stabilized by noncovalent or covalent interactions.[72,73] The unique functions exhibited by these natural materials are a derivative of their shape, which is imparted by the precise folding process. Consequently, the development of synthetic polymers with the same degree of structural organization has

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2–14

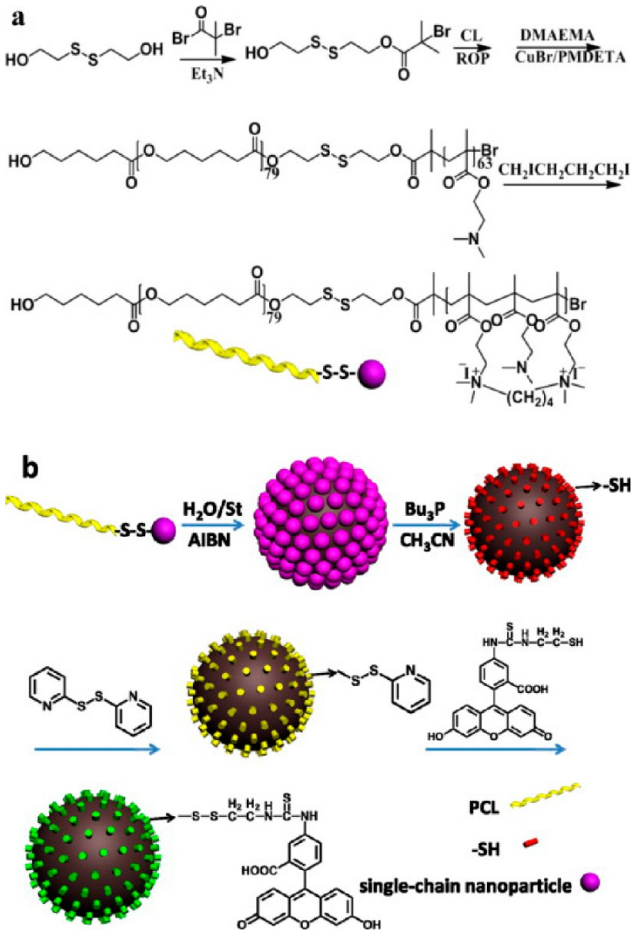


Figure 4. Outline for the synthesis of cleavable monotethered single-chain nanoparticles (SCNP). (b) Schematic depiction of the preparation of surface-tunable PS colloidal particles by using the monotethered SCNP as surfactants. Reproduced with permission from ref 70. Copyright 2015 Elsevier.

been the focus of a considerable amount of research. In order to fabricate functional soft nanomaterials that more closely mimic folded biomolecules in structure and activity, the effect of the chain length, placement of cross-links within a polymer chain, and rigidity of the polymer backbone require further studies. The effect of these parameters on the three-dimensional structure of SCNP has not been fully explored.

In a recent study, Stals and co-workers utilized UPy dimerization to examine the effect of several variables in SCNP formation (Figure 7). 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 SCNP, while solvent played an important role in disrupting or facilitating quadruple hydrogen bond association.[23] When using hydrogen bonding as a means to cross-link polymer chains to form SCNP the solvent choice is evidently going to play a large role in SCNP formation, but the approach that Stals and co-workers took in this study could be applied to SCNP systems with different types of cross-linking chemistries. When using covalent and dynamic covalent systems, the influence of polymer backbone rigidity and placement of cross-linking species within the polymer could have a profound effect on SCNP formation and should be explored for the variety of SCNP systems that have been developed. While an overwhelming number of different types of chemistries to form SCNP have been developed, the literature lacks studies in which systematic approaches have been used to identify the effects of exchangeable parameters in SCNP systems.

Advanced polymer synthesis used to control monomer sequence or functional group placement will help to work toward the structural precision demonstrated by natural folded macromolecules. Work has been done by Perrier,[74] Lutz,[75] and Whittaker[76] to develop systems that can achieve precisely controlled microstructures within a linear polymer chain. Lutz and co-workers demonstrated the use of their technique to introduce a versatile strategy for preparing foldable linear polymer chains using ATRP (Figure 8).[77] The controlled addition of discrete amounts of protected maleimide at precise times during the synthesis enabled the formation of polystyrene chains that contained positionable reactive alkyne groups. Different types of covalently folded polymer chains were obtained through intramolecular reactions between these functions. A variety of folded polymers were established including tadpole (P-shaped), pseudocyclic (Q-shaped), bicyclic (8-shaped), and knotted (α-shaped) macromolecular origamis. Well-defined chain-growth methods and step-growth chemistries such as ADMET[78] and the use of multicomponent reactions[79] are potentially advantageous for the fabrication of advanced SCNP architectures.

Challenge 3: Functionalization. Imparting functionality that allows for the installment of structural features after nanoparticle formation is an advantage inherent to SCNP formation. However, establishing more versatile strategies is required to enable their application to a variety of SCNP systems. Xie et al. recently prepared SCNP with exactly one functional group exposed on their surface (Figure 9).[80] These SCNP were synthesized by grafting block copolymers of (poly(ethylene oxide)-*block*-poly(2-cinnamoylethyl methacrylate)) to the surface of silica spheres. Steric repulsion led to sparsely grafted particles, and SCNP were formed through intramolecular cross-linking by UV irradiation. When the SCNP were removed from the silica particles, the chain end was exposed for potential modification. Performing azide–alkyne click reactions at the end group revealed the possibility of functionalizing these SCNP with a variety of azide species. Applying a grafting strategy ensures the end groups will be open for further functionalization and eliminates the potential for end groups being embedded within the globular SCNP. Many examples involve imparting functionalization during SCNP formation rather than afterward. The grafting strategy designed by Xie and co-workers offers a new technique for functionalization that has the potential to be applied to other SCNP systems.

Similarly, Oria and co-workers used a RAFT copolymerization process to obtain poly(4-chloromethylstyrene-*co*-2-methylacrylic acid 3-trimethylsilanyl-prop-2-ynyl ester) copolymer. Subsequent partial replacement of the chloride groups with azide moieties and intramolecular "click" coupling at room temperature resulted in SCNP.[81] The PS-NPs retain the Cl^- functionality after their synthesis, which were then used as nanostructured multifunctional initiators for ATRP. Azide functionalization of the remaining Cl^- groups was also shown followed by coupling with triazole–benzene–triazole units. While the technique is not particularly versatile, as it requires a pendent alkyl halide, this study is an example of pendant cross-

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2–14

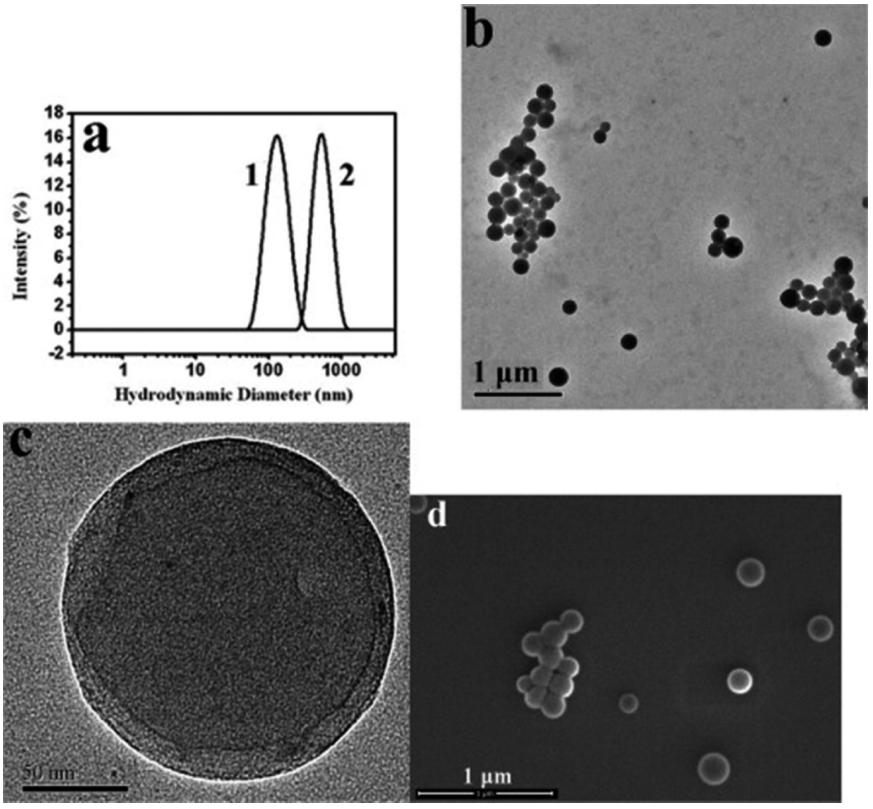


Figure 5. (a) Dynamic light scattering curves of PS colloidal particles prepared by suspension polymerization with 3% (curve 1) and 1.5% (curve 2) monotethered SCNP. (b, c) TEM images of PS colloidal particles prepared by suspension polymerization with 3% monotethered SCNP at low and high magnifications. (d) SEM image of PS colloidal particles prepared by suspension polymerization with 3% monotethered SCNP. Reproduced with permission from ref 70. Copyright 2015 Elsevier.

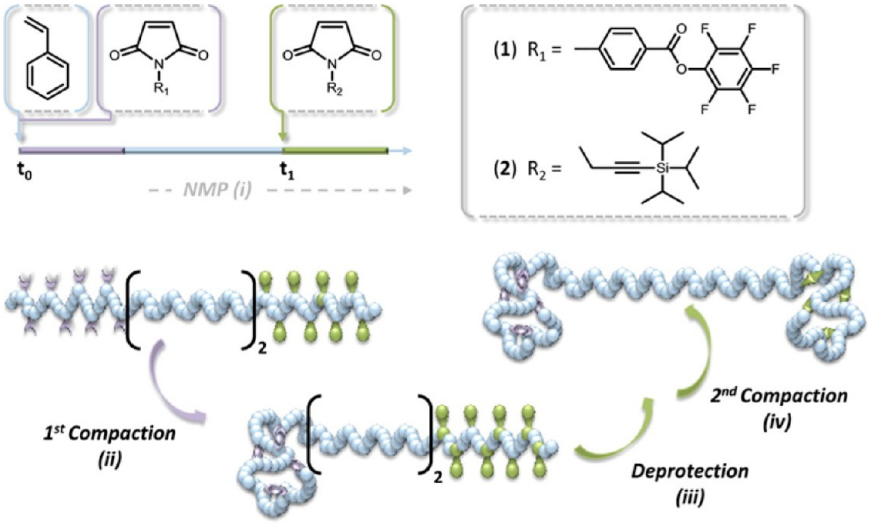


Figure 6. General concept used for preparing compartmentalized single-chain objects. A sequence-controlled precursor was first prepared by NMP of styrene with N-substituted maleimides **1** and **2** (top). Afterward, this polymer was compacted in dilute conditions using a stepwise folding process (bottom). Reproduced with permission from ref 71.

linking groups with a dual purpose used to impart further functionalization.

Functionality can also be incorporated into cross-linking species. Perez-Baena and co-workers described a method for introducing Gd(III) ions into a polymer by incorporating them into the cross-linker to form SCNP as potential MRI contrast agents.[42] They synthesized azide-functionalized acrylic polymers that were cross-linked with a dialkyne-functionalized Gd(III) diethylenetriaminepentaacetic acid. 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 example demonstrates that SCNP have the potential to make higher relaxivity complexes, but these systems must be optimized. Applying complex polymer designs will help to

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

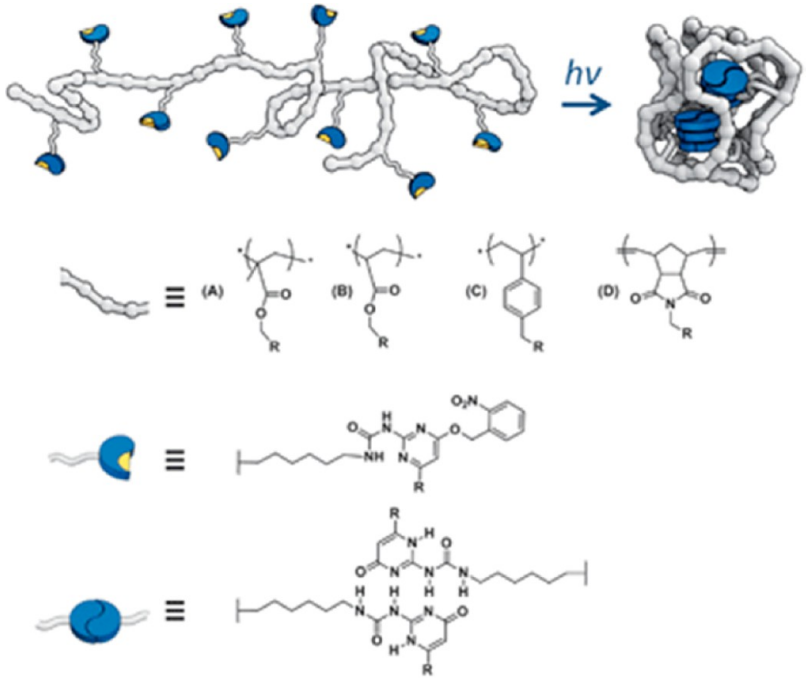


Figure 7. Schematic representation of the collapse of UPy containing polymers. Reproduced with permission from ref 23. Copyright 2013 the Royal Society of Chemistry.

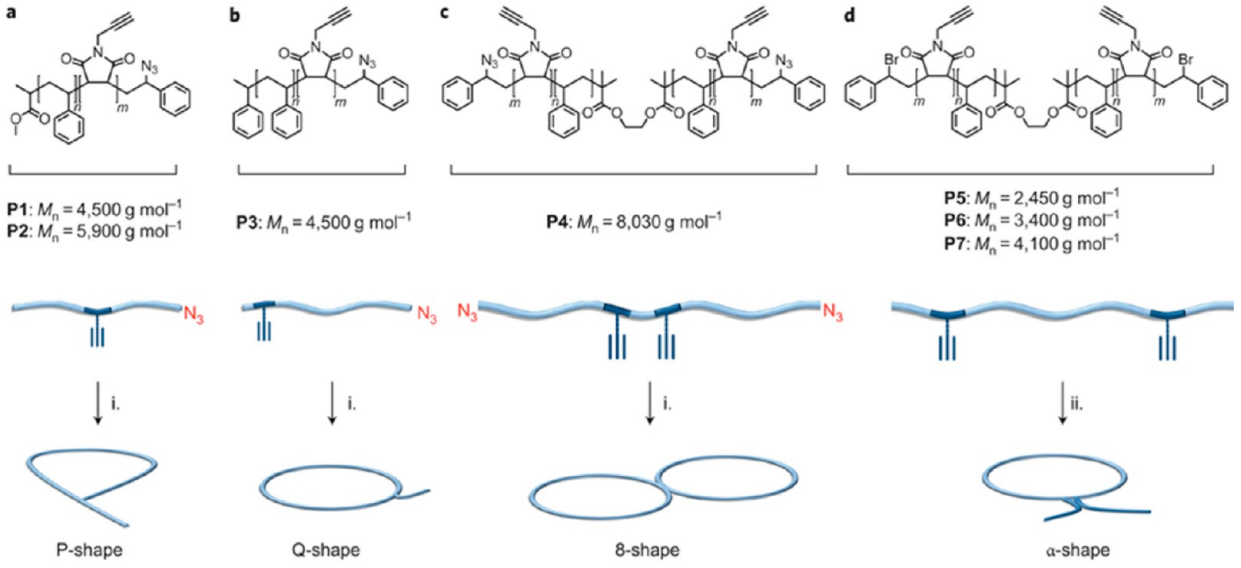


Figure 8. Covalent folding of linear synthetic polymer chains. This approach leads to a variety of controlled shapes: (a) tadpole (P-shaped), (b) pseudocyclic (Q-shaped), (c) bicyclic (8-shaped), and (d) knotted (a-shaped). Reproduced with permission from ref 77. Copyright 2011 the Nature Publishing Group.

optimize conditions while validating the utility of SCNP. More recently, Adkins and co-workers synthesized functionalized water-soluble semiconducting nanoparticles from ABA triblock copolymers.[82] Ethylene oxide-modified polyfluorene served as the center B block, and the A blocks were polyacrylate. Low-temperature benzocyclobutene cross-linking groups were attached to initiate collapse. The resulting nanoparticles were further modified by pegylation to enhance solubility while catechol groups were added to provide complexing sites for MRI reagents such as gadolinium. The resulting system showed high relaxivity values of approximately 10 mM^{-1} s^{-1}, a significant increase from the system Perez-Baena and co-workers described. While the SCNP designed by Adikins et al. involved a more complicated synthesis, the utility of their system significantly increased, validating that complex design of SCNP can allow for not only functionalization but also a broad range of capabilities. There is value in looking back at literature examples of functionalized SCNP to explore the design of more complex polymers like block copolymers and applying postpolymerization modifications to expand their utility.

Functionalization of SCNP with catalytic moieties has been achieved, introducing the beneficial use of SCNP in catalysis.[19,20,28,31,32,50] SCNP can be modeled from efficient catalytic systems with similar polymer backbone design and

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

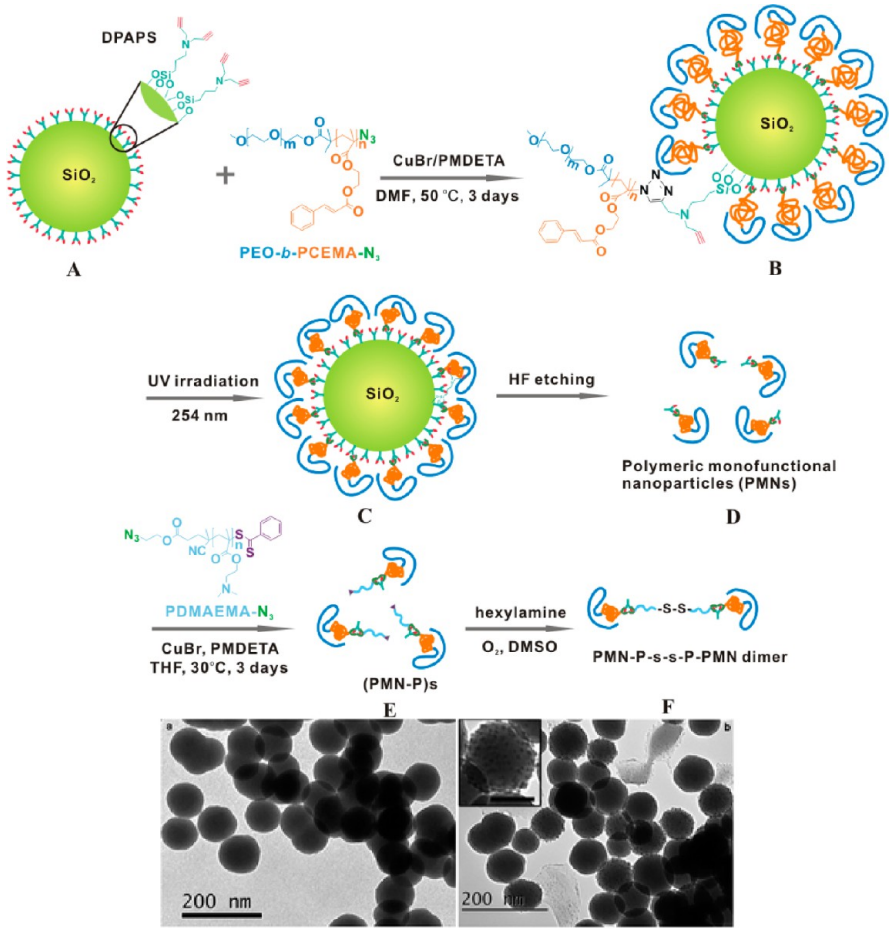


Figure 9. (top) Processes for preparation of polymeric monofunctional nanoparticles and their reactions. (bottom) TEM images of (a) the silica spheres before polymer grafting and (b) the PEO-*b*-PCEMA grafted silica spheres after photo-cross-linking of the PCEMA block stained by RuO_4. The scale bar in the inset is 50 nm. Reproduced with permission from ref 80. Copyright 2015 the Royal Society of Chemistry.

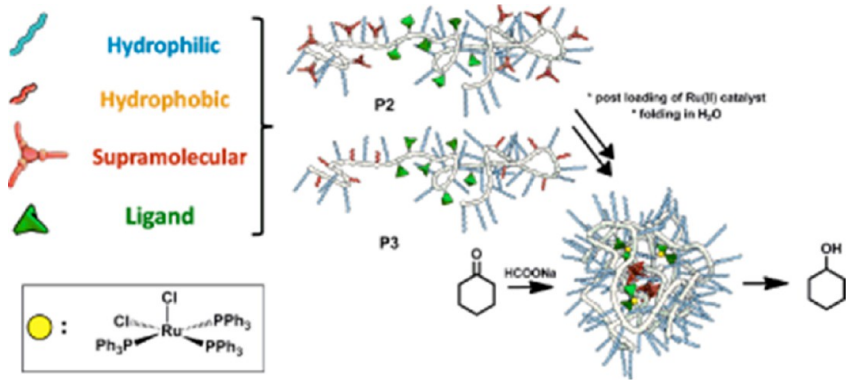


Figure 10. Design of catalytically active SCPNs for transfer hydrogenation of ketones in water. Reproduced with permission from ref 20. Copyright 2013 John Wiley and Sons.

control over properties including polymer solubility, increased accessibility to a larger library of substrates, and increased turnover frequency (TOF). They can be designed to contain hydrophobic pockets, as seen in enzymes, and the catalyst containing polymer can be easily removed, overcoming the challenge of the separation of catalysts and products with similar solubility. Artar et al. demonstrated the synthesis of catalytically active SCNP in which RAFT was used to form amphiphilic copolymers that comprised oligo (ethylene glycol) side chains to impart water solubility, BTA and/or lauryl side chains to induce hydrophobicity, and diphenylphosphinostyrene (SDP) units in the middle section as a ligand to bind a ruthenium catalyst. The Ru(II) loaded SCNP were tested in the transfer hydrogenation of cyclohexanone (Figure 10).[20] While the folding induced by BTA units was determined to be nonessential to the catalysis, the SCNP-bound catalyst was proven more effective than the free catalyst. In a prior publication from the same researchers, the self-assembled α-helices of a similar system were shown to withstand aqueous catalytic conditions (0.4 M HCOONa and substrate at 40 °C),

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

and cyclohexanone was efficiently reduced to cyclohexanol (86% reduced after 18 h).[19] The turnover frequency for this SCNP-based catalytic system was comparable to similar catalytic systems (1−20 h^{-1}). Recently, Tooley and co-workers demonstrated the first SCNP-based model of a metalloenzyme bearing a single, bio-inspired active site (Figure 11).[100] They synthesized SCNP functionalized with a single diiron cluster by thiol−ene click chemistry. This system has the potential to catalyze the production of H_2.

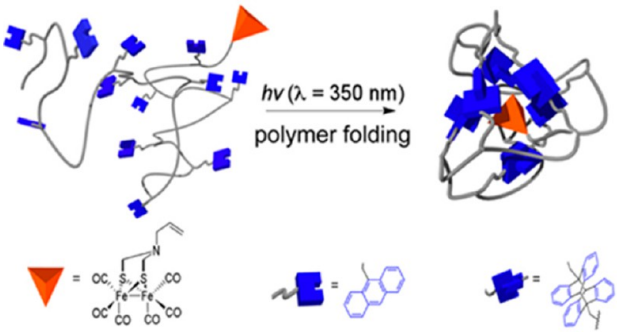


Figure 11. Schematic representation of polymer-bound Fe_2-H_2ase complex folding into a SCNP. Reproduced with permission from ref 100. Copyright 2015 the Royal Society of Chemistry.

Challenge 4: Scalable Methods. A limitation in most SCNP syntheses is the requirement of dilute polymer solutions. Highly dilute conditions reduce the practicality of large-scale industrial production of SCNP. Some effort has been made to solve this problem, but currently these solutions are not widely applicable. Hawker and co-workers demonstrated a continuous addition method as a way to circumvent this issue. They synthesized random copolymers of 4-vinylbenzocyclobutene with various vinyl monomers via nitroxide-mediated free radical polymerization. SCNP formation was achieved via intramolecular dimerization of benzocyclobutene groups in which a concentrated polymer solution (2.5 mg mL^{-1}) could be used to obtain SCNP while still preventing intermolecular coupling.[35] Wong and co-workers developed a system to form SCNP at high polymer concentration (ca. 100 mg mL^{-1}) via organo-catalyzed ring-opening polymerization of linear polymer precursors functionalized with pendent polymerizable caprolactone moieties.[65] Terashima et al. reported the synthesis of a styrene-based copolymer containing PEG, BTA, and diphenylphosphinostyrene using ruthenium-catalyzed CRP that self-assemble and form SCNP in an aqueous environment at a concentration of 1.0−5.0 mg/mL.[19] Each of these examples demonstrates that SCNP have the ability to be produced at higher concentrations, but these examples are applicable to particular systems and cannot be universally applied.

Another approach is to sparsely graft linear chains onto other nanoparticles such as silica particles, preventing intermolecular coupling while allowing for an increase in concentration. Xie et al. grafted block copolymers to the surface of silica spheres and could intramolecularly cross-link the copolymers at a silica mass concentration of 10 mg mL^{-1}.[80] This system has the potential to be applied to a variety of SCNP systems although it adds the steps of grafting to silica particles and purification to isolate the SCNP. Clearly, if SCNP are to be accepted as a viable method to obtain nano-sized objects, more research must be done to find practical scalable methods.

Challenge 5: Self-Assembly. Despite what the term "self-assembly" implies, the process is highly dependent on environmental conditions such as solvent, pH, and temperature. Considering these variables, SCNP folding must be examined under a variety of conditions. Some groups have shown that linear polymers can self-assemble into SCNP through dynamic covalent bonds such as single[16,83] or multiple hydrogen bonding pairs.[22] For SCNP, the microstructure of the parent polymer may be considered analogous to a protein's primary structure. Thereby, SCNP may be considered analogous to secondary structures, which could potentially be assembled into hierarchical so-called tertiary structures. While literature contains many reports of protein-like polymers that adopt pseudo-secondary structures using BTA units, to the best of our knowledge no examples are shown to have control over the tertiary structure. In the past, the alteration of the environment in which nanoparticles form has shown to provide significant control in the structure of the self-assembly of various linear polymers.[84] Recently, Gröschel and co-workers demonstrated an approach to a guided hierarchical coassembly of soft patchy nanoparticles built from triblock terpolymers.[85] Specifically, they synthesized monodisperse colloidal building blocks with suitably anisotropic interaction patterns that form well-ordered nanoparticles and compartmentalized materials in different environments. If this type of control on nanoparticle formation can be gained through the alteration of the environment in which a terpolymer exists, this same technique could potentially be applied to SCNP formation. SCNP may serve as building blocks for more compartmentalized nanomachinery, comparable to complex biomacromolecules.

A stepwise solvent-directed self-assembly has been achieved for linear polymers containing solvaphilic and solvaphobic units, but limited work has been done to apply this same concept to SCNP. Unlike block copolymers, which first self-assemble into micelles, SCNP have an architecture that inherently gives them a compact sometimes spherical-like structure before self-assembly. The initial forced compact structure of SCNP could aid to achieve more controlled hierarchical self-assembled structures currently unattainable by linear polymers. There have been some examples in the literature demonstrating the self-assembling abilities of SCNP, indicating that they have potential in the development of a bottom-up design of hierarchical self-organization, but synthetic polymers are still far from the precise control seen in biomacromolecules.

Wen et al. developed self-assembling monotethered SCNP shape amphiphiles based on poly(2-(dimethylamino)ethyl methacrylate)-*block*-polystyrene (PDMAEMA-*b*-PS). The tertiary amine block was cross-linked with 1,4-diiodobutane via the Menschutkin reaction to form "tadpole"-shaped amphiphiles.[52,86] 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 conditions. The diameters of these micelles were between 30 and 80 nm. Similar work has been shown by Tao and Liu[87] and Kim et al.[88]

Polymer scientists can use Nature's ability to self-assemble as a guideline for the synthesis of complex artificial materials. Current man-made self-assemblies are far from the well-defined structure of natural materials due to the lack in uniformity at the molecular level (10−100 nm) that amplifies throughout hierarchical structures. In order to gain control over the self-assembly of SCNP, a linear polymer precursor with precise

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

monomer sequence as seen in a proteins primary structure should be utilized. As mentioned before, studies by Perrier,[74] Lutz,[75] and Whitaker[76] demonstrated that precisely controlled microstructures with in a linear polymer chain are achievable. These methods have yet to be applied to polymer precursors of SCNP, as most examples use random copolymers. If control over the placement of cross-links or cross-linkable units as well as solvaphillic and solvaphobic moieties within a polymer chain can be achieved, this uniformity at the molecular level could aid to develop control over hierarchical structures.

Challenge 6: Using Simulation To Drive Experimental Design. Given the challenge of experimental characterization of SCNP, simulations may help to unravel the folding process and resulting morphology. Molecular dynamic (MD) simulations performed have indicated that SCNP have the potential to control their folding process and mimic globular proteins when using amphiphilic precursors.[90,94] Unfortunately, many MD simulations show a variety of SCNP systems are far from the goal of obtaining compact protein like nanoparticles and rather point to noncompact, nonglobular morphologies.[50,68,90,91] Historically, the characterization of protein folding was also a great challenge, and atomistic simulations have been able to fully characterize the folding process of a variety of small proteins.[92] Lui and co-workers were some of the first to use an atomistic model to study intramolecular cross-linking of benzocyclobutene/styrene copolymers.[89] They were able to study the effects of the concentration of cross-linkers, the ambient temperature, and the rigidity. The cross-linking rate showed to have a quadratic dependence on the number of free cross-linkers. A quantitative dependence of the systems rigidity threshold on its Kuhn length was also found. The morphologies of the precursor polymers had a profound effect on their intramolecular cross-linking and could be easily altered by the quality of solvent and the ambient temperature. Using different reaction conditions by simply altering the solvent and temperature during SCNP formation could be more widely applied to experimental studies. Most SCNP literature apply one particular solvent and temperature combination for intramolecular cross-linking, but a series of different conditions could be beneficial to a variety of systems. Recently Danilov et al. performed all-atom implicit-solvent Monte Carlo simulations of single a,ω-donor−acceptor chains with different linker length and temperature (Figure 12).[93] Atom simulations for a "selective point folding" system have demonstrated that polymers below a defined chain length fold into a near-unique closed conformation with a precision which is normally only reached for well-structured proteins. These studies reveal the potential of SCNP to mimic globular proteins, but experimentally the field is still far from this goal. Simulations could aid with the design of polymer systems that can fold into unique conformations. Lo Verso et al. confirmed the use of computer simulations to obtain two routes for the design of SCNP.[94] Full three-dimensional modeling of SCNP that includes a wider range of collapsing chemistries and polymer backbones is still needed to allow for mathematical and computational methods to be sufficiently applicable to SCNP characterization.

Challenge 7: Improved Characterization. While some simulations reveal that SCNP have the potential to fold into a single unique conformation rather than disordered noncompact particles, experimentally this concept has yet to be fully demonstrated. Characterization of SCNP through the use of multiple techniques has provided proof of their formation and

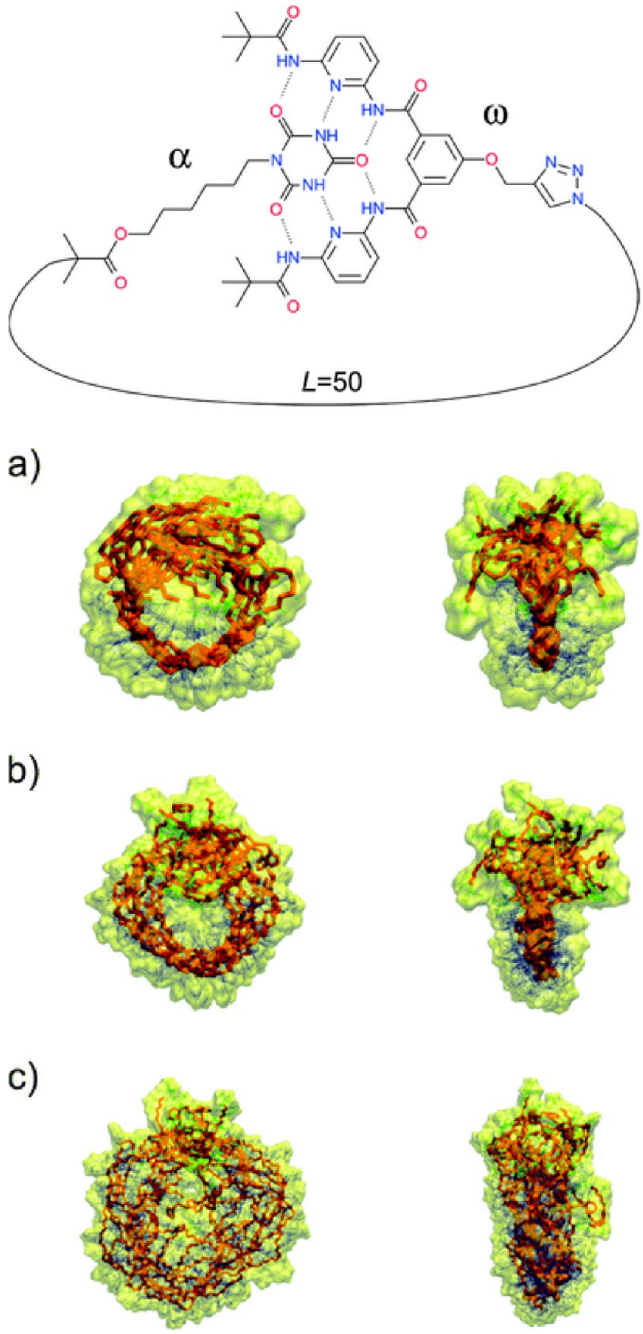


Figure 12. Model system of a polystyrene precision-designed polymer with a,ω-donor−acceptor for single chain self-assembly. Front and side views of ten closed conformations obtained from uncorrelated closed intervals for polymers with (a) L = 10 (RMSD = 0.95 Å), (b) L = 20 (RMSD = 2.44 Å), and (c) L = 50 (RMSD = 6.40 Å). Reproduced with permission from ref 93. Copyright 2015 the Royal Chemical Society.

probed their conformation and morphology, but little is known experimentally about their internal folding structure. In particular, size exclusion chromatography has been vital to SCNP characterization. Qualitative data based on standards can provide insight into inter- versus intramolecular coupling. A decrease in apparent molecular weight and hydrodynamic radius and an increase in retention time from the polymer precursor to SCNP have been observed.[35,47] This has also been confirmed using dynamic light scattering (DLS).[37,46,97] More

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

quantitative measurements are becoming popular by using multiple in-line detectors such as multiangle light scattering (MALS) and viscometry.[96] A MALS detector can prove the molecular weight is consistent from parent polymer to SCNP.[33,40] As mentioned before, these experimental techniques provide evidence of SCNP formation, but can their folding conformation be deciphered? Nuclear magnetic resonance spectroscopy could aid to answer some of these questions. In the past, it has been used to monitor the formation of cross-links by the appearance or disappearance of signals from cross-linkers, but more advanced analyses using NMR are emerging. From the parent polymer to the SCNP spin−spin relaxation time (T_2) decreases,[32,66] and DOSY experiments indicate decreases in hydrodynamic volume.[43] DOSY experiments determine the diffusion coefficient of a polymer in solution, and this parameter is inversely proportional to the hydrodynamic radius. The intramolecular collapse leads to an increase of the diffusion coefficient, as evidence of the formation of single chain particles. More complex NMR studies have the potential to gain insight into the nature of SCNP as they have proven to provide for certain protein systems. The application of 2D NMR studies could help with SCNP analysis through analyzing detailed through-bond and through-space interactions.

The characterization of the morphology of SCNP has been studied using solution-free microscopy techniques, primarily atomic force microscopy (AFM) and transmission electron microscopy (TEM), which have provided information on the size, shape, and aggregation of SCNP. Unfortunately, SCNP behavior in the absence of solvent and their interactions with substrates are still not entirely understood, so studies have been done to observe SCNP in solution. Characterization of SCNP solution morphology has been achieved using small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) by measuring the radius of gyration and observing form factors.[22,50,68,91,98] Pomposo and co-workers analyzed a large number of SCNP in solution using a combination of SAXS and SANS.[95] Polystyrene (PS)-based systems with 30 different precursors and 11 different cross-linking chemistries including covalent, dynamic covalent, and noncovalent were studied in comparison to PS globules of the same molecular weight. Results indicated that the SCNP stray from compact globular proteins and rather take on a nonglobular, noncompact structure resembling intrinsically disordered proteins. The field would benefit from more comparative studies such as this with a large series of SCNP. While solution-based techniques provide insight into the morphology and folding conformation of SCNP, insufficient experimental evidence has been obtained to gain understanding of the conformation and their internal arrangement. Hosono and co-workers recently demonstrated an innovative technique utilizing AFM to acquire information about the internal folding structure of SCNP (Figure 13).[99] They synthesized polyacrylate-based polymers carrying 2-ureido-4-[1*H*]-pyrimidinone (UPy) or benzene-1,3,5-tricarboxamide (BTA) pendants that induce an intramolecular chain collapse into nanoparticles consisting of one polymer chain only via internal supramolecular cross-linking. The polymers were end-functionalized with dithiolane groups to anchor them between a gold surface and a gold-coated cantilever of the AFM. The SCNP was then stretched by the AFM cantilever to mechanically unfold it, allowing for the measurement of force−extension profiles. This is a unique method to access the internal structure of SCNP. Performing

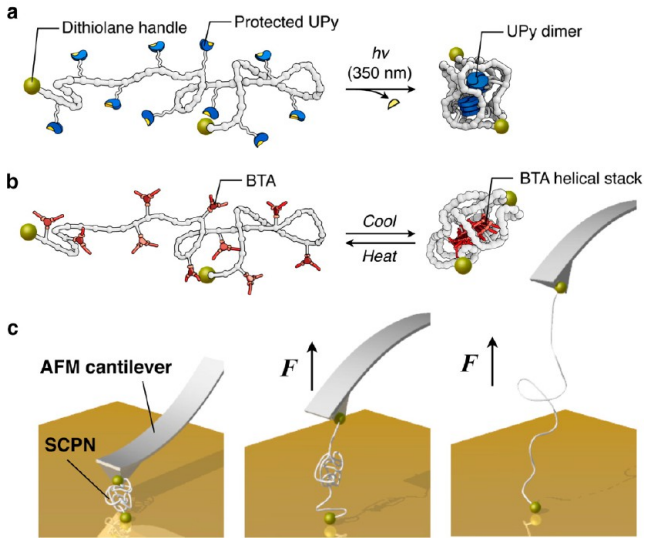


Figure 13. Schematic representations of folding polymer with (a) UPy modules P[UPyn] and (b) BTA modules P[BTAn], which self-assemble into dimer and helical columnar aggregates, respectively, resulting in SCPN formation. (c) Schematic illustrations of the mechanical unfolding experiment on SCPN. Reproduced with permission from ref 96.

experimental characterization techniques that probe the internal folding structure of SCNP will help to fully characterize these systems.

■ OUTLOOK

While synthetically mimicking natural biomolecules seems like a daunting task, examining simplified synthetic systems will lead to a better understanding of natural systems and the development of synthetic analogues. The synthesis and characterization of SCNP, although fundamental in nature, represents an important initial step toward advanced nano-architectures that are capable of mimicking the complexity found in natural systems. This Perspective highlights key areas where ongoing investigation and insight are necessary in order to continue moving forward. Specifically, the clever use of analytical techniques to characterize SCNP morphology and the folding process; the application of synthetic methods toward forming new structural features in SCNP, forming SCNP from different polymer precursors, and forming hierarchical structures with SCNP as structural units; the continued investigation of SCNP properties with benefits for various applications; and the challenges associated with scaling up and moving away from the ultradilute conditions required for SCNP formation.

Most examples of SCNP are derived from random copolymers or homopolymers with pendant functionality and the ability to internally or externally cross-link in an intramolecular fashion. More complex designs of polymer precursors using block copolymers or multiple orthogonal cross-linking methods are beginning to surface; however, these examples are relatively few, and the potential to incorporate various architectures and cross-linking methods leaves questions unanswered.

A critical challenge involves limitations of characterization techniques for both the kinetics of polymer collapse and the morphology of the final single-chain nanoparticle product. Efforts in characterization have allowed for evidence of SCNP

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

formation and information regarding their conformation and morphology, but full characterization of their internal structure has yet to be obtained. Clever methods, both computational and experimental, are required for continuing discovery to this end.

The scalability of SCNP is another issue that requires attention from researchers, as current ultradilute syntheses require an impractical volume of solvent to produce an industrially relevant quantity of material. Adaptation of chemistries that are already capable of SCNP formation at abnormally high polymer concentrations to continuous addition or flow systems could potentially begin to confront this problem.

Polymer science broadly, and SCNP chemistry in general, is still far from the ultimate goal of mimicking natural biomolecules. Regardless, by stripping away all of the complex interactions that dictate the behavior of biomacromolecules and learning how to control the conformation of a well-solvated single polymer coil in solution, using a variety of chemistry, and effectively characterizing this process, we have already taken an enormous step forward. As synthetic chemistry and analytical methods continue to evolve, we will continue to make progress in this regard. The versatility and ease of incorporating multiple functionalities in SCNP demonstrate their viability as candidates for critical technological needs, especially those that require materials in the same size regime as single polymer chains. So the evolution of single-chain nanoparticle chemistry continues, and improving simultaneously with it is the prospect of taking these fascinating materials to functional fruition.

AUTHOR INFORMATION

Corresponding Author

*E-mail Erik.Berda@unh.edu; Fax +603 862 4278; Tel +603 862 1762 (E.B.B.).

Notes

The authors declare no competing financial interest.

Biographies

Ashley Hanlon completed her B.S. in chemistry at Salve Regina University in Newport, RI, in 2011. She joined the Berda Research group as a PhD student at the University of New Hampshire in 2013. Her research is focused on using unexplored synthetic routes for controlling the spatial arrangement of chemistry on the surface of polymeric nanostructures.

Christopher Lyon obtained his B.S. in chemistry from the University of Massachusetts Lowell in 2011 and joined the Berda Research group as a PhD student at the University of New Hampshire in Autumn of the same year. His research interests include investigating intramolecular radical polymerization techniques used to synthesize single-chain nanoparticles.

Erik Berda received a BS in Chemistry from Penn State in 2003 where he was introduced to polymer science through research with Prof. Harry Allcock. After a completing a PhD in organic chemistry with Prof. Ken Wagener at the University of Florida (2008) and postdoctoral training with Prof. Dr. E. W. "Bert" Meijer at TU/e (Eindhoven, NL), Erik joined the faculty at the University of New Hampshire in 2010. He is currently an Associate Professor of Chemistry and Materials Science.

ACKNOWLEDGMENTS

We graciously acknowledge financial support from the University of New Hampshire, the Army Research Office for support through award W911NF-14-1-0177, and NIST for support through award 70NANB15H060.

REFERENCES

(1) Trinh, T. T.; Laure, C.; Lutz, J. F. *Macromol. Chem. Phys.* **2015**, *216*, 1498−1506.
(2) Gutekunst, W. R.; Hawker, C. J. *J. Am. Chem. Soc.* **2015**, *137*, 8038−8041.
(3) Gao, H. *Macromol. Rapid Commun.* **2012**, *33*, 722−734.
(4) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665−1688.
(5) Bachler, P.; Wagener, K. *Monatsh. Chem.* **2015**, *146*, 1053−1160.
(6) Gonzalez-Burgos, M.; Latorre-Sanchez, A.; Pomposo, J. *Chem. Soc. Rev.* **2015**, *44*, 6122−6142.
(7) Kuhn, W.; Balmer, G. *J. Polym. Sci.* **1962**, *57*, 311−319.

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

(8) Longi, P.; Greco, F.; Rossi, U. *Makromol. Chem.* **1968**, *116*, 113−121.
(9) Longi, P.; Greco, F.; Rossi, U. *Makromol. Chem.* **1969**, *129*, 157−164.
(10) Martin, J. E.; Eichinger, B. E. *Macromolecules* **1983**, *16*, 1345−1350.
(11) Martin, J. E.; Eichinger, B. E. *Macromolecules* **1983**, *16*, 1350−1358.
(12) Davankov, V. A.; Tsyurupa, M. P. *React. Polym.* **1990**, *13*, 27−42.
(13) Tsyurupa, M. P.; Mrachkovskaya, T. A.; Maslova, L. A.; Timofeeva, G. I.; Dubrovina, L. V.; Titova, E. F.; Davankov, V. A.; Menshov, V. M. *React. Polym.* **1993**, *19*, 55−66.
(14) Davankov, V. A.; Ilyin, M. M.; Tsyurupa, M. P.; Timofeeva, G. I.; Dubrovina, L. V. *Macromolecules* **1996**, *29*, 8398−8403.
(15) Altintas, O.; Lejeune, E.; Gerstel, P.; Barner-Kowollik, C. *Polym. Chem.* **2012**, *3*, 640−651.
(16) Altintas, O.; Rudolph, T.; Barner-Kowollik, C. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 2566−2576.
(17) Foster, E. J.; Berda, E. B.; Meijer, E. W. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 118−126.
(18) Foster, E. J.; Berda, E. B.; Meijer, E. W. *J. Am. Chem. Soc.* **2009**, *131*, 6964−6966.
(19) Terashima, T.; Mes, T.; De Greef, T. F.; Gillissen, M. A.; Besenius, P.; Palmans, A. R.; Meijer, E. W. *J. Am. Chem. Soc.* **2011**, *133*, 4742−4745.
(20) Artar, M.; Terashima, T.; Sawamoto, M.; Meijer, E. W.; Palmans, A. R. *J. Polym. Sci., Part A: Polym. Chem.* **2014**, *52*, 12−20.
(21) Gillissen, M. A. J.; Voets, I. K.; Meijer, E. W.; Palmans, A. R. *Polym. Chem.* **2012**, *3*, 3166−3174.
(22) Hosono, N.; Gillissen, M. A.; Li, Y.; Sheiko, S. S.; Palmans, A. R.; Meijer, E. W. *J. Am. Chem. Soc.* **2013**, *135*, 501−510.
(23) Stals, P. J. M.; Gillissen, M. A. J.; Nicolaÿ, R.; Palmans, A. R.; Meijer, E. W. *Polym. Chem.* **2013**, *4*, 2584−2597.
(24) Mes, T.; van der Weegen, R.; Palmans, A. R.; Meijer, E. W. *Angew. Chem., Int. Ed.* **2011**, *50*, 5085−5089.
(25) Ouchi, M.; Badi, N.; Lutz, J. F.; Sawamoto, M. *Nat. Chem.* **2011**, *3*, 917−924.
(26) Berda, E. B.; Foster, E. J.; Meijer, E. W. *Macromolecules* **2010**, *43*, 1430−1437.
(27) Seo, M.; Beck, B. J.; Paulusse, J. M. J.; Hawker, C. J.; Kim, S. Y. *Macromolecules* **2008**, *41*, 6413−6418.
(28) Huerta, E.; Stals, P. J.; Meijer, E. W.; Palmans, A. R. *Angew. Chem., Int. Ed.* **2013**, *52*, 2906−2910.
(29) Jeong, J.; Lee, Y.; Kim, B.; Kim, B.; Jung, K.; Paik, H. *Polym. Chem.* **2015**, *6*, 3392−3397.
(30) Mavila, S.; Diesendruck, C. D.; Linde, S.; Amir, L.; Shikler, R.; Lemcoff, N. G. *Angew. Chem., Int. Ed.* **2013**, *52*, 5767−5770.
(31) Sanchez-Sanchez, A.; Arbe, A.; Colmenero, J.; Pomposo, J. *ACS Macro Lett.* **2014**, *3*, 439−443.
(32) He, J.; Tremblay, L.; Lacelle, S.; Zhao, Y. *Soft Matter* **2011**, *7*, 2380−2386.
(33) Frank, P. G.; Tuten, B. T.; Prasher, A.; Chao, D.; Berda, E. B. *Macromol. Rapid Commun.* **2014**, *35*, 249−253.
(34) Croce, T. A.; Hamilton, S. K.; Chen, M. L.; Muchalski, H.; Harth, E. *Macromolecules* **2007**, *40*, 6028−6031.
(35) Harth, E.; Van Horn, B.; Lee, V. Y.; Germack, D. S.; Gonzales, C. P.; Miller, R. D.; Hawker, C. J. *J. Am. Chem. Soc.* **2002**, *124*, 8653−8660.
(36) Dobish, J. N.; Hamilton, S. K.; Harth, E. *Polym. Chem.* **2012**, *3*, 857−860.
(37) Altintas, O.; Willenbacher, J.; Wuest, K. N. R.; Oehlenschlaeger, K. K.; Krolla-Sidenstein, P.; Gliemann, H.; Barner-Kowollik, C. *Macromolecules* **2013**, *46*, 8092−8101.
(38) Tuteja, A.; Mackay, M. E.; Hawker, C. J.; Van Horn, B.; Ho, D. L. *J. Polym. Sci., Part B: Polym. Phys.* **2006**, *44*, 1930−1947.
(39) Dukette, T. E.; Van Horn, B.; Wooley, K. L.; Drockenmuller, E.; Malkoch, M.; Hawker, C. J. *Nano Lett.* **2005**, *5*, 1704−1709.
(40) Tuten, B. T.; Chao, D.; Lyon, C. K.; Berda, E. B. *Polym. Chem.* **2012**, *3*, 3068−3071.
(41) Ryu, J.; Chacko, R. T.; Jiwpanich, S.; Bickerton, S.; Babu, R. P.; Thayumanavan, S. *J. Am. Chem. Soc.* **2010**, *132*, 17227−17235.
(42) Perez-Baena, I.; Loinaz, I.; Padro, D.; García, I.; Grande, H. J.; Odriozola, I. *J. Mater. Chem.* **2010**, *20*, 6916−6922.
(43) Ormategui, N.; García, I.; Padro, D.; Cabañero, G.; Grande, H. J.; Loinaz, I. *Soft Matter* **2012**, *8*, 734−740.
(44) Ruiz de Luzuriaga, A.; Ormategui, N.; Grande, H. J.; Odriozola, I.; Pomposo, J. A.; Loinaz, I. *Macromol. Rapid Commun.* **2008**, *29*, 1156−1160.
(45) Ruiz de Luzuriaga, A.; Perez-Baena, I.; Montes, S.; Loinaz, I.; Odriozola, I.; García, I.; Pomposo, J. A. *Macromol. Symp.* **2010**, *296*, 303−310.
(46) Stevens, D. M.; Tempelaar, S.; Dove, A. P.; Harth, E. *ACS Macro Lett.* **2012**, *1*, 915−918.
(47) Davankov, V. A.; Ilyin, M. M.; Tsyurupa, M. P.; Timofeeva, G. I.; Dubrovina, L. V. *Macromolecules* **1996**, *29*, 8398−8403.
(48) Beck, J. B.; Killops, K. L.; Kang, T.; Sivanandan, K.; Bayles, A.; Mackay, M. E.; Wooley, K. L.; Hawker, C. J. *Macromolecules* **2009**, *42*, 5629−5635.
(49) Dirlam, P. T.; Kim, H. J.; Arrington, K. J.; Chung, W. J.; Sahoo, R.; Hill, L. J.; Costanzo, P. J.; Theato, P.; Char, K.; Pyun, J. *Polym. Chem.* **2013**, *4*, 3765−3773.
(50) Perez-Baena, I.; Barroso-Bujans, F.; Gasser, U.; Arbe, A.; Moreno, A. J.; Colmenero, J.; Pomposo, J. A. *ACS Macro Lett.* **2013**, *2*, 775−779.
(51) Sanchez-Sanchez, A.; Asenjo-Sanz, I.; Buruaga, L.; Pomposo, J. A. *Macromol. Rapid Commun.* **2012**, *33*, 1262−1267.
(52) Wen, J.; Yuan, L.; Yang, Y.; Liu, L.; Zhao, H. *ACS Macro Lett.* **2013**, *2*, 100−106.
(53) Zhu, B.; Qian, G.; Xiao, Y.; Deng, S.; Wang, M.; Hu, A. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 5330−5338.
(54) Murray, B. S.; Fulton, D. A. *Macromolecules* **2011**, *44*, 7242−7252.
(55) Whitaker, D. E.; Mahon, C. S.; Fulton, D. A. *Angew. Chem., Int. Ed.* **2013**, *52*, 956−959.
(56) Sanchez-Sanchez, A.; Fulton, D. A.; Pomposo, J. A. *Chem. Commun.* **2014**, *50*, 1871−1874.
(57) Jiang, J.; Thayumanavan, S. *Macromolecules* **2005**, *38*, 5886−5891.
(58) Mecerreyes, D.; Lee, V.; Hawker, C. J.; Hedrick, J. L.; Wursch, A.; Volksen, W.; Magbitang, T.; Huang, E.; Miller, R. D. *Adv. Mater.* **2001**, *13*, 204−208.
(59) Cherian, A. E.; Sun, F. C.; Sheiko, S. S.; Coates, G. W. *J. Am. Chem. Soc.* **2007**, *129*, 11350−11351.
(60) Zhu, B.; Ma, J.; Li, Z.; Hou, J.; Cheng, X.; Qian, G.; Liu, P.; Hu, A. *J. Mater. Chem.* **2011**, *21*, 2679−2683.
(61) Buruaga, L.; Pomposo, J. A. *Polymers* **2011**, *3*, 1673−1683.
(62) Jiang, X.; Pu, H.; Wang, P. *Polymer* **2011**, *52*, 3597−3602.
(63) Hansell, C. F.; Lu, A.; Patterson, J. P.; O'Reilly, R. K. *Nanoscale* **2014**, *6*, 4102−4107.
(64) Lyon, C. K.; Prasher, A.; Hanlon, A. M.; Tuten, B. T.; Tooley, C. A.; Frank, P. G.; Berda, E. B. *Polym. Chem.* **2015**, *6*, 181−197.
(65) Wong, E. H. H.; Qiao, G. G. *Macromolecules* **2015**, *48*, 1371−1379.
(66) Willenbacher, J.; Altintas, O.; Trouillet, V.; Knöfel, N.; Monteiro, M. J.; Roesky, P. W.; Barner-Kowollik, C. *Polym. Chem.* **2015**, *6*, 4358−4365.
(67) Sanchez-Sanchez, A.; Pomposo, J. A. *J. Nanomater.* **2015**, *2015*, 1−7.
(68) Moreno, A. J.; Lo Verso, F.; Sanchez-Sanchez, A.; Arbe, A.; Colmenero, J.; Pomposo, J. A. *Macromolecules* **2013**, *46*, 9748−9759.
(69) Chao, D.; Jia, X.; Tuten, B.; Wang, C.; Berda, E. B. *Chem. Commun.* **2013**, *49*, 4178−4180.
(70) Zhang, Y.; Zhao, H. *Polymer* **2015**, *64*, 277−284.
(71) Roy, R. K.; Lutz, J. F. *J. Am. Chem. Soc.* **2014**, *136*, 12888−12891.
(72) Anfinsen, C. B. *Science* **1973**, *181*, 223−230.

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14

(73) Dobson, C. M. *Nature* **2003**, *426*, 884−890.
(74) Gody, G.; Maschmeyer, T.; Zetterlund, P. B.; Perrier, S. *Nat. Commun.* **2013**, *4*, 1−9.
(75) Lutz, J.; Ouchi, M.; Liu, D. R.; Sawamoto, M. *Science* **2013**, *341*, 628−636.
(76) Anastasaki, A.; Nikolaou, V.; Pappas, G. S.; Zhang, Q.; Wan, C.; Wilson, P.; Davis, T. P.; Whittaker, M. R.; Haddleton, D. M. *Chem. Sci.* **2014**, *5*, 3536−3542.
(77) Schmidt, B.; Fechler, N.; Falkenhagen, J.; Lutz, J. *Nat. Chem.* **2011**, *3*, 234−238.
(78) Berda, E. B.; Wagener, K. B. In *Complex Macromolecular Architecture: Synthesis, Characterization, and Self- Assembly*; Hadjichristidis, N., Hirao, A., Tezuka, Y., Du Prez, F., Eds.; John Wiley & Sons (Asia) Pte Ltd.: Singapore, 2011; Chapter "Precision Polyolefins".
(79) Zhang, Z.; You, Y.; Wu, D.; Hong, C. *Macromolecules* **2015**, *48*, 3414−3421.
(80) Xie, M. X.; Jiang, L.; Xu, Z. P.; Chen, D. Y. *Chem. Commun.* **2015**, *51*, 1842−1845.
(81) Oria, L.; Aguado, R.; Pomposo, J. A.; Colmenero, J. *Adv. Mater.* **2010**, *22*, 3038−3041.
(82) Adkins, C. T.; Dobish, J. N.; Brown, S.; Harth, E. *ACS Macro Lett.* **2013**, *2*, 710−714.
(83) Romulus, J.; Weck, M. *Macromol. Rapid Commun.* **2013**, *34*, 1518−1523.
(84) Sun, J.; Hong, C.; Pan, C. *Polym. Chem.* **2013**, *4*, 873−881.
(85) Groschel, A. H.; Walther, A.; Lobling, T. I.; Schacher, F. H.; Schmalz, H.; Muller, A. H. *Nature* **2013**, *503*, 247−251.
(86) Wen, J.; Zhang, J.; Zhang, Y.; Yang, Y.; Zhao, H. *Polym. Chem.* **2014**, *5*, 4032−4038.
(87) Tao, J.; Liu, G. *Macromolecules* **1997**, *30*, 2408−2411.
(88) Kim, Y.; Pyun, J.; Fréchet, J. M. J.; Hawker, C. J.; Frank, C. W. *Langmuir* **2005**, *21*, 10444−10458.
(89) Liu, J. W.; Mackay, M. E.; Duxbury, P. M. *Macromolecules* **2009**, *42*, 8534−8542.
(90) Yoshikawa, K.; Yoshinaga, N. *J. Phys.: Condens. Matter* **2005**, *17*, S2817−S2823.
(91) Sanchez-Sanchez, A.; Akbari, S.; Etxeberria, A.; Arbe, A.; Gasser, U.; Moreno, A. J.; Colmenero, J.; Pomposo, J. A. *ACS Macro Lett.* **2013**, *2*, 491−495.
(92) Lindorff-Larsen, K.; Piana, S.; Dror, R. O.; Shaw, D. E. *Science* **2011**, *334*, 517−520.
(93) Danilov, D.; Barner-Kowollik, C.; Wenzel, W. *Chem. Commun.* **2015**, *51*, 6002−6005.
(94) Lo Verso, F.; Pomposo, J. A.; Colmenero, J.; Moreno, A. J. *Soft Matter* **2015**, *11*, 1369−1375.
(95) Pomposo, J. A.; Perez-Baena, I.; Lo Verso, F.; Moreno, A. J.; Arbe, A.; Colmenero, J. *ACS Macro Lett.* **2014**, *3*, 767−772.
(96) Frank, P.; Prasher, A.; Tuten, B.; Chao, D.; Berda, E. *Appl. Petrochem. Res.* **2015**, *5*, 9−17.
(97) Appel, E. A.; del Barrio, J.; Dyson, J.; Isaacs, L.; Scherman, O. A. *Chem. Sci.* **2012**, *3*, 2278−2281.
(98) Lundberg, P.; Lynd, N. A.; Zhang, Y.; Zeng, X.; Krogstad, D. V.; Paffen, T.; Malkoch, M.; Nystrom, A. M.; Hawker, C. J. *Soft Matter* **2013**, *9*, 82−89.
(99) Hosono, N.; Kushner, A. M.; Chung, J.; Palmans, A. R.; Guan, Z.; Meijer, E. W. *J. Am. Chem. Soc.* **2015**, *137*, 6880−6888.
(100) Tooley, C. A.; Pazicni, S.; Berda, E. B. *Polym. Chem.* **2015**, *6*, 7646.

DOI: 10.1021/acs.macromol.5b01456
Macromolecules 2016, 49, 2−14