Reexamining single-chain nanoparticles.

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ABSTRACT: Single-chain nanoparticles (SCNP) are a class of polymeric nanoparticles obtained from the intramolecular crosslinking of polymers bearing reactive pendant groups. The development of SCNP has drawn tremendous attention since the fabrication of SCNP mimics the self-folding behavior in natural biomacromolecules, and are highly desirable for a variety of applications ranging from catalysis, nanomedicine, nanoreactors and sensors. The versatility of novel chemistries available for SCNP synthesis has greatly expanded over the past decade. Significant progress was also made in the understanding of structure-property relationship in the single chain folding process. In this Viewpoint, we discuss the effect of precursor polymer topology on single polymer folding. We summarize progress in SCNP of complex architectures, and highlight unresolved issues in the field, such as scalability and topological purity of SCNP.

Polymeric nanoparticles have been utilized in an increasing number of fields over the past two decades due to their unique properties such as design flexibility and good biocompatibility. Despite various techniques available to produce polymer nanoparticles, the preparation of small nanoparticles with customized functions in the sub 20 nm dimension remains challenging. Inspired by the self-organizing behavior of natural biomacromolecules, a class of materials referred to as "single-chain nanoparticles" (SCNP) are synthesized featuring biomimicry and ultrafine size. These nanoparticles are prepared from self-folding of polymer precursors bearing reactive pendant groups. Linear polymer precursors bearing reactive pendant groups undergo intramolecular cross-linking when the solution concentration is below the polymer's overlap concentration c*. After intramolecular cross-linking, the polymer folds into a well-defined nanosized object with potential functions such as catalysis and sensing.[1-4] (Fig.1A) In that sense, SCNP partially mimics the natural folding process of peptide chains that affords complex hierarchical protein structures, but can be made using a variety of monomers and polymerization techniques available. An immense number of synthetic methodologies have been applied to the formation of SCNP, including covalent, dynamic covalent and noncovalent cross-linking.[1-4] The main approaches to achieve chain folding can be classified into homofunctional cross-linking, heterobifunctional cross-linking and cross-linker mediated chain collapse. (Fig. 1B)[5]

Over the past decade, due to the advancement of controlled polymerization techniques as well as utilization of efficient synthetic strategies such as photo-mediated reactions and organometallic reactions,[6-9] the scientific community has witnessed a surge in the study of SCNP. New characterization methods also contribute significantly to the expansion of SCNP studies. [4, 10-20] The synthesis, characterization and potential use of SCNP were extensively covered in a handful of review articles.[1, 3, 5, 21-28] While the library of chemistry used to obtain SCNP continues to expand, a great amount of work emerged offering deeper fundamental studies on the structure-property relationship in the single chain folding process. Efforts were also made in synthesizing SCNP with complex architectures or exhibiting protein-like catalytic behaviors. In this Viewpoint, we highlight a selection exciting recent SCNP work, and discuss areas of question that remain unresolved in this field.

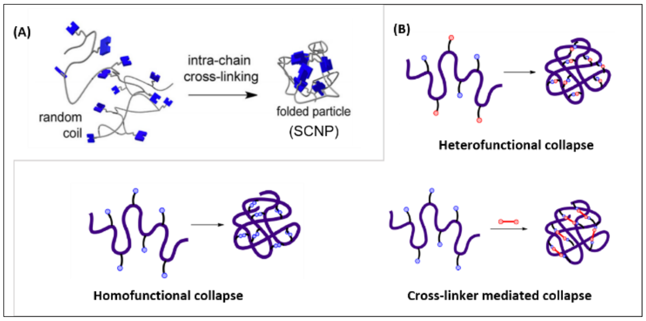

Figure 1. (A) Schematic representation of SCNP synthesis;[4] (B) Modes for intramolecular cross-linking of a polymer chain.[5] Adapted with permission from Ref. 5. Copyright 2016 American Chemical Society.

Effect of precursor topology on single chain folding

The majority of SCNP systems begin with a simple linear polymer precursor. Utilizing non-linear polymer precursors, such as cyclic, graft, star and dendritic polymer precursors for SCNP synthesis has been reported, but their effect on the single chain folding process is rarely explored. SCNP based on linear polymer precursors often exhibit a sparse, non-globular conformation in solution, as revealed by both SAXS, SANS data and computer simulations.[29] A recent molecular dynamics simulation study by Formanek and Moreno[30] predicted that using cyclic polymers as precursors would enhance intramolecular cross-linking of distant functional groups and lead to SCNP with more compact and globular conformation. Experimental work by Rubio-Cervilla and coworkers[31] validated this prediction. They synthesized a single ring polymer *via* hetero Diels-Alder reaction of chain ends on the linear polymer precursor. The resulting cyclic polymer was subsequently activated by azidation, then subjected to collapse *via* CuAAC in the presence of an external dialkyne cross-linker. (Fig.2A) As a comparison, an SCNP from direct cross-linking of the linear polymer precursor was synthesized using the same reaction sequence without the ring-closing step. It was found that the SCNP from ring polymer precursor had a higher compaction degree than the SCNP from linear precursor. This phenomenon was attributed to the conformation of the polymer precursors: In the case of linear polymer precursor, cross-linking of functional groups pre-aligned within the chain contributes little to the level of compaction. By cyclization, the chain conformation has a distinct rearrangement, resulting in a higher degree of compaction upon collapse.

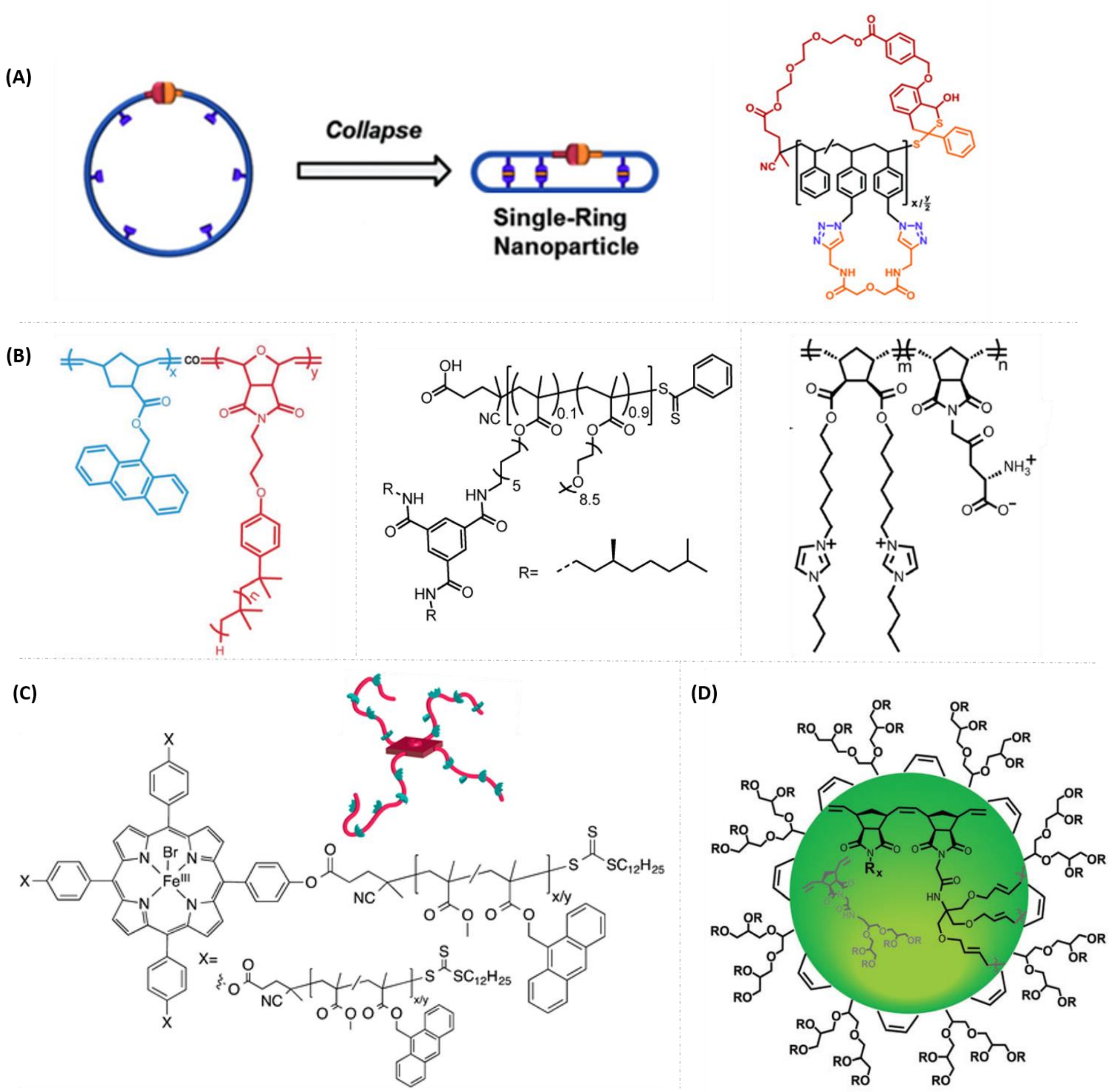

Figure 2. (A) Schematic illustration of the synthesis of a single-ring nanoparticle from a cyclic polymer precursor;[31] (Adapted with permission from Ref. 31. Copyright 2018 Wiley) (B) Examples of polymer precursors with grafted side chains: polyisobutylene side chains soluble in THF and hexanes;[32] water soluble linear PEG side chains;[33] water soluble alkyl side chains containing imidazolium groups;[34] (C) Porphyrin-cored star polymer precursor;[35] (Reprinted with permission from Ref. 35. Copyright 2016 American Chemical Society) (D) Crosslinked dendronized polymer.[36] (Reprinted with permission from Ref. 36. Copyright 2016 The Royal Society of Chemistry.

Some SCNP were synthesized from linear polymer precursors with long side chains, i.e., polymer grafts. Polymer grafts carrying cross-linkable functional groups may aid the intramolecular reaction, since the increased length and flexibility of side chains allows cross-linking of sites separated by long contour distance. This design is widely found in literature but quantitative evaluation of graft length on polymer folding is scarcely reported. A recent study in our group[37] explored the length of methacryloyl-functionalized side chain in relation to the folding of a poly(oxanorbornene) polymer precursor. We compared the difference of a 2-carbon and a 6-carbon spacer between the polymer backbone and the pendant cross-linkable group. Our results showed that within the length scales we studied, side chain length does not significantly affect polymer folding by intramolecular radical polymerization.

Polymer grafts that are not functionalized with cross-linkable groups may contribute to the overall solubility of SCNP. During the synthesis of SCNP, intramolecular cross-linking of the functional groups leads to collapsed inner compartments. Polymer grafts not carrying functional groups could be made solvophilic or hydrophilic. They exhibit an extended conformation in solution, shielding the core of SCNP and stabilizing the SCNP solution by repulsive forces between coronas. A study by Liu and coworkers[32] reported intramolecular cross-linking of comb polymers with large aliphatic polyisobutylene grafts, which have affinity for non-polar aliphatic solvents. They found that switching the solvent from THF to hexanes has pronounced effect on the chain collapse, indicating that disparate solvency could be a choice to tune chain collapse of a single comb copolymer. Besides hydrophobic polymer grafts, hydrophilic grafts are widely applied in the synthesis of water soluble SCNP. These hydrophilic grafts on the polymers could be water soluble PEG chains,[33] or ionic species embedded in an alkyl chain. (Fig. 2B)[34]

Graft polymers precursors with densely grafted polymeric branches adopt a persistent cylindrical shape in solution, when the polymeric side chains are much shorter than the backbone.[38-39] Intramolecular cross-linking of such polymers does not led to single chain compaction, but instead the cylindrical shape remains. Therefore, they were used as templates for the preparation of nanotubes. The Rzayev group developed a series of core-shell bottlebrush copolymers that exhibit cylindrical shape in solution and used them as single molecule templates.[40-45] The polymer precursors were subjected to intramolecular cross-linking of the shell layer followed by selective removal of the core. The size and shape of the polymer precursor was preserved after these transformations, leading to organic nanotubes. The polymer backbone could be methacrylate or norbornene-based, and a variety of cross-linking chemistries have been utilized, including photodimerization of coumarin functionalities,[40-41] oxidative coupling of mercapto groups,[42] cross metathesis of olefin groups,[43, 45] and oxidative polymerization of pyrrole groups.[44]

Intramolecular cross-linking of star-shaped and dendritic polymer precursors were scarcely reported in the literature. The first example of intramolecular cross-linking of a star polymer was reported by Van Renterghem and coworkers in 2008.[46] They synthesized star-shaped poly(isobornyl acrylate) precursors with methacrylate end groups and converted them to unimolecular nanoparticles by intramolecular polymerization. The nanoparticles were used as viscosity modifiers in the polymerization of acrylate monomers and changed the visco-elastic properties of the polymer by noncovalent chain entanglements. Ding and colleagues[47] synthesized polymer nanoparticles from intramolecular cross-linking of reactive azo-containing star polymer precursors. The nanoparticles show circular transformations when the azo chromophores undergo trans-cis isomerization cycles. A porphyrin-cored four-arm star polymer was synthesized by Rodriguez et al[35] and transformed to a nanoparticle by intramolecular anthracene dimerization. (Fig. 2C) The resulting porphyrin-cored polymer nanoparticle displayed redox and ligand-binding reactivity and was used as a macromolecular model for heme proteins.

Dendrimers with a high degree of branching adopt a three-dimensional globular conformation with a large number of functional groups at the periphery.[48] Intramolecular cross-linking of high-generation ($g \geq 3$) dendronized polymer precursor was scarcely found in the literature. In 2002 Zimmerman and coworkers[49] reported the synthesis of macromolecular hosts for porphyrin guests based on dendrimer precursors. An allyl-functionalized dendrimer was synthesized containing a porphyrin core as the templating agent. The dendrimer was subjected to intramolecular ring-closing metathesis of the peripheral allyl groups, leading to a cross-linked unimolecular nanoparticle. A subsequent study by Lemcoff et al[50] showed that a dendrimer's size reduction is linearly dependent on the extent of intramolecular cross-linking, irrespective of the number of dendrons used. Encapsulating a hydrophobic, fluorescent dye inside the core of a water-soluble shell-crosslinked dendrimer provides solubility of the dye in aqueous media.[51] The cross-linked dendritic shell of the small and rigid nanoparticle enhanced the photostability of the fluorescent dye in water. A similar approach of solubilizing and stabilizing fluorophores was applied on linear dendronized polymers.[36] Linear dendronized polymers contain densely packed dendrons along the polymer backbone and adopt a semi-rod-like structure, hence intramolecular cross-linking occurs primarily on short range, leading to lower degree of compaction. (Fig. 2D)

SCNP with novel topologies

Tadpole-shaped SCNP are composed of a flexible polymer attached to an intramolecularly folded SCNP. They are commonly synthesized from diblock linear polymer precursors containing a reactive block and an inert block. A mathematical study by Asenjo-Sanz and coworkers[52] demonstrated that the size of a tadpole-shaped SCNP is related to its tail length and could be predicted prior to the synthesis of SCNP. Tadpole-shaped SCNP exhibit surfactant-mimicking structures and have been used as nano building blocks to self-assemble to higher ordered structure.[53-58]

Zhang and coworkers[59] synthesized linear diblock copolymers with diol pendants, and used a boronic acid cross-linker to afford tadpole-shaped SCNP. The crosslinker exhibited an isoelectric point so the head of the tadpole undergoes hydrophobic/hydrophilic transition based on pH. The SCNP self-assembled into spherical multimeric morphology and the assembly could be dissociated by varying the environmental pH or adding glucose. Thanneeru et al[60] prepared amphiphilic diblock copolymers poly(PDMA-*b*-

PS) from N,N’-dimethylacrylamide and styrene monomers. Photo-crosslinkable cinnamoyl groups reside in either the hydrophilic PDMA block or the hydrophobic PS block. Intramolecular photo-cross-linking of cinnamoyl groups led to the formation of tadpole-shaped SCNP containing either a hydrophobic or hydrophilic self-collapsed head. It was found that in both cases, the size and morphology of the self-assembled SCNP are related to the degree of intramolecular cross-linking and could be controlled from a single polymer precursor. A systematic study on the self-assembly behavior of tadpole-shaped SCNP consisting liquid crystalline (LC) properties was reported by Wen and coworkers.[61] (Fig. 3A) By varying the UV irradiation time or hydrophilic block chain length, a library of assembled morphologies were afforded, including tubular assemblies, bowl-like particles, saddle-shaped lamellae and spheres.

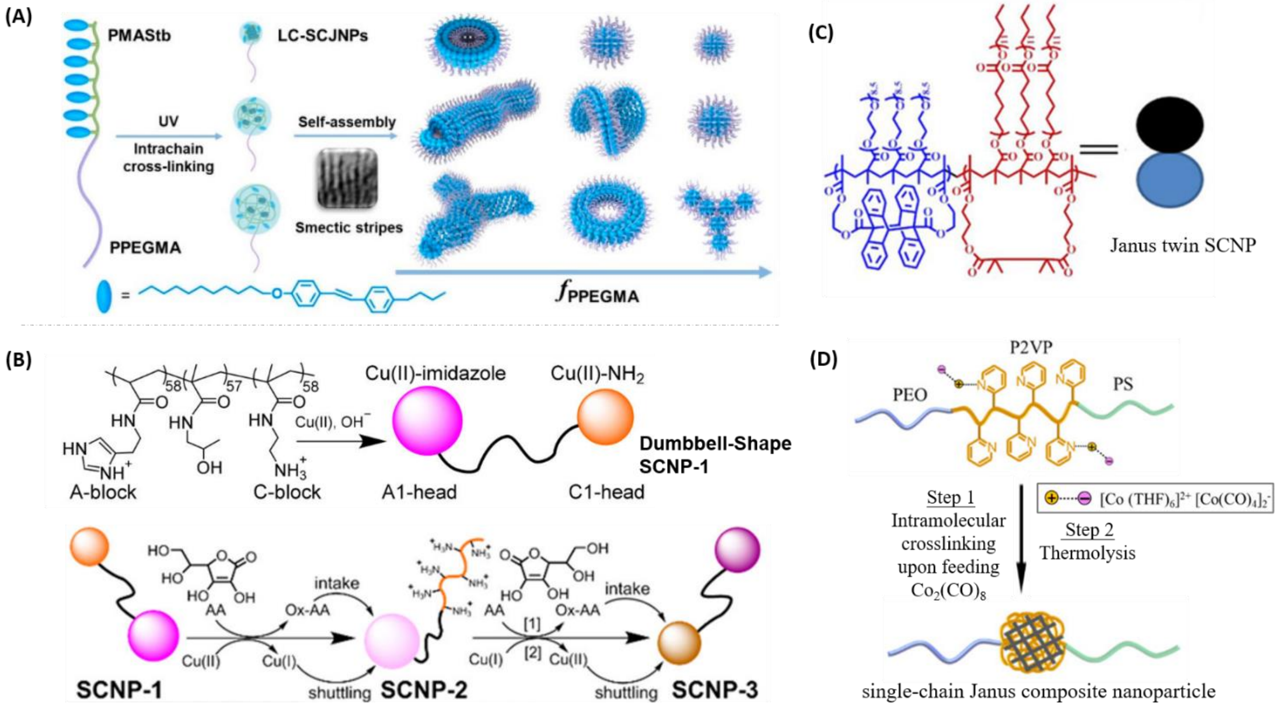

Figure 3. (A) Synthesis of SCNP with tunable liquid crystalline properties via intrachain photo cross-linking stilbene-containing amphiphilic block copolymers and the self-assembly behavior of SCNP in solution;[61] (Reprinted with permission from Ref. 61. Copyright 2019 American Chemical Society) (B) Schematic illustration of the formation of dumbbell-shaped SCNP1 and suggested molecular shuttling mechanism of SCNP;[62] (Reprinted with permission from Ref. 62. Copyright 2018 American Chemical Society) (C) Janus twin SCNP;[63] (Adapted with permission from Ref. 63. Copyright 2018 Wiley) (D) Synthesis of single-chain Janus composite nanoparticle from PEO-*b*-P2VP-*b*-PS.[64] (Adapted with permission from Ref. 64. Copyright 2020 American Chemical Society)

Another pathway to tadpole-shaped SCNP is to synthesize a self-cross-linked nanoparticle first, then attach a tail in a separate step. Recently, a set of photoligations that can be carried out orthogonally either by a sequence of decreasing or increasing wavelengths was reported by the Barner-Kowollik group.[65] They synthesized a polymer precursor containing a styrylpyrene chain end and 9-triazolylanthracene units distributed along the backbone. The polymer can be ligated with a PEG chain through [2+2] cycloaddition of styrylpyrene at the chain ends, and folded into an SCNP through [4+4] cycloaddition of the anthracene units along the polymer backbone. The two sets of reactions could occur in either order controlled by irradiation wavelength, and both lead to tadpole-shaped SCNP.

Orthogonal self-folding of a diblock copolymer bearing reactive groups within each block leads to a double compartment SCNP. Matsumoto and coworkers[66] synthesized an amphiphilic random block copolymer containing PEG and hydrophobic dodecyl, benzyl and olefin pendants. The copolymer undergoes orthogonal self-assembly of hydrophobic dodecyl and benzyl pendants in water, followed by covalent crosslinking, forming an SCNP carrying double yet distinct hydrophobic nanocompartments. Another study by Kozawa and coworkers[67] used amphiphilic ABA random triblock copolymers as precursors bearing PEG and hydrophobic dodecyl pendants as the A-segments, and a hydrophilic poly(ethylene oxide) (PEO) as the middle B-segment. It was found that the copolymers could self-assemble into PEO-linked double core unimer micelles, PEO-looped unimer or dimer micelles, or multimeric micelles, depending on the composition of the polymer precursor.

Claus and coworkers[68] employed a PMMA-based ABC triblock copolymer precursor to form dual compacted SCNP. The middle B-block was a nonfunctional spacer block. The outer A- and C-blocks carry phenacyl sulfide and α-methylbenzaldehyde moieties respectively and could be sequentially compacted with external dithiol or diacrylate cross-linkers within each block. It was found that the hydrodynamic diameter of the polymer was significantly reduced after the first compaction. The second compaction revealed a far less pronounced reduction in size, due to the reduced degrees of freedom available after the first compaction. A dumbbell-shaped SCNP was synthesized by Cui and coworkers[69] using an ABC triblock copolymer bearing protonated imidazolium motifs in the A- block, NH_3^+ motifs in the C-block, and hydroxyl motifs in the B-block. (Fig. 3B) The outer blocks underwent stepwise coordination to copper ions, taking advantage of the difference in the basicity and coordination of imidazole and NH_2 motifs. In a follow-up study[62], the dumbbell-shaped SCNP was subjected to ascorbic acid reduction and air oxidation. Unidirectional molecule shuttling between discrete double heads was found, leading to dumbbell-*to*-tadpole-*to*-dumbbell configurational transition and the intake of oxidized ascorbic acid into as-reassembled heads.

Multi-block SCNP were synthesized by Zhang and coworkers[70] using a stepwise folding-chain extension-folding process. A multi-block copolymer was made by RAFT polymerization containing hydroxyl pendants. The hydroxyl groups were cross-linked using isocyanate cross-linkers, leading to compaction of the block. Subsequent chain extension added a spacer block and a further hydroxyl-decorated block, and folding was repeated to generate SCNP with segregated compacted domains. A pentablock copolymer was synthesized by this approach bearing three individually folded subdomains with an overall dispersity of 1.21.

Janus SCNP are prepared from double cross-linking of A-*b*-B diblock polymer precursors. They are a type of compartmented SCNP with distinct hydrophilicities between the compartments. Ji and coworkers[63] reported the synthesis of an amphiphilic Janus twin SCNP by two-step intramolecular cross-linking reactions including anthracene photodimerization and atom transfer radical coupling reaction. (Fig. 3C) The amphiphilic nanoparticles possess surfactant properties and reduced the surface tension of water. In aqueous solution, the nanoparticles self-assemble into vesicles with hydrophobic moieties in the inner walls and hydrophilic parts on the surface. Self-assembly behavior of the double cross-linked Janus SCNP was different from its linear precursor polymer.

Another type of Janus SCNP was reported by Xiang and coworkers[64] and contains a cross-linked core with hydrophilic and hydrophobic tails on the opposite sides of the core. (Fig. 3D) A linear polymer of polyethylene oxide-*block*-poly(2-vinylpyridine)-*block*-polystyrene (PEO-*b*-P2VP-*b*-PS) was first pretreated with $Co_2(CO)_8$ to introduce positive charges to the P2VP chain. The pretreated polymer was then cross-linked by metal complexation, followed by thermolysis to afford Janus SCNP containing cobalt within the core. The amphiphilic Janus nanoparticles worked as functional emulsifiers that could deliver metallic cobalt toward emulsion interfaces. They also exhibit high catalytic capability and recyclability in the reduction of nitrobenzene to aniline at the oil-water emulsion interface.

Scale up methods

SCNP synthesis generally requires ultra-dilute condition which limits the scalability of SCNP. Besides continuous addition strategy,[71] some other methods have been developed targeting intramolecular cross-linking at higher concentrations. These approaches include steric stabilization by polymer brushes,[72] introducing electrostatic interaction along polymer chains,[73] and internalizing the cross-linking groups.[74] Generalized methods for efficient SCNP synthesis at higher concentration is still required.

A study by ter Huurne and coworkers[75] found that steric stabilization by polymer grafts allowed SCNP synthesis at high concentrations. They created copolymers containing dendritic polyglycerol grafts and found that the dendritic hydrophilic grafts can provide steric stabilizing effect to efficiently isolate the cross-linking groups, preventing interparticle coupling events. As a result, the synthesis of SCNP could be carried out at concentrations as high as 100 mg/mL.[72]

Introducing electrostatic repulsion along polymer chains is another way for intramolecular cross-linking polymers in concentrated solutions. Xiang and coworkers[73] fabricated polystyrene-block-poly(4-vinylpyridine)-block poly(ethylene oxide) (PS-b-P4VP-b-PEO) copolymers and used 1,5-diiodopentane as a modification agent and a crosslinker. They found that intramolecular cross-linking was achieved at 300 mg/mL.

Topological purity of SCNP

Due to the random conformation of polymer chains in solution and the kinetically controlled cross-linking process[76-77], there are large structural and conformational deviations among SCNP produced in the same cross-linking system. The mixture of SCNP of different size and morphology may lead to unpredictable properties of the material. Characterization and purification of the topologically impure SCNP is needed to achieve the same level of precision and complexity as natural biomacromolecules.

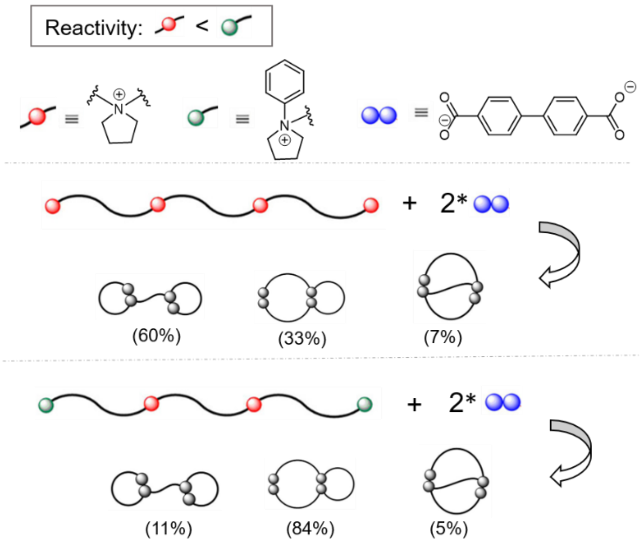

Figure 4. Construction of dicyclic polymer topologies (manacle-form, 8-form and θ-form through the folding of a tetrafunctional telechelic precursor.[78] (Adapted with permission from Ref. 78. Copyright 2019 American Chemical Society)

Recently, programmed folding of a pair of linear polymer precursors having four linking units was reported by Kyoda and coworkers.[78] (Fig. 4) The polymers contain four linking units. By adding two equivalents of the difunctional crosslinker, the linear polymer folds into dicyclic isomers of manacle-, 8-, and θ-forms. Different ratios of the three isomers were obtained when adjusting the reactivities of the four linking units. The results indicated that the polymer folding process is directed either by spatial distance between functional points, or by the chemical reactivity of the functional points in the telechelic precursor. Topologically controlled folding of a dendritic polymer precursor having six reactive end groups was demonstrated by Suzuki and coworkers.[79] Reacting the polymer with two equivalents of the trifunctional cross-linkers led to nanoparticles of $K_{3,3}$ graph topology and another constitutional isomer having a tetracyclic ladder form. The $K_{3,3}$ graph isomer was found to be remarkably contracted in comparison with the ladder form isomer in solution.

The fabrication of pure Janus-type SCNP is reported by Jiang et al.[77] They synthesized a diblock copolymer A-*b*-B containing alkyne and tertiary amine pendants within the A- and B-blocks. Two-step intramolecular cross-linking *via* Glaser coupling and amine quaternization led to a mixture of single chain Janus nanoparticles, multichain particles and irregular single-chain particles. Under appropriate conditions, the single-chain Janus nanoparticles in the mixture exclusively self-assembled to form regularly structured macroscopic assemblies that crystallized out of the suspension. The exclusive self-assembly behavior of pure Janus SCNP is rationalized by the highly uniform size, shape and surface structures matching those of the surrounding SCNP.

Outlook

In the past five years since our last perspective on this topic, there has been an amazing number of important advances as is evident by the excellent work highlighted above. Perhaps most critical is the effort by members of our community to simultaneously and deeply investigate both basic fundamental aspects of SCNP precursor synthesis, chain collapse processes, characterization, etc., along with an increased emphasis on functional applications. In the next five years we look forward to seeing these applied aspects of SCNP continue to progress as the synthetic methods and characterization of these materials become almost routine. One thing is certainly clear: research on SCNP is not a passing curiosity but a vibrant and intricately evolving subfield of polymer chemistry and will remains so for the foreseeable future.

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Notes

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REFERENCE

(1) Lyon, C. K.; Prasher, A.; Hanlon, A. M.; Tuten, B. T.; Tooley, C. A.; Frank, P. G.; Berda, E. B. A Brief User's Guide to Single-Chain Nanoparticles. *Polym. Chem.* **2015,** *6*, 181-197.

(2) Hanlon, A. M.; Lyon, C. K.; Berda, E. B. What Is Next in Single-Chain Nanoparticles? *Macromolecules* **2015,** *49*, 2-14.

(3) Cole, J. P.; Hanlon, A. M.; Rodriguez, K. J.; Berda, E. B. Protein-Like Structure and Activity in Synthetic Polymers. *J. Polym. Sci., Part A: Polym. Chem.* **2017**, *55*, 191-206.

(4) Frank, P.; Prasher, A.; Tuten, B.; Chao, D.; Berda, E. Characterization of Single-Chain Polymer Folding Using Size Exclusion Chromatography with Multiple Modes of Detection. *Appl. Petrochem. Res.* **2014,** *5*, 9-17.

(5) Mavila, S.; Eivgi, O.; Berkovich, I.; Lemcoff, N. G. Intramolecular Cross-Linking Methodologies for the Synthesis of Polymer Nanoparticles. *Chem. Rev.* **2016,** *116*, 878-961.

(6) Chao, D.; Wang, S.; Tuten, B. T.; Cole, J. P.; Berda, E. B. Densely Functionalized Pendant Oligoaniline Bearing Poly(Oxanorbornenes): Synthesis and Electronic Properties. *Macromolecules* **2015,** *48*, 5054-5057.

(7) Tuten, B. T.; De Keer, L.; Wiedbrauk, S.; Van Steenberge, P. H. M.; D'hooge, D. R.; Barner-Kowollik, C. Visible-Light-Induced Passerini Multicomponent Polymerization. *Angew. Chem. Int. Ed.* **2019,** *58*, 5672-5676.

(8) Tuten, B. T.; Bloesser, F. R.; Marshall, D. L.; Michalek, L.; Schmitt, C. W.; Blanksby, S. J.; Barner-Kowollik, C. Polyselenoureas Via Multicomponent Polymerizations Using Elemental Selenium as Monomer. *ACS Macro Lett.* **2018,** *7*, 898-903.

(9) Menzel, J. P.; Feist, F.; Tuten, B.; Weil, T.; Blinco, J. P.; Barner-Kowollik, C. Light-Controlled Orthogonal Covalent Bond Formation at Two Different Wavelengths. *Angew. Chem. Int. Ed.* **2019,** *58*, 7470-7474.

(10) Pomposo, J. A. *Single-Chain Polymer Nanoparticles: Synthesis, Characterization, Simulations, and Applications*, Wiley: 2017.

(11) Engelke, J.; Brandt, J.; Barner-Kowollik, C.; Lederer, A. Strengths and Limitations of Size Exclusion Chromatography for Investigating Single Chain Folding – Current Status and Future Perspectives. *Polym. Chem.* **2019,** *10*, 3410-3425.

(12) Steinkoenig, J.; Rothfuss, H.; Lauer, A.; Tuten, B. T.; Barner-Kowollik, C. Imaging Single-Chain Nanoparticle Folding Via High-Resolution Mass Spectrometry. *J. Am. Chem. Soc.* **2017,** *139*, 51-54.

(13) Blasco, E.; Tuten, B. T.; Frisch, H.; Lederer, A.; Barner-Kowollik, C. Characterizing Single Chain Nanoparticles (Scnps): A Critical Survey. *Polym. Chem.* **2017,** *8*, 5845-5851.

(14) Arbe, A.; Rubio-Cervilla, J.; Alegría, A.; Moreno, A. J.; Pomposo, J. A.; Robles-Hernández, B.; Malo de Molina, P.; Fouquet, P.; Juranyi, F.; Colmenero, J. Mesoscale Dynamics in Melts of Single-Chain Polymeric Nanoparticles. *Macromolecules* **2019,** *52*, 6935-6942.

(15) González-Burgos, M.; Arbe, A.; Moreno, A. J.; Pomposo, J. A.; Radulescu, A.; Colmenero, J. Crowding the Environment of Single-Chain Nanoparticles: A Combined Study by Sans and Simulations. *Macromolecules* **2018,** *51*, 1573-1585.

(16) Oberdisse, J.; González-Burgos, M.; Mendia, A.; Arbe, A.; Moreno, A. J.; Pomposo, J. A.; Radulescu, A.; Colmenero, J. Effect of Molecular Crowding on Conformation and Interactions of Single-Chain Nanoparticles. *Macromolecules* **2019,** *52*, 4295-4305.

(17) Lo Verso, F.; Pomposo, J. A.; Colmenero, J.; Moreno, A. J. Simulation Guided Design of Globular Single-Chain Nanoparticles by Tuning the Solvent Quality. *Soft Matter* **2015,** *11*, 1369-75.

(18) Hosono, N.; Kushner, A. M.; Chung, J.; Palmans, A. R.; Guan, Z.; Meijer, E. W. Forced Unfolding of Single-Chain Polymeric Nanoparticles. *J. Am. Chem. Soc.* **2015,** *137*, 6880-8.

(19) Li, Z.; Li, Y.; Zhao, Y.; Wang, H.; Zhang, Y.; Song, B.; Li, X.; Lu, S.; Hao, X. Q.; Hla, S. W.; Tu, Y.; Li, X. Synthesis of Metallopolymers and Direct Visualization of the Single Polymer Chain. *J. Am. Chem. Soc.* **2020,** *142*, 6196-6205.

(20) Steinkoenig, J.; Nitsche, T.; Tuten, B. T.; Barner-Kowollik, C. Radical-Induced Single-Chain Collapse of Passerini Sequence-Regulated Polymers Assessed by High-Resolution Mass Spectrometry. *Macromolecules* **2018,** *51*, 3967-3974.

(21) Huurne, G. M. t.; Palmans, A. R. A.; Meijer, E. W. Supramolecular Single-Chain Polymeric Nanoparticles. *CCS Chem.* **2019,** *1*, 64-82.

(22) Gonzalez-Burgos, M.; Latorre-Sanchez, A.; Pomposo, J. A. Advances in Single Chain Technology. *Chem. Soc. Rev.* **2015,** *44*, 6122-42.

(23) Kroger, A. P. P.; Paulusse, J. M. J. Single-Chain Polymer Nanoparticles in Controlled Drug Delivery and Targeted Imaging. *J. Controlled Release* **2018,** *286*, 326-347.

(24) Rothfuss, H.; Knofel, N. D.; Roesky, P. W.; Barner-Kowollik, C. Single-Chain Nanoparticles as Catalytic Nanoreactors. *J. Am. Chem. Soc.* **2018,** *140*, 5875-5881.

(25) Altintas, O.; Barner-Kowollik, C. Single Chain Folding of Synthetic Polymers by Covalent and Non-Covalent Interactions: Current Status and Future Perspectives. *Macromol. Rapid Commun.* **2012,** *33*, 958-71.

(26) Altintas, O.; Barner-Kowollik, C. Single-Chain Folding of Synthetic Polymers: A Critical Update. *Macromol. Rapid Commun.* **2016,** *37*, 29-46.

(27) Latorre-Sánchez, A.; Pomposo, J. A. Recent Bioinspired Applications of Single-Chain Nanoparticles. *Polym. Int.* **2016,** *65*, 855-860.

(28) Verde-Sesto, E.; Blazquez-Martin, A.; Pomposo, J. A. Advances in the Phototriggered Synthesis of Single-Chain Polymer Nanoparticles. *Polymers* **2019,** *11*, 1903.

(29) Pomposo, J. A.; Perez-Baena, I.; Lo Verso, F.; Moreno, A. J.; Arbe, A.; Colmenero, J. How Far Are Single-Chain Polymer Nanoparticles in Solution from the Globular State? *ACS Macro Lett.* **2014,** *3*, 767-772.

(30) Formanek, M.; Moreno, A. J. Effects of Precursor Topology and Synthesis under Crowding Conditions on the Structure of Single-Chain Polymer Nanoparticles. *Soft Matter* **2017,** *13*, 6430-6438.

(31) Rubio-Cervilla, J.; Frisch, H.; Barner-Kowollik, C.; Pomposo, J. A. Synthesis of Single-Ring Nanoparticles Mimicking Natural Cyclotides by a Stepwise Folding-Activation-Collapse Process. *Macromol. Rapid Commun.* **2019,** *40*, e1800491.

(32) Liu, C. H.; Dugas, L. D.; Bowman, J. I.; Chidanguro, T.; Storey, R. F.; Simon, Y. C. Forcing Single-Chain Nanoparticle Collapse through Hydrophobic Solvent Interactions in Comb Copolymers. *Polym. Chem.* **2020,** *11*, 292-297.

(33) Stals, P. J. M.; Gillissen, M. A. J.; Paffen, T. F. E.; de Greef, T. F. A.; Lindner, P.; Meijer, E. W.; Palmans, A. R. A.; Voets, I. K. Folding Polymers with Pendant Hydrogen Bonding Motifs in Water: The Effect of Polymer Length and Concentration on the Shape and Size of Single-Chain Polymeric Nanoparticles. *Macromolecules* **2014,** *47*, 2947-2954.

(34) Chen, J.; Wang, J.; Bai, Y.; Li, K.; Garcia, E. S.; Ferguson, A. L.; Zimmerman, S. C. Enzyme-Like Click Catalysis by a Copper-Containing Single-Chain Nanoparticle. *J. Am. Chem. Soc.* **2018,** *140*, 13695-13702.

(35) Rodriguez, K. J.; Hanlon, A. M.; Lyon, C. K.; Cole, J. P.; Tuten, B. T.; Tooley, C. A.; Berda, E. B.; Pazicni, S. Porphyrin-Cored Polymer Nanoparticles: Macromolecular Models for Heme Iron Coordination. *Inorg. Chem.* **2016,** *55*, 9493-9496.

(36) Li, Y.; Bai, Y.; Zheng, N.; Liu, Y.; Vincil, G. A.; Pedretti, B. J.; Cheng, J.; Zimmerman, S. C. Crosslinked Dendronized Polyols as a General Approach to Brighter and More Stable Fluorophores. *Chem. Commun.* **2016,** *52*, 3781-4.

(37) Chen, R.; Benware, S. J.; Cawthern, S. D.; Cole, J. P.; Lessard, J. J.; Crawford-Eng, I. M.; Saxena, R.; Berda, E. B. Assessing Structure/Property Relationships and Synthetic Protocols in the Fabrication of Poly(Oxanorbornene Imide) Single-Chain Nanoparticles. *Eur. Polym. J.* **2019,** *112*, 206-213.

(38) Hokajo, T.; Terao, K.; Nakamura, Y.; Norisuye, T. Solution Properties of Polymacromonomers Consisting of Polystyrene V. Effect of Side Chain Length on Chain Stiffness. *Polym. J.* **2001,** *33*, 481-485.

(39) Lecommandoux, S.; Chécot, F.; Borsali, R.; Schappacher, M.; Deffieux, A.; Brûlet, A.; Cotton, J. P. Effect of Dense Grafting on the Backbone Conformation of Bottlebrush Polymers: Determination of the Persistence Length in Solution. *Macromolecules* **2002,** *35*, 8878-8881.

(40) Onbulak, S.; Rzayev, J. Synthesis and One-Dimensional Assembly of Cylindrical Polymer Nanoparticles Prepared from Tricomponent Bottlebrush Copolymers. *J. Polym. Sci., Part A: Polym. Chem.* **2017,** *55*, 3868-3874.

(41) Onbulak, S.; Rzayev, J. Cylindrical Nanocapsules from Photo-Cross-Linkable Core–Shell Bottlebrush Copolymers. *Polym. Chem.* **2015,** *6*, 764-771.

(42) Huang, K.; Johnson, M.; Rzayev, J. Synthesis of Degradable Organic Nanotubes by Bottlebrush Molecular Templating. *ACS Macro Lett.* **2012,** *1*, 892-895.

(43) Huang, K.; Rzayev, J. Charge and Size Selective Molecular Transport by Amphiphilic Organic Nanotubes. *J. Am. Chem. Soc.* **2011,** *133*, 16726-9.

(44) Huang, K.; Canterbury, D. P.; Rzayev, J. Organosoluble Polypyrrole Nanotubes from Core-Shell Bottlebrush Copolymers. *Chem. Commun.* **2010,** *46*, 6326-8.

(45) Huang, K.; Rzayev, J. Well-Defined Organic Nanotubes from Multicomponent Bottlebrush Copolymers. *J. Am. Chem. Soc.* **2009,** *131*, 6880-5.

(46) Van Renterghem, L. M.; Lammens, M.; Dervaux, B.; Viville, P.; Lazzaroni, R.; Du Prez, F. E. Design and Use of Organic Nanoparticles Prepared from Star-Shaped Polymers with Reactive End Groups. *J. Am. Chem. Soc.* **2008,** *130*, 10802-11.

(47) Ding, L.; Wang, C.; Jiang, R.; Wang, L.; Song, W. Preparation of Small and Photoresponsive Polymer Nanoparticles by Intramolecular Crosslinking of Reactive Star Azo-Polymers. *React. Funct. Polym.* **2016,** *109*, 56-63.

(48) Thota, B. N.; Urner, L. H.; Haag, R. Supramolecular Architectures of Dendritic Amphiphiles in Water. *Chem. Rev.* **2016,** *116*, 2079-102.

(49) Zimmerman, S. C.; Wendland, M. S.; Rakow, N. A.; Zharov, I.; Suslick, K. S. Synthetic Hosts by Monomolecular Imprinting inside Dendrimers. *Nature* **2002,** *418*, 399-403.

(50) Lemcoff, N. G.; Spurlin, T. A.; Gewirth, A. A.; Zimmerman, S. C.; Beil, J. B.; Elmer, S. L.; Vandeveer, H. G. Organic Nanoparticles Whose Size and Rigidity Are Finely Tuned by Cross-Linking the End Groups of Dendrimers. *J. Am. Chem. Soc.* **2004,** *126*, 11420-1.

(51) Yang, S. K.; Zimmerman, S. C. Polyglycerol-Dendronized Perylenediimides as Stable, Water-Soluble Fluorophores. *Adv. Funct. Mater.* **2012,** *22*, 3023-3028.

(52) Asenjo-Sanz, I.; Verde-Sesto, E.; Pomposo, J. A. Valuable Structure-Size Relationships for Tadpole-Shaped Single-Chain Nanoparticles with Long and Short Flexible Tails Unveiled. *Phys. Chem. Chem. Phys.* **2019,** *21*, 10884-10887.

(53) Li, W.; Thanneeru, S.; Kanyo, I.; Liu, B.; He, J. Amphiphilic Hybrid Nano Building Blocks with Surfactant-Mimicking Structures. *ACS Macro Lett.* **2015,** *4*, 736-740.

(54) Zhou, F.; Xie, M.; Chen, D. Structure and Ultrasonic Sensitivity of the Superparticles Formed by Self-Assembly of Single Chain Janus Nanoparticles. *Macromolecules* **2013,** *47*, 365-372.

(55) Li, W.; Kuo, C.-H.; Kanyo, I.; Thanneeru, S.; He, J. Synthesis and Self-Assembly of Amphiphilic Hybrid Nano Building Blocks Via Self-Collapse of Polymer Single Chains. *Macromolecules* **2014,** *47*, 5932-5941.

(56) Zhang, Y.; Zhao, H. Surface-Tunable Colloidal Particles Stabilized by Mono-Tethered Single-Chain Nanoparticles. *Polymer* **2015,** *64*, 277-284.

(57) Wen, J.; Zhang, J.; Zhang, Y.; Yang, Y.; Zhao, H. Controlled Self-Assembly of Amphiphilic Monotailed Single-Chain Nanoparticles. *Polym. Chem.* **2014,** *5*, 4032.

(58) Wen, J.; Yuan, L.; Yang, Y.; Liu, L.; Zhao, H. Self-Assembly of Monotethered Single-Chain Nanoparticle Shape Amphiphiles. *ACS Macro Lett.* **2013,** *2*, 100-106.

(59) Zhang, J.; Tanaka, J.; Gurnani, P.; Wilson, P.; Hartlieb, M.; Perrier, S. Self-Assembly and Disassembly of Stimuli Responsive Tadpole-Like Single Chain Nanoparticles Using a Switchable Hydrophilic/Hydrophobic Boronic Acid Cross-Linker. *Polym. Chem.* **2017,** *8*, 4079-4087.

(60) Thanneeru, S.; Li, W.; He, J. Controllable Self-Assembly of Amphiphilic Tadpole-Shaped Polymer Single-Chain Nanoparticles Prepared through Intrachain Photo-Cross-Linking. *Langmuir* **2019,** *35*, 2619-2629.

(61) Wen, W.; Huang, T.; Guan, S.; Zhao, Y.; Chen, A. Self-Assembly of Single Chain Janus Nanoparticles with Tunable Liquid Crystalline Properties from Stilbene-Containing Block Copolymers. *Macromolecules* **2019,** *52*, 2956-2964.

(62) Cui, Z.; Huang, L.; Ding, Y.; Zhu, X.; Lu, X.; Cai, Y. Compartmentalization and Unidirectional Cross-Domain Molecule Shuttling of Organometallic Single-Chain Nanoparticles. *ACS Macro Lett.* **2018,** *7*, 572-575.

(63) Ji, X.; Zhang, Y.; Zhao, H. Amphiphilic Janus Twin Single-Chain Nanoparticles. *Chem. Eur. J.* **2018,** *24*, 3005-3012.

(64) Xiang, D.; Jiang, B.; Liang, F.; Yan, L.; Yang, Z. Single-Chain Janus Nanoparticle by Metallic Complexation. *Macromolecules* **2020,** *53*, 1063-1069.

(65) Frisch, H.; Bloesser, F. R.; Barner-Kowollik, C. Controlling Chain Coupling and Single-Chain Ligation by Two Colours of Visible Light. *Angew. Chem. Int. Ed.* **2019,** *58*, 3604-3609.

(66) Matsumoto, M.; Terashima, T.; Matsumoto, K.; Takenaka, M.; Sawamoto, M. Compartmentalization Technologies Via Self-Assembly and Cross-Linking of Amphiphilic Random Block Copolymers in Water. *J. Am. Chem. Soc.* **2017,** *139*, 7164-7167.

(67) Kozawa, S. K.; Matsumoto, K.; Suzuki, A.; Sawamoto, M.; Terashima, T. Self - Assembly of Amphiphilic Aba Random Triblock Copolymers in Water. *J. Polym. Sci., Part A: Polym. Chem.* **2018,** *57*, 313-321.

(68) Claus, T. K.; Zhang, J.; Martin, L.; Hartlieb, M.; Mutlu, H.; Perrier, S.; Delaittre, G.; Barner-Kowollik, C. Stepwise Light-Induced Dual Compaction of Single-Chain Nanoparticles. *Macromol. Rapid Commun.* **2017,** *38*, 1700264.

(69) Cui, Z.; Cao, H.; Ding, Y.; Gao, P.; Lu, X.; Cai, Y. Compartmentalization of an Abc Triblock Copolymer Single-Chain Nanoparticle Via Coordination-Driven Orthogonal Self-Assembly. *Polym. Chem.* **2017,** *8*, 3755-3763.

(70) Zhang, J.; Gody, G.; Hartlieb, M.; Catrouillet, S.; Moffat, J.; Perrier, S. Synthesis of Sequence-Controlled Multiblock Single Chain Nanoparticles by a Stepwise Folding–Chain Extension–Folding Process. *Macromolecules* **2016,** *49*, 8933-8942.

(71) Harth, E.; Van Horn, B.; Lee, V. Y.; Germack, D. S.; Gonzales, C. P.; Miller, R. D.; Hawker, C. J. A Facile Approach to Architecturally Defined Nanoparticles Via Intramolecular Chain Collapse. *J. Am. Chem. Soc.* **2002,** *124*, 8653-60.

(72) Basasoro, S.; Gonzalez-Burgos, M.; Moreno, A. J.; Verso, F. L.; Arbe, A.; Colmenero, J.; Pomposo, J. A. A Solvent-Based Strategy for Tuning the Internal Structure of Metallo-Folded Single-Chain Nanoparticles. *Macromol. Rapid Commun.* **2016,** *37*, 1060-5.

(73) Xiang, D.; Chen, X.; Tang, L.; Jiang, B.; Yang, Z. Electrostatic-Mediated Intramolecular Cross-Linking Polymers in Concentrated Solutions. *CCS Chem.* **2019,** *1*, 407-430.

(74) Schultz, L. G.; Zhao, Y.; Zimmerman, S. C. Synthesis of Cored Dendrimers with Internal Cross-Links. *Angew. Chem. Int. Ed.* **2001,** *40*, 1962-1966.

(75) Huurne, G. M.; Vantomme, G.; Bersselaar, B. W. L.; Thota, B. N. S.; Voets, I. K.; Palmans, A. R. A.; Meijer, E. W. The Effect of Dendritic Pendants on the Folding of Amphiphilic Copolymers Via Supramolecular Interactions. *J. Polym. Sci., Part A: Polym. Chem.* **2018,** *57*, 411-421.

(76) Gröschel, A. H.; Schacher, F. H.; Schmalz, H.; Borisov, O. V.; Zhulina, E. B.; Walther, A.; Müller, A. H. E. Precise Hierarchical Self-Assembly of Multicompartment Micelles. *Nat. Commun.* **2012,** *3*, 710.

(77) Jiang, L.; Xie, M.; Dou, J.; Li, H.; Huang, X.; Chen, D. Efficient Fabrication of Pure, Single-Chain Janus Particles through Their Exclusive Self-Assembly in Mixtures with Their Analogues. *ACS Macro Lett.* **2018,** *7*, 1278-1282.

(78) Kyoda, K.; Yamamoto, T.; Tezuka, Y. Programmed Polymer Folding with Periodically Positioned Tetrafunctional Telechelic Precursors by Cyclic Ammonium Salt Units as Nodal Points. *J. Am. Chem. Soc.* **2019,** *141*, 7526-7536.

(79) Suzuki, T.; Yamamoto, T.; Tezuka, Y. Constructing a Macromolecular K(3,3) Graph through Electrostatic Self-Assembly and Covalent Fixation with a Dendritic Polymer Precursor. *J. Am. Chem. Soc.* **2014,** *136*, 10148-55.