Polymer 66 (2015) A11–A21

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Feature article

Single-chain polymer nanoparticles: Mimic the proteins

Meng Huo [a, b], Niejun Wang [a], Tommy Fang [a], Mengzhen Sun [a], Yen Wei [b], Jinying Yuan [a, *]

[a] Key Lab of Organic Optoelectronics and Molecular Engineering of Ministry of Education, Department of Chemistry, Tsinghua University, Beijing 100084, PR China

[b] Key Lab of Bioorganic Phosphorus Chemistry & Chemical Biology of Ministry of Education, Department of Chemistry, Tsinghua University, Beijing 100084, PR China

ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form
28 March 2015
Accepted 3 April 2015
Available online 15 April 2015

Keywords:
Single chain polymer nanoparticles
High-order structures
Protein mimicry

ABSTRACT

With recent development in controlled/"living" polymerization and sequence-specific polymeric architecture design, polymeric materials with accurately designed primary structure can be to some extent designed and prepared. Such development has actuated the design of single chain polymer nanoparticles (SCPNs) with tunable high-order structures from well-defined polymeric precursors. Until now, various synthetic strategies for SCPNs have been proposed and some possible applications, especially protein mimicry, have demonstrated the SCPNs' attracting prospect. After briefly introducing the origin of SCPNs, this feature article reviewed the recent development of SCPNs in terms of different crosslinking strategies as well as their potential applications. Meanwhile, we highlighted some important characterization techniques for SCPNs study.

1. Introduction

Inspiration from nature has always been one of the most significant contributors to the development of chemistry. Proteins, constituted with one or several folded peptides, have been found to function under unique conformations. It is their complex and delicate self-folded structures and dynamic self-adaptability that endow proteins with various functions. Enzyme catalysis is one of the most important functions, and it can promote the reaction rate by a factor of 10^{19} [1]. As a result, the design of artificial enzyme that possess high catalytic efficiency has been the intense subject of study. Generally, there are two approaches to artificial enzymes: the first one is to copy the structure of the enzyme active center and the second one is to mimic the catalytic function of the enzymes regardless of their structures [2]. As the spatial conformation of the active center plays a significant role in enzyme catalysis, the strategy of structure mimic has attracted much attention.

On the other hand, numerous man-made polymers with intricate topological structures have been realized with the progress in "living"/controlled polymerization and sequence-specific polymeric architecture design [3–7]. As the similarity between native proteins and polymers, the controlled folding of delicate designed polymers may provide a "bottom-up" approach to artificial enzymes. Despite promising applications in artificial enzymes and smart nanomaterials, polymers with designable advanced structures are now only in their primary stage. Up to date, the so-called "self-folding polymers" actually means single-chain polymer nanoparticles (SCPNs) formed by intramolecular polymeric chain collapse [8]. Different from the foldmers, SCPNs are usually prepared with single high molecular weight polymer chain precursor and are thought to have high-order structures like protein. Until now, various synthetic strategies for SCPNs have been proposed and some potential applications have demonstrated the SCPNs' attracting prospect [9–12]. Different from other artificial enzymes, SCPNs are designed to mimic the whole enzyme in consideration of both the conformation and the chemical entity of the reactive center, which is similar to molecular imprinting. However, one important difference between them is that for SCPNs the shape of the active center is formed by direct folding, while for molecular imprinting the formation of the artificial active center is by virtue of imprinting molecules. As SCPNs have designable and tunable active center, they have been studied to load catalyst to act as artificial enzyme mimic. Furthermore, due to their ultra-small size, SCPNs have also been used as a novel platform for drug delivery, imaging and sensing. In this feature article, we first summarized the synthetic routes and potential applications of SCPNs, and then we highlighted some important techniques used for SCPN characterization.

* Corresponding author. Tel.: +86 10 62783668.
E-mail address: yuanjy@mail.tsinghua.edu.cn (J. Yuan).

http://dx.doi.org/10.1016/j.polymer.2015.04.011
0032-3861/

2. Synthetic routes and applications of SCPNs

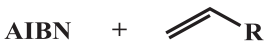
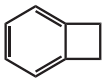
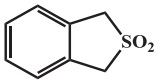
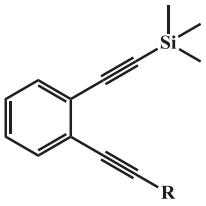
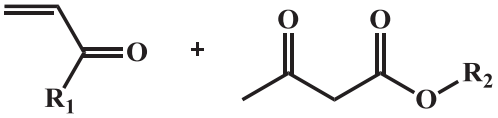
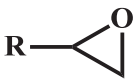
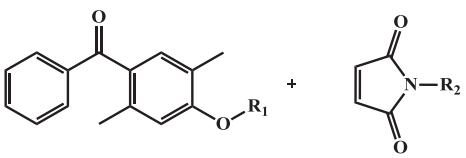
Although Martin [13,14] first synthesized discrete polymeric nanoparticles via intramolecular polymeric collapse in 1983, the ultra-dilute condition for intramolecular crosslinking remained a restriction until 2002 when Hawker's group [15] proposed to crosslink polymeric precursor with a continuous addition strategy that permitted the successful preparation of discrete SCPNs in multi-gram quantities. Other than the breakthrough in the yield, there were a variety of crosslinking methods developed for the SCPNs. In this section, we reviewed the progress of SCPN synthesis from the following aspects: covalent crosslinking, non-covalent crosslinking and dynamic covalent crosslinking strategy.

2.1. Covalent crosslinking strategy

Crosslinking is the most significant step towards the SCPNs as the crosslinking density and the crosslinking groups all contribute to the morphology of the SCPNs. As a result, the function of the SCPNs is dependent on the choice of crosslinking agents. Among these crosslinking methods, covalent crosslinking is the most studied. Table 1 summarized the recent covalent crosslinking methods used for the SCPN fabrication.

In 2002, Hawker's group developed a novel approach for preparing SCPNs in multigram quantities [15]. They first copolymerized benzocyclobutene with styrene to obtain benzocyclobutene-functionalized polystyrene (PS). Under 200 °C, these linear precursors were able to undergo intramolecular crosslinking by continuous adding strategy. SCPN formation was confirmed by the reduction of the hydrodynamic radius, which could be easily detected by gel permeation chromatography (GPC). Moreover, they selectively crosslinked one block of the copolymer and fabricated a nanoparticle-coil copolymer, which may extend the scope of macromolecular amphiphiles. To study these novel nanoparticle-coil amphiphiles, atomic force microscopy (AFM) was used to visualize their conformation [18]. The difference in architecture further led to the dramatically different interfacial assembly morphology: Langmuir–Blodgett (LB) film assemblies showed

Table 1
Recent crosslinking methods used for the SCPNs fabrication.

Functional groups	Crosslinking chemistry	References
	Radical reaction	[16,17]
	Benzocyclobutane dimerization	[15,18,19]
	Benzothiophene dimerization	[20–22]
	Olefin metathesis	[23]
	CuAAC	[24–26]
	Bergman cyclization	[27,28]
	Isocyanate chemistry	[29]
	Glaser–Hay coupling	[30]
	Michael addition	[31,32]
	Thiol–Ene click	[33]
	Ring-Opening polymerization of glycidylic group	[34]
	UV-Induced Diels–Alder reaction	[35]

disc-like surface assemblies for the linear block copolymer precursors, while it showed "long worm" morphology for those nanoparticle-coil copolymers [19].

On the other hand, Harth et al. [20] have exploited alternative crosslinking chemistry for PS-based SCPNs. Benzothiophene derivative was used to copolymerize with styrene and the resulting copolymer precursors were crosslinked by heating at 250 °C. Benzothiophene has been reported to undergo coupling to generate dibenzocyclooctadiene, which is the same product as that of the benzocyclobutane chemistry. To further explore its potential applications, ABA triblock copolymers were prepared by nitroxide-mediated living free radical polymerization with fluorene homopolymers or fluorene/thiophene copolymers as the macro-initiator and the PS/poly(vinylbenzosulfone) as the A block [21]. After intracellular collapse of the linear precursors, the isolated semiconducting polymeric nanoparticles showed increased quantum efficiency depending on the A/B ratio. Considering their ultra-small size, Harth et al. prepared poly(acrylic acid)-based SCPNs and conjugated dendritic molecular transporter molecules onto them to explore their potential application in peptide delivery [22]. As shown in Fig. 1, SCPNs were first modified with trifluoroacetyl-protected amine and succinimide group, then the dendritic molecular transporter was conjugated onto the SCPN, after which trifluoroacetyl groups were removed to bear the amine groups. The resulting nanoparticle **10** (in Fig. 1) could be further labeled with Alexa Fluor 568 dye and fluorescein isothiocyanate-labeled peptides to study the intracellular uptake of the nanoparticle conjugates. The uptake experiment indicated that the conjugated peptides could be rapidly delivered into the cytoplasm of 3T3 cells. As for their extremely small size and the protein-like structure, these bioconjugates could reduce their accumulation in liver and spleen while avoiding the immunogenetic reactions caused by viral capsid carrier.

However, both the benzocyclobutane and benzosulfone chemistry need to be conducted under rather high temperature, which may not be suitable to (meth)acrylate polymers. Moreover, solvent with high boiling point used during the SCPN synthesis may become a problem to remove. To solve these problems, Harth's group added an electron-donating group on the cyclobutene ring of benzocyclobutane to lower the activation temperature [36]. They modified the crosslinking group onto the poly(methyl methacrylate) (PMMA) and successfully prepared the PMMA-based SCPNs in dimethylformide (DMF) at 150 °C. Employing the crosslinking agent above, they further ameliorated the synthetic strategy for the ABA-triblock copolymer based SCPNs mentioned above. Ethylene oxide was used to increase the hydrophilicity of 2,7-dibromofluorene monomer, which was then used as the macro-initiator for *t*-butyl acrylate (Fig. 2) [37]. Crosslinking agent 2-(1,2-dihydrocyclobutabenzen-1-yloxy)-ethanamine could be easily conjugated onto the resulting ABA triblock copolymer by post-polymerization. After thermal crosslinking, the SCPNs were successfully prepared with abundant carboxylates, which could be further modified with catechol for gadolinium complexation. These SCPNs showed higher fluorescence quantum efficiency compared with the linear precursors. Due to their high water solubility, quantum efficiency and relaxivity, this kind of SCPNs may be used as an integrated diagnosis agent.

The trend of the research on covalent bonding SCPNs was to seek mild crosslinking strategy. To prepare PMMA-based SCPN, Aiguo Hu's group has developed a novel crosslinking strategy using Bergman cyclization by continuous addition or under ultra-dilute condition [27]. Enediyne was employed as the crosslinking precursor, and the crosslinking temperature was quite low. Recently, they further employed light as the trigger for SCPN formation [28]. Compared with heat, light is more specific and controllable, thus it may provide a convenient way to crosslink the SCPN stepwise. To endow the linear polymer precursor with photo-responsiveness, they substituted the triple bond of the enediyne with phenyl group and copolymerize the enediyne-containing acrylate with n-butyl acrylate. After UV-irradiation for 6 h, SCPNs with size less than 20 nm were confirmed by AFM.

The crosslinking strategies used above were all based on single-component crosslinker, which is relatively stable while they need to be activated under quite high temperature or intense UV light. However, the use of this kind of crosslinker was limited, and the crosslinking efficiency was generally low. As a result, double- or multi-component crosslinker systems with higher crosslinking

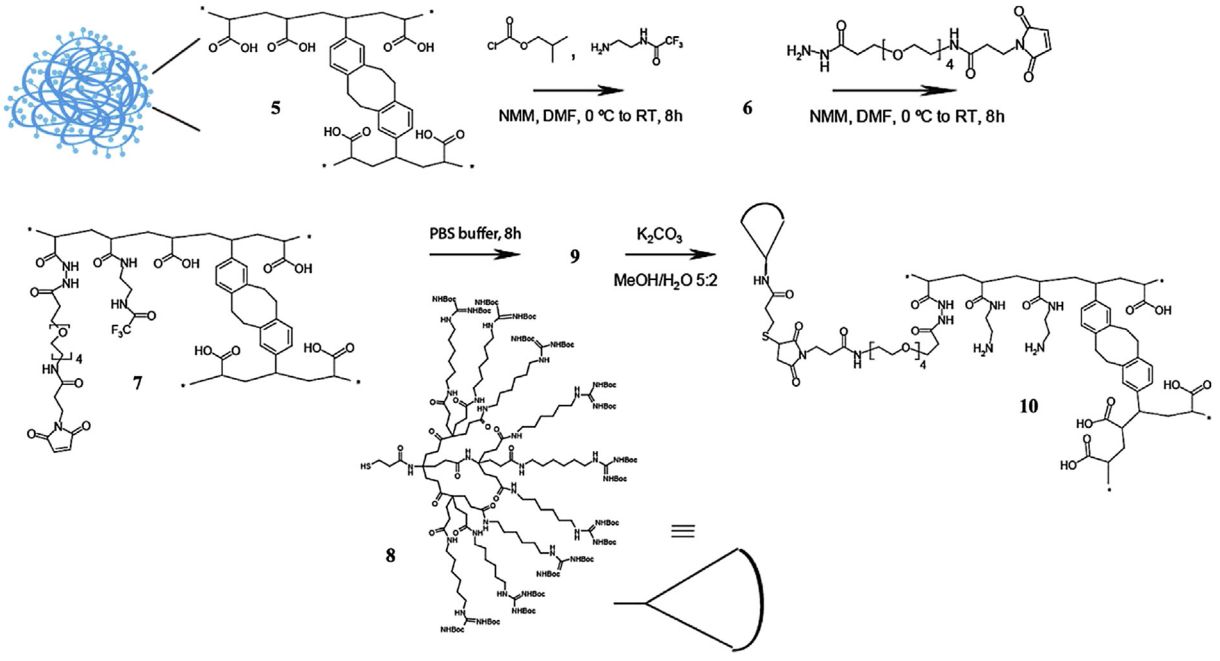


Fig. 1. Synthetic scheme of the poly(acrylic acid)-based SCPN-transporter conjugates. Reprinted with permission from Ref. [22]; Copyright 2009 American Chemical Society.

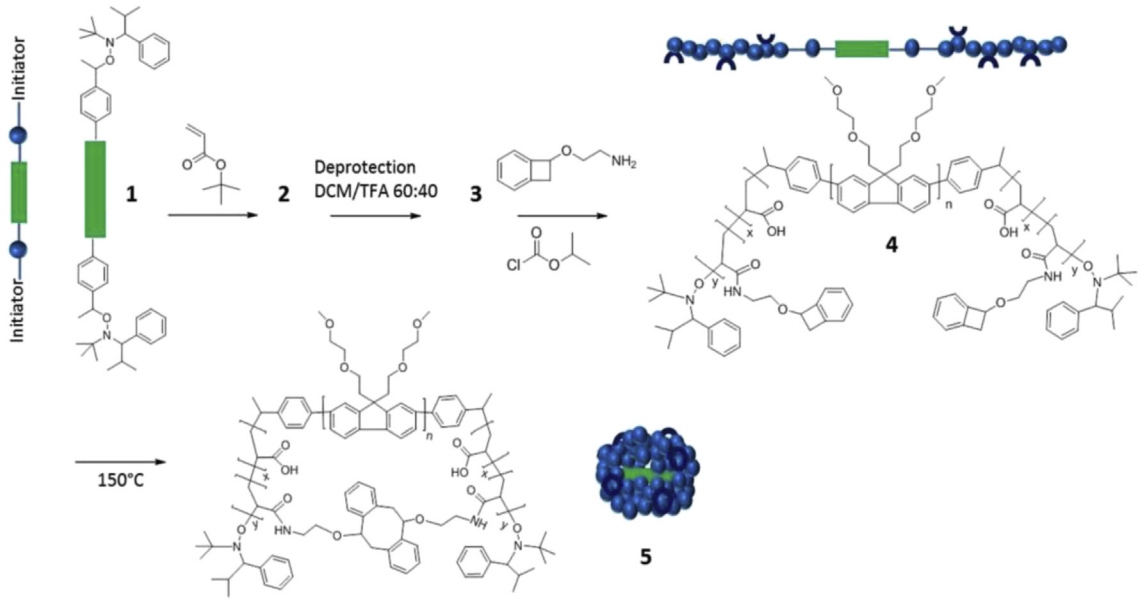


Fig. 2. PMMA-based SCPNs crosslinked by activated benzocyclobutane. Reprinted with permission from Ref. [37]; Copyright 2013 American Chemical Society.

efficiency are required. "Click chemistry" was proposed by Sharpless in 2001, which referred to those modular chemical reactions with high yield, wide range and only inoffensive byproducts [38]. Among these click chemistry reactions, copper(I)-catalyzed alkyne-azide cycloaddition reaction (CuAAC) was the most studied and has been used in polymer modification, bioconjugation, *in vivo* labeling and surface modification [39–45]. Due to its quantification, modularization, orthogonality and robustness, CuAAC has recently been exploited as a facile way to prepare SCPNs. In 2008, Loinaz et al. first applied CuAAC for intracellular crosslinking of the copolymer precursors [24]. The copolymer was composed of methyl methacrylate, 3-azidopropyl methacrylate and 3-trimethylsilyl-propyn-1-yl methacrylate, which underwent intramolecular collapse in the presence of copper(I) salt. The as-formed SCPNs could be readily designed with azide group or triple bond just by adjusting the initial monomer ratio, which was useful for further decoration. Similarly, Pomposo et al. modified the styrene with triple bonds or azide groups and obtained PS-based SCPNs of 4.2 nm in THF, which was in agreement with the theoretical value calculated from the following formula:

$$D_p = \left(\frac{6M_n}{\pi N_A \rho}\right)^{\frac{1}{3}}$$

where D_p represents the diameter of the SCPNs, M_n represents the number-average molecular weight of the precursor, N_A is the Avogadro constant and ρ is the density of the polymer precursors [25]. Though CuAAC has provided an efficient approach for facile and versatile SCPNs, research towards their applications is still rare. As the polymer precursors could be easily functionalized with azide groups, a diethylenetriaminepentaacetic acid-derived dialkyne was used as the crosslinker for poly(acrylic acid)-based SCPNs, which were subsequently complexed with Gd(III) by simply stirring at pH 6 and subsequently explored as MRI contrast agents [26].

However, one drawback of CuAAC is the tedious process for copper removal, which may limit its application in catalysis and biomedicine. Hence, "click chemistry" with no copper ions has attracted more and more attention [46–48]. As an efficient click chemistry, photo-induced D-A ligation has been used for the preparation of SCPNs under ambient temperature [35]. Post-Polymerization of poly(styrene-co-4-chloromethylstyrene) with 4-hydroxy-2,5-dimethylbenzophenone (DMBP) and an N-maleimide (Mal) derivative enabled the photo-responsive functional polymer nanoparticle precursors. After irradiation under 320 nm light for 30 min, the D-A ligation of DMBP and Mal resulted in the SCPNs with size depending on the functional group density. Besides click chemistry, olefin metathesis, isocyanate chemistry and Michael addition have been explored to efficiently prepare SCPNs [23,29,31]. However, their application in enzyme mimicry has been rarely studied.

Although covalent bond crosslinking methods for SCPNs were the most studied strategy, they have some intrinsic problems that limit their further applications. Unlike the enzymes with disulfide and non-covalent interactions, covalent bond crosslinked SCPNs have difficulty in responding to the environment and adjusting their conformations. To endow the SCPNs with enzyme-mimic functions, non-covalent and dynamic covalent chemistry should be incorporated into the SCPNs.

2.2. Non-covalent crosslinking strategy

Compared with covalent bond, non-covalent interaction enables the construction of self-adaptive, self-replicating and self-healing materials that mimic the natural smart systems [49,50]. Consequently, SCPNs crosslinked by supramolecular interaction are more akin to the natural protein regarding dynamic adaptability. Learning from the natural enzymes, these non-covalent bonding SCPNs may be decorated with metallorganic or organic catalytic center to improve the catalytic efficiency and stereo-selectivity. Due to their adaptability, these artificial enzymes may be more intelligent through the regulation of stimuli signals, which may help to exert more control towards the catalytic reactions.

The research of non-covalent bonding SCPNs started from the study of the conformation transformation influenced by the supramolecular interactions. In fact, Vincent M. Rotello's group has developed the early concept of non-covalent interaction based SCPNs in 1990s [51–54]. They functionalized PS with anthracene derivative, and the SCPNs were prepared by dissolving the resulting polymer into nonpolar solvent [51]. Molecular dynamics calculations predicted that the conformation of the SCPN was highly folded

and these polymer globules could be swelled by picric acid. These interesting results could be explained by the π-π interaction between the anthracene groups. Moreover, the variable-temperature fluorescence and GPC were used to study the stability of the polymer globule and their complexion with picric acid. Similar to the protein and nucleic acid folding, these polymer globules showed thermal-induced cooperative unfolding, while the polymer-picric acid showed more stability. Later, they modified the same polymer precursor with triazine and studied the folding and unfolding process mediated by hydrogen bonding [52]. Variable-temperature ^{1}H NMR titrations were performed to quantify the energetics during the interactions between triazine-containing polymers and two different receptors: flavin and 6-ferrocenyluracil (guest **2** in Fig. 3) [54]. Compared with flavin/polymer system, the 6-ferrocenyluracil/polymer system showed almost 13-fold enhancement in association constant. Such obvious difference was caused by the internal binding model, where flavin bound with the polymeric globule by unfolding and binding, while 6-ferrocenyluracil bound with the polymer by encapsulation. Such encapsulation mechanism provided a biomimetic approach to the site isolation of the metalloenzymes. These seminal studies have proven the significance of SCPNs and provided several promising applications, despite they have not provided enough evidence for the formation of SCPNs.

Recently, Meijer's group has developed the concept of noncovalent bond crosslinking SCPNs by 2-ureido-pyrimidinone (UPy) and benzene-1,3,5-tricarboxamide (BTA) groups. They first linked the UPy group to the norborn-5-en-2-yl-methanol monomer, then protected the UPy with UV-responsive 2-nitro-benzyl group [55]. These protected monomers were later copolymerized with norbornene by ring-opening metathesis polymerization to obtain the SCPN precursor (Fig. 4). Interestingly, these copolymers collapsed into globular nanoparticles of ~20 nm upon UV exposure owing to the dimerization of UPy groups. As these SCPNs were crosslinked by the hydrogen bonding between UPy groups, their size in dilute solution was larger than those crosslinked by covalent bonds. In a similar approach, they conjugated the *o*-nitro-benzyl protected BTA onto the poly(isobornyl methacrylate) backbone by click chemistry [56]. The *o*-nitro-benzyl was shed by the UV light and the resulting polymers self-assembled due to the BTA, which self-assembled into helical stacks in nonpolar solvent (Fig. 5). As BTA is chiral, circular

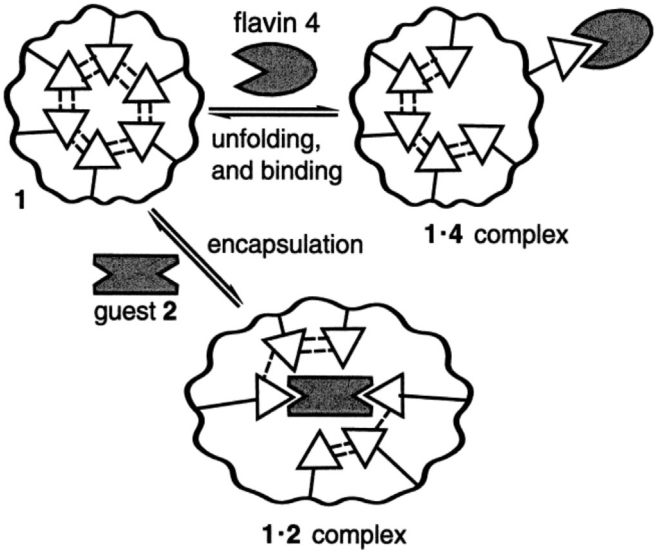


Fig. 3. Different binding mechanisms of SCPN/flavin and SCPN/6-ferrocenyl uracil. Reprinted with permission from Ref. [54]; Copyright 2000 American Chemical Society.

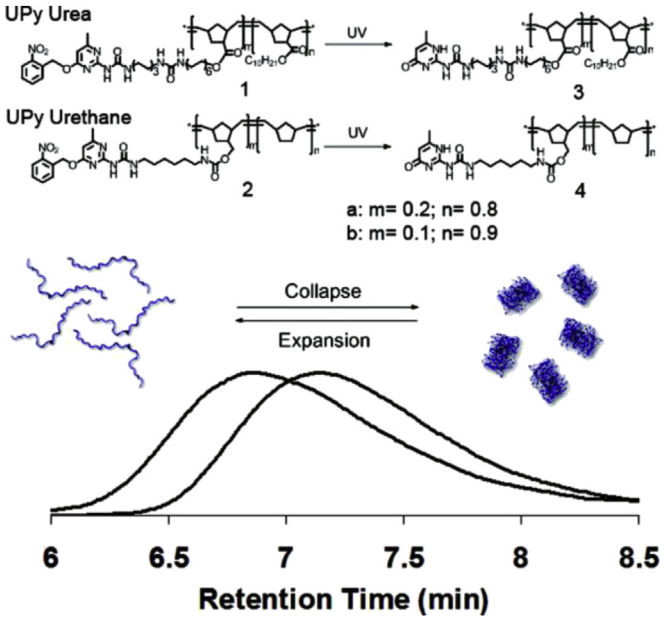


Fig. 4. UV-induced chain collapse of the UPy-containing poly(norbornene). Reprinted with permission from Ref. [55]; Copyright 2009 American Chemical Society.

dichroism (CD) spectrum could provide details about the collapse process. The BTA self-assembly process was monitored by heating the solution to 80 °C, then cooling to ambient temperature slowly. Typical Cotton effect at $\lambda = 225$ nm was found during the cooling process, which was attributed to the helical hydrogen bonded BTA aggregates. Different from the free BTA molecules, BTA groups on the polymer chains self-assembled with the onset of aggregation concentration-independent, which means that the onset of aggregation relied on the local BTA concentration, not the overall concentration. Moreover, the "sergeants and soldiers" principle has not been found for the BTA on the polymer chains, which further proved the intramolecular folding of the BTA-containing polymers. Though the SCPNs have been studied for decades, this was the first time that these nanoparticles were proven to be constructed through intramolecular collapse of single polymer chain.

To fulfill their functions, proteins are intrinsically dynamic and adaptive, which are similar to the manmade supramolecular systems. As a result, SCPNs need more "intelligence", that is to respond to various circumstances. Combining the above two SCPN systems, Meijer et al. [57] have successfully synthesized a series of intricate and delicate ABA triblock copolymers with UPy in both "A" block and the chiral BTA groups in the middle block through atom transfer radical polymerization (ATRP) and post-polymerization (Fig. 6). Importantly, these copolymers would undergo sequential and orthogonal folding under successive thermal and light trigger, which were confirmed by variable-temperature NMR, CD spectroscopy, GPC and AFM. Such orthogonal control of the SCPNs system would definitely benefit the study of artificial enzyme and protein mimicry. In fact, many stimuli such as redox, pH, light, gas and heat could be chosen to design multiple-responsive SCPNs systems that are more akin to the natural protein [58–61].

Instead of hydrogen bonding, Scherman's group has developed a SCPN system in water based on the host-guest interaction between cucurbit [8]uril and viologen (MV) and napthyl group [62]. The MV and napthyl group were reported to form ternary complex with association constant up to 10^{11} M^{-1}, which is large enough to form stable supramolecular polymers. They first conjugated the MV and napthyl groups onto the polymer backbone and crosslinked them

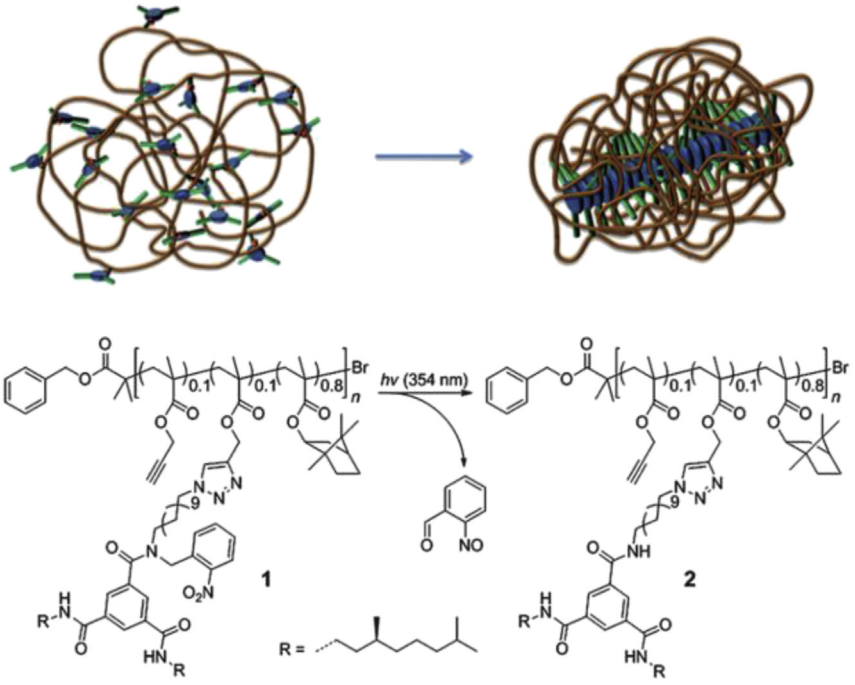


Fig. 5. UV-induced chain collapse of the BTA-containing copolymer precursor. Reprinted with permission from Ref. [56]; Copyright 2011 WILEY-VCH.

by adding cucurbit [8]uril in water to form SCPNs. To examine the dynamic properties, 1-adamantanamine solution was instantaneously added into the nanoparticle solution and the stopped-flow photophysical measurements were performed to monitor the expansion of the SCPNs upon competitive guest dilution. The intrinsic property of supramolecular linkers could resemble the nature of proteins, which was in an intricate equilibrium between different conformations. Later, they simplified this system and conjugated only MV onto the polymer backbone and used nor-secoCB [10] as the crosslinker [63]. As nor-secoCB [10] had two CB cavities, both of them formed host-guest complex with MV and ultimately the SCPNs were prepared.

Akashi et al. modified the poly(γ-glutamic acid) with various grafting ratio of hydrophobic phenylalanine groups and found that the aggregation behavior of these amphiphilic copolymers was strongly influenced by the graft ratio of phenylalanine groups [64].

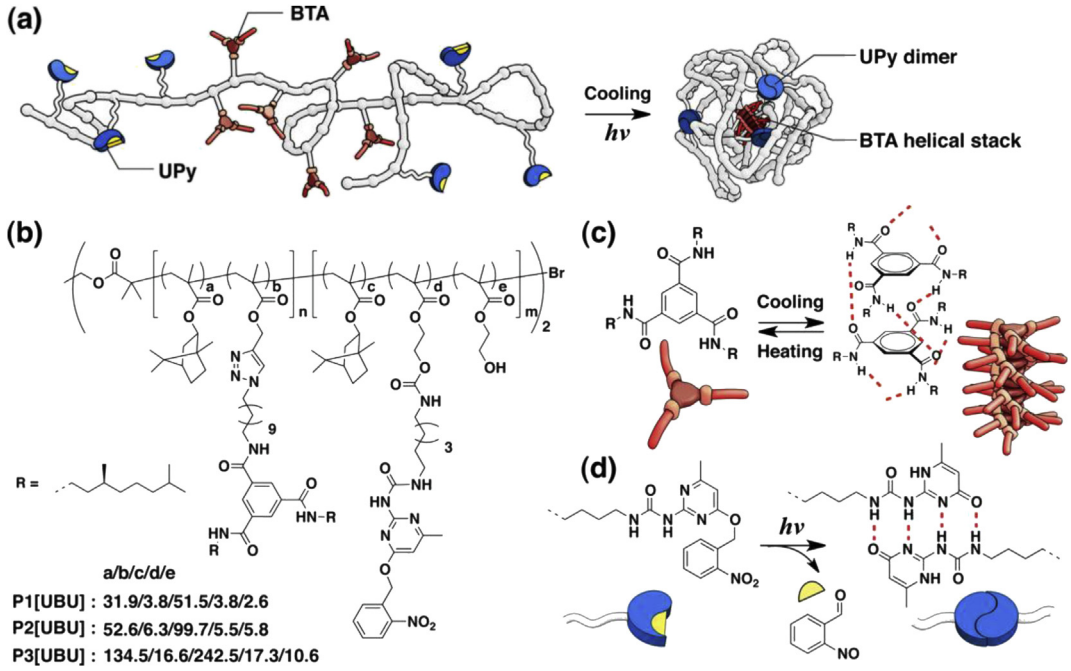


Fig. 6. The chemical composition and response mechanism of the thermal- and UV-responsive SCPNs. Reprinted with permission from Ref. [57]; Copyright 2012 American Chemical Society.

SCPNs could be obtained using copolymers with the molecular weight of γ-PGA over 140 kDa and the graft ratio ranged from 27 to 42%. Such biodegradable polymers may find applications in drug delivery.

2.3. Dynamic covalent crosslinking

Dynamic covalent chemistry is a reversible chemical reaction that is controlled by equilibrium [65]. Since dynamic covalent chemistry is controlled by thermodynamics, it shows "proof" and "correction" properties, which are the essential requirements of smart materials. Meanwhile, the bond energy of dynamic covalent bonds is usually weaker than that of common covalent bonds, while stronger than that of supramolecular interactions. Due to its reversibility, dynamic covalent chemistry presents characteristics of both covalent chemistry and supramolecular chemistry, thus has attracted more and more attention [66–70]. Recently, a variety of dynamic covalent bonds, such as imine, acylhydrozone, disulfide and trithiocarbonate, have been studied to construct molecular cages, rotors, sensors and self-healing materials [71–75].

The earliest research work dealing with SCPNs and dynamic covalent crosslinking dates back to 1997 when Guojun Liu and his co-workers synthesized PS-*block*-poly(2-cinnamoylethyl methacrylate) and found that in THF/cyclohexane mixture these copolymers self-assembled into micelles accompanied with tadpole-shaped unimers [76]. They crosslinked these unimers by UV-induced cinnamates dimerization, which has been reported as one member of the dynamic covalent chemistry toolbox. However, they did not study the dynamic properties of this dynamic covalent chemistry. In fact, it was in 2011 that Fulton et al. first prepared SCPNs by dynamic covalent acylhydrazone [77]. They synthesized poly(vinylbenzaldehyde)s (PVBAs) by reversible addition–fragmentation chain transfer (RAFT) polymerization and crosslinked them with a bis-hydrazide crosslinker. To prove the self-adaptive property of the hydrazine-crosslinked SCPNs, they first synthesized linear PVBAs with all the aldehyde groups functionalized with monoacylhydrazides, after which they collapsed these PVBAs with bis-hydrazide crosslinkers in the presence of a catalytic amount of trifluoroacetic acid. Moreover, they further prepared aldehyde-containing copolymers poly[oligo (ethylene glycol) methacrylate-co-*p*-(2-methacryloxyethoxy) benzaldehyde] [P(OEGMA-co-MAEBA)] by RAFT polymerization and crosslinked them with succinic dihydrazide intramolecularly to obtain SCPNs with thermo-responsiveness (Fig. 7) [78]. Interestingly, these thermo-responsive nanoparticles were found to constitutionally reorganized into macroscopic hydrogels after heating to above their lower critical solution temperature (LCST) at pH 4.5 and redissolved slowly to SCPNs after cooled to below their LCST. Both of these states could be fixed by switching the temperature and pH, manifesting the dynamic adaptability of the acylhydrozone bond. To illustrate the mechanism, ^{1}H NMR, GPC and dynamic laser scattering (DLS) were used to follow the transition process, from which they inferred that the hydrophilicity to hydrophobicity transformation of the POEGMA segment may increase the local concentration of the acylhydrozone and facilitated the SCPN-to-hydrogel transformation.

In another case, Zhao et al. synthesized copolymers poly(N,N-dimethylaminoethyl methacrylate-co- 4-methyl-[7-(methacryloyl) oxy-ethyl-oxy]coumarin) [P(DMAEMA-co-CMA)] with different CMA ratios and crosslinked them simply by UV illumination [79]. At

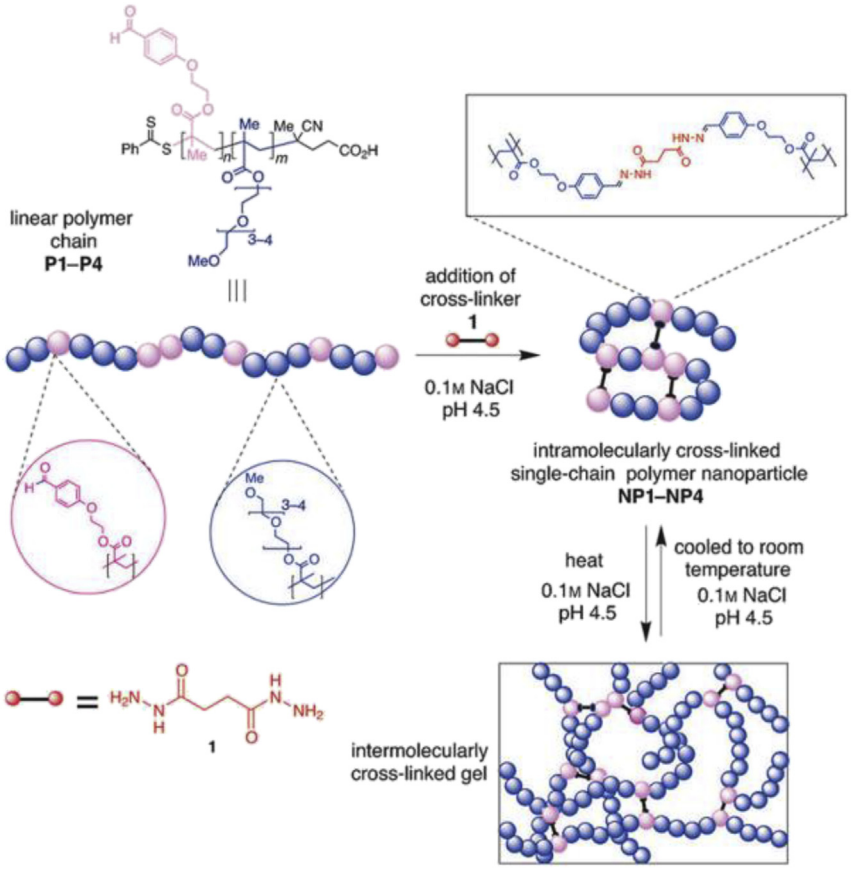


Fig. 7. Thermal- and pH-responsive SCPN-gel transition. Reprinted with permission from Ref. [78]; Copyright 2013 WILEY-VCH.

suitable CMA ratio, these copolymers could be crosslinked intramolecularly to form SCPNs. The ^{1}H spin–spin relaxation time (T_2) of DMAEMA protons, a parameter that is sensitive to the molecular motion, was elegantly measured to monitor the conformation changes during the crosslinking process, which showed a random coil to globule transformation. Since the coordination ability of tertiary amine of PDMAEMA segment with Au nanoparticles, these SCPNs were exploited as the nanoreactors for Au nanoparticles preparation. Interestingly, the formation rate of Au nanoparticles increased as the crosslinking density improved, which can be readily tuned by UV exposure time.

Meanwhile, SCPNs formed through disulfide bond, enamine bond and naphthalene dimerization have also been studied [80–82]. However, their applications have been less studied compared with the covalent crosslinked SCPNs and supramolecular interaction based SCPNs. More effort should be paid to expand the dynamic covalent chemistry based SCPNs library as well as their applications in biomimetic catalysis.

2.4. Protein mimicry by SCPNs

To mimic the proteins, especially the enzymes, the top priority is to understand the proteins. Thanks to the computer simulation and the large Protein Data Bank, scientists learn enzymes more and more. They have found that proteins were composed with sequence-specific amino chain, which folded into a unique 3D structure with hydrophobic compartments and hydrophilic shell. For enzymes, the 3D conformation of the active center is stabilized with a synergistic action of hydrogen bonding, van der Waals interaction, electrostatic interaction, hydrophobic interaction and so on [83]. Similarly, by SCPNs formation, the polymer precursors can collapse into 3D structures with hydrophobic compartments stabilized with the hydrophilic periphery. Moreover, the artificial active center can be constructed by incorporating organic or organometallic catalysts and be stabilized by covalent or non-covalent crosslinking strategies. Accordingly, SCPNs may supply an intriguing possibility to the field of protein mimicry.

Among the three crosslinking strategies for SCPNs, supramolecular interactions and dynamic covalent bonds may give access to the artificial enzyme because of their dynamic adaptivity. Dynamic covalent bonds can be found in many proteins, since disulfide bonds in many cases contribute to the stability of proteins. Though SCPNs crosslinked with disulfide bonds have been prepared, their application in protein mimicry has not been studied [81]. Up to now, the most studied SCPNs for protein mimicry is those crosslinked by supramolecular interaction.

Meijer's and Palmans' group have exerted a great deal of effort to endow SCPNs with enzyme-mimic catalytic properties. They first loaded ruthenium-based catalyst, an important organometallic catalyst, onto the ABA triblock copolymers (Fig. 8) [84]. The BTAs on the polymer backbone self-assembled into a helical secondary structure that created a hydrophobic compartment for the ruthenium-based catalyst. These folded SCPNs were used as the catalyst for the transfer hydrogenation of cyclohexanone and acetophenone. CD spectrum indicated that the reaction condition did not obstruct the self-assembly of the SCPNs in water. With 1‰ ruthenium loaded, the substrates quantitatively transformed into the corresponding alcohol. The hydrophobic segment poly(lauryl methacrylate) played a critical role in stabilizing the SCPNs after loading the ruthenium-based catalyst [85]. Surprisingly, the copolymers without BTA segments showed similar catalytic results as that catalyzed by the copolymers with BTA. In another case, they modified the BTA-containing polymer with L-proline, which is an organic catalyst for the Aldol reaction [86]. The resulting catalyst-loaded polymers collapsed into SCPNs by dint of the BTA self-

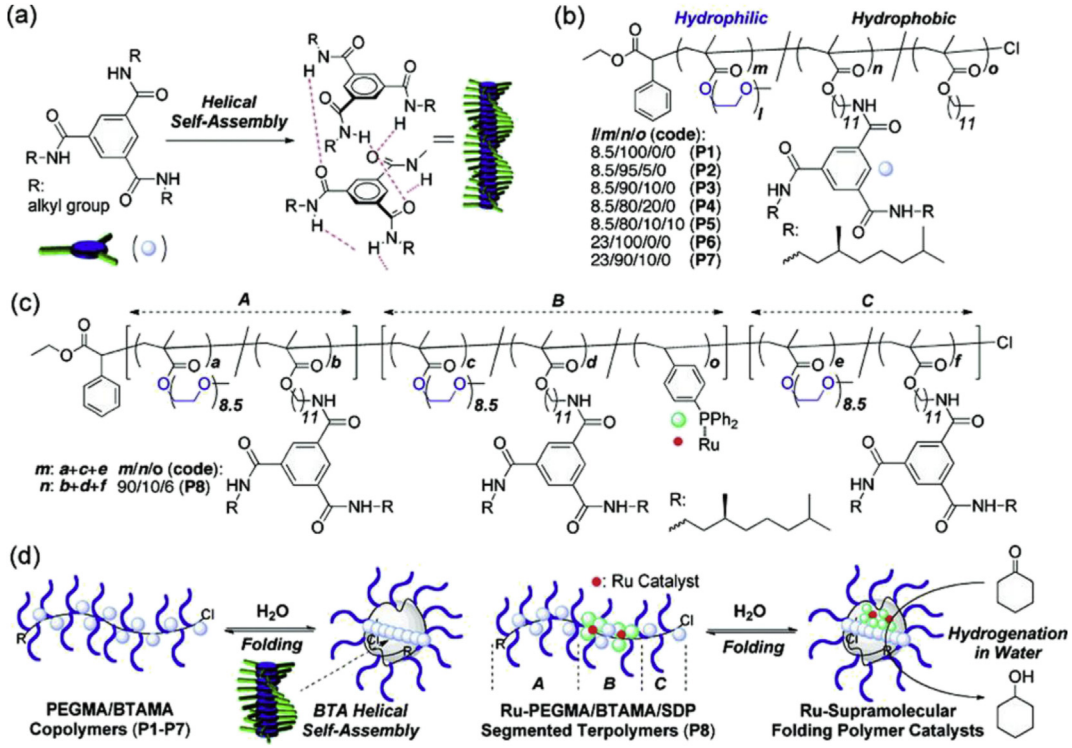


Fig. 8. Ru-Loaded SCPNs as the enzyme-mimic catalyst. Reprinted with permission from Ref. [84]; Copyright 2011 American Chemical Society.

assembly and were added to catalyze the reaction between cyclohexanone and p-nitrobenzaldehyde in water. With the loading of catalytic polymers as low as 0.5 mol% and rather low substrate concentration, this catalytic reaction could still reach extremely high conversion, while the unfolded state did not show any catalytic activity.

However, the detailed functions of the conformation in the catalytic process need more experimental evidence. Additionally, more studies should be focused on the connection between the selectivity of certain catalytic reaction and different SCPN conformations. Only after the structure–effect relationship between the conformation of SCPN and the catalytic reaction is understood, the design and adjustment of the catalytic reactions by SCPN conformation could be realized.

3. Characterization of SCPNs

Compared with the common self-assemblies that are composed of thousands of polymer chains, SCPN is formed through intramolecular crosslinking of only one polymer chain. As a result, one primary issue of SCPN is to verify that the nanoparticle is composed of only one polymer chain. There are many characterization techniques for SCPN study, among which DLS is an important tool for determining the SCPN formation. However, it is to some extent empirical, where the size of SCPN crosslinked by covalent bond is normally 3–10 nm varying with the solvent quality, while that crosslinked by non-covalent bond is usually larger. Hence, these observations can only be used as corroborative evidence for SCPN formation. It was only recently that Meijer's and Scherman's group used CD and stopped-flow photophysics respectively, to prove that the SCPN they prepared was composed of only one polymer chain [56,62]. Other than the primary structure determination, the inner compartmental structure and the conformation transformation of SCPN are the prerequisites to their application as enzyme-mimic catalysis. Static laser light scattering (SLS), small angle X-ray scattering (SAXS), small angle neutron scattering (SANS) and CD may provide the details about the inner structure of SCPN. In this section, we focus on the recent progress in SCPN characterization by highlighting some important achievements. Readers who are interested in the basic principles of these equipment are suggested to read more specific books and reviews.

AFM is an effective tool for determining the surface information and 3D structure of ultra-small nanoparticles. Its X resolution varies with the size of the tip while Z resolution can be up to atomic level [87]. Compared with TEM, AFM provides a robust technique for the ultra-small nanoparticles regardless of the contrast. Moreover, liquid AFM enables the visualization of the nanoparticles in water, which is more accurate than performed in dry state. For SCPN characterization, an additional advantage is that the morphology changes could be visualized after carefully tuning the concentration of the SCPN solutions. For example, Stals et al. have synthesized a cylindrical polymer brush with extremely complex structures (see Fig. 9) with the molecular weight up to 10^5 Da, which was large enough to be visualized by the AFM [88]. Upon UV illumination, the protected UPy groups dimerized intramolecularly and the whole structure transformed into a more compact conformation. To quantify the morphology change, the statistical radius of gyration of the polymers before and after chain collapse could be extracted and compared. Despite the direct visualization of the morphology transformation, the in-depth details of the collapsed SCPNs have not been obtained because of the limit of the X resolution. Besides, the collapse process has not been observed directly in solution by AFM. Instead, the polymers were absorbed on the mica, which may increase the difficulty in determining the real morphology change. Moreover, the concentration of the SCPNs should be carefully tuned, otherwise the dry-induced self-assembly and aggregation may disturb or even destroy the original SCPNs morphology.

To determine the morphology and evolutional change in solution, a growing number of studies have exploited SLS, SAXS and SANS to examine the size and morphology of SCPNs in solution at different levels. The basic principle of SLS, SAXS and SANS is to detect the intensity of the scattering wave at a certain position when the wave irradiates through the solution. Their difference lies in the incident wave: the wave used for SLS is visible light, for SAXS is X-ray, while for SANS is neutron. Generally, the overall size and aggregation number of the assemblies can be derived from SLS, while the local structure and the conformation information of the macromolecules can be obtained from SAXS and SANS, which are of great significance in the study of the structure–performance relationship of SCPNs. Taking advantage of SANS, Pomposo et al. have found that SCPNs formed through "Michael" addition resembled those intrinsic disorder protein like $p67^{phox}$ instead of those globular proteins, which showed amazing contrast to that observed by TEM in dry state [31]. These SCPNs were prepared by crosslinking the β-ketoester-containing polymers with trimethylolpropane triacrylate, and TEM showed compact globules with narrow distribution. However, Kratky plot of the SCPNs showed the characteristics of partially folded protein, which was verified by the molecular dynamic simulations. In another SCPN system, Gillissen et al. have studied how the secondary structure driven by BTA self-assembly affects the conformation of the SCPNs in solution through combining SANS with circular dichroism spectroscopy [89]. They found that the SCPNs were ellipsoidal particles in water at room temperature, which was in close relationship with the BTA self-assembly. Because of the evaporative self-assembly during the TEM sample preparation, it was important to introduce SANS into SCPN characterization. Unfortunately, to get the conformation information, experimental results need to be compared with the existing models, where the coincidence between experimental and theoretical curves only meant they have the same scattering profile while it did not mean the same internal structure. Moreover, the accessibility of this equipment made it difficult to be used extensively.

Other characterization techniques paid more attention to the intrinsic characteristics of the polymer precursors, like fluorescence, chirality *etc*. These characteristics may help to study the SCPNs, although they could only be applied to some systems. For those polymers with fluorescent groups, stopped-flow fluorescence measurement may help to study the folding kinetics. As mentioned above, Scherman's group has fabricated SCPNs by crosslinking the polymer precursors by cucurbit [8]uril and used stopped-flow fluorescence to monitor the dynamic of the crosslinking process [62]. Within 0.5 s, the fluorescence intensity of naphthyl groups decreased to a plateau, which suggested that the nanoparticles were formed. Instantaneous dilution of the as-formed nanoparticle solution by 1-admanetanamine solution and monitored the emission intensity may provide a robust approach to verify the dynamic property of the supramolecular linkage. As can be seen from Fig. 10, the fluorescence intensity increased upon adding 1-admanetanamine solution, indicating the dissociation of the nanoparticles. Other than fluorescence, chirality was another property that can be exploited to characterize the structure of the SCPNs. For those polymers or assemblies with chirality, CD could provide the chirality change during the self-assembly or conformation changes. For the SCPNs formed by the chiral BTAs self-assembly, the chirality change during the collapse process could be monitored in solution. The o-nitrobenzyl protected BTAs showed no Cotton effect, while the BTAs after UV illumination for 1 h showed increasing Cotton effect, which indicated that the BTA groups appended on the polymeric chain self-assembled into helical stacks upon UV illumination. Furthermore, CD spectroscopy may provide evidence for

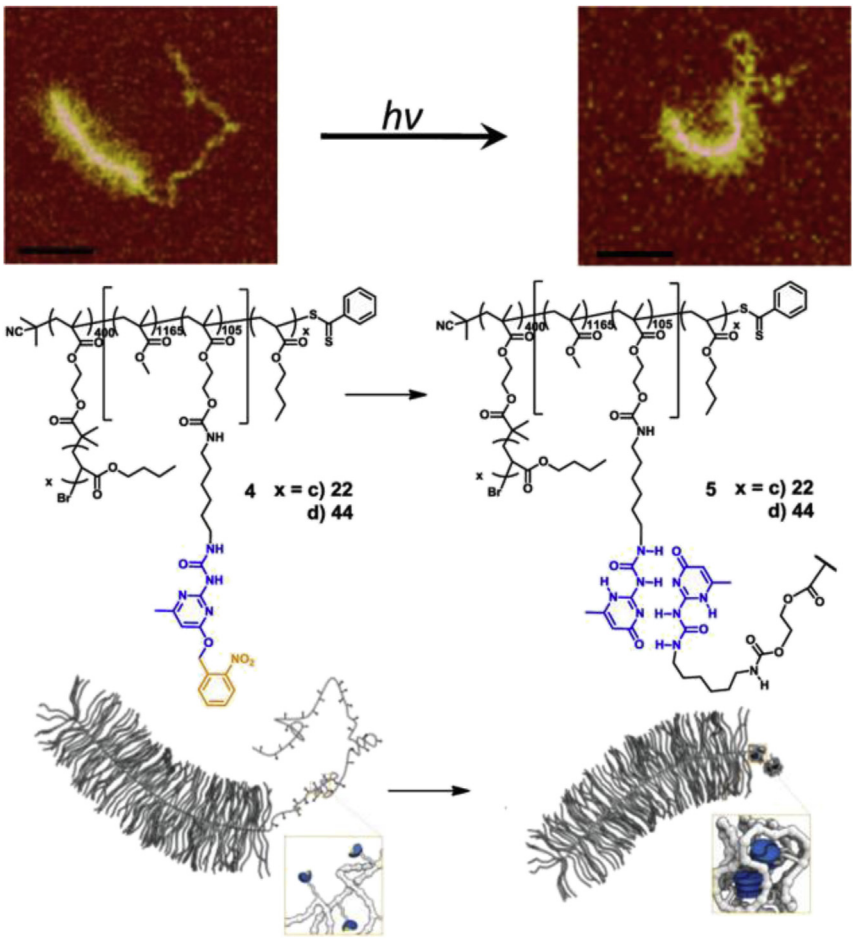


Fig. 9. AFM characterization of the intramolecular folding of the brush copolymers. Reprinted with permission from Ref. [88]; Copyright 2013 American Chemical Society.

the intramolecullar collapse of SCPNs, as the onset of aggregation is concentration-independent for intramolecular collapse. Through the two-state folding model, the CD spectroscopy could be quantitatively analyzed. As the grafting ratio of BTA increased, the melting temperature of the supramolecular assemblies increased, while it showed no dependence on the concentration of the nanoparticles, which verified the intramolecular folding of the polymer precursors.

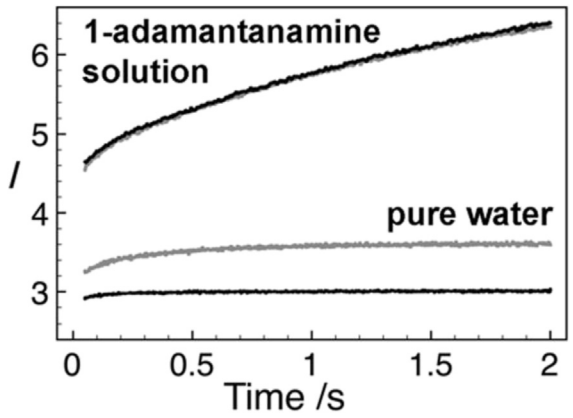


Fig. 10. Fluorescence changes upon instantaneously diluting the SCPN solution by 1-adamantanamine and pure water (black line). Reprinted with permission from Ref. [62]; Copyright 2012 WILEY-VCH.

4. Conclusions

Owing to the development of controlled/"living" polymerization and supramolecular chemistry, various SCPNs have been designed and their potential in enzyme mimicry indicated a further step towards the artificial enzyme with designable compartments. Despite these significant progresses, the goal of enzyme mimicry is far from reached. To realize more complex compartmentation, more effort should be contributed towards precise polymer synthesis and orthogonal crosslinking groups. Additionally, the regulation of SCPN's conformation needs more diversified triggers and advanced characterization techniques so as to achieve and characterize this process accurately, which may provide a toolbox for the tunable enzyme-mimic catalysis in the future. To enrich the characterization techniques, characterization methods used for intrinsic disorder proteins such as single-molecule fluorescence spectroscopy, single-molecule force spectroscopy and single-nanopore-sensing skills may be exploited to study the SCPNs [90]. It is only after the inner compartments of the SCPNs have been studied clearly, that the programmable enzyme-mimic catalysis can become a reality.

Acknowledgments

The authors gratefully acknowledge financial support from the National Natural Science Foundation of China (21174076, 21374053, 21134004), and Tsinghua University Initiative Scientific Research Program (2012z023998).

References

[1] Garcia-Viloca M, Gao J, Karplus M, Truhlar DG. Science 2004;303(5655):186–95.
[2] Raynal M, Ballester P, Vidal-Ferran A, van Leeuwen PWNM. Chem Soc Rev 2014;43(5):1734–87.
[3] Matyjaszewski K, Xia J. Chem Rev 2001;101(9):2921–90.
[4] Hawker CJ, Bosman AW, Harth E. Chem Rev 2001;101(12):3661–88.
[5] Moad G, Rizzardo E, Thang SH. Aust J Chem 2012;65(8):985–1076.
[6] Lutz J-F. Acc Chem Res 2013;46(11):2696–705.
[7] Lutz J-F, Ouchi M, Liu DR, Sawamoto M. Science 2013;341(6146):1238149.
[8] Ouchi M, Badi N, Lutz J-F, Sawamoto M. Nat Chem 2011;3(12):917–24.
[9] Lyon CK, Prasher A, Hanlon AM, Tuten BT, Tooley CA, Frank PG, et al. Polym Chem 2015;6(2):181–97.
[10] Altintas O, Barner-Kowollik C. Macromol Rapid Commun 2012;33(11):958–71.
[11] Aiertza M, Odriozola I, Cabañero G, Grande H-J, Loinaz I. Cell Mol Life Sci 2012;69(3):337–46.
[12] Pomposo JA. Polym Int 2014;63(4):589–92.
[13] Martin JE, Eichinger B. Macromolecules 1983;16(8):1345–50.
[14] Martin JE, Eichinger B. Macromolecules 1983;16(8):1350–8.
[15] Harth E, Horn BV, Lee VY, Germack DS, Gonzales CP, Miller RD, et al. J Am Chem Soc 2002;124(29):8653–60.
[16] Mecerreyes D, Lee V, Hawker CJ, Hedrick JL, Wursch A, Volksen W, et al. Adv Mater 2001;13(3):204–8.
[17] Jiang J, Thayumanavan S. Macromolecules 2005;38(14):5886–91.
[18] Pyun J, Tang C, Kowalewski T, Fréchet JMJ, Hawker CJ. Macromolecules 2005;38(7):2674–85.
[19] Kim Y, Pyun J, Fréchet JMJ, Hawker CJ, Frank CW. Langmuir 2005;21(23):10444–58.
[20] Croce TA, Hamilton SK, Chen ML, Muchalski H, Harth E. Macromolecules 2007;40(17):6028–31.
[21] Adkins CT, Muchalski H, Harth E. Macromolecules 2009;42(15):5786–92.
[22] Hamilton SK, Harth E. ACS Nano 2009;3(2):402–10.
[23] Cherian AE, Sun FC, Sheiko SS, Coates GW. J Am Chem Soc 2007;129(37):11350–1.
[24] de Luzuriaga AR, Ormategui N, Grande HJ, Odriozola I, Pomposo JA, Loinaz I. Macromol Rapid Commun 2008;29(12–13):1156–60.
[25] Oria L, Aguado R, Pomposo JA, Colmenero J. Adv Mater 2010;22(28):3038–41.
[26] Perez-Baena I, Loinaz I, Padro D, Garcia I, Grande HJ, Odriozola I. J Mater Chem 2010;20(33):6916–22.
[27] Zhu B, Ma J, Li Z, Hou J, Cheng X, Qian G, et al. J Mater Chem 2011;21(8):2679–83.
[28] Zhu B, Qian G, Xiao Y, Deng S, Wang M, Hu A. J Polym Sci Part A Polym Chem 2011;49(24):5330–8.
[29] Beck JB, Killops KL, Kang T, Sivanandan K, Bayles A, Mackay ME, et al. Macromolecules 2009;42(15):5629–35.
[30] Sanchez-Sanchez A, Asenjo-Sanz I, Buruaga L, Pomposo JA. Macromol Rapid Commun 2012;33(15):1262–7.
[31] Sanchez-Sanchez A, Akbari S, Etxeberria A, Arbe A, Gasser U, Moreno AJ, et al. ACS Macro Lett 2013;2(6):491–5.
[32] Sanchez-Sanchez A, Akbari S, Moreno AJ, Verso FL, Arbe A, Colmenero J, et al. Macromol Rapid Commun 2013;34(21):1681–6.
[33] Chao D, Jia X, Tuten B, Wang C, Berda EB. Chem Commun 2013;49(39):4178–80.
[34] Perez-Baena I, Barroso-Bujans F, Gasser U, Arbe A, Moreno AJ, Colmenero J, et al. ACS Macro Lett 2013;2(9):775–9.
[35] Altintas O, Willenbacher J, Wuest KNR, Oehlenschlaeger KK, Krolla-Sidenstein P, Gliemann H, et al. Macromolecules 2013;46(20):8092–101.
[36] Dobish JN, Hamilton SK, Harth E. Polym Chem 2012;3(4):857–60.
[37] Adkins CT, Dobish JN, Brown S, Harth E. ACS Macro Lett 2013;2(8):710–4.
[38] Kolb HC, Finn MG, Sharpless KB. Angew Chem Int Ed 2001;40(11):2004–21.
[39] Meldal M, Tornøe CW. Chem Rev 2008;108(8):2952–3015.
[40] Binder WH, Sachsenhofer R. Macromol Rapid Commun 2007;28(1):15–54.
[41] Li N, Binder WH. J Mater Chem 2011;21(42):16717–34.
[42] Tang W, Becker ML. Chem Soc Rev 2014;43(20):7013–39.
[43] Qin A, Lam JWY, Tang BZ. Chem Soc Rev 2010;39(7):2522–44.
[44] Xi W, Scott TF, Kloxin CJ, Bowman CN. Adv Funct Mater 2014;24(18):2572–90.
[45] Such GK, Johnston APR, Liang K, Caruso F. Prog Polym Sci 2012;37(7):985–1003.
[46] Stenzel MH. ACS Macro Lett 2012;2(1):14–8.
[47] Becer CR, Hoogenboom R, Schubert US. Angew Chem Int Ed 2009;48(27):4900–8.
[48] Hoyle CE, Bowman CN. Angew Chem Int Ed 2010;49(9):1540–73.
[49] Philp D, Stoddart JF. Angew Chem Int Ed 1996;35(11):1154–96.
[50] Grzybowski BA, Wilmer CE, Kim J, Browne KP, Bishop KJM. Soft Matter 2009;5(6):1110–28.
[51] Ilhan F, Gray M, Blanchette K, Rotello VM. Macromolecules 1999;32(19):6159–62.
[52] Deans R, Ilhan F, Rotello VM. Macromolecules 1999;32(15):4956–60.
[53] Clavier G, Ilhan F, Rotello VM. Macromolecules 2000;33(25):9173–5.
[54] Galow TH, Ilhan F, Cooke G, Rotello VM. J Am Chem Soc 2000;122(15):3595–8.
[55] Foster EJ, Berda EB, Meijer EW. J Am Chem Soc 2009;131(20):6964–6.
[56] Mes T, van der Weegen R, Palmans ARA, Meijer EW. Angew Chem Int Ed 2011;50(22):5085–9.
[57] Hosono N, Gillissen MAJ, Li Y, Sheiko SS, Palmans ARA, Meijer EW. J Am Chem Soc 2012;135(1):501–10.
[58] Huo M, Yuan J, Tao L, Wei Y. Polym Chem 2014;5(5):1519–28.
[59] Zhou K, Wang Y, Huang X, Luby-Phelps K, Sumer BD, Gao J. Angew Chem Int Ed 2011;50(27):6109–14.
[60] Jochum FD, Theato P. Chem Soc Rev 2013;42(17):7468–83.
[61] Yan Q, Yuan J. Chem J Chin Univ 2012;33(09):1877–85.
[62] Appel EA, Dyson J, del Barrio J, Walsh Z, Scherman OA. Angew Chem Int Ed 2012;51(17):4185–9.
[63] Appel EA, del Barrio J, Dyson J, Isaacs L, Scherman OA. Chem Sci 2012;3(7):2278–81.
[64] Piyapakorn P, Akagi T, Hachisuka M, Onishi T, Matsuoka H, Akashi M. Macromolecules 2013;46(15):6187–94.
[65] Rowan SJ, Cantrill SJ, Cousins GR, Sanders JK, Stoddart JF. Angew Chem Int Ed 2002;41(6):898–952.
[66] Maeda T, Otsuka H, Takahara A. Prog Polym Sci 2009;34(7):581–604.
[67] Jackson AW, Fulton DA. Polym Chem 2013;4(1):31–45.
[68] Xin Y, Yuan J. Polym Chem 2012;3(11):3045–55.
[69] Wojtecki RJ, Meador MA, Rowan SJ. Nat Mater 2010;10(1):14–27.
[70] Jin Y, Yu C, Denman RJ, Zhang W. Chem Soc Rev 2013;42(16):6634–54.
[71] Brantley JN, Wiggins KM, Bielawski CW. Science 2011;333(6049):1606–9.
[72] Murphy EB, Wudl F. Prog Polym Sci 2010;35(1):223–51.
[73] Moulin E, Cormos G, Giuseppone N. Chem Soc Rev 2012;41(3):1031–49.
[74] Belowich ME, Stoddart JF. Chem Soc Rev 2012;41(6):2003–24.
[75] Sanchez-Sanchez A, Pomposo JA. Part Part Syst Charact 2014;31(1):11–23.
[76] Tao J, Liu G. Macromolecules 1997;30(8):2408–11.
[77] Murray BS, Fulton DA. Macromolecules 2011;44(18):7242–52.
[78] Whitaker DE, Mahon CS, Fulton DA. Angew Chem Int Ed 2013;52(3):956–9.
[79] He J, Tremblay L, Lacelle S, Zhao Y. Soft Matter 2011;7(6):2380–6.
[80] Buruaga L, Pomposo JA. Polymers 2011;3(4):1673–83.
[81] Tuten BT, Chao D, Lyon CK, Berda EB. Polym Chem 2012;3(11):3068–71.
[82] Sanchez-Sanchez A, Fulton DA, Pomposo JA. Chem Commun 2014;50(15):1871–4.
[83] Dill KA, MacCallum JL. Science 2012;338(6110):1042–6.
[84] Terashima T, Mes T, De Greef TFA, Gillissen MAJ, Besenius P, Palmans ARA, et al. J Am Chem Soc 2011;133(13):4742–5.
[85] Artar M, Terashima T, Sawamoto M, Meijer EW, Palmans ARA. J Polym Sci Part A: Polym Chem 2014;52(1):12–20.
[86] Huerta E, Stals PJM, Meijer EW, Palmans ARA. Angew Chem Int Ed 2013;52(10):2906–10.
[87] Zhang J, Chen P, Yuan B, Ji W, Cheng Z, Qiu X. Science 2013;342(6158):611–4.
[88] Stals PJM, Li Y, Burdyńska J, Nicolaÿ R, Nese A, Palmans ARA, et al. J Am Chem Soc 2013;135(31):11421–4.
[89] Gillissen MAJ, Terashima T, Meijer EW, Palmans ARA, Voets IK. Macromolecules 2013;46(10):4120–5.
[90] Brucale M, Schuler B, Samorì B. Chem Rev 2014;114(6):3281–317.

Mr. Meng Huo received his B.Sc. from the college of chemistry, Sichuan University in 2013. He is now studying for his Ph.D degree under the supervision of Prof. Yen Wei and Prof. Jinying Yuan at the Department of Chemistry, Tsinghua University. His research interests are focused on developing new stimuli for biomedical applications and exploring biomimetic applications of stimuli-responsive polymers and supramolecular systems.

Dr. Jinying Yuan received her B.Sc from the Department of Applied Chemistry, University of Science and Technology of China (USTC) in 1987, and received her Ph.D. in the Department of Polymer Science and Engineering, USTC in 2000. After two years postdoctoral research at the College of Chemistry and Molecular Engineering, Peking University, she joined the Department of Chemistry, Tsinghua University in 2002, where she became a full professor in 2011. Her research interests are focused on methodology of polymer synthesis and functional polymer materials, especially on controlled polymerization and stimuli-responsive polymers.