HIGHLIGHT

Shell Crosslinked Polymer Assemblies: Nanoscale Constructs Inspired from Biological Systems

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ABSTRACT: The general approach involving the organization of polymers into micellar assemblies followed by stabilization through covalent intramicellar crosslinking of the assemblies has emerged as a powerful method for the production of well-defined nanostructured materials, having an amphiphilic core-shell morphology. When the covalent crosslinks are limited to the chain segments that compose the polymer micelle shell, then shell crosslinked knedel-like (SCK) nanostructures result. The shell composition dictates the interactions of the SCKs with external agents, forms a barrier layer over the core domain, and provides robust character to the nanoparticle. Because of the stability that the crosslinked shell provides, the core domain can be of dramatically different compositions and properties—glassy, fluidlike, and crystalline polymer chains have been employed for the core material and the effects that each contributes to the overall nanostructure properties have been examined. Most notably, the shell crosslinks allow for complete removal of the core to generate hollow (solvent-filled) nanoscale cagelike structures. © 2000 John Wiley & Sons, Inc. J Polym Sci A: Polym Chem 38: 1397–1407, 2000

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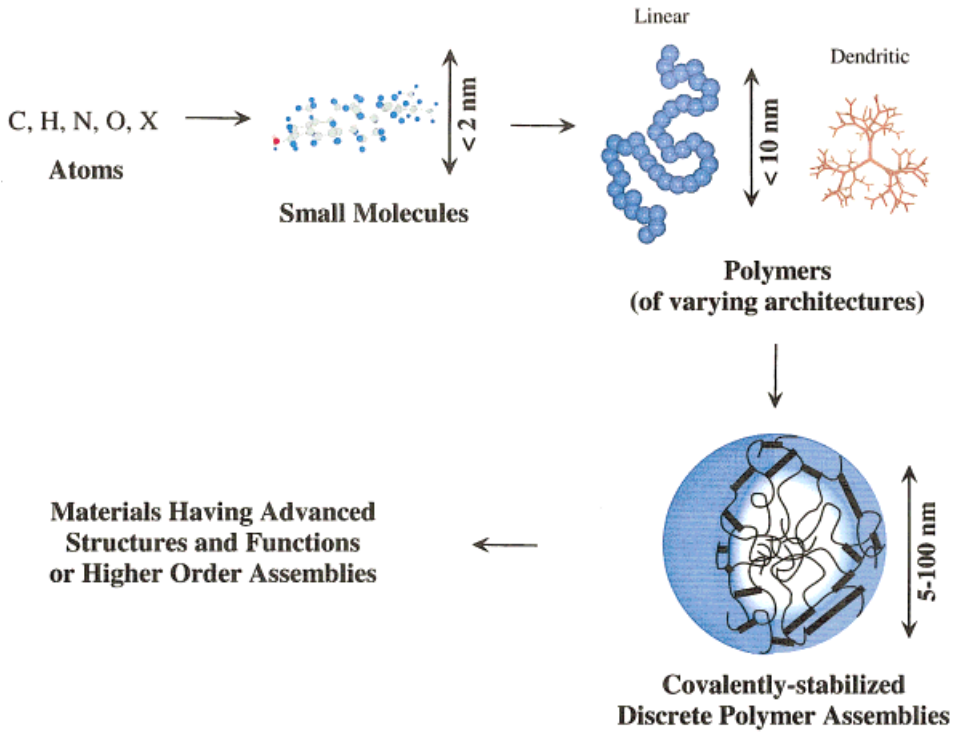


Figure 1. The dimensional evolution of synthetic organic chemistry.

INTRODUCTION

Reactivity and *creativity* are two words that differ only in the placement of a "c," and therefore, they are essentially isomers (regioisomers). As with chemical isomers, the characteristics and ramifications of these isomeric (word) forms are quite different. Chemists have long been interested in the study of chemical *reactivity*, and the investigation of new methods for chemical transformations continues.[1] The greatest scientific and societal benefits of chemistry then follow from the *creativity* exercised in the controlled application of chemical *reactivity* knowledge. As is expressed in Figure 1, chemists have gained exquisite control over the three-dimensional composition, regiochemistry, stereochemistry and, therefore, the properties and function of small molecules.

There are several length scales at which the behavior of chemical systems and the ability to manipulate those systems are important. For traditional synthetic organic chemistry, that scale is limited to tens of Angstroms. However, biology most often operates with systems having dimensions of tens of nanometers. Generally, as the sizes of the individual molecules increase, their inherent rigidity decreases, and their degrees of freedom increase, which complicates the specific placement of functionalities in three-dimensional space. Therefore, creative approaches toward polymer chemistry are required to accommodate higher degrees of control at larger dimensions, and this need has lead to a synthetic evolution, with significant advances occurring over increasing dimensions in the past twenty years. This evolution is partly the result of a shift of focus from consideration of macromolecules as polymer chains to emphasis on the construction of unique macromolecular architectures and well-defined three-dimensional nanostructures. This emphasis has generated a new perspective, considering such materials as macromolecular objects.[2] As is represented by the schematic drawings of Figure 1, the progression from small molecules of accurate structure to polymers of varying architectures[3] extends the ability to control composition, structure, and function of molecular species nearly an order of magnitude (to the dimensions of small proteins, for example). The development of synthetic methodologies that afford discrete nanoscale structures having diameters from 10 to 100 nm and precise features requires added complexity.

The challenges associated with the preparation of well-defined nanoscopic objects by a facile route are being addressed with rapidly increasing attention and progress. From the initial stages, it was recognized that the construction of such large objects (on the molecular scale) could not involve the direct, singular use of small molecule precursors, but rather that such syntheses would be most easily facilitated by the selective ordering

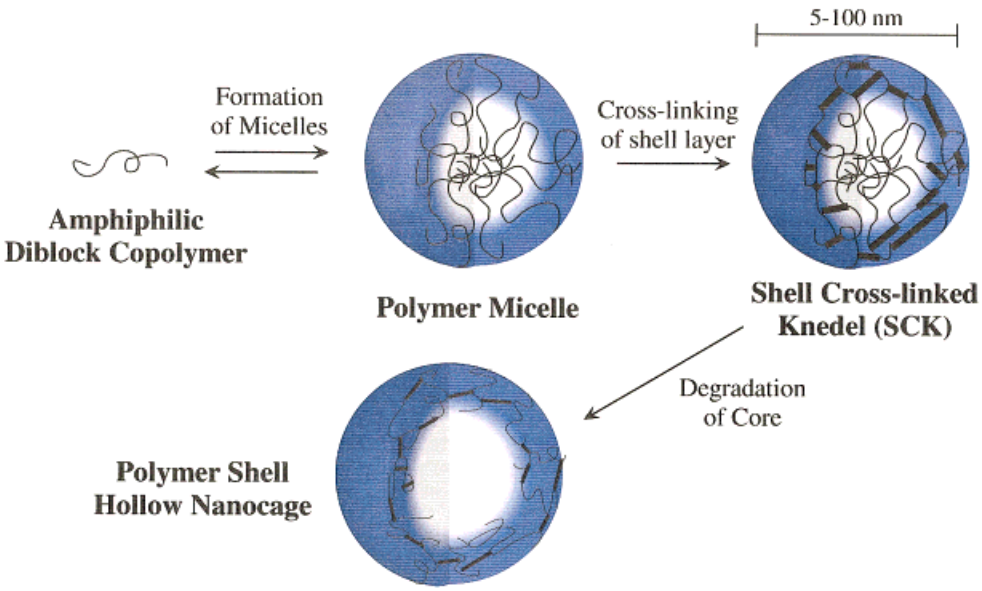


Figure 2. The general synthetic approach for the preparation of shell crosslinked knedel-like (SCK) polymer assemblies involves a combination of self-assembly and covalent stabilization. This provides robust nanostructured materials from supramolecular, dynamic precursors.

and cementing of macromolecular building blocks. Therefore, the general theme that has been adopted in the current synthetic methods is to rely upon the self-assembly processes of multi-block copolymers, along with covalent crosslinking to provide reinforcement and produce individual, crosslinked, nanoparticulate entities.

This article focuses upon shell crosslinked knedel-like[4] (SCK) polymer assemblies, which are nanoparticles having an amphiphilic core-shell morphology with stabilizing crosslinks being isolated to the peripheral shell layer.[5] The development of methodologies that allow for the preparation of SCKs will be discussed, some details of recent advances in the characterization of their structure, properties, and function will be highlighted, and example studies that are designed to address the interactions of SCKs with biological macromolecules and to examine the capabilities of the SCKs to mimic biological systems will be given. The intent is to provide the reader with a brief history about the creation and development of one class of nanostructured materials and to forecast on the key issues of the near future, which are expected to advance this technology to the preparation of nanoscale materials having highly sophisticated structures and functions. For interesting advances in the preparation and study of other discrete crosslinked assemblies, previous literature reviews[6–8] and reports[2,9–12] can be consulted.

GENERAL SYNTHETIC METHODOLOGY

The generally applicable synthetic approach for the preparation of SCK nanoparticles involves the multimolecular aggregation of amphiphilic block copolymers in aqueous solution, followed by crosslinking reactions limited to the chain segments composing the hydrophilic shell domain (Fig. 2). This procedure draws analogy from the fundamental aspects of quaternary protein structures, which rely upon weak intramolecular and intermolecular interactions for folding and assembly of linear, amphiphilic polypeptide chains, coupled with disulfide bridges between cysteine residues that provide added stability. By this simple methodology, discrete nanoscale macromolecules are produced, with differentiation of composition, structure and function progressing from the external particle surface to the core domain. Important features include dimensions ranging from 5 to 100 nm diameters, narrow size distributions, core-shell morphology with the core and shell chemically attached while possessing differing physical and chemical properties, the ability to tailor the core diameter and shell thickness, the use of aqueous-based chemistry, and the straightforward synthetic approach.

There are several advantages associated with the covalent crosslinks being regiospecifically located in the shell layer (as opposed to the core or interface regions). By "tying together" the periphery of the nanostructure, the particle surface consists of a reinforcing network that

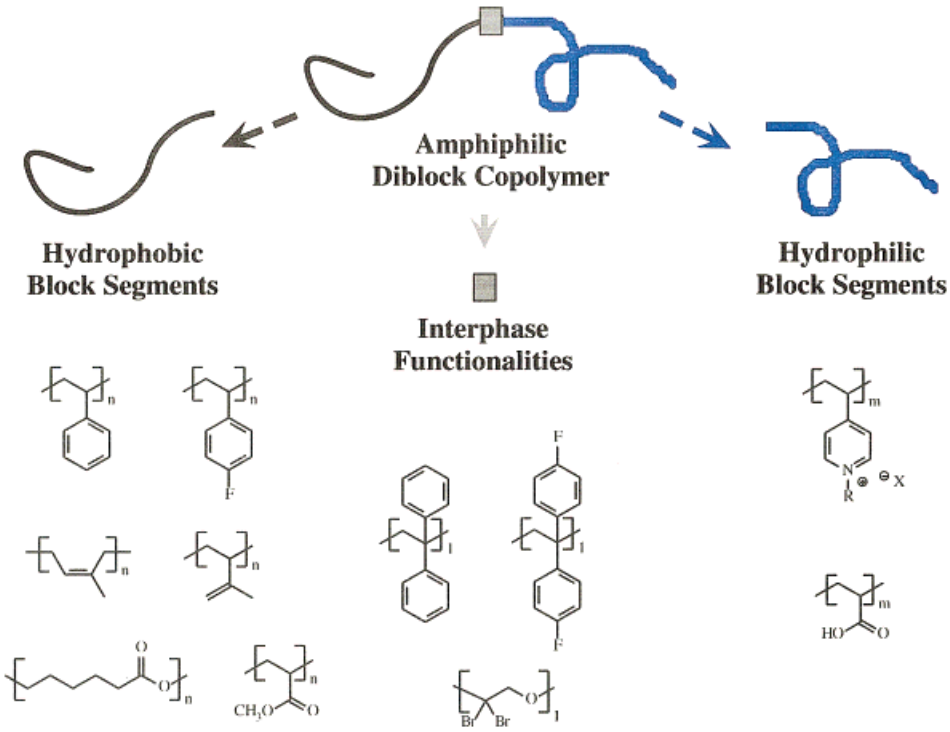


Figure 3. Several combinations of hydrophobic and hydrophilic monomer repeat units have composed the amphiphilic polymer surfactant used for the preparation of SCKs. Some examples are shown here. In some cases, an interphase unit is present from the chemistry employed during the diblock copolymer synthesis.

provides robust character and stability under the influence of changing environmental conditions. This network also serves as a membrane layer, the permeability of which can be tailored to control the transport of guests to and from the particle core. The core is essentially a nanoscale domain of surface-grafted polymer chains. Furthermore, the lack of crosslinks in the core region maintains chain mobility and access to the core volume. Perhaps the most significant aspect of the shell crosslinking, however, is the opportunities that are then available by subsequent modification of the initial SCK nanostructures. As is illustrated in Figure 2, nucleation of the insoluble chain segments is required to form the polymer micelles, but once the covalent bonds have been established throughout the shell, the backbones of the core chains can be cleaved and the degradation products extracted to leave a crosslinked polymer shell or nanocage.

The critical parameter for the overall synthetic approach is the composition and structure of the amphiphilic block copolymer, as this dictates the self-assembly process, the nature of the polymer assembly that serves as the precursor to the crosslinked nanoparticle, as well as the physical and chemical properties of the SCK. Most often, diblock copolymers with one segment that is hydrophobic and the other that is hydrophilic have been employed, and their assembly has occurred in an aqueous solution. The selections of hydrophobic and hydrophilic block segments have been based upon combinations of the monomeric repeat units included in Figure 3. The preparation of diblock and triblock copolymers composed of these monomer repeat units has been accomplished by either living anionic or living free radical polymerization methods, the details of which will not be provided herein. Instead, focus is on the utilization of such linear polymer chains as the starting materials in order to provide nanostructured materials with control over the basic elements highlighted in Figure 4. Specifically, the properties of the SCK surface, shell, interface, and core are of interest. Because of their nanoscale dimensions, the SCKs are intermediate between macromolecules (e.g., dendrimers)[5] and particles prepared by standard emulsion polymerization techniques, and as such, they possess a very high surface area: volume ratio, while maintaining macromolecular qualities associated with the ability to access and chemically derivatize the surface and other regions within the SCKs. The composition and crosslink density of the SCK shell determine the extent of swelling, control the interactions with the environment, and affect the ability of the membrane-like shell layer to conceal or expose the core domain. The

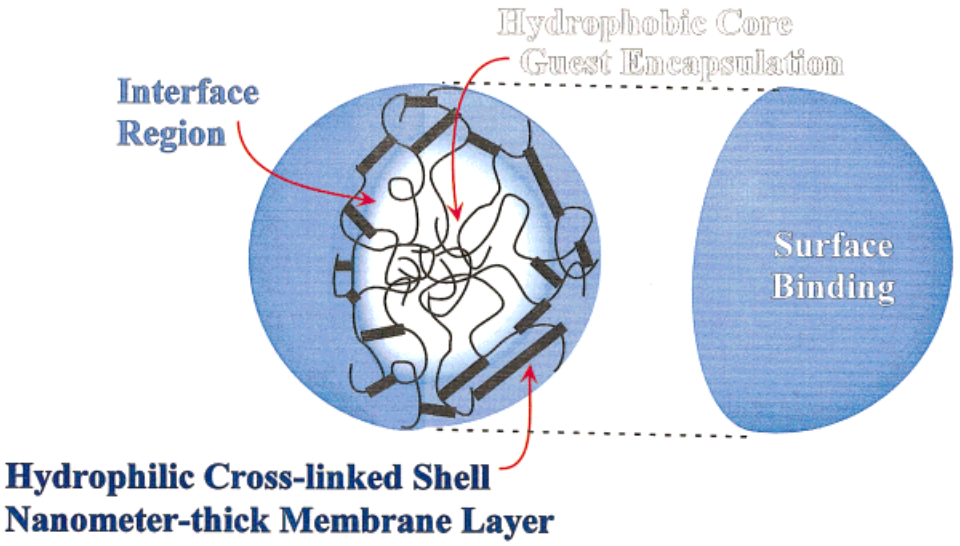


Figure 4. The amphiphilic core shell morphology of the SCKs imparts interesting partitioned properties proceeding from the nanoparticle surface, through the shell, across the interface, and into the core domain.

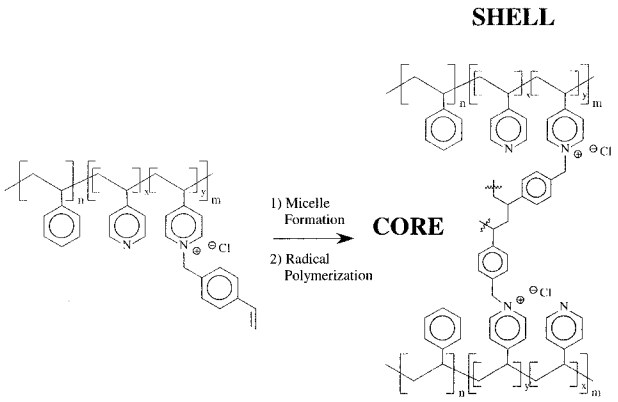
core provides a unique nanoenvironment for the sequestration of guests that are able to cross the shell barrier, or for other purposes. In addition, the properties of the core contribute significantly to the overall nanoparticle properties, for example shape adaptability, and the core properties can be affected by its isolation to nanoscopic dimensions.

The title of this article refers to the SCKs as nanoscale constructs that are inspired from biology. This reference is based upon the application of fundamental construction tools of biology (self-assembly through weak interactions combined with covalent bonds for stability) toward their production, added with the finding that these SCKs can serve as nanoscale scaffolds which are readily modifiable to mimic several biological systems, for example globular proteins, lipoproteins, and viral capsids. The effects that have been demonstrated through alteration of the shell composition are first discussed, followed by descriptions of a broad range of core compositions that have been studied, and the influence that each exerts on the overall behavior of the SCKs. Those cores that conveniently allow for transformation to the nanocages are noted. Throughout the text, reference will be made to efforts directed toward adaptation of the SCKs to accomplish these rather ambitious goals of broadly targeted biomimicry.

EFFECTS OF SHELL COMPOSITION

The first family of shell crosslinked knedel-like nanoparticles (SCKs) were assembled from amphiphilic diblock copolymers based upon polystyrene (PS) as the hydrophobic, inert segment from which the core nucleated, and poly(4-vinyl pyridine) (P4VP) that was partially quaternized with *p*-chloromethylstyrene (ClMeS).[13] The overall degrees of polymerization were typically 100–130 and the quaternization extents ranged from 10–50%. The quaternization procedure introduced water-soluble salts along the P4VP(ClMeS) backbone, and also introduced reactive styrenyl side chain moieties that provided for crosslinking of the P4VP(ClMeS) chain segments. Therefore, assembly of the PS-*b*-P4VP(ClMeS) diblocks in a mixture of 30% tetrahydrofuran (THF)/H_2O at a concentration of $10^{-5}M$ yielded spherical polymer micelles (critical micelle concentration (cmc) of about $10^{-7}M$), which were then stabilized through crosslinking reactions between the styrenyl groups within the peripheral shell by reaction with a water-soluble radical initiator, 4,4′-azobis(4-cyanovaleric acid), under irradiation at 254 nm for 24 h (Scheme 1).

The intramicellar, shell-crosslinking reactions were confirmed by the observations that no cmc was detectable for the SCKs, along with interesting behaviors exhibited in ^{1}H NMR, atomic force microscopy (AFM) and analytical ultracentrifuge (AU) measurements, in comparison with the polymer micelle precursors. In ^{1}H NMR spectra, only the core PS chains of the SCKs were visible, and only upon the addition of THF (to a solution of the SCKs in D_2O) as an organic solvent to penetrate the crosslinked shell, permeate the core domain and solvate the PS chains. AFM revealed that the polymer micelles, although possessing a glassy PS core domain, experienced slight deformation through favorable elec-



Scheme 1. Poly(styrene-*b*-vinyl pyridine) diblocks containing *p*-chloromethyl styrene side groups allow for the preparation of SCKs having a glassy polystyrene core and positively charged shell and surface groups.

trostatic interactions between the P4VP(ClMeS) surface groups and mica (the substrate upon which the polymer micelles were deposited from H_2O solution). In contrast, the SCKs retained their height and appeared to be of more uniform spherical shape (following deconvolution from the AFM tip effects in making lateral measurements). Analytical ultracentrifuge experiments proved invaluable for the determination of molecular weights and aggregation numbers for the SCKs.[14] However difficulties were experienced for the polymer micelles due to nonideal effects in the solution properties, attributable to an inherently less stable structure of the micelle, which was subject to deformation and distortion under ultracentrifugation forces.

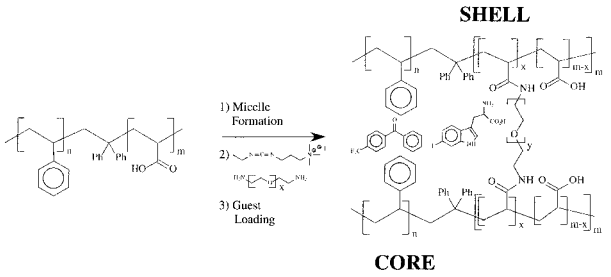
Study of this initial family of SCKs offered a significant amount of information regarding the methods of preparation, the stability imparted by the shell crosslinking, and also the parameters that allowed for variation of the particle size.[15] It was found that alteration of the hydrophobic/hydrophilic balance affords the greatest range of control over SCK dimensions. For instance, three block copolymers (each having a degree of polymerization of 100–130) with styrene:vinyl pyridine molar compositions of 1 : 2, 1 : 1.2, and 1.9 : 1 gave SCKs of 9 ± 3, 15 ± 2 and 27 ± 5 nm solid-state diameters, respectively by AFM height analysis, and solution-state hydrodynamic diameters of 14 ± 1, 21 ± 1, 33 ± 2 nm for >95 vol % of the samples by dynamic light scattering (DLS) measurement. These SCKs have corresponding molecular weights (M_w) of 244 ± 36, 1046 ± 78, and 6336 ± 75 kg/mol and aggregation numbers (N_{agg}, number of linear polymer chains that originally assembled to yield the polymer micelles and then were "trapped" through shell crosslinking) of 12 ± 2, 71 ± 5, and 439 ± 5, respectively, as determined by AU.[14]

The presence of the positively charged pyridinium salts on the surface of the SCKs was confirmed by measurements of electrophoretic mobility and zeta potential via electrophoretic light scattering,[14] which indicated a partial burial of charge beneath the particle surface. The accessibility of the positively charged groups for interaction with small molecules was evaluated by UV-vis analysis of aqueous solutions of the SCKs and a negatively charged dye, either coomassie blue or copper (II) phthalocyaninetetrasulfonic acid, tetrasodium salt. The SCK-dye complexes were soluble at low and high amounts of dye, however, insoluble complexes precipitated near the theoretical charge balance.[16]

From consideration of the diameter of the SCKs, combined with the stable structure and positively charged surface groups, analogies were then drawn to the globular protein assembly that constitutes the histone core (a disk-shaped assembly with a diameter of 11 nm and thickness of 6 nm, possessing positively charged residues for electrostatic interaction with the phosphodiester backbone of DNA) found in nucleosome units of chromatin.[17] Therefore, studies were undertaken to evaluate the potential of SCKs to serve as synthetic mimics of histones.[18] As is shown in the AFM images of Figure 5(a), an SCK sample with a 16 nm volume-averaged mean particle diameter in aqueous solution (by DLS), can effectively condense DNA. The plasmid DNA condensation is seen here occurring *in situ* under buffer solution, as the SCK/DNA aggregates deposit on the mica substrate, with increasing SCK:DNA quantities increasing from 20 : 1, 50 : 1, and 100 : 1, proceeding down the three panels. In the uppermost image, SCK nanoparticles are observed as being bound to the DNA coiled on the mica substrate. The second panel reveals small segments or coils of DNA extending from the SCK/DNA aggregate, and finally in the third panel, the DNA is buried within the aggregate and no longer visible by AFM. Comparable aggregation events were observed as occurring in solution by DLS measurements. It is interesting to note that once the SCK/DNA aggregate formed, the DNA was no longer digested by enzymatic action. Moreover, enzymatic cleavage with small amounts of SCK present (incomplete aggregation) was inhibited, requiring longer digestion times and yielding increased lengths of DNA segments than for similar enzymatic processes in the absence of SCKs. Continued studies are directed toward the development of the SCKs for packaging and transport of DNA and RNA, for control over transcription, translation or replication, and for use in gene therapy or other biomedical applications.

Coincident with many of the accomplishments that had been made with the PS-*b*-P4VP(ClMeS) system, it

was realized that the importance of the shell composition and crosslinking density warranted alternative methods for the production of SCKs, utilizing different diblock compositions and also different crosslinking chemistries. As is illustrated in Scheme 2, replacement of the PVP segment of the diblock copolymer with poly(acrylic acid) (PAA), allowed for the shell crosslinking to be performed through amidation chemistry, providing crosslinks through a condensation mechanism and incorporating modifiable shell and surface properties through selection of the crosslinking agent. Following micelle formation and activation of the carboxylic acid side



Scheme 2. The micellar assembly of poly(styrene-*b*-acrylic acid) diblock copolymers is followed by condensation reactions with diamino crosslinking agents to form the covalent nanostructured SCK. Loading with guests is then performed in the aqueous solution, and it has been found by REDOR solid-state NMR experiments that hydrophobic guests migrate to the hydrophobic core domain, whereas amphiphilic guests locate near the core-shell interface.

chain groups by reaction with a water-soluble carbodiimide, 1-(3-dimethylamino)propyl)-3-ethylcarbodiimide methiodide, a diamino crosslinker is then added to effect the intramicellar crosslinking of the shell layer. A number of diamino (and polyamino) crosslinkers have been studied and found to be effective in the formation of SCKs.[19] The nature of the crosslinker can have dramatic effects upon the SCK shell properties. For example, when a short diamino(triethylene oxide) linker is used, then the shell is barely visible by transmission electron microscopy (TEM), whereas a diamino(polyethylene oxide) crosslinker greatly increases the shell thickness and volume, relative to the PS core domain, and it is then clearly distinguishable by TEM [Figure 5(b)].[20] This control over the shell thickness and composition may provide for control over the facial, surface–surface interactions of SCKs with other species. As an example, increasing the amount and length of poly(ethylene glycol) incorporated during the crosslinking process is expected to limit the degree of protein adsorption. Alter-

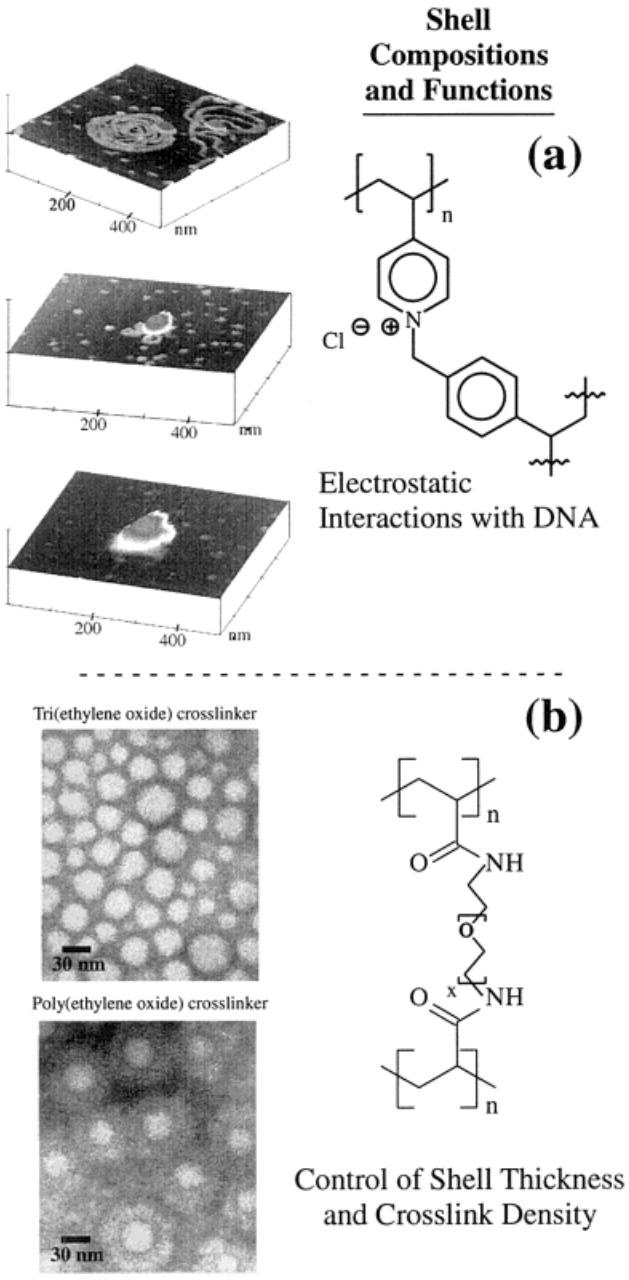


Figure 5. The surface functionality regulates binding events, for example (a) the incorporation of positively charged quaternized pyridyl groups throughout the shell and on the surface allows for electrostatic binding with the negatively charged phosphodiester backbone of DNA. Tapping-mode AFM under solution allows for *in situ* observation of the SCK/DNA aggregation. (b) The shell thickness, crosslink density and overall properties are conveniently tailored through the selection of a crosslinking agent. Transmission electron microscopy (negatively stained with uranyl acetate) provides visualization of the SCK corona versus core.

natively, functionalities that are known to offer specific binding, for example, to cell surfaces, can offer a method by which to tailor the SCK targeting.

The length of the crosslinker as well as the amount used (molar ratio in comparison to acrylic acid repeat units present) has allowed for tailoring of the shell crosslink density. Although it is presently not possible to measure an exact crosslink density, due to difficulties distinguishing intrachain "loops" from interchain crosslinks within the SCKs, the overall extent of amidation can be determined by ^{13}C and ^{15}N solid-state NMR, and has been found to reach a maximum at about 70% conversion to amides with remaining carboxylic acid and primary amino groups. Loading of guests within the SCKs is modeled after lipoproteins, which are composites of cholesterol, cholesteryl ester, phospholipids, and protein, to form biological structures of core-shell morphology and overall diameters of 10–100 nm, whose role is to transport insoluble esterified cholesteryl ester. Some of the most important aspects of any transport device are the loading capacity and the mechanism of uptake and release, both of which rely upon the location of the guests within the host system. The locations of guests within the SCK nanostructure were accurately determined by rotational-echo double-resonance (REDOR) NMR, taking advantage of the unique ^{13}C of the PS core, the ^{15}N of the crosslinkers in the shell, and the placement of a ^{19}F label on the guest molecules. By measuring the dipolar coupling of the ^{19}F-labeled guest, independently for the core and shell nuclei, it was found that the amphiphilic small molecule, 6-fluorotryptophan, resides at the amphiphilic region of the SCK, near the core-shell interface,[21] whereas the hydrophobic guest, migrates to the hydrophobic PS core domain (Scheme 2).[22] Moreover, it was found that at high amidation extents (an indirect measure of the crosslinking density), the guests were prohibited from passing across the shell membrane layer, and were not taken up into the SCKs. All loading experiments were performed in aqueous solutions containing the SCKs, in some cases with added THF.

INFLUENCE OF CORE PROPERTIES

It is fairly obvious that the nature of the SCK shell governs their direct physical interactions with the surrounding environment, but less obvious is the influence that the core domain exerts over the properties of the entire nanostructure. To illustrate the modifications in nanoparticle behavior as a function of core composition, four very different core compositions will be briefly discussed, having compositions including: glassy polystyrene, fluidlike polyisoprene, crystalline poly(ε-caprolactone), and hollow (water-filled) nanocage structures. In each example, the shell consists of poly(acrylic acid) crosslinked through amidation chemistry to yield poly(acrylic acid-*co*-acrylamide) hydrogel material.

The SCKs having the composition shown in Scheme 2 possess a glassy polystyrene core domain, and this offers rigidity to the overall nanoparticle. The AFM particle heights agreed very well with the TEM diameters, indicating that the particles are essentially spherical, and that they do not undergo deformation upon adsorption onto a solid substrate.[19] This is an excellent property for applications that require hard spheres, however, there are many instances where shape adaptability could be important. In addition, a glassy core does not offer much void space or available free volume for loading with guest molecules, and it also limits the extent that the nanostructure can expand, for example by swelling of the shell. In the case of noncrosslinked polymer micelles, glassy cores are often beneficial in the added stability that is offered to the supramolecular assemblies,[23] however, the decreased core mobility also results in lower rates of guest diffusion.[24] Since the SCKs owe their stability to crosslinks throughout the shell, a greater degree of variability in core composition is possible, without compromising the nanostructure integrity.

Polymer micelles prepared from poly(isoprene-*b*-acrylic acid) (PI-*b*-PAA) with predominantly *cis*-4,1-isoprene microstructure and block lengths of 130 isoprene and 170 acrylic acid repeat units, are difficult to handle due to the fluidlike character of the polyisoprene core domain ($T_g = -75$ °C).[25] In fact, upon adsorption onto a polar mica substrate, the micellar assembly is destroyed, and AFM analysis reveals only a nonuniform spreading of polymer material across the substrate surface, with no identifiable morphology or order [Fig. 6(a)]. In contrast, crosslinking of the PAA shell of the PI-*b*-PAA micelles to form the corresponding SCK (Scheme 3) prior to deposition onto mica and imaging by AFM confirms the stabilization that is provided through the covalent crosslinking to produce robust nanostructured materials. As is seen in Figure 6(b), the SCKs deform on the substrate, due to the fluid core behavior ($T_g = -65$ °C), however the core can flow only to the point that the shell allows. This demonstrates that once the shell is crosslinked, it serves as a nanoscale containment device, in this case it is a fluid-filled membrane. As such, it exhibits shape adaptability and this characteristic is currently being exploited in our laboratories.

The extreme case of a fluid-filled nanostructure is a solvent-filled cavity, which we have recently generated from the SCK template. The hydrophobic block segments are required for the initial self-assembly to form the polymer micelles, however once the crosslinks are established throughout the shell layer, the core is no longer required. The PI core material is conveniently

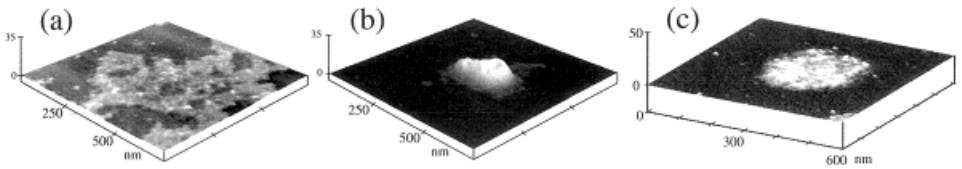
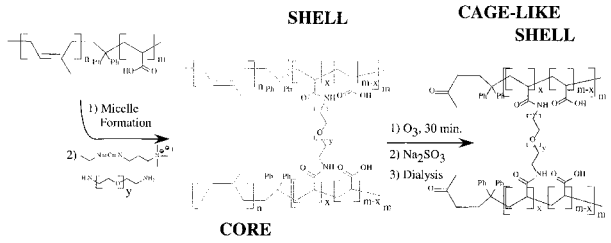


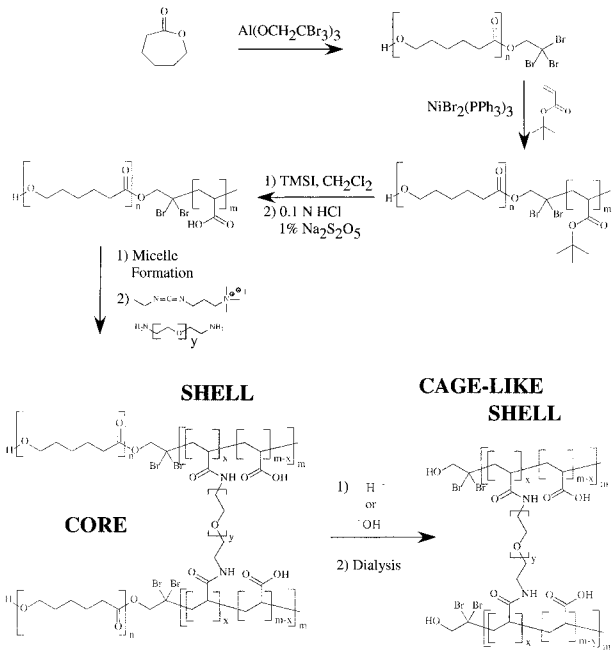
Figure 6. Tapping-mode AFM imaging of samples deposited from aqueous solution onto a mica substrate indicate the differences in properties for (a) poly(isoprene-*b*-acrylic acid) micelles, (b) poly(isoprene-*b*-acrylic acid-*co*-acrylamide) SCKs (micelles and SCKs are each composed of low T_g core material), and (c) the nanocages that result from SCK core degradation by ozonolysis.

broken down into small molecule byproducts through ozonolytic cleavage of the carbon–carbon double bonds, without detriment to the crosslinked shell layer, and these small molecules are easily removed from the system through dialysis. This results in the production of a nanocage structure, composed of the poly(acrylic acid-*co*-acrylamide) hydrogel material originally present as the shell layer of the SCK. Since the SCK structure is maintained through a combination of the hydrophobic interactions within the core domain (which initially provided for the micellar assembly) and the reinforcing covalent shell crosslinks, removal of the core material results in a significant expansion of the hydrogel shell material to give an overall diameter for the nanocage that is much greater than that occupied by the SCK. For the SCK that contains tri(ethylene oxide) crosslinkers, the SCK of 27 ± 9 nm hydrodynamic diameter expands to a nanocage of 133 ± 1 nm hydrodynamic diameter, as measured by DLS in aqueous solution. This is believed to occur due to a filling of the nanocage with water, and expansion of the structure to the extent that the crosslinked network allows. The porosity and nanocage shell thickness are presently unknown. However, as is observed by AFM [Fig. 6(c)], the nanocage structures do flatten upon adsorption onto the mica substrate, to a greater degree than the SCK, supporting the loss of the core material. In addition, the diameter by AFM is greater for the nanocage than for the SCK, again suggesting that the nanocage is an extended nanocavity, which collapses upon the solid surface. A fascinating possibility results from the chemistry employed in the preparation of these nanocages, in that the ozonolysis of the PI core leaves ketones (and aldehydes) on the inner nanocage surface, whereas the remainder of the nanocage is composed of carboxylic acid and amide functional groups. This offers the ability to chemically derivatize specific regions of the nanocage, in other words, regiochemical control may be possible on a nanometer scale.



Scheme 3. The crosslinking of poly(isoprene-*b*-acrylic acid) diblock copolymers affords fluid-filled membranes of nanometer-scale dimensions (see also Fig. 6). Subsequent cleavage of the carbon–carbon double bonds along the polyisoprene chain segments and extraction of the degradation products produces a cage-like nanostructure. Note also that ozonolysis of the polyisoprene core leaves ketone (and possibly aldehyde) groups on the inner wall of the nanocage, which can be utilized for treatment uniquely to the inside of the nanocage.

An alternative strategy for the preparation of nanocage structures, involving the hydrolytic degradation of a poly(ϵ-caprolactone) core domain, lead to very interesting findings related to the properties of a crystalline polymer confined to the SCK core of nanoscale volume.[26] Scheme 4 outlines the synthetic approach that was followed for the preparation of the poly(ε-caprolactone-*b*-acrylic acid).[27] The ring opening polymerization of ε-caprolactone initiated from $Al(OCH_2CBr_3)_3$, a dual-action, difunctional initiating species, then allowed for growth of *tert*-butyl acrylate by atom transfer radical polymerization, and finally selective hydrolysis of the *tert*-butyl esters to yield the PCL-*b*-PAA diblock copolymers, with accurate control over each of the block segment lengths. As in the previous cases, assembly of these diblock copolymers into polymer micelles in water was followed by crosslinking of the shell layer through amide coupling chemistry. However, these SCKs exhibit different behavior, relating to their flattened, lamellar shape from the crystalline PCL core domain (T_m = 45–60 °C), which gave them a high aspect ratio in solution and upon adsorption onto a substrate. Characteristic features included diameter to height ratios from AFM measurements of about 10 : 1, which agreed with the solution-



Scheme 4. The crosslinking of poly(ε-caprolactone-*b*-acrylic acid) diblock copolymers affords SCKs with nanoscale domains of crystalline poly(ε-caprolactone) within the cores. Subsequent hydrolytic cleavage of the poly(ε-caprolactone) chain segments and extraction of the degradation products produces a cagelike nanostructure.

state hydrodynamic diameters from DLS measurements that were about ten-fold greater than the nanoparticle heights measured by AFM. The isolation of the PCL chain segments to the nanoscale core domains affected their lamellar growth potential, and it was found that the melting transition temperatures decreased with decreasing particle volume. Further studies are directed at more extensive characterization of the crystal growth process (kinetics and thermodynamics) and crystal structure as a function of confinement space. Hydrolytic cleavage of the PCL core is facilitated by either acid catalysis or base promotion, and is complete within a few hours to again allow for the production of nanocage-like structures (Scheme 4). Liu et al. have also examined the use of crosslinked precursors as templates for the preparation of hollowed nanostructures.[28]

CONCLUSIONS

Since their introduction in 1996, SCKs have begun shaping the future of nanoscopic structures, by essentially providing simple pathways to prepare synthetic analogs of many important biological systems and methods to generate nanoscale constructs, from which nanotechnological devices may be built. Particular synthetic targets have included rigid SCKs of 10–15 nm diameters with a glassy core and positively charged surface functionalities as a model of the histone core of nucleosomes, fluid-filled SCKs to accommodate high degrees of guest loading in a similar fashion to the encapsulation, transport, and delivery processes of lipoproteins, and hollow nanocages have been produced with differential inner- and outer-cage chemistries for the construction of viral capsid mimics. The inherent versatility in the structure, composition, and function of the basic SCK nanomaterials suggest that they could also find applicability in nanotechnology, for example as pH- or temperature-responsive actuators in microfluidic devices, as scaffolds or components for nanofabrication, and so forth.

Although the emphasis of this article has been the assembly of diblock copolymers into spherical polymer micelles, several variations upon this general theme have already been realized. For example, triblock copolymers have been used, inverse micelles have been formed, and alternative assembled morphologies have been utilized to generate crosslinked nanostructures of varying shapes, compositions, dimensions, and properties. Additionally, a significant advance by S. P. Armes[29] and coworkers has overcome one of the limitations of the general assembly/crosslinking approach, that of the requirement that the crosslinking reactions be performed under fairly dilute conditions to avoid intermicellar reactions and thus retain the individual crosslinked nanostructures. The approach demonstrated by Armes takes advantage of steric effects for stabilization of the polymer micelles by an outermost nonreactive block segment, while the crosslinking is performed at an intermediate layer within the polymer micelles, assembled from triblock copolymers. It is also important to note that although our research has primarily involved amphiphilic nanostructures prepared in an aqueous environment, to mimic biological structures in basic structural elements and potentially their functions, entirely hydrophobic[30] or entirely hydrophilic[31] and zwitterionic[32] shell crosslinked polymer assemblies are also of interest. With the synthetic tools that have been established, combined with the current demonstration of their abilities to generate complex nanostructured materials, it is expected that only a short period of time will be required before these nanostructures find roles in some of the more obvious biomedical and nanotechnological applications, as well as in the development of as yet unforeseen devices.

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REFERENCES AND NOTES

1. See for example, Larock, R. C. Comprehensive Organic Transformations: A Guide to Functional Group Preparations, 2nd ed.; Wiley-VCH: New York, 1999.
2. Zubarev, E. R.; Pralle, M. U.; Li, L.; Stupp, S. I. Science 1999, 283, 523.
3. (a) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. Chem Rev 1999, 99, 1665; (b) Percec, V.; Ahn, C.-H.; Bera, T. K.; Ungar, G.; Yeardley, D. J. P. Chem Eur J 1999, 5, 1070; (c) Percec, V.; Ahn, C.-H.; Ungar, G.; Yeardley D. J. P.; Möller, M.; Sheiko, S. S. Nature 1998, 391, 161; (d) Emrick, T.; Fréchet, J. M. J Curr Opin Colloid Interface Sci 1999, 4, 15; (e) Fréchet, J. M. J. Science 1994, 263, 1710; (f) Newkome, G. R.; He, E.; Moorefield, C. N. Chem Rev 1999, 99, 1689; (g) Fischer, M.; Vögtle, F. Angew Chem Int Ed 1999, 38, 885; (h) Gibson, H. W.; Gong, C.; Liu, S.; Nagvekar, D. Macromol Symp 1998, 128, 89; (i) Raymo, F. M.; Stoddart, J. F. Chem Rev 1999, 99, 1643; (j) Prince, R. B.; Okada, T.; Moore, J. S. Angew Chem Int Ed 1999, 38, 233; (k) Moore, J. S. Acc Chem Res 1997, 30, 402; (l) Gauthier, M.; Tichagwa, L.; Downey, J. S.; Gao, S. Macromolecules 1996, 29, 519; (m) Malmström, E. E.; Hawker, C. J. Macromol Chem Phys 1998, 199, 923.
4. Knedel is a Polish term for dumpling; the etymology of this term can be found in Latterman, G. Chem Eur J 1997, 3, 2081.
5. Wooley, K. L. Chem Eur J 1997, 3, 1397.
6. Clark, Jr., C. G.; Wooley, K. L. Curr Opin Colloid Interface Sci 1999, 4, 122.
7. Liu, G. Curr Opin Colloid Interface Sci 1998, 3, 200.
8. Stupp, S. I. Curr Opin Colloid Interface Sci 1998, 3, 20.
9. Procházka, K.; Baloch, M. K. Macromol Chem 1979, 180, 2521.
10. Ishizu, K.; Saito, R. Polym Plast Technol Eng 1992, 31(7,8), 607.
11. Won, Y.-Y.; Davis, H. T.; Bates, F. S. Science 1999, 283, 960.
12. Li, J.; Swanson, D. R.; Qin, D.; Brothers, H. M.; Piehler, L. T.; Tomalia, D.; Meier, D. J. Langmuir 1999, 15(21), 7347.
13. Thurmond, II, K. B.; Kowalewski, T.; Wooley, K. L. J Am Chem Soc 1996, 118, 7239.
14. Remsen, E. E.; Thurmond, II, K. B.; Wooley, K. L. Macromolecules 1999, 32, 3685.
15. Thurmond, II, K. B.; Kowalewski, T.; Wooley, K. L. J Am Chem Soc 1997, 119, 6656.
16. Thurmond, II, K. B.; Wooley, K. L. ACS Symposium Series on Materials for Controlled Release Applications; American Chemical Society: Washington, DC, 1998; Chapter 13.
17. Luger, K.; Mäder, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. Nature 1997, 389, 251.
18. Thurmond, II, K. B.; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. Nuc Acids Res 1999, 27, 2966.
19. Huang, H.; Kowalewski, T.; Remsen, E. E.; Gertzmann, R.; Wooley, K. L. J Am Chem Soc 1997, 119, 11653.
20. Huang, H.; Remsen, E. E.; Wooley, K. L. Chem Commun 1998, 1415.
21. Baugher, A. H.; Goetz, J. M.; McDowell, L. M.; Huang, H.; Wooley, K. L.; Schaefer, J. Biophys J 1998, 75, 2574.
22. Schaefer, J.; Huang, H.; Wooley, K. L. ACS Polym Prepr 1999, 40(1), 460.
23. Kataoka, K.; Kwon, G. S.; Yokoyama, M.; Okano, T.; Sakurai, Y. J Controlled Release 1993, 24, 119.
24. Arca, E.; Tian, M.; Webber, S. E.; Munk, P. Int J Polym Anal Charact 1995, 2, 31.
25. (a) Huang, H.; Wooley, K. L. ACS Polym Prepr 1998, 39(1), 239; (b) Wooley, K. L.; Huang, H.; Kowalewski, T. ACS PMSE Prepr 1999, 80, 13.
26. Zhang, Q.; Wooley, K. L. ACS Polym Prepr 1999, 40(2), 986.
27. (a) Hawker, C. J.; Hedrick, J. L.; Malmström, E. E.; Trollsås, M.; Mecerreyes, D.; Moineau, G.; Dubois, P.; Jérôme, R. Macromolecules 1998, 31, 213; (b) Moineau, G.; Minet, M.; Dubois, P.; Teyssie, P.; Senninger, T.; Jérôme, R. Macromolecules 1999, 32, 27; (c) Mecerreyes, D.; Moineau, G.; Dubois, P.; Jérôme, R.; Hedrick, J. L.; Hawker, C. J.; Malmström, E. E.; Trollsås, M. Angew Chem Int Ed 1998, 37, 1274.
28. (a) Stewart, S.; Liu, G. Chem Mater 1999, 11, 1048; (b) Ding, J.; Liu, G. Chem Mater 1998, 10, 537; (c) Ding, J.; Liu, G. J Phys Chem B 1998, 102, 6107.
29. Bütün, V.; Wang, X.-S.; de Paz Báñez, M. V.; Robinson, K. L.; Billingham, N. C.; Armes, S. P. Macromolecules 2000, 33, 1.
30. Ding, J.; Liu, G. Macromolecules 1998, 31, 6554.
31. Bütün, V.; Billingham, N. C.; Armes, S. P. J Am Chem Soc 1998, 120, 12135.
32. Bütün, V.; Lowe, A. B.; Billingham, N. C.; Armes, S. P. J Am Chem Soc 1999, 121, 4288.