Preparation of orthogonally-functionalized core Click cross-linked nanoparticles†

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The preparation of well-defined core cross-linked polymeric nanoparticles is reported, utilizing multi-functional dendritic cross-linkers that allow for the effective stabilization of supramolecular polymer assemblies and the simultaneous introduction of reactive groups within the core domain. Amphiphilic diblock copolymers of poly(acrylic acid)-*b*-poly(styrene) (PAA-*b*-PS) that contained alkynyl functionality, partially incorporated throughout the hydrophobic PS block segment, were utilized as Click-readied precursors for the formation of polymer micelles. Divergently-grown dendrimers of the zero, first, second and third generations, having increasing numbers of azide terminating groups ($(N_3)_2$-[G-0], $(N_3)_4$-[G-1], $(N_3)_8$-[G-2], and $(N_3)_{16}$-[G-3], respectively), were investigated as cross-linkers *via* Click reactions with the polymer alkynyl groups to form covalent linkages throughout the micellar core domains, thus forming core cross-linked nanoparticles. All four generations of dendrimers were found to cross-link the micelle core effectively and afford robust nanostructures, whilst simultaneously introducing Click-readied functionalities throughout the hydrophobic domain, which are proposed to be readily available for further chemical modification.

Introduction

The controlled assembly of amphiphilic block copolymers has attracted much attention in the field of polymer science due to their potential biomedical application as novel imaging agents, scavengers and/or drug delivery systems.[1] These amphiphilic block copolymers, containing large solubility differences between hydrophobic and hydrophilic segments, are known to assemble supramolecularly when placed in block-selective solvents, to afford a wide range of nano- or microstructures,[2] including micelles with core-shell architectures, which can be spherical,[3] tubular,[4] or vesicular[5] in morphology. The inherent dynamic nature of these self-assembling polymer-based nanostructures is influenced by various conditions such as concentration, solvent/non-solvent, and temperature, and by the chemical structure of the polymer.[6] However, in many applications, it is advantageous to have a covalently-stabilized nanostructure, which is robust and maintains its integrity under a variety of conditions. As a result, there has been significant effort in recent years towards the stabilization of self-assembled polymeric nanostructures. This has been realized by cross-linking either the core[7] or the shell[8,9] domains of the polymer micelles through various methods, including the incorporation of polymerizable[10] or photo cross-linkable groups,[11] the introduction of cross-linking reagents,[12] the chain extension of the block copolymers with a cross-linking agent,[13] and the application of an external stimulus.[14]

Stabilized block copolymer micelles are of interest in medical and other applications[15] due to their potential for loading guest molecules, by either physical entrapment[16] or chemical attachment,[17] within the hydrophobic core domain, allowing for the encapsulation, transportation, and potential delivery of hydrophobic molecules in concentrations that exceed their intrinsic water-solubility. The hydrophilic shell, which consists of a brush-like protective corona, not only stabilizes the micelles in aqueous solution and protects the contents of the hydrophobic core from hydrolysis or degradation, but can also be modified selectively to present functional moieties on the surface. These include detection elements or targeting ligands that would allow for selective delivery of the packaged therapeutic to diseased areas.[18]

A critical aspect in the design and synthesis of these multifunctional polymer micelles is the selective incorporation of orthogonally reactive groups in the hydrophobic core domain of the micelle, which will allow for both the attachment of small molecules and cross-linking of the core. Previously, we have examined the application[19] of copper(I) catalyzed Huisgen 1,3-dipolar cycloaddition reactions between azides and alkynes to yield triazoles as a highly efficient synthetic tool in materials chemistry.[20] This quantitative "Click" reaction of

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terminal alkynes and azides, which forms 1,2,3-triazoles regiospecifically, has been demonstrated to be highly efficient, selective, and to proceed under benign reaction conditions.[21] This cycloaddition reaction has also shown excellent compatibility with a wide range of functional groups, and, as a result, the application of this efficient and compatible chemistry is of great interest at the interface of materials science, chemistry, and biology.[22]

We have previously reported the synthesis of shell Click cross-linked nanoparticles using a multi-functional dendritic cross-linker.[23] However, the hydrophobic nature of the dendrimer proved to be incompatible with the hydrophilic nature of the micelle corona within the aqueous reaction conditions and, as a result, dendrimers of generations greater than one, did not behave as effective cross-linkers. This incompatibility can be exploited if the regiochemical site of cross-linking is changed from the hydrophilic shell to the hydrophobic core of the polymer micelle. The modular nature of Click chemistry can be adapted to include novel polymer micelles with poly (acrylic acid) shells and alkynyl-functionalized poly(styrene) cores, which are easily prepared by the self-assembly of the corresponding amphiphilic block copolymer. The hydrophobic nature of the multivalent dendrimers then places these cross-linkers into the hydrophobic core domains to produce nanoparticles having core domains cross-linked *via* Click chemistry.[24] The cross-linking strategy, employing a polyvalent dendritic cross-linker, is significant as it allows for the stabilization of the micelle and also permits orthogonal incorporation of Click readied functionality, within the core domain of the robust micelle, which can undergo subsequent Click reactions. The cross-linking reactions to form polymeric nanoparticles were characterized by IR spectroscopy, differential scanning calorimetry (DSC), temperature dependent dynamic light scattering (DLS) measurements, and atomic force microscopy (AFM).

Results and discussion

A new synthetic strategy for the direct transformation of self-assembled block copolymer micelles into core-shell nanoparticles *via* a controlled cross-linking reaction within the core domain leading to both covalent stabilization and the incorporation of reactive functional groups is described. The methodology involves the reaction of block copolymer micelles that present reactive side chain functionalities in the hydrophobic domain, with a polyvalent dendritic cross-linker. Dendritic macromolecules are ideal for utilization as cross-linkers due to their polyvalency and well defined functionality with the utilization of Click chemistry leading to the retention of active functionality within the cross-linked domain if a slight excess of dendrimer is used. In this study, azide-terminated dendritic cross-linking units of generations zero, one, two, and three were allowed to undergo reaction with amphiphilic diblock copolymer micelles that had been partially functionalized throughout the core with alkynyl moieties to establish core Click cross-linked polymer nanoparticles in aqueous solution.

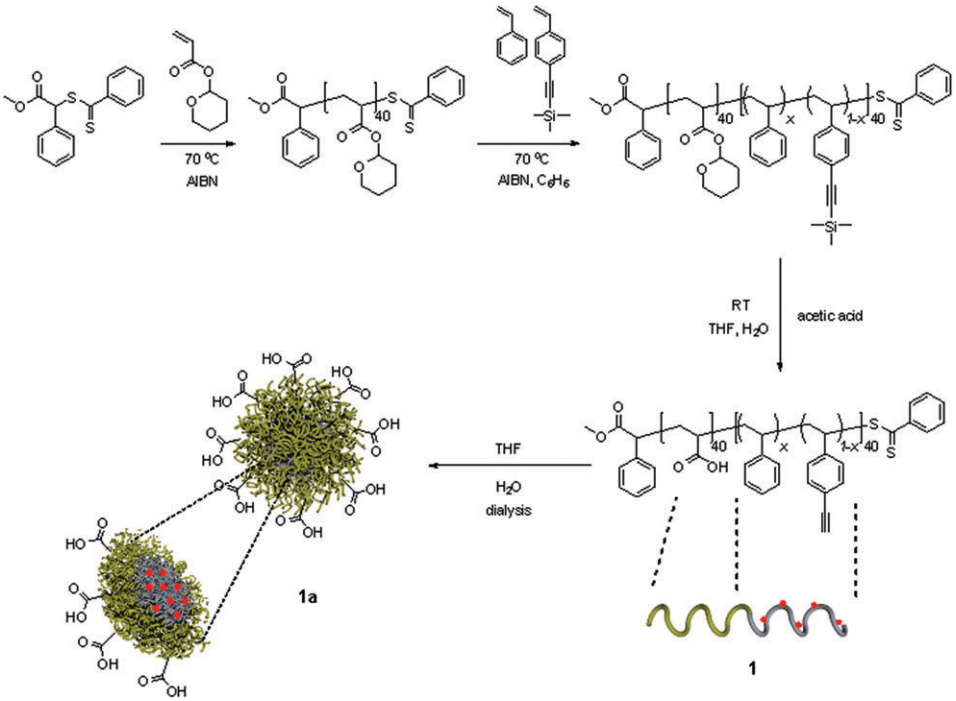
Core alkynyl-functionalized micelles were constructed from the solution self-assembly of amphiphilic diblock copolymers, which were functionalized partially throughout the hydrophobic core domain with alkynyl groups.[24] The synthesis of alkynyl functionalized amphiphilic block copolymers involved the initial synthesis of the diblock copolymer, $PTHPA_{40}$-*b*-$[PS_{0.9}$-*co*-$PSC{\equiv}CTMS_{0.1}]_{40}$ by the reversible addition fragmentation chain transfer (RAFT)[25] polymerization of tetrahydropyran acrylate (THPA)[26] using a dithioester initiator[27] and then subsequent chain extension using a mixture of styrene and trimethylsilylacetylene styrene ($SC{\equiv}CTMS$).[28] Following the simultaneous deprotection of the ester[29] and acetylene functionalities, the amphiphilic block copolymer, PAA_{40}-*b*-$[PS_{0.9}$-*co*-$PSC{\equiv}CH_{0.1}]_{40}$, **1**, was isolated.[30]

The micellar organization of these linear amphiphilic polymer chains was performed *via* dropwise addition of an equal volume of water to a solution of the diblock in THF.[31] Following extensive dialysis of the micelle solution, against water, alkynyl core functionalized micelles, **1a**, were isolated and characterized (Scheme 1). IR spectroscopic analysis of the micelles **1a** highlighted the presence of the terminal alkynyl groups (absorbances observed at *ca.* 2150 and 3300 cm^{-1}) within the structure, DLS analysis confirmed that the micelles had a uniform hydrodynamic diameter, and their shape was confirmed by TEM and AFM analyses.

The hydrophobic core of micelle **1a** was then swollen by dialysis for 3 days into a solution of 20 vol% THF in buffered water, to afford micelle **1b**. The calculated concentration of the polymer micelle solution was determined by measurement of the final volume of micelles obtained together with the initial mass of the polymer precursors. The DLS analysis of micelles of **1** before and after dialysis into THF (**1a** and **1b**, respectively) highlights the expected swelling effect of the core upon dialysis into a good solvent for this block (D_h(**1a**) = 42 ± 2 nm *vs.* D_h(**1b**) = 54 ± 2 nm). It was expected that swelling of the micelle core would assist in solublizing the hydrophobic dendrimer molecules within the core domain of the micelle and also allow Click chemistry to be performed within the solvent filled core of the micelle.

The Click cross-linking of micelle **1b** was accomplished with the azide-functionalized dendrimers generations 0th, 1st, 2nd, and 3rd (Scheme 2) in the presence of an organo-soluble copper(I) source, $CuBr(PPh_3)_3$, diisopropylethylamine, and a small amount of copper wire (Scheme 3), with the copper wire being added to assist in preventing oxidation of the copper(I) catalyst in the organic/aqueous reaction media. The azide-functionalized dendrimers, in THF solution, were added slowly to a stirred THF–water solution of the Click catalyst and micelles. Unlike the previously reported shell cross-linking case, precipitation of the dendrimers was not observed in the reaction mixture, which demonstrates that the poly(benzyl ether) based dendritic cross-linkers are better suited as core cross-linkers, due to their hydrophobic nature. The Click reaction was allowed to proceed, with stirring, for 3 days at ambient temperature and the product was purified by exhaustive dialysis against deionized water and THF (5 : 1) for 4 d and then dialysis into Nanopure water for 4 d, to ensure removal of catalyst and any unreacted dendrimers.

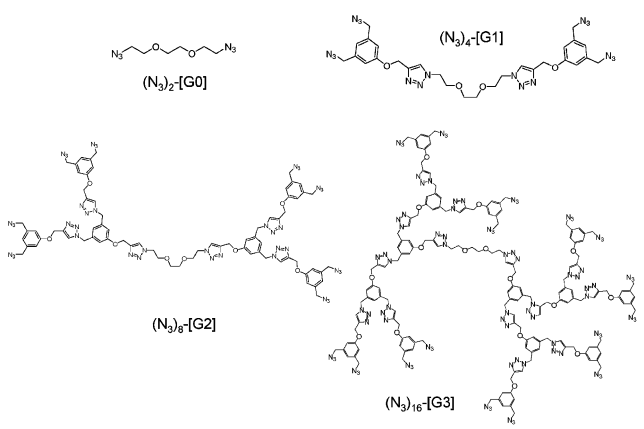
After dialysis, the nanoparticles obtained from the cross-linking reactions were characterized by DLS, with the number-averaged hydrodynamic diameter (D_h) measured as a function of temperature (20 to 60 °C) and compared with the starting



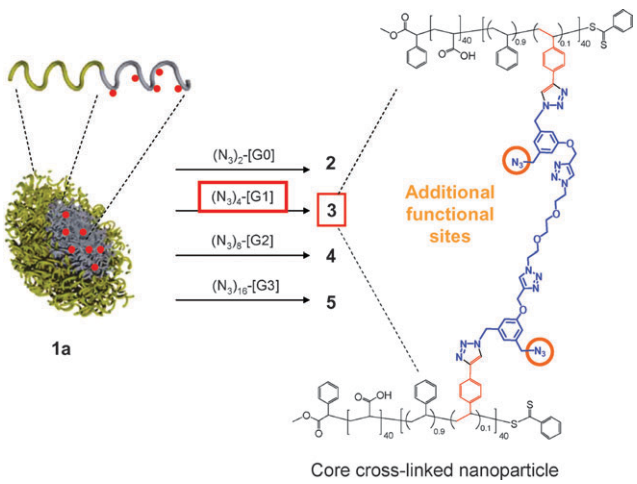
Scheme 1 Schematic representing the synthesis of block copolymer **1**, using RAFT polymerization techniques, and subsequent self assembly into polymeric micelles, **1a**.

polymer micelles. In aqueous solution, the dynamic polymer micelle structures, **1a**, decreased in stability with increasing temperature, and no self-assembled structures were detectable at 60 °C due to thermodynamically favored dissociation of the polymer chains. In contrast, the core cross-linked polymer nanoparticles, **2–5**, maintained a stable particle size at 60 °C upon heating from 20 °C (Fig. 1). These results strongly suggest that all dendritic cross-linkers, from generation 0 to 3, behaved successfully as cross-linkers within the cores of the micelles, which also further demonstrates the efficiency of the Click reaction as the $G = 0$ dendrimer is a simple difunctional cross-linking agent. Significantly, DLS analysis showed that the cross-linked nanoparticles maintained the approximate size of the starting micelles, indicating that the cross-linking reaction occurred intramicellarly within the core of the micelles rather than intermicellarly between aggregates.

The sizes and shapes of micelles **1a** and polymer nanoparticles (**2–5**) were measured in the solid state by AFM. The large diameter values (D_{av}(**2–5**) = 80–110 nm) obtained from AFM measurements in comparison to those from DLS analysis at ambient temperature reflect, in part, the difference in the sizes of the particles in solution and the solid state, but also indicate



Scheme 2 Structures of dendritic cross-linkers of generations zero, one, two and three.



Scheme 3 Synthesis of polymer nanoparticles from alkynyl-functionalized micelles and azido-terminated dendrimers. The example shown is for the 1st generation dendrimer, which must undergo at least two coupling reactions to construct a cross-link. Each reaction was performed with 1.0 equiv. of azide, $CuBr(PPh_3)_3$ (0.30 equiv.), *N*,*N*′-diisopropylethylamine (DIPEA) (3.0 equiv.), and Cu wire. All equivalences are relative to alkynyl functionalities. The dendrimers were added as solutions in THF to the micelles in aqueous solutions (1 : 4, THF–H_2O).

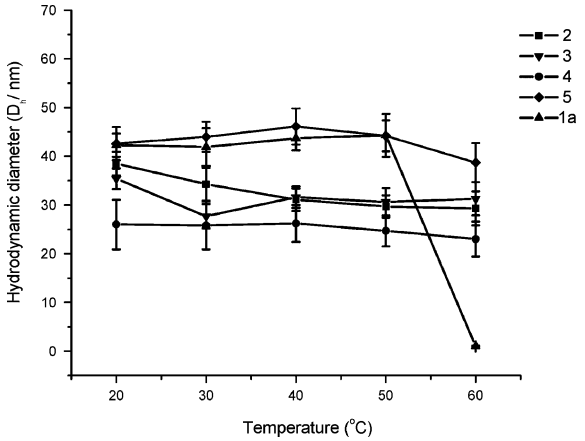


Fig. 1 Number-averaged hydrodynamic diameter (D_h) values determined from DLS experiments as a function of temperature, for the block copolymer micelles, **1a**, (▲) and for the core cross-linked nanoparticles afforded from the micelles being allowed to undergo reaction with the dendrimers $(N_3)_2$-[G-0], (■) **2**, $(N_3)_4$-[G-1] (▼) **3**, $(N_3)_8$-[G-2] (●) **4**, and $(N_3)_{16}$-[G-3] (◆) **5**.

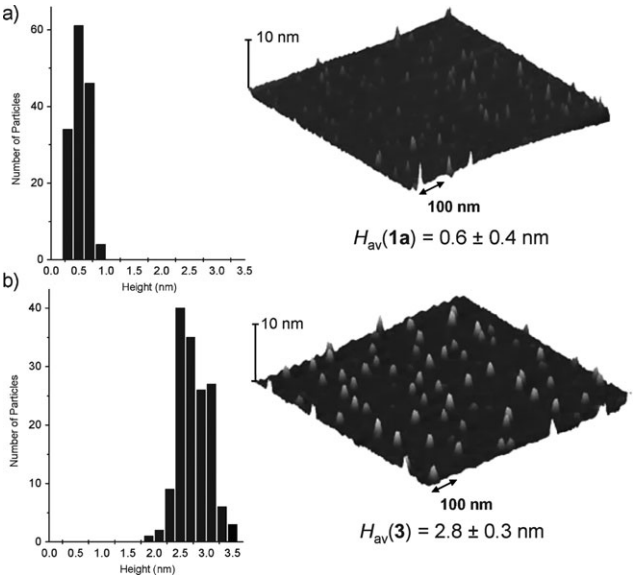


Fig. 2 Representative tapping-mode AFM height images of (a) micelles **1a** and (b) core-cross-linked nanoparticles **3** prepared by Click cross-linking **1a** with a 1st generation azido-terminated dendrimer. Average diameters are shown with the corresponding distribution and a representative image. Samples were prepared by drop deposition onto freshly cleaved mica and allowed to dry under ambient conditions.

deformation of the particles on the hydrophilic mica surface, the substrate for AFM characterization; however, these measurements are distorted due to the finite size of the AFM tip. The small heights (H_{av}) of the core cross-linked nanoparticles, **2**–**5**, indicate that significant deformation from the spherical shape observed in solution occurred upon absorption onto mica. Despite this deformation, AFM analysis can be utilized to demonstrate the increased stability of the nanoparticles compared to the starting micelles, **1a**. This can be inferred by comparing the smaller height of the micelles **1a** (H_{av} = 0.6 ± 0.4 nm) to the nanoparticles (H_{av}(**2**) = 2.6 ± 0.7 nm, H_{av}(**3**) = 2.8 ± 0.3 nm, H_{av}(**4**) = 3.1 ± 0.6 nm, H_{av}(**5**) = 2.6 ± 0.5 nm), indicating that the core-cross-linked structures undergo less deformation on the mica surface compared to the starting micelle, suggesting a more stable and robust structure (Fig. 2).

By maintaining the appropriate ratio of alkynyl groups within the micelle to dendritic cross-linker, the ratio of alkyne to azide functionality could be controlled and varying excesses of azide groups incorporated into the final polymeric nanoparticle. The incorporation of the azido functionality within the core of the cross-linked nanostructure from these unconsumed functionalities of the dendritic cross-linkers was evaluated qualitatively using IR spectroscopic analysis, while at the same time confirming consumption of the alkynyl groups. For instance, the availability of residual azide functionality post cross-linking was confirmed by IR analysis of Click core cross-linked micelle **4** in which absorbances were clearly visible in the region *ca.* 2100 cm^{-1}. In addition, the consumption of the alkynyl functionality was confirmed by the disappearance of the absorbances at *ca.* 2150 and 3300 cm^{-1}, in comparison with the IR spectrum for the starting micelles **1a**. These data further confirm that the attempted cross-linking reaction of the azido dendrimer and alkynyl-functionalized micelle was indeed successful and that this methodology allowed for the incorporation of further Click readied azido-functionality within the core of the cross-linked polymer nanoparticle.

Experimental

Hydrodynamic diameters (D_h) and size distributions for the micelles and polymer nanoparticles in aqueous solutions were determined by DLS. The DLS instrumentation consisted of a Brookhaven Instruments Limited (Worcestershire, UK) system, including a model BI-200SM goniometer, a model BI-9000AT digital correlator, a model EMI-9865 photomultiplier, and a model 95-2 Ar ion laser (Lexel, Corp.; Farmindale, NY) operated at 514.5 nm. Initial measurements were made at 20 ± 1 °C. For the temperature dependent study, the temperature of the DLS bath was set and then allowed to reach this temperature over 60 min. After this time, the solution was placed in the instrument and allowed to equilibrate at this temperature for 30 min before data collection was performed. Prior to analysis, solutions were centrifuged in a model 5414 microfuge (Brinkman Instruments, Inc.; Westbury, NY) for 4 min to remove dust particles. Scattered light was collected at a fixed angle of 90 °C. The digital correlator was operated with 522 ratio spaced channels, and an initial delay of 0.1 μs, a final delay of 5.0 μs, and a duration of 15 min. A photomultiplier aperture of 200 μm was used, and the incident laser intensity was adjusted to obtain a photon counting of between 200 and 300 kcps. Only measurements in which the measured and calculated baselines of the intensity autocorrelation function agreed to within 0.1% were used to calculate particle size. The calculations of the particle size distributions and distribution averages were performed with the ISDA software package (v. 3.19, Brookhaven Instruments Company, Holtsville, NY), which employed single-exponential fitting, cumulants analysis, and CONTIN particle size distribution analysis routines. All determinations were made in triplicate.

The height measurements and distributions for the nanoparticles were determined by tapping-mode AFM under ambient conditions in air. The AFM instrumentation consisted of a Nanoscope III BioScope system (Digital Instruments, Veeco Metrology Group; Santa Barbara, CA) and standard silicon tips (type, OTESPA-70; L, 160 μm; normal spring constant, 50 N m^{-1}; resonance frequency, 246–282 kHz). The sample solutions were prepared for AFM analysis by dilution (typical concentrations between 0.002–0.0002 mg mL^{-1}) and deposition of a drop (2 μL) onto freshly cleaved mica, allowed to dry freely in air. The number-average particle height (H_{av}) and diameter (D_{av}) values and standard deviations were generated from the sectional analysis of 150 particles from at least five different analysis regions.

The modulated differential scanning calorimetry (DSC) measurements were performed with a TA Instruments, DSC 2920 and with a ramp rate of 4° min^{-1}. The glass-transition temperatures (T_g) were taken as the midpoint of the inflection tangent, upon the third heating scan. Infrared spectra (IR) were obtained on Perkin-Elmer Spectrum BX FT-IR system using diffuse reflectance sampling accessories.

All materials were used as received from Sigma-Aldrich Company (St. Louis, MO) unless otherwise stated. Supor 25 mm 0.1 μm Spectra/Por Membrane tubes (molecular weight cut-off (MWCO) 6–8 kDa, Spectrum Medical Industries Inc., Laguna Hills, CA) were used for dialysis. $CuBr(PPh_3)_3$ was synthesized according to literature methods.[32] The alkynyl functionalized amphiphilic block copolymer [PAA_{40}-*b*-[$PS_{0.9}$-*co*-PSC≡$CH_{0.1}$]$_{40}$], **1**,[24] and azido terminated dendrimers[9] were synthesized as previously reported.

Preparation of PAA_{40}-*b*-[$PS_{0.9}$-*co*-PSC≡$CH_{0.1}$]$_{40}$ micelles in H_2O, 1a

A round-bottom flask equipped with a stirrer bar was charged with PAA_{40}-*b*-[$PS_{0.9}$-*co*-PSC≡$CH_{0.1}$]$_{40}$, (M_n^{NMR} = 7000 g mol^{-1}; 0.50 g, 2.96 mmol of acrylic acid groups). THF (500 mL) was added and the solution was allowed to stir at RT for 30 min to ensure the mixture was homogenous. Deionized water (500 mL) was added *via* a metering pump at the rate of 18 mL h^{-1}. After all of the water had been added, the bluish micelle solution was transferred to dialysis tubing (MWCO *ca.* 6–8 kDa), and dialyzed against deionized water for 4 d, to remove all of the THF. The final volume of **1a** was 1.6 L, affording a polymer concentration of *ca.* 0.30 mg mL^{-1}. D_h (DLS) = 42 ± 2 nm; D_{av} (TEM): 33 ± 1 nm; D_{av} (AFM): 85 ± 16 nm; H_{av} (AFM): 0.6 ± 0.4 nm. Lyophilization gave **1a** as a white solid. DSC: $(T_g)_{PAA}$ = 132 °C, $(T_g)_{PS\text{-}co\text{-}PSC\equiv CH}$ = 100 °C. IR: 3309, 2985, 2153, 2099, 1702, 1643, 1563, 1556, 1537, 1459, 1413, 1320, 1292, 1174, 1087, 865, 840 cm^{-1}.

Preparation of PAA_{40}-*b*-[$PS_{0.9}$-*co*-PSC≡$CH_{0.1}$]$_{40}$ micelles in THF–H_2O, 1b

A solution of micelles **1a** (1.5 L, 0.30 mg mL^{-1}) was transferred to pre-soaked dialysis membrane tubes (MWCO *ca.* 6–8 kDa), and dialyzed against a 1 : 4 THF and buffered H_2O (50 mM sodium phosphate, 1.0 M sodium chloride, pH 7.3) mixture for 3 d. This afforded a micelle solution of **1b** of concentration *ca.* 0.50 mg mL^{-1}. D_h (DLS) = 54 ± 2 nm.

General synthesis of core Click cross-linked nanoparticles using gen = 0 dendrimers (2)

To a stirred solution of polymer micelles, **1b**, (250 mL, 0.075 mmol of alkynyl groups) was added $CuBr(PPh_3)_3$ (0.0209 g, 0.0225 mmol), DIPEA (0.0290 g, 0.225 mmol), Cu wire (*ca.* 100 mg), and the $(N_3)_2$-[G-0] dendrimer (7.5 mg, 3.73 × 10^{-5} mol, in 0.25 mL THF). The reaction was allowed to stir at RT for 3 d, and was then transferred to pre-soaked dialysis tubing (MWCO 6–8 kDa) and allowed to dialyze against deionized water and THF (5 : 1) for 4 d and then into Nanopure water (18 MΩcm) for 4 d. D_h (DLS) = 39 ± 2 nm; D_{av} (AFM): 84 ± 11 nm; H_{av} (AFM): 2.6 ± 0.7 nm. Lyophilization of an aliquot of the solution gave a sample of **2** as a white solid for characterization. DSC: $(T_g)_{PS}$ = 102 °C. IR: 2953, 2100, 1734, 1701, 1602, 1571, 1554, 1459, 1437, 1405, 1260, 1163, 1118, 1080, 865, 840, 830, 672 cm^{-1}.

Synthesis of nanoparticles using gen = 1 dendrimers (3)

This sample was prepared using the general procedure described above from a stirred solution of micelles, **1b**, (250 mL, 0.075 mmol of alkynyl groups), $CuBr(PPh_3)_3$ (0.0209 g, 0.0225 mmol), DIPEA (0.0290 g, 0.225 mmol), Cu wire (*ca.* 100 mg), and the $(N_3)_4$-[G-1] dendrimer (12.8 mg, 1.86 × 10^{-5} mol, in 0.65 mL THF). D_h (DLS) = 35 ± 2 nm; D_{av} (AFM): 81 ± 10 nm; H_{av} (AFM): 2.8 ± 0.3 nm. Lyophilization of an aliquot of the solution gave a sample of **3** as a white solid for characterization. DSC: $(T_g)_{PS}$ = 104 °C. IR: 2952, 2101, 1735, 1702, 1648, 1560, 1438, 1407, 1261, 1196, 1163, 1119, 1054, 970, 827 cm^{-1}.

Synthesis of nanoparticles using gen = 2 dendrimers (4)

This sample was prepared using the general procedure described above from a stirred solution of micelles, **1b**, (250 mL, 0.075 mmol of alkynyl groups), $CuBr(PPh_3)_3$ (0.0209 g, 0.0225 mmol), DIPEA (0.0290 g, 0.225 mmol), Cu wire (*ca.* 100 mg), and the $(N_3)_8$-[G-2] dendrimer (15.4 mg, 9.32 × 10^{-6} mol, in 0.63 mL THF. D_h (DLS) = 26 ± 5 nm; D_{av} (AFM): 100 ± 21 nm; H_{av} (AFM): 3.1 ± 0.6 nm. Lyophilization of an aliquot of the solution gave a sample of **4** as a white solid for characterization. DSC: $(T_g)_{PS}$ = 99 °C. IR: 2952, 2100, 1739, 1701, 1654, 1560, 1438, 1406, 1263, 1194, 1171, 1120, 1064, 828, 751, 699, 629 cm^{-1}.

Synthesis of nanoparticles using gen = 3 dendrimers (5)

This sample was prepared using the general procedure described above from a stirred solution of micelles, **1b**, (250 mL, 0.075 mmol of alkynyl groups), $CuBr(PPh_3)_3$ (0.0209 g, 0.0225 mmol), DIPEA (0.0290 g, 0.225 mmol), Cu wire (*ca.* 100 mg), and the $(N_3)_{16}$-[G-3] dendrimer (16.9 mg, 4.68 × 10^{-6} mol, in 0.65 mL THF). D_h (DLS) = 43 ± 3 nm; D_{av} (AFM): 110 ± 28 nm; H_{av} (AFM): 2.6 ± 0.5 nm. Lyophilization of an aliquot of the solution gave a sample of **5** as a white solid for characterization. DSC: $(T_g)_{PS}$ = 100 °C. IR: 2920, 2850, 2099, 1752, 1701, 1646, 1551, 1534, 1450, 1412, 1098, 1026, 1080, 843, 821, 697 cm^{-1}.

Conclusions

Click chemistry between alkynyl core-functionalized block copolymer micelles and azido-terminated dendrimers was employed to construct cross-links between the polymer chain segments within the micelle core domain, to yield core Click cross-linked polymer nanoparticles. The $(N_3)_2$-[G-0], $(N_3)_4$-[G-1], $(N_3)_8$-[G-2], and $(N_3)_{16}$-[G-3] dendrimers were each successfully utilized as cross-linkers within the hydrophobic core domain and the success of the cross-linking reactions was analyzed by temperature dependent DLS experiments, DSC, AFM, and IR spectroscopy. The facile nature of the Click chemistry used for cross-linking allowed the size and structure of the dynamic polymer micelles to be maintained during covalent stabilization, and also permitted the remaining azido functionalities located on the dendrimer cross-linker to be detected by IR spectroscopic analysis. It is proposed that these groups could be used as versatile Click-readied handles for the further functionalization of these stabilized micelles. In addition, the shell layer, consisting of poly(acrylic) acid groups, is available for amidation chemistry for further tailoring of these nanoparticles with targeting ligands or probes. This cross-linking strategy and simultaneous incorporation of Click-readied functionality provides a facile route for the future development of these nanostructures in both materials and biomedical applications.

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