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Shell Click-Crosslinked (SCC) Nanoparticles: A New Methodology for Synthesis and Orthogonal Functionalization

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Abstract: A new methodology for the preparation of well-defined core−shell nanoparticles was developed, based upon the employment of a multifunctional crosslinker to coincidently stabilize supramolecular polymer assemblies and imbed into the shell unique chemical functionalities. Amphiphilic diblock copolymers of poly(acrylic acid)$_{80}$-*b*-poly(styrene)$_{90}$ that had been assembled into micelles and partially functionalized throughout the corona with alkynyl groups were utilized as Click-readied nanoscaffolds for the formation of shell Click-crosslinked nanoparticles (SCCs). 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 crosslinkers via Click reactions with the alkynyl groups to form covalent linkages throughout the block copolymer micelle corona, thus forming a crosslinked shell. The crosslinking reactions were characterized by ^{1}H NMR and IR spectroscopies, differential scanning calorimetry (DSC), and dynamic light scattering (DLS) measurements. Only the first generation dendrimer ((N$_3$)$_4$-[G-1]) possessed a sufficient balance of polyvalency and water solubility to achieve crosslinking and establish a robust nanostructure. The resulting SCC was further characterized with atomic force microscopy (AFM), transmission electron microscopy (TEM), and analytical ultracentrifugation (AU). The dendritic crosslinker is important as it also allows for the incorporation of excess functionality that can undergo complementary reactions. Within the shell of this SCC the remaining azide termini of the dendrimer crosslinker were then consumed in a secondary Click reaction with an alkynyl-functionalized fluorescein to yield a fluorescently labeled SCC that was characterized with DLS, AFM, TEM, AU, UV−vis, and fluorescent measurements as a function of pH.

Introduction

Nature's power to drive systems toward a desired architecture or order has been elegantly harnessed in the synthesis of self-assembled architectures. Synthetic chemistry has evolved to model such natural systems, and engineer polymer chains, such that their assembly into supramolecular architectures via non-covalent interactions has become tunable through control of the polymer composition and the physiological conditions under which assembly is conducted.[1] Architectures that have been accessed include toroids,[2] helices,[3,4] rods,[5−7] spheres,[8−10] disks,[11] vesicles,[12−14] fibers,[15−18] tubes,[19−22] and other shapes.[23,24] The ability to control self-assembly provides exciting opportunities to manufacture unique materials that demonstrate properties that are not otherwise accessible. Self-assembled nanoparticles and self-assembled arrays of nanoparticles have great potential to serve as multifunctional platforms for delivery of therapeutics,[25−31] smart materials,[32−35] and nanosized devices.[36−40] Often this diverse spectrum of applications requires both robust

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structures and the covalent attachment of functional molecules to the nanoparticle scaffold.

Crosslinking the individual components of self-assembled nano-objects after assembly has emerged as a viable strategy for imparting greater stability to self-assembled structures by providing covalent linkages between the self-assembled polymer chains. The robustness created by the covalent crosslinks allows expansion of the applications for self-assembled materials, and this strategy has been employed to create core crosslinked nanoparticles,[41−46] shell crosslinked nanoparticles,[47−51] shell crosslinked rods,[52] nanocages,[47,51,53,54] nanotubes,[55−57] and nanoporous films.[58,59] The potential uses of these crosslinked materials is expanded by their stability toward more environments than their non-crosslinked precursors; in addition, the covalent attachment of moieties to the crosslinked self-assembled architectures further extends the potential employment of these materials. Strategic attachment of ligands to nanoscaffolds has been executed through various chemical reactions and has evolved to allow for regiospecific functionalization of the self-assembled structure such that either the core or shell is modified. Further expansion of the methodologies for functionalization of nanoparticles is reported herein and combines both crosslinking of the self-assembled polymer chains and incorporation of unique sites upon which further chemistry can be conducted.

Recently, SCKs have been investigated as targeted drug delivery systems, since therapeutics, imaging agents and targeting ligands can be attached to the nanoparticle.[60] Various methods have been employed for the covalent attachment of these molecules to the SCKs and include chain-end attachment of the molecule to a polymer precursor,[61,62] carbodiimide mediated coupling to the acrylic acid groups of the shell in solution[63−65] and on a solid support,[66] and Click addition to functional groups along the backbone of the polymer precursor post particle formation.[67] Although these methods have proven successful and valuable, a unique and orthogonal strategy was developed that would expand the types of chemistry that could be performed on the nanoparticle. In addition, an orthogonal approach would not require the creation of a new functionalized initiator and polymer for each functionality that was desired and would be tolerant to both the functional groups present in the SCK and any further chemistry performed on the SCK. Due to all of these requirements, Click chemistry was employed for the combined crosslinking and functionalization of the nanoparticle and coupled with amidation chemistry.

Common characteristics of the different reactions that qualify as Click chemistry are essentially quantitative yields, with easily isolated products, regiospecificity, and easily obtained starting materials.[68−71] Click reactions have recently been employed to advance drug discovery,[72−79] functionalize monolayers,[80−82] and

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synthesize and functionalize various polymers[83−86] and dendrimers.[87,88] These examples illustrate the diverse range of functional groups and conditions that are tolerated by Click chemistry and demonstrate the powerful possibilities provided by placing Click functional groups throughout the shell, core, and surface of a nanoparticle. We recently reported[67] the Huisgen 1,3-dipolar cycloaddition (between azides and terminal alkynes) Click reaction[89] as a method for the functionalization of the shells of SCKs, and it is this Click chemistry that was utilized to implement the new nanoparticle preparation and functionalization strategy. In the previously reported method, Click-readied functionalities located in the shell or core of the nanoparticle were allowed to react with Click-readied small molecules. In these cases, the shell crosslinking of the SCK was performed, using the traditional carbodiimide mediated amidation reaction, prior to the Click functionalization reactions.[67] The new strategy, reported herein, allows the reaction between Click-readied functional groups in the shell of block copolymer micelles and Click-readied termini of a dendrimer to effect crosslinking. The nonconsumed dendrimer termini were then available for reaction with Click-readied fluorescently active molecules.

Experimental Section

Materials. Tetrahydrofuran (99%), methanol (99%), copper sulfate·$5H_2O$, and sodium ascorbate were used as received from Sigma-Aldrich Company (St. Louis, MO). Spectra/Por membranes (Spectrum Medical Industries, Inc., Laguna Hills, CA) used for dialysis were obtained from Fisher Scientific (Pittsburgh, PA). The alkynyl functionalized micelle, alkynyl functionalized fluorescein derivative, and azido terminated dendrimers were synthesized as previously reported.[67,88]

Instrumentation. Infrared spectra were obtained on a Perkin-Elmer Spectrum BX FT-IR system using diffuse reflectance sampling accessories. Nuclear magnetic resonance (^{1}H and ^{13}C) spectra were acquired on a Bruker AVANCE 400 FT-NMR spectrometer with the solvent signal as reference. The modulated differential scanning calorimetry (DSC) measurements were performed with a TA Instruments, DSC 2920 and with a ramp rate of 4°/min. The glass-transition temperatures (T_g) were taken as the midpoint of the inflection tangent, upon the third heating scan.

Hydrodynamic diameters (D_z, D_n) and size distributions for the SCKs in aqueous solutions were determined by dynamic light scattering (DLS). The DLS instrumentation consisted of a Brookhaven Instruments Limited (Holtsville, NY) 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. Measurements were made at 20 ± 1 °C. 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°. The digital correlator was operated with 522 ratio spaced channels, 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 200 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 (Brookhaven Instruments Company), which employed single-exponential fitting, cumulants analysis, and nonnegatively constrained least-squares particle size distribution analysis routines.

The height measurements and distributions for the SCCs 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; resonance frequency, 224−272 kHz). The sample solutions were drop (2 μL) deposited onto freshly cleaved mica and allowed to dry freely in air.

Transmission electron microscopy samples were diluted in water (9:1) and further diluted with a 1% phosphotungstic acid (PTA) stain (1:1). Carbon grids were prepared by a plasma treatment to increase the surface hydrophilicity. Micrographs were collected at 100 000× magnification and calibrated using a 41 nm polyacrylamide bead standard from NIST. Histograms of particle diameters were generated from the analysis of a minimum of 150 particles from at least three different micrographs.

Sedimentation equilibrium experiments were conducted on a Beckman Instruments, Inc. (Fullerton, CA) model Optima XL-I analytical ultracentrifuge fitted with a model An60-Ti four-hole rotor and Epon charcoal-filled, six-channel centerpiece sample cells with matched sapphire windows. All data were recorded using the instrument's Rayleigh interferometric (refractive index) detection optics at 20 °C and 3000, 4000, and 5000 rpm, respectively, with a centrifugation time of 3−5 days to reach sedimentation equilibrium. The solution volume was 110 μL, and the optical path length was 12 mm. A Mettler-Parr model DMA 602 high-precision digital density meter was employed to determine the density at 20.0 °C for all solutions. All densities were an average of 5 runs, with measurements of 100 periods per run. The molecular weight and weight-average degree of aggregation, N_{agg}, was computed as previously reported,[62] except that the dendrimer crosslinker was taken into account for calculation of N_{agg} (5 dendrimers per chain since 0.25 equiv of dendrimer was reacted with 1 equiv of alkyne). Following sedimentation at 5000 rpm, the UV−vis spectra (400−600 nm) of the dye-SCC were collected at the top, middle, and bottom of the sample cell with water as the blank.

UV−vis spectroscopy data were acquired on a Varian Cary 1E UV−vis spectrophotometer. Nanoparticle solutions before and after attachment of the fluorescein derivative (concentrations ca.. 0.20 mg/mL) were made in deionized water prior to the absorbance measurements. A calibration curve was determined for the fluorescein dye using first derivative UV−vis spectroscopy,[90] and this was then utilized to quantitatively determine the amount of dye present in the Click nanoparticles. Fluorescence spectroscopy data were acquired on a Varian Cary Eclipse Fluorescence spectrophotometer. Each sample was

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prepared independently from a nanoparticle stock solution at ca. 0.20 mg/mL. Sample solutions at various pH values from 3.7 to 9.5 were excited at $\lambda_{ex} = 488$ nm, and the fluorescence emission spectra ($\lambda_{em} = 512$ nm) in the range 500−630 nm were recorded.

Micelle Formation (1). A round-bottom flask equipped with a stirrer bar was charged with PAA_{80}-*b*-PS_{90}, ($M_n^{NMR} = 15\,600$ g/mol; 0.70 g, 3.6 mmol of acrylic acid groups), THF (700 mL) was added, and the solution was allowed to stir at rt for 30 min. After this time deionized water (700 mL) was added via a metering pump at the rate of 20 mL/h. After all 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 days, to remove all of the THF. To a stirred solution of these micelles (700 mL, 0.31 mg/mL, 1.4 mmol of acrylic acid) in a round-bottomed flask was added a solution of propargylamine (0.019 g, 0.35 mmol) in deionized water (5.0 mL). The solution was allowed to stir for 2 h at rt. To this reaction mixture was added dropwise, via a metering pump at the rate of 20 mL/h, a solution of 1-[3′-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (0.12 g, 0.42 mmol) dissolved in deionized water (100 mL). The reaction mixture was allowed to stir overnight at RT and was then transferred to presoaked dialysis membrane tube (MWCO ca. 3.5 kDa), and dialyzed against deionized water for 4 days to remove small molecule contaminants. The final concentration of the solution of **1** was ca. 0.27 mg/mL. D_h (DLS) = 45 ± 2 nm; D_{av} (TEM): 32 ± 6 nm; D_{av} (AFM): 52 ± 10 nm; H_{av} (AFM): 0.9 ± 0.3 nm. Lyophilization of an aliquot of this solution gave a sample of **1** as a white solid for characterization. DSC: $(T_g)_{PAA} = 144$ °C, $(T_g)_{PS} = 96$ °C. IR: 3510−3300, 3292, 3025, 2925, 2109, 1705, 1652, 1610, 1558, 1499, 1452, 1417, 1274, 1151, 1078, 1029, 796, 763, 698 cm^{-1}.

Shell Click-Crosslinked Nanoparticle Synthesis (2). To a stirred solution of micelles, **1**, (200 mL, 1.49×10^{-4} mol alkyne) was added $CuSO_4 \cdot 5H_2O$ (9.13 mg, 3.66×10^{-5} mol), sodium ascorbate (14.5 mg, 7.42×10^{-5} mol, 5 wt % in H_2O), and the $(N_3)_4$-[G-1] dendrimer (50.8 mg, 7.42×10^{-5} mol, in 5.60 mL of THF). The reaction was allowed to stir at rt for 3 d and was then transferred to presoaked dialysis tubing (MWCO 6000−8000 Da) and allowed to dialyze against Nanopure water (18 MΩ·cm) for 4 d. D_h (DLS) = 35 ± 2 nm; D_{av} (TEM) = 32 ± 5 nm; D_{av} (AFM) = 104 ± 17 nm; H_{av} (AFM) = 2.4 ± 0.5 nm. Lyophilization of an aliquot of the solution gave a sample of **2** as a white solid for characterization. DSC: $(T_g)_{PS} = 99$ °C. IR: 2926, 2099, 1602, 1556, 1493, 1554, 1542, 1452, 1413, 1327, 1262, 1087, 1029, 850, 758, 698 cm^{-1}.

Fluorescently Labeled Shell Click-Crosslinked Nanoparticle Synthesis (3). To an aqueous solution of the SCC (75 mL, 4.2×10^{-5} mol azide) was added $CuSO_4 \cdot 5H_2O$ (2.6 mg, 1.0×10^{-5} mol), sodium ascorbate (4.1 mg, 2.1×10^{-5} mol, in 0.08 mL of H_2O), and a solution of alkyne-functionalized fluorescein (21.3 mg, 4.99×10^{-5} mol, in 0.6 mL of MeOH, 5.0 mL of H_2O, pH 8). The reaction was allowed to stir for 2 d and was then transferred to presoaked dialysis tubing (MWCO 6000−8000 Da) and dialyzed against Nanopure water for 4 d. D_h (DLS) = 38 ± 3 nm; D_{av} (TEM) = 21 ± 1 nm; D_{av} (AFM) = 102 ± 19 nm; H_{av} (AFM) = 6.2 ± 1.6 nm. Lyophilization of an aliquot of the solution gave a sample of **3** as a pale yellow solid for characterization. DSC: $(T_g)_{PS} = 103$ °C. IR: 2925, 1655, 1611, 1556, 1493, 1457, 1452, 1413, 1327, 1252, 1090, 1024, 850, 759, 698 cm^{-1}.

Results and Discussion

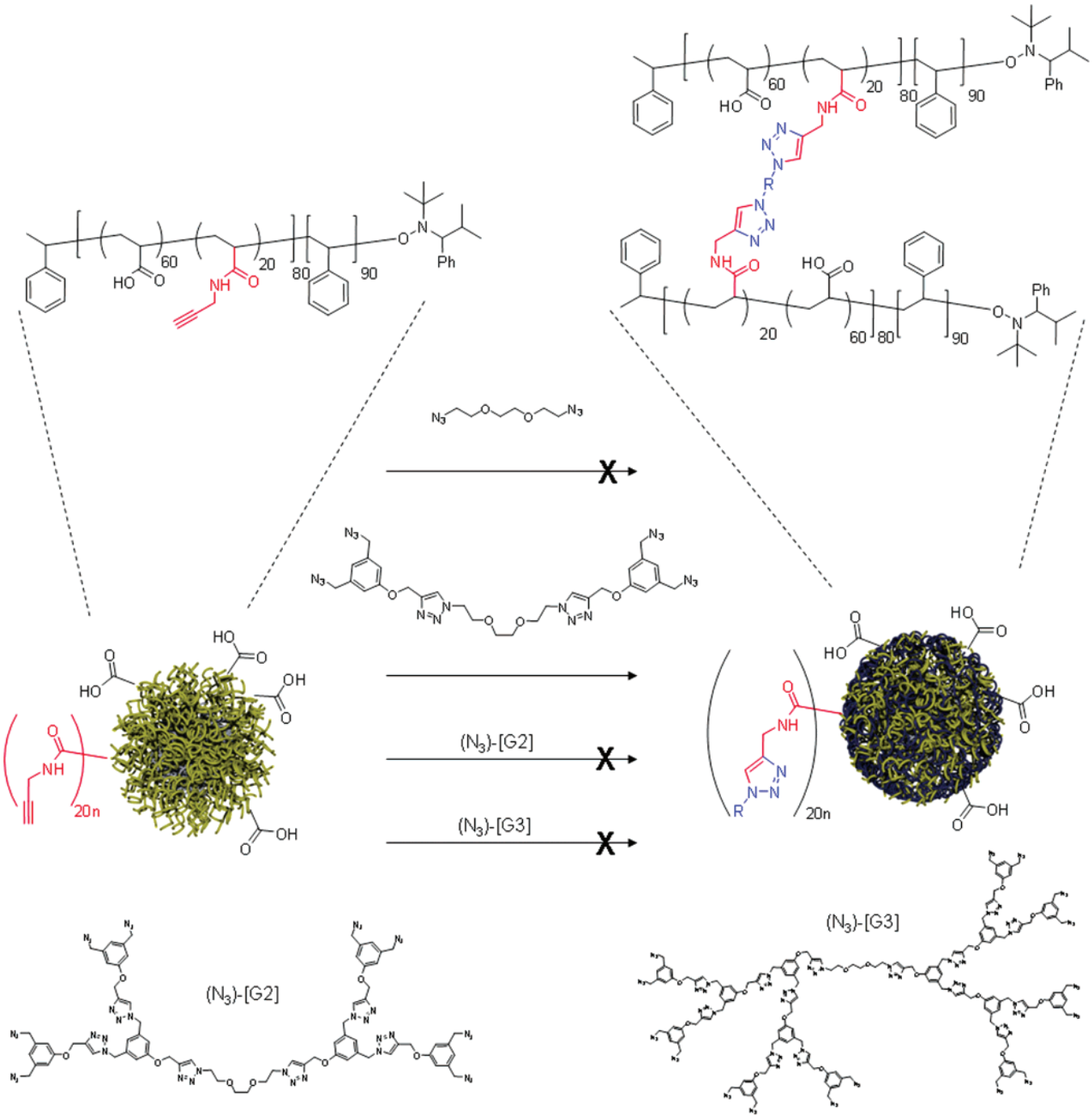
This new methodology for the preparation of well-defined, amphiphilic core−shell nanostructures involved the direct transformation of supramolecularly assembled block copolymer micelles into robust materials via reaction with polyvalent crosslinking units. The shell crosslinking of block copolymer micelles that present large numbers of reactive side chain functionalities has been studied extensively;[63−67] however, in this latest development, the multifunctionality of the crosslinker was also explored as a strategy to complete the crosslinking process and also incorporate unique functional groups. Dendritic macromolecules are ideal structures for investigation of the effects of polyvalency, as they are constructed in a stepwise approach that provides control over the numbers of chain termini. In this study, therefore, azide-terminated dendritic crosslinking 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 shell with alkynyl moieties to establish shell Click-crosslinked (SCC) nanoparticles in aqueous solution.

The SCC synthesis was designed to probe the effects of polyvalency while also balancing the molecular size of the crosslinker and its solubility in the aqueous medium. Beginning with assembly of the previously reported[67] poly(acrylic acid)$_{80}$-*b*-poly(styrene)$_{90}$ into an aqueous solution of micelles, alkynyl functionalites were placed throughout the micelle corona through a carbodiimide-mediated coupling and then utilized to form crosslinks with azide terminated dendrimers.[88] Click-crosslinking was attempted with the zeroth, first, second, and third generation azide terminated dendrimers in the presence of copper sulfate and sodium ascorbate (Scheme 1).

Each of the attempted crosslinking reactions was characterized by DLS measurements, with the number average diameter (D_{av}) measured as a function of sample concentration. Successfully crosslinked micelles retain a constant diameter independent of concentration, whereas the diameter of noncrosslinked micelles is concentration dependent; consequently, the micelle breaks apart into unassembled polymer chains at concentrations lower than the critical micelle concentration (cmc). As shown in Figure 1, only the first generation dendrimer was successful in crosslinking the micelle corona, since it was the only sample for which the number average diameter remained constant upon dilution. Certainly, for the second and third generation dendrimers, poor solubility in the aqueous micelle solution prevented successful crosslinking. The bis(azide) zeroth generation dendrimer was soluble in aqueous solution, but it failed to produce crosslinked micellar structures that maintained integrity upon dilution beyond the cmc. It is hypothesized that this difunctional small molecule is ineffective because of a lack of sufficient functionality, combined with water solubility and a lack of attractive interactions with the block copolymer micelle assemblies. Previous crosslinking chemistries involving amidation of the acrylic acid residues within micelle shells composed of PAA by reaction with bifunctional amines indicates that preassociation (in this case via acid−base interactions) of the crosslinker with the micelle enhances the bimolecular coupling and also suggests that the failure of the Click chemistry is not due to insufficient branching and limited chain length. Only the first generation dendrimer balanced the degree of branching and solubility properties to perform as a crosslinker for the synthesis of SCC nanoparticles.

Further evidence of SCC formation with $(N_3)_4$-[G-1] was afforded from ^{1}H NMR spectroscopy and DSC experiments. A new resonance at 8.25 ppm was evident in the ^{1}H NMR spectrum of an SCC sample that had been lyophilized and then suspended in DMSO-d_6, due to a proton corresponding to the triazole linkage formed upon the Click crosslinking between the polymer chain and dendrimer crosslinker. The new proton resonance was integrated relative to the dendrimer triazole

Scheme 1. Synthesis of SCC Nanoparticles from Click-Readied Micelles and Dendrimers, Where R Represents the Dendritic Crosslinking Unit, Having the Possibility of Multiple Crosslinkages and Remaining Azido Functionalities[a]



[a] Each reaction was performed with 0.50 equiv of azide, $CuSO_4 \cdot 5H_2O$ (0.25 equiv) and sodium ascorbate (0.50 equiv, 5 wt % soln. in H_2O). All equivalences are relative to alkyne functionalities. The $(N_3)_4$-[G-1], $(N_3)_8$-[G-2], and $(N_3)_{16}$-[G-3] dendrimers were added as solutions (10 mg/mL) in THF.

proton (ratio 1.7:1.0) to provide a quantitative estimate of the degree of crosslinking of the SCC. Since this ratio is greater than 0.5:1.0 and less than 2.0:1.0, it was concluded that the dendrimers performed a high degree of crosslinking while retaining unreacted azides for further reactivity, respectively. In addition, IR analysis confirmed the presence of unreacted azido groups on the dendrimer termini (ca. 2100 cm^{-1}) and demonstrated the complete consumption of the alkynyl functionality. The observation of only one glass transition temperature (T_g) at 99 °C for the SCC polystyrene core domain vs two glass transitions observed at 144 °C and 96 °C for the poly(acrylic acid) and polystyrene, respectively, of the Click-readied micelle also indicated that covalent crosslinking within the poly(acrylic acid) shell had occurred.

Upon confirmation of crosslinking via the Click reaction with the $(N_3)_4$-[G-1], the nonconsumed azide functionalities of the dendrimer were used as a handle for the attachment of an alkynyl functionalized fluorescein derivative. The functionalization proceeded under the copper sulfate, sodium ascorbate, Click conditions as shown in Scheme 2, and the product was characterized by AU sedimentation equilibrium and UV−vis spectroscopy. The UV−vis spectra of the nanoparticle before

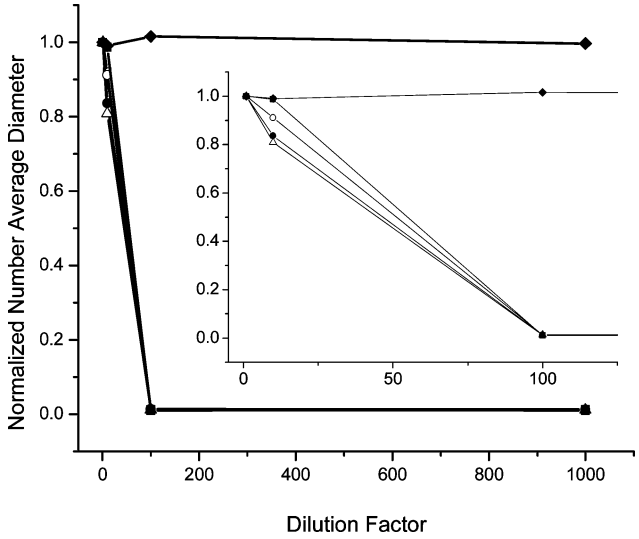


Figure 1. Normalized number average diameter determined from DLS experiments as a function of dilution, for the block copolymer micelles (●) and those after being allowed to undergo reaction with the $(N_3)_2$-[G-0] (△), $(N_3)_4$-[G-1] (◆), $(N_3)_8$-[G-2] (○), and $(N_3)_{16}$-[G-3] (■).

and after attachment of the fluorescein derivative were recorded in solution from 400 to 600 nm. Absorbance was observed after reaction with the dye, shown in Figure 2, plot I. Upon sedimentation of the nanoparticles, as monitored by interference, the absorbance from 400 to 600 nm was measured at the meniscus of the solution in the sample cell. Absorbance at the meniscus indicated the presence of free dye; however, repetition of the same set of measurements after extensive dialysis proved that all of the free dye had been removed (see Figure 2, plot II).

Table 1. Characterization Data for Alkynyl Functionalized Micelles, **1**, SCCs, **2**, Fluorescently Labeled SCCs, **3**, and SCK Nanoparticle Formed Using Amidation Chemistry

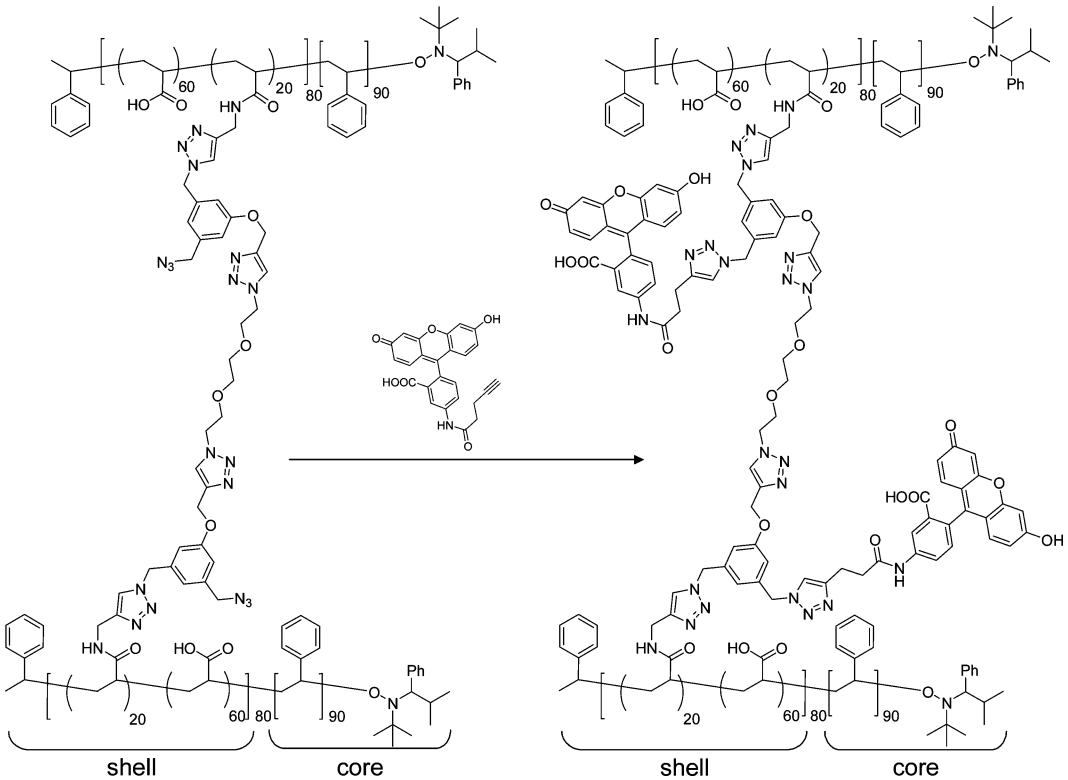
	DLS	AFM		TEM
nanoparticle	D_{av} (nm)	H_{av} (nm)	D_{av} (nm)	D_{av} (nm)
1	45 ± 2	0.9 ± 0.3	52 ± 10	32 ± 6
2	35 ± 2	2.4 ± 0.5	104 ± 17	32 ± 5
3	38 ± 3	6.2 ± 1.6	102 ± 19	21 ± 2
SCK	36 ± 2	2.8 ± 0.3	133 ± 17	34 ± 4

The amount of attached dye was quantified from the first derivative of UV−vis measurements, as reported.[67] From this analysis, it was determined that the Click reaction had proceeded quantitatively between the SCC and alkynyl dye (it was known from ^{1}H NMR the amount of azide groups remaining on the dendrimer crosslinker and the number of azides remaining per particle from the SCC aggregation number).

Fluorescent measurements were also collected for **3** at various pH values. Since the fluorescent intensity of fluorescein is pH dependent, and upon attachment to the nanoparticle shell this dependence is buffered,[67] it was not surprising that the fluorescence intensity of the fluorescein dye attached to the SCC decreased with decreasing pH and that this decrease was less dramatic than that observed for the free dye. The pH dependences of the free alkynyl-functionalized dye and **3** are plotted in Figure 3.

The micelles, SCCs, and fluorescently labeled SCCs were further characterized with AFM, TEM, and AU sedimentation equilibrium experiments (Table 1). As was observed for the conventionally crosslinked shell functionalized nanoparticles,[67] a decrease in hydrodynamic diameter, D_h, as determined by DLS

Scheme 2. Attachment of Fluorescein Molecules to SCCs via Click Reactions between Remaining Azides of the Dendrimer and Alkynes of the Fluorescent Molecules[a]



[a] The reaction was performed with 1.0 equiv of alkynyl-functionalized fluorescein, $CuSO_4 \cdot 5H_2O$ (0.50 equiv), and sodium ascorbate (0.25 equiv, 5 wt % in H_2O). The product shown represents only one structural possibility.

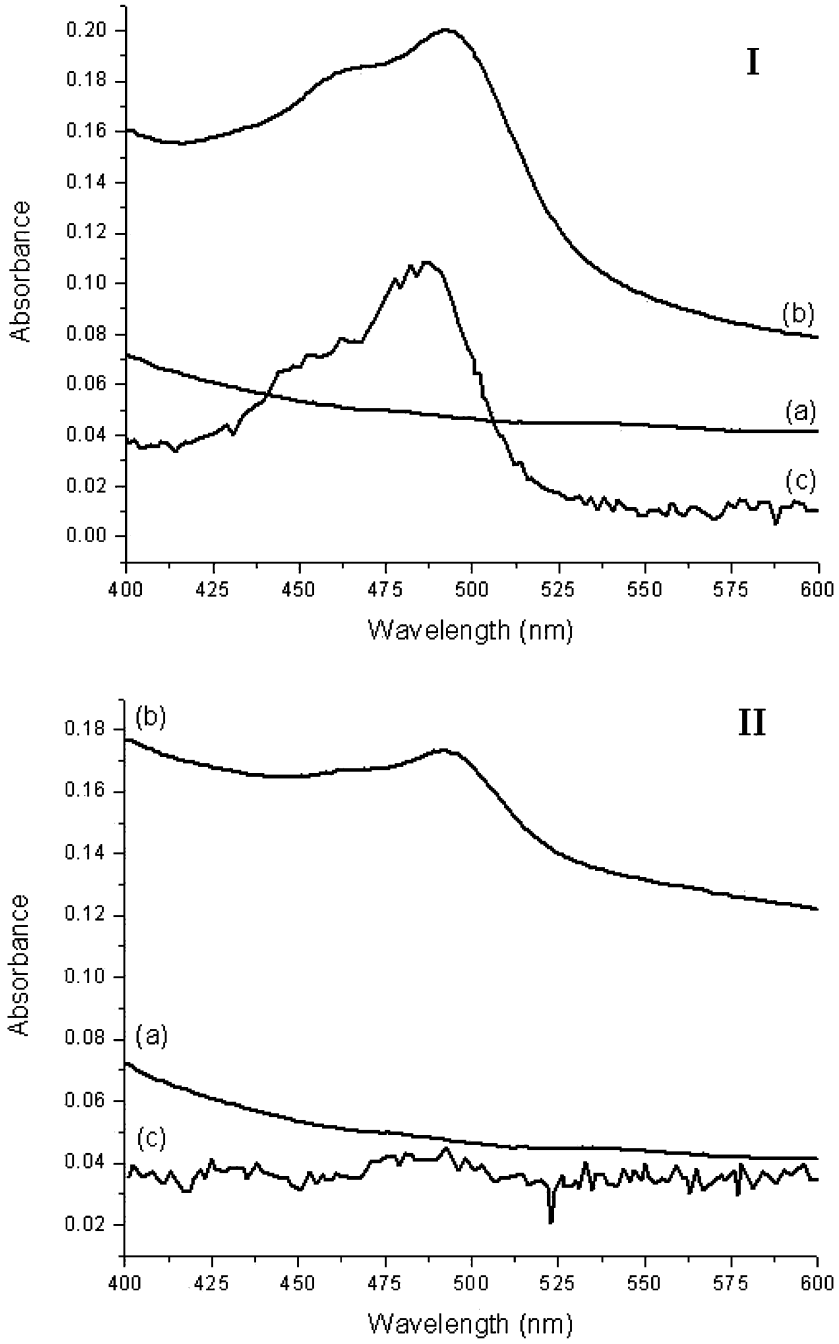


Figure 2. (I) UV−vis spectra of (a) **2** as a bulk solution, (b) **3** as a bulk solution prior to dialysis, and (c) **3** prior to dialysis, with data acquired at the meniscus of the AU sample after sedimentation. (II) UV−vis spectra of (a) **2** in bulk solution, (b) **3** in bulk solution after exhaustive dialysis, and (c) **3** at the meniscus of the AU sample after exhaustive dialysis and sedimentation.

analysis, was observed upon crosslinking. Each type of particle flattened onto the mica surface when drop deposited and allowed to dry freely in air, as shown by the small heights acquired by AFM measurements. The flattening was also observed by the large diameters from AFM measurements, but these data are distorted due to the width of the AFM tip. For comparison, micelle **1** was crosslinked using conventional amidation chemistry to approximately the same degree as the SCC **2** (ca. 20%), and the characterization data for this SCK are shown in Table 1. Interestingly, the crosslinking of the micelle and functionalization of the SCC with the fluorescein derivative seemed to provide some rigidity to the particle as the height for the SCC is larger than that of the micelle, **1**, and the height of **3** is larger than that of **2**. Similar diameters were measured for **1** and **2** by TEM; however, **3** gave a smaller diameter than those of the other nanostructures, which is in agreement with the observation of greater height and less deformation of **3** upon adsorption onto the substrate.

The molecular weight of **2** was determined by AU sedimentation equilibrium and density measurements to be 6 000 000 ± 1 000 000 g/mol, and the aggregation number was calculated to be 320 ± 70 chains/particle. The molecular weight and aggregation number are not available for the micelle since the particles undergoes constant reorganization, and concentrating

(91) Remsen, E. E.; Thurmond, K. B., II; Wooley, K. L. *Macromolecules* **1999**, *32*, 3685−3689.

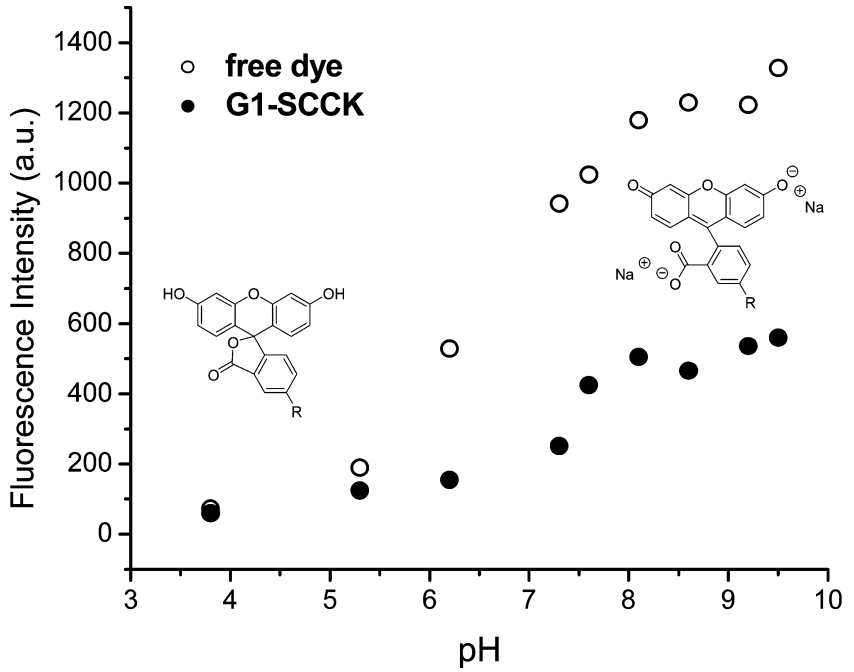


Figure 3. Fluorescence intensity of free dye (○) and **3** (●) as a function of pH (λ_{ex} = 488 nm, λ_{em} = 512 nm).

the sample for analysis would alter the size of the micelle,[91] whereas the data for **3** were not determined.

Conclusions

Click chemistry between an alkynyl shell functionalized block copolymer micelle and an azido terminated dendrimer was employed to construct crosslinks between the polymer chain segments within the micelle corona, to yield shell Click-crosslinked (SCC) nanoparticles. Even though the $(N_3)_2$-[G-0], $(N_3)_4$-[G-1], $(N_3)_8$-[G-2], and $(N_3)_{16}$-[G-3] dendrimers were attempted as crosslinkers to form SCCs, only the $(N_3)_4$-[G-1] dendrimer successfully crosslinked the hydrophilic shell of the alkynyl functionalized micelle. The success of the crosslinking reactions was analyzed by DLS experiments, DSC, ^{1}H NMR, and IR spectroscopy. The remaining azido functionalities located on the dendrimer crosslinker were then consumed in the attachment of an alkyne functionalized fluorescein derivative under Click chemistry conditions, which served as a model system for any Click-readied small molecule that would be attached to the nanoparticle. The fluorescein-functionalized nanostructures were analyzed by AU and UV−vis spectroscopy, to determine that the dye was covalently attached to the nanoparticle, indicating that the dendritic crosslinker provided available sites for chemical modification of the nanostructures. This type of nanoparticle crosslinking and subsequent functionalization provides a facile route for the future attachment of biologically important molecules such as reporter molecules, therapeutics, and targeting ligands onto the nanoparticle.

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