62 *Macromolecules* **2010**, *43*, 62–70
DOI: 10.1021/ma901840n

Macromolecules
ARTICLE

Preparation of Organosoluble Silica−Polypeptide Particles by "Click" Chemistry

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Received August 18, 2009; Revised Manuscript Received October 28, 2009

ABSTRACT: The synthesis of organo-soluble hybrid particles with a hydrophobic, α-helical polypeptide shell and silica core has been achieved by combining ring-opening polymerization and click chemistry. Alkyne end-terminated poly(γ-stearyl-α-L-glutamate) (alkyne-PSLG) was prepared by the ring-opening polymerization of the *N*-carboxyanhydride of γ-stearyl-α-L-glutamate using propargylamine as initiator. The molecular weight and structure of this polypeptide were characterized by GPC and MALDI, and the α-helical nature was established by ^{1}H NMR and FTIR. Azide-functionalized silica particles (azido-silica) were prepared by the functionalization of silica particles with 3-bromopropyltrichlorosilane followed by nucleophilic substitution with sodium azide. The azide functionalization was confirmed by FTIR and XPS. Alkyne-PSLG was coupled to azido-silica by click reaction in tetrahydrofuran or toluene in the presence of pentamethyldiethylenetriamine and copper(I) bromide. Further characterization of the product using XPS, FTIR, and ^{1}H NMR revealed that the grafted polypeptide retained its α-helical nature and formed colloidal particles that readily dispersed in organic solvents. These hydrophobic, polypeptide-functionalized particles can serve as model systems in studies of colloid dynamics and/or crystallization. They may also find use in investigations designed to model enzyme activation or the properties of hydrophobic proteins in cell membranes.

Introduction

The recent development of hybrid particles made of various inorganic cores and inorganic, organic, polymeric, or biological shells results in properties that cannot be obtained by the core particles or polymers alone.[1−5] Silica particles are popular candidates for these kinds of hybrid structures because of their ease of synthesis, low cost, and facile surface modification possibilities. Compared to gold nanoparticles, where the gold−sulfur bond normally used to attach the shell is easily photo-oxidized and may suffer damage at higher temperatures and with some organic solvents, the silica surface is more stable for the covalent attachment of monolayers, polymers, or biomolecules. Such particles have already proved their usefulness in drug delivery, biosensing, separation, and purification of biological molecules and in the preparation of photonic crystals. Several reviews have appeared.[1,3−7]

Synthetic poly(γ-alkyl-α-L-glutamates) are polypeptides known to exist in a well-defined α-helical structure and retain this conformation even in solution.[8−11] The α-helical structure of many polyglutamates is stabilized by intramolecular hydrogen bonding which gives the polypeptide a rodlike structure. The grafting of such polypeptides to different surfaces has been explored[12−27] in association with various applications, including the separation of optical isomers,[19,27] and membrane permeability control through the helix-to-coil transition.[26,27] Poly(γ-stearyl-α-L-glutamate) (PSLG) is less often studied than poly(γ-benzyl-α-L-glutamate) (PBLG) or poly(ε-carbobenzoxy-L-lysine) (PCBL); unlike these mainstays, it has the desirable feature of being soluble in nonpolar organic solvents. PSLG features an α-helical backbone surrounded by long, flexible side chains and is often called a hairy rodlike polymer.[8,28−30] The stearyl chains impart good solubility to this polypeptide, which shows both lyotropic and thermotropic properties.[31−33] PSLG-grafted membranes have been shown to increase the activity retention and thermal stability of lipases immobilized on them, which is due to the enhanced hydrophobic interaction between the lipase and the long stearyl chains.[34,35] Sackmann suggested that hairy rod polymers such as PSLG can be deposited on substrates, which can be further used to support biomimetic membranes by a self-assembly process.[36] Sohn et al. demonstrated the self-assembly of PSLG on mica sheets by atomic force microscopy, which shows that these polypeptides with long alkyl chains self-assemble on the surface producing a flowerlike morphology.[37]

The attachment of α-helical polypeptides to colloidal silica is fundamentally interesting, as the core−shell composite particles call to mind naturally existing, protein-caged materials like viruses. Several methods can be used to immobilize a polymer or a biomacromolecule onto a colloidal particle surface. They mainly are the *grafting from* approach in which the particles' surface is functionalized with initiator molecules from which the polymerization occurs after addition of monomer and the *grafting to* approach in which the particles' surface is functionalized with binding groups that have affinity for the anchoring groups of a preformed polymer. Both methods have advantages and disadvantages. The *grafting from* approach can result in higher grafting densities and thicker films with fewer steps, but the characterization of the grafted polymer is often challenging. Earlier publications from this group reported the grafting of PBLG and PCBL polypeptides from colloidal silica using the *grafting from* approach. The polypeptide chains were grown from primary amine initiators attached to the surface of the colloidal silica.[38,39] This robust method resulted in high grafting density, but one of the issues encountered was finding the molecular weight of the grafted polypeptide chains. Typically in order to determine the molecular weight and polydispersity index

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 Published on Web 11/19/2009

(M_w/M_n) of the grafted polymer, it needs to be cleaved from the silica. For that, either the initiator should have cleavable groups (e.g., esters) or the silica core may be etched using solution or complexes of hydrofluoric acid (HF) to collect the polymer separately.[40−45] In the case of hydrophobic polypeptide chains on silica, both these methods are unfavorable; HF not only etches the silica but also damages the polypeptide chain.

In the present work, the *grafting to* approach utilizing Huisgen 1,3-dipolar cycloaddition,[46] widely known as click chemistry, was chosen to attach PSLG to silica particles. Even though the *grafting to* approach may yield comparatively lower grafting densities,[47] it has advantages in terms of precise control of molecular weight for all the chains attached to the surface and hence produces uniform layer thickness.[48] Two important details to consider when choosing the *grafting to* approach to obtain reasonable grafting density are[47] (1) selection of the end group of the preformed polymer and the reactive functional groups on the surface (they should not interfere with any other functional groups on the polymer or on the surface) and (2) use of a preformed polymer with highly reactive end groups (this makes the grafting step faster than nonreactive surface coverage by the polymer chains). These two requirements can be achieved by click chemistry. Introduced by Sharpless, click chemistry involves cycloaddition of alkyne and azide groups to form a very stable 1,2,3-triazole in presence of Cu(I) salt as catalyst. This chemoselective and fast reaction is well known for its tolerance to a variety of functional groups.[49−52] Several reports and reviews describe the use of click chemistry to couple preformed polymers or biomolecules to other synthetic polymers, nucleic acids, peptides, sugars, proteins, or even viruses and cells.[53−55] A few reports describe the use of click chemistry to make block copolymers of homopolypeptides.[56−62] Very few studies have been conducted to attach polymers to silica nanoparticles by the click coupling approach.[63−67] Recently, it was reported that azido-functionalized, silica-coated magnetic particles showed site-specific immobilization of alkyne-functionalized proteins, and the binding activity was higher than the random amide formation.[68] However, not much work has been attempted to explore the attachment of biomacromolecules or biosimilar molecules to silica nanoparticles using click chemistry.[69] The present work demonstrates the attachment of PSLG homopolypeptide to silica nanoparticles by click chemistry and the characterization of the peptide-modified particles in terms of structure, solubility in organic media, α-helical nature, and thermal stability.

Experimental Section

Materials. Stearyl alcohol (octadecanol) (99%), L-glutamic acid (99%), *tert*-butanol (99.5%), triethylamine (99.5%), triphosgene (98%), anhydrous dichoromethane, propargylamine (98%), trifluoroacetic acid (TFA), tetraethoxysilane (98%), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA) (99%), and CuBr (99.99%) were obtained from Aldrich and used as received. Dry tetrahydrofuran (THF) was obtained from a PureSolv solvent purification system. (3-Bromopropyl)trichlorosilane was obtained from Gelest. All other chemicals were reagent grade and used without further purification.

Synthetic Methods. *Synthesis of γ-Stearyl-α-L-glutamate (SLG).* SLG was synthesized using a reported procedure.[70] 8 g (0.0543 mol) of L-glutamic acid, 58.8 g (0.217 mol) of stearyl alcohol, and 85 mL of *tert*-butanol were added to a flask fitted with an addition funnel and a condenser. The stirred mixture was heated to 40 °C, and 6 mL of concentrated H_2SO_4 was slowly added. Once the addition was complete, the mixture was heated to 65 °C and stirred for 1 h. A clear solution was obtained. To this, 6 mL (0.042 mol) of triethylamine was added (resulted in cloudiness) followed by the addition of 10 mL of water, 150 mL of ethanol, and 17 mL (0.121 mol) of triethylamine. A white precipitate was formed which was stirred for 30 min. Hot methanol was then added to make a slurry, which was filtered on a Büchner funnel. The precipitate was washed with more hot methanol and then with diethyl ether. It was then dried in vacuo until constant mass was obtained. The crude ester obtained was recrystallized using water:*n*-butanol (1:1) mixture; mp = 168−173 °C, yield 60%.

Preparation of γ-Stearyl-α-L-glutamate N-Carboxyanhydride (SLG-NCA).[71] 5 g (0.0125 mol) of dried γ-stearyl-α-L-glutamate and 75 mL of dry THF were added to a flask equipped with a stirring bar, condenser, and an adapter connected to a nitrogen bubbler in-line to keep the inert atmosphere and to release any pressure formed during reaction. The flask was heated to 50 °C using a water bath, and 1.26 g (0.004 25 mol) of triphosgene was added to this mixture and stirred at that temperature for 1 h. The completely clear solution obtained was concentrated to 1/3 of its volume and poured into twice its volume of dry *n*-hexane. A white precipitate was formed. The flask was refrigerated to complete crystallization. The product was then filtered by suction filtration and washed with cold hexane. The crystals were redissolved in dichloromethane and shaken with a small amount of sodium carbonate and filtered through a pad of Celite on cotton. The filtrate was concentrated, poured into hexane, and refrigerated. Recrystallization was performed thrice by redissolving in dry THF and precipitating in dry hexane. Finally the filtered product was dried in vacuum and used immediately for polymerization. *Triphosgene is toxic, so this reaction should be carried out in a hood and the HCl/triphosgene from the nitrogen bubbler should be passed through an ammonium hydroxide solution. The triphosgene should be weighed inside a hood.* Filtration and recrystallization steps were done in a nitrogen atmosphere inside a glovebag. Yield 85%. ^{1}H NMR: δ 0.88 (t, terminal CH_3), 1.26, 1.6 (methylene groups of stearyl chain) 2.2−2.4 (m, β-CH_2) 2.6 (m, γ-CH_2), 4.10 (t, $-CH_2$ group adjacent to O═C−O− group) 4.4 (m, α-CH_2) 6.2 (−NH); mp = 77−79 °C.

Synthesis of Alkyne-Terminated Poly(γ-stearyl-α-L-glutamate) (Alkyne-PSLG). This was prepared by the ring-opening polymerization of the *N*-carboxyanhydride of γ-stearyl-α-L-glutamate (SLG-NCA).[71] 3.72 g (0.0087 mol) of SLG-NCA was weighed into a dry 50 mL round-bottomed flask and capped with a rubber septum and connected to an argon line using a syringe needle. To this, 37.2 mL of dry dichloromethane was injected. The solution was stirred and kept in a water bath at 30 °C. Then (49 μL, 7.22×10^{-4} mol) propargylamine in dichloromethane was added using a syringe. The reaction was allowed to continue for 3 days and was concentrated and precipitated in acetone. The polypeptide so obtained was filtered and dried in a vacuum oven. The yield was 77%.

Preparation of Silica Nanoparticles. Silica nanoparticles were prepared by the Stöber method[72] using 30 mL of concentrated ammonium hydroxide, 500 mL of ethanol, and 10 mL of tetraethoxysilane. The particles were centrifuged and redispersed four times in ethanol to remove unreacted reagents. The particles were then dispersed in toluene, and the solution was subjected to azeotropic distillation to remove any traces of water.

Preparation of Azide-Functionalized Silica (Azido-silica) Nanoparticles. The silica nanoparticles in toluene were functionalized with bromo groups by reaction with (3-bromopropyl)trichlorosilane.[65] The bromofunctionalized particles (3 g) were then centrifuged and redispersed in toluene two times. After the third centrifugation the isolated bromo-functionalized particles were subjected three times to centrifugation and redispersion in dimethylformamide (DMF). Subsequently, 2 g of NaN_3 and 50 mg of tetrabutylammonium iodide were added to these bromo-functionalized silica particles in DMF. The solution was heated at 80 °C for 24 h. The obtained azide-functionalized particles were centrifuged and dispersed in DMF and then in toluene.

Surface Grafting of Alkyne-PSLG on Azido-silica Nanoparticles by Huisgen's 1,3-Dipolar Cycloaddition (Click Reaction). 0.05 g of azido-silica was dispersed in 10 mL of dry toluene inside a three-neck flask which was attached to a water condenser and a nitrogen line. This dispersion was degassed for 10 min, and a degassed solution of 1 g alkyne-PSLG in 10 mL of toluene was added to it. CuBr (0.0915 g) was weighed in another vial and dissolved in 10 mL of toluene, which was closed with a septum. 0.26 mL of PMDETA was added to this CuBr solution using a syringe. This stirred solution was degassed for 10 min and injected into the three-neck flask containing azido-silica and alkyne-PSLG. The mixture was stirred and heated to 60 °C for 1 h and at 40 °C for 24 h. The reaction mixture was cooled, and the composite particles were recovered by centrifugation and further redispersed and centrifuged three times in toluene to remove unreacted polypeptide. After the final centrifugation, the particles were dispersed in chloroform and extracted with water, a solution of the sodium salt of EDTA, and finally with water to remove the copper. The washed colloidal suspension of silica-*click*-PSLG was centrifuged and dispersed three times in chloroform. Removal of chloroform gave modified nanoparticles for further characterization. The polymerization was also repeated using THF as solvent.

Characterization Methods. The molecular weight of the alkyne-PSLG was measured by gel permeation chromatography with multiangle light scattering (GPC-MALS). The separations were carried out using an Agilent 1100 solvent degasser, Agilent 1100 pump, and Agilent 1100 autosampler. A 10 μm, 50 × 7.8 mm guard column and two Phenogel 300 × 7.8 mm columns (Phenomenex, Torrance, CA) were used for the separation. The columns used were (1) 10 μm, 10^5 Å (10K−1000K) and (2) 10 μm, MXM (100−10000K). For detection, a Wyatt Dawn DSP-F multiangle light scattering detector, with a He−Ne laser, and a Hitachi model L-7490 differential refractive index detector (32 × 10^{-5} RIUFS) were used. All separations were done using an injection volume of 100 μL. THF (1 mL/min) stabilized with 250 ppm of BHT was used as the solvent. The specific refractive index increment (dn/dc) of PSLG was taken as 0.080 ± 0.002 mL/g.[28] The matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry MALDI-TOF spectrum of the alkyne-PSLG was obtained on a Bruker PROFLEX III MALDI-TOF mass spectrometer. Dithranol was used as a matrix, and the solution was prepared in chloroform. ^{1}H NMR spectra were recorded on a Bruker AV-400 using a solution of samples in deuterated chloroform and in some cases in a mixture of deuterated chloroform and trifluoroacetic acid (TFA). Fourier transform infrared spectra (FTIR) were recorded using a Bruker Tensor 27 FT-IR instrument with a Pike Miracle single-bounce attenuated total reflectance (ATR) cell equipped with a ZnSe single crystal. Thermogravimetric analysis (TGA) of the samples was recorded on a TA Instruments TGA Q50 with a heating rate of 10 °C min^{-1} under nitrogen flow. The surface composition of the nanoparticles at different stages was obtained using a Kratos Analytical Axis 165 X-ray photoelectron spectrometer (XPS) with Al Kα X-ray radiation with an energy of 1486.6 eV and takeoff angle of 90°. Peak locations were corrected based on the C (1s) signal at 285 eV. A survey spectrum and a high-resolution spectrum of individual elements were recorded with pass energies of 80 and 40 eV, respectively. Transmission electron microscopy (TEM) images of the particles were obtained using a JEOL 100-CX. A small drop of the sample solution was placed on the top of a 400 mesh carbon-coated grid (Electron Microscopy Sciences) and dried. Samples were investigated with an accelerating voltage of 80 kV at different magnifications. The size of the nanoparticles was determined by dynamic light (DLS) scattering using a Malvern Zeta-sizer Nano ZS.

Results and Discussion

Azido-Functionalized Silica Core Particles. The average diameter of the silica nanoparticles prepared by the Stöber method was found to be 140 ± 3 nm by DLS. A TEM image of these particles is shown in Figure 1. In order to make azide-functionalized silica particles, first bromo-functionalized particles were prepared by the condensation of (3-bromopropyl)trichlorosilane onto silica particles.

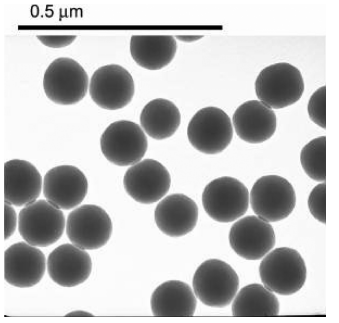


Figure 1. TEM image of silica nanoparticles.

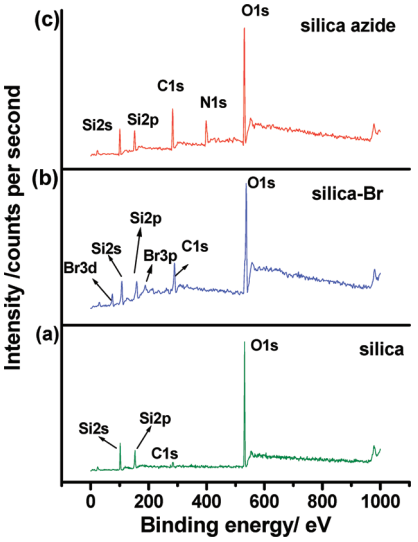


Figure 2. XPS survey scans of (a) silica, (b) bromo-functionalized silica, and (c) azide-functionalized silica nanoparticles.

The presence of bromine groups on the particle surface was confirmed using XPS (Figure 2). The survey scan of the silica nanoparticles (Figure 2a) shows peaks corresponding to Si 2s, Si 2p, C 1s, and O 1s. The survey spectrum of the bromo-functionalized particles (Figure 2b) shows the presence of a Br 3d peak at 70 eV and Br (3p) peak at 188 eV along with the other peaks present on bare silica.

The bromine groups were then converted to azide groups by a nucleophilic substitution reaction with NaN_3 in DMF using tetrabutylammonium iodide as catalyst. The substitution of the bromo groups with azide groups was clearly established using the XPS spectrum (Figure 2c), where the disappearance of bromine peaks and the emergence of the azide N 1s peak at ~400 eV are evident. Additional information on the molecular structure of azide-terminated silica nanoparticles was obtained from the XPS high-resolution N 1s scan (Figure 3). It shows the characteristic double peak structure of azide groups at 400.2 and 403.8 eV.[73,74] The less intense peak at 404.2 eV is due to the presence of electron-deficient nitrogen in the azide group.[75,76]

Further confirmation of azide functionalization was obtained from the FTIR spectra. Figure 4 shows the FTIR spectra of silica and azide-functionalized silica particles. The spectrum of the silica particles shows the characteristic absorption around 1100 cm^{-1} due to Si−O−Si and at

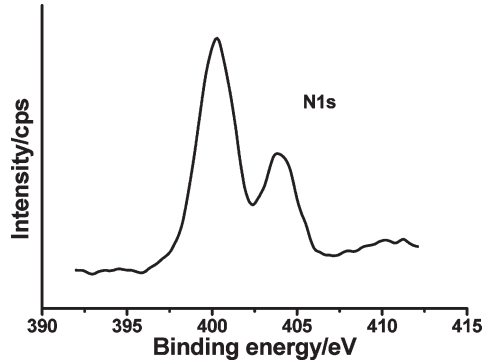


Figure 3. XPS high-resolution N 1s peak of azido-silica nanoparticle.

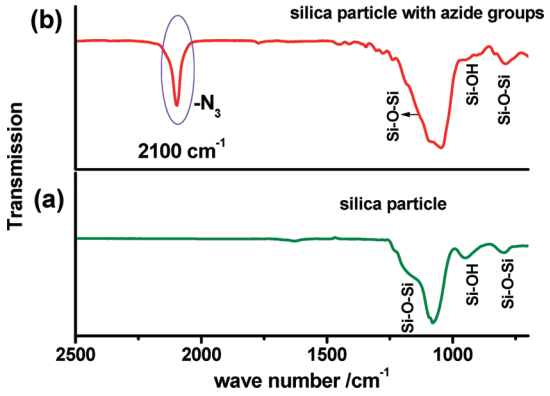


Figure 4. FTIR spectra of (a) silica nanoparticle and (b) azido-silica particle.

935 cm^{-1} due to Si−OH. The spectrum of the azide-functionalized silica particles showed a new absorption peak at 2100 cm^{-1} corresponding to azide groups, silica absorption at 1100 cm^{-1}, and a decrease in Si−OH peak at 935 cm^{-1}, which confirms the azide functionalization (Figure 4).

Alkyne-Terminated PSLG Shell Polymer. An alkyne-terminated PSLG (alkyne-PSLG) was synthesized by the ring-opening polymerization of *N*-carboxyanhydride of γ-stearyl-α-L-glutamate (SLG-NCA) using propargylamine as the initiator and dichloromethane as the solvent.[71] The SLG-NCA was synthesized by the cyclization of γ-stearyl-α-L-glutamate amino acid by direct phosgenation using triphosgene.[71] The polymerization of *N*-carboxyanhydride (NCA) is known to yield the corresponding poly(α-amino acid) or polypeptide. The use of primary amine initiator normally forces the reaction via an amine-activated mechanism,[10] which is shown in Scheme 1. GPC/MALS analysis (see Figure 5) of the alkyne-PSLG in THF (2% solution) returned a number-average molecular weight, M_n, of 7380 and weight-average molecular weight, M_w, of 8480 (M_w/M_n = 1.15). These values exceed the expectation (~4600) based on the monomer/initiator ratio, as if to suggest that some initiators were inactive or quickly led to dead chains. There is just a hint of low-*M* material in the LS trace, but the DRI trace suggests it is low in amount.

The MALDI-TOF spectrum of the alkyne-PSLG is shown in Figure 6. A single series of peaks is observed from *m*/*z* 1580 to 4250 Da, with a mass difference of 381 *m*/*z*, matching the molecular weight of the stearyl-L-glutamate (SLG) monomeric repeat unit. The mass at each peak is calculated to be the sum of the masses of the corresponding SLG units, mass of the initiator molecule and a proton adduct. For example, the peak at 2344 corresponds to the proton adduct of the hexamer with an incorporated initiator propargylamine group. The mass values

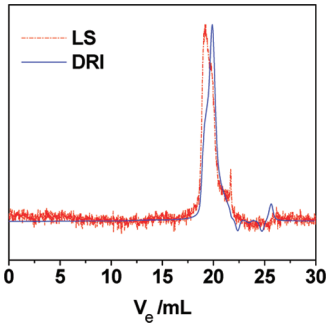


Figure 5. GPC chromatogram of alkyne-terminated PSLG prepared by ring-opening NCA polymerization using propargylamine as initiator; differential refractive index (DRI) and 90° light scattering (LS) traces are shown.

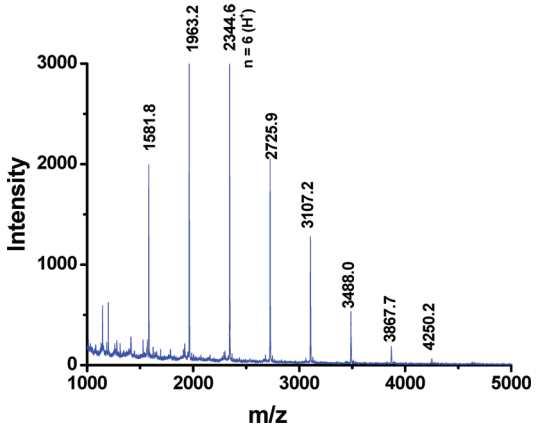
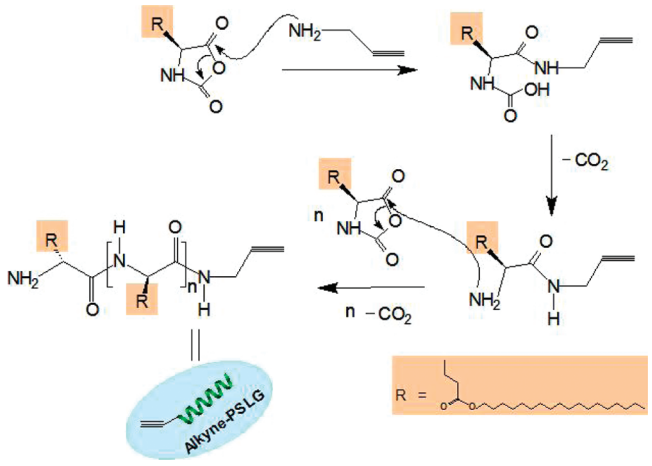


Figure 6. MALDI-TOF MS of poly(γ-stearyl-α-L-glutamate) synthesized with propargylamine.

Scheme 1. Polymerization of γ-Stearyl-α-L-glutamate *N*-Carboxyanhydride Initiated by Propargylamine To Form Alkyne End-Terminated PSLG (Alkyne-PSLG)



also indicate that the polymer chains contain an alkyne end group and thus formed via the primary amine mechanism.[77,78] The MALDI spectrum was used only for confirming the amine-initiated mechanism because the detector response as a function of molecular weight in this system is uncalibrated. A similar approach was adopted by other researchers.[10]

The secondary structure of a polypeptide can depend on environmental parameters such as solvent and temperature.[9,10] PSLG is known to form a right-handed α-helix in appropriate solvents (THF and chloroform) irrespective

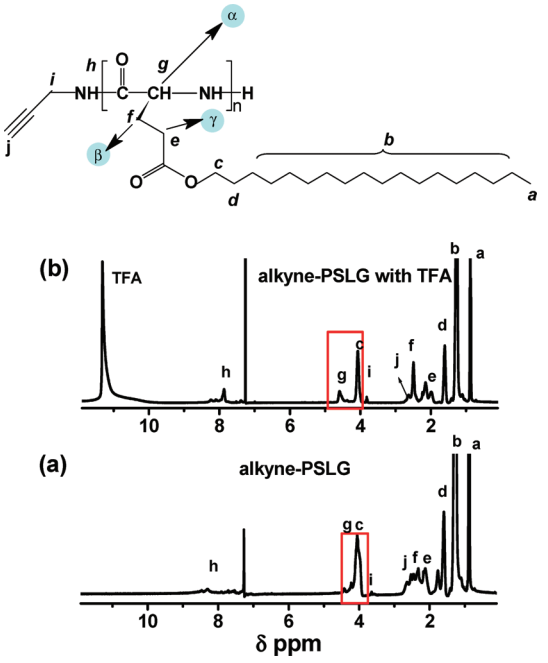


Figure 7. ^{1}H NMR of alkyne-PSLG in (a) chloroform (helical confirmation) and (b) in a mixture of chloroform and TFA (random coil conformation) showing its helix-to-coil conformation.

of its long side chains.[28,56] The bare helix has a diameter of 5.6 Å; a single turn requires 3.6 monomers and projects a distance of 5.4 Å. When considering the stearyl chains, the width of the polymer can be between 18 and 36 Å depending on the definition of diameter, method of measurement, and calculation.[28,79] This helical structure, formed by the intramolecular hydrogen bonding between the N−H and C=O groups, gives the molecule a rodlike behavior. Mitchell et al.[80] have shown that an average of more than 10 monomer units can form a stable α-helix structure. In this work, with a repeating unit weight of 382 and GPC molecular weight of 8480, the degree of polymerization of the PSLG is 18−19, which is sufficient to support the formation of a stable α-helix. Recently, Sanda et al.[56] reported the α-helical formation of low-molecular-weight PSLG on a helical polyacetylene main chain.

PSLG is known to exist as an α-helix in chloroform solution, which can be confirmed from its ^{1}H NMR spectrum by observing the α-CH and peptide NH proton resonances as the conformation of polypeptide changes from helix to random coil form. Figure 7a shows the ^{1}H NMR of the alkyne-PSLG in $CDCl_3$. The peak at 4.0 ppm is an overlap of α-CH (g) and $-OCH_2$- peak (c) of the side chain. The β-CH_2 (f) and γ-CH_2 (e) absorptions are weak and overlapped; they occur from 2 to 3 ppm. Addition of trifluoroacetic acid (TFA) to a solution of polypeptide triggers the transformation from an ordered α-helical to random coil structure.[28,81−83] TFA competes with the intermolecular hydrogen bonding; it interrupts the stiff helical structure and forces the polypeptide to assume a random coil structure. The second spectrum (Figure 7b) shows the effect of addition of 10% TFA (w/v) to the chloroform solution of alkyne-PSLG (1.5%) on the helical structure of the polypeptide. It shows that β-CH_2 (f) and γ-CH_2 (e) are resolved, and the α-CH peak (g) shifts to 4.61 ppm. The shift of the α-CH (which is a part of the main chain) peak from 4.0 to 4.6 ppm reflects the change in the conformation of the whole backbone in response to the change in solvent composition.[28,84] The high-field peak at 4.0 ppm corresponds to the helix, and the low-field peak at 4.6 ppm corresponds to the random-coil conformation.[28] This clearly shows that the alkyne-PSLG assumed the α-helical conformation in $CDCl_3$. An attempt was made to calculate the molar mass by comparing the stearyl group's methyl proton intensity to that of the initiator's methylene protons;[57] however, the difficulty with this is knowing whether the methylene protons are fully represented, even with added TFA.

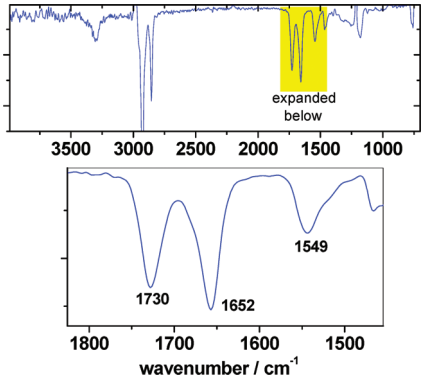


Figure 8. FTIR spectrum of alkyne-PSLG and the expanded region from 1825 to 1550 cm^{-1} showing the α-helical conformation of alkyne-PSLG.

Scheme 2. Schematic Representation of the Click Reaction between Azido-silica and Alkyne−Polypeptide

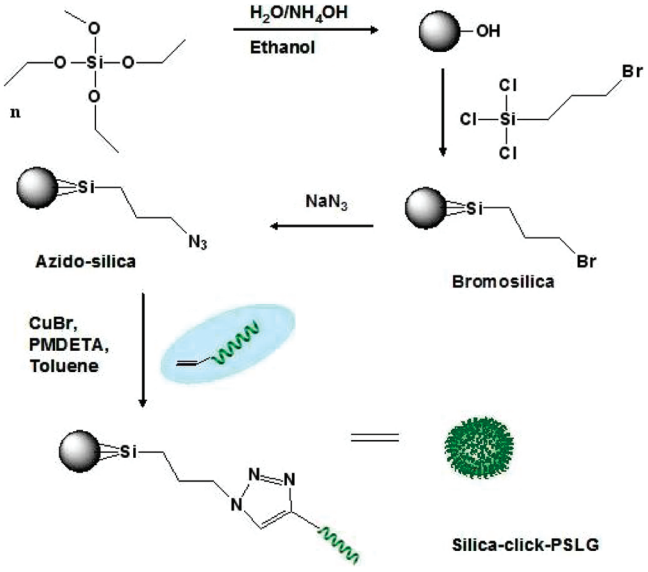


Figure 8 shows the FT-IR spectrum of the alkyne-PSLG in chloroform solution. It shows the characteristic N−H stretching of amide I at 3200−3400 cm^{-1}, along with other characteristic absorptions of the PSLG polypeptide structure. The absorption of alkyne end group occurs at 3290 cm^{-1} which appeared to be overlapped by the N−H stretching. The peaks between 2800 and 3000 cm^{-1} correspond to the symmetric and asymmetric stretching mode of CH_2 side chains, and the one at 1740 cm^{-1} corresponds to the C=O stretching of ester of polypeptide. Further evidence of helical conformation of the polypeptide was obtained from the IR spectrum. The polypeptide shows absorption bands of amide I at 1652 cm^{-1} and amide II at 1549 cm^{-1} which correspond to the absorptions of amide α-helical structure.

Silica−Polypeptide Composite Hybrid Particles. To attach the PSLG to the silica particles, the alkyne-terminated PSLG was reacted with azide-terminated silica nanoparticles in toluene using CuBr/PMDETA as the catalyst system. PMDETA is an aliphatic ligand known to increase the rate of copper-catalyzed azide−alkyne cycloaddition in organic media.[85]

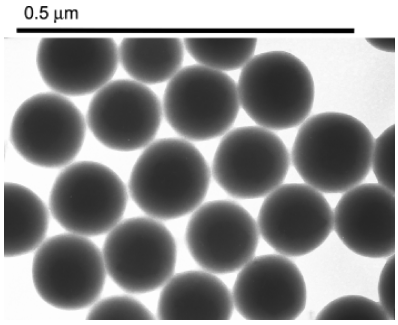


Figure 9. TEM image of silica-*click*-PSLG.

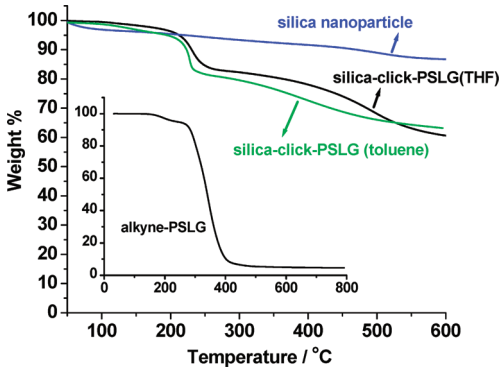


Figure 10. TGA curves for silica nanoparticle and silica-*click*-PSLG prepared in THF and toluene. The inset shows the TGA response of alkyne-PS.

After the click reaction, the polypeptide-coated nanoparticles (silica-*click*-PSLG) were collected by centrifugation from dispersions in either THF or toluene. These particles were further washed several times by resuspension in these solvents and centrifugation to remove any unreacted polypeptide present in the mixture. The trace amount of copper was removed by extracting the colloidal solution of the polypeptide in chloroform with an aqueous solution of the sodium salt of EDTA. The obtained grafted particles are highly dispersible in organic solvents like chloroform, dichloromethane, toluene, and THF, which are also good solvents for PSLG. A TEM image of these particles is shown in Figure 9. An easily visible change in the particle size, compared to Figure 1, is not expected, as the molecular weight of PSLG is small; even so, DLS data in chloroform showed the diameter of the click product was 160 ± 10 nm, significantly above the core diameter of 140 ± 3 nm.

Figure 10 displays TGA traces of silica nanoparticles before functionalization, silica-*click*-PSLG prepared in THF, and silica-*click*-PSLG prepared in toluene; the inset shows the TGA of alkyne-PSLG. In the case of the click product, weight loss from room temperature to 200 °C is due to water and unreacted azide groups.[86,87] The decomposition from 200 to 540 °C corresponds to the grafted PSLG. It shows that ~20% of the particle is covered with PSLG chains. Separate curves for the products obtained in toluene and THF show that a solvent change during the click reaction did not alter the grafting efficiency significantly.

The TGA results were converted to the average number of polymers per particle using a modification of the equation proposed by Bartholome et al.[88] and two other approaches, as described in the Supporting Information. The result is 57 000 PSLG chains per particle (±16%). Despite PSLG's rodlike conformation, this does not differ greatly from values reported for *grafting to* attachment of random coil polymers using click chemistry.[64] The coverage is very dense: combining 57 000 polymers/particle and the surface area of 620 000 Å^2 (see Supporting Information) show that only ~110 Å^2 of surface is devoted to each PSLG rod, suggesting a very congested surface and virtually requiring the alkyl side chains to interpenetrate.

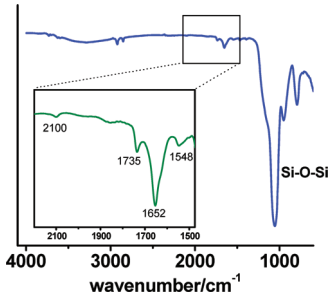


Figure 11. FTIR spectrum of silica-*click*-PSLG. The inset shows the expanded region from 2200 to 1400 cm^{-1} showing the α-helical peaks of the peptide.

The FTIR spectrum of silica-*click*-PSLG (Figure 11) shows the characteristic absorption of Si−O−Si between 984 and 1245 cm^{-1}. The peaks corresponding to PSLG were very weak, which may be due to the low molecular weight of the attached polypeptide. A broad N−H stretch around 3100−3500 cm^{-1}, asymmetric and symmetric stretching modes of the C−H groups of the side chains from 2810 to 3020 cm^{-1}, and the ester and amide absorption peaks of PSLG between 1700 and 1500 cm^{-1} were observed. The inset graph of Figure 11 shows the expansion of the peaks between 2000 and 1400 cm^{-1} which clearly shows the amide I peak at 1652 cm^{-1}, the amide II peak at 1548 cm^{-1}, and the ester peak at 1735 cm^{-1}. The presence of these peaks points to the α-helical nature of the attached polypeptide. There was a small azide peak at 2100 cm^{-1} which reveals that some reactive azide sites remain on the surface. This is expected for a *grafting to* approach, where not all groups on the surface take part in the reaction due to the steric hindrance of the grafted chains. This α-helical nature of the grafted polypeptide by click chemistry is similar to the other reports of the *grafting-from* approach of alkyl L-glutamates to substrates such as silicon wafers.[15−17,89,90]

The particles were further analyzed by XPS (see Figure 12). Compared to the survey scan of azide-functionalized silica (Figure 2c), there is a very substantial increase in the carbon peak after attaching PSLG (Figure 12d). This increase directly shows the immobilization of polypeptide following the click reaction. The high-resolution scan of the C 1s region of alkyne-PSLG (Figure 12c) and that of silica-*click*-PSLG (Figure 12f) look very similar. The major peak at 284.8 eV corresponds to C−C, and the peak at 286 eV corresponds to C−O and C−N bond and the small peak around 288 eV corresponds to the C═O from amide and ester of the PSLG. The presence of these peaks on the C 1s spectrum of silica-*click*-PSLG further confirmed the attachment of this polypeptide on the silica surface.

The microstructure of the silica-*click*-PSLG was also confirmed by analyzing the samples using ^{1}H NMR. As the particles form a highly stable colloidal solution, the ^{1}H NMR of the polypeptide-clicked particles could be measured in solution, revealing the characteristic peaks of the polypeptide. Figure 13a shows the NMR spectrum of Si-*click*-PSLG in $CDCl_3$ and Figure 13b in $CDCl_3$ with 10% TFA. The CH_2 and CH_3 protons of the polypeptide are seen in the range from 1.5 to 2.5 ppm and are very close to the PSLG polymer shown in Figure 7. The α-CH protons (designated

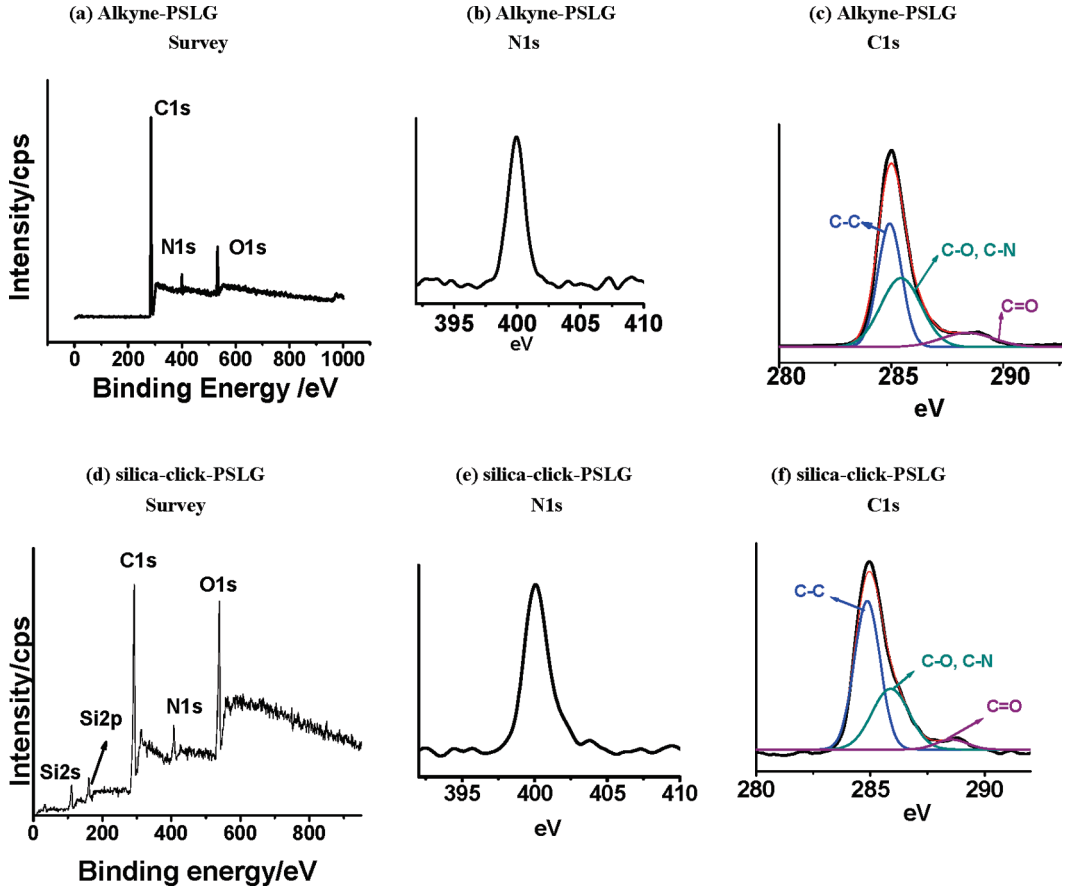


Figure 12. (a) XPS survey scan of alkyne-PSLG. (b, c) High-resolution scan of alkyne PSLG's N 1s and C 1s, respectively. (d) XPS survey scan of silica-*click*-PSLG. (e, f) High-resolution scan of the N 1s and C 1s peaks, respectively, of silica-*click*-PSLG.

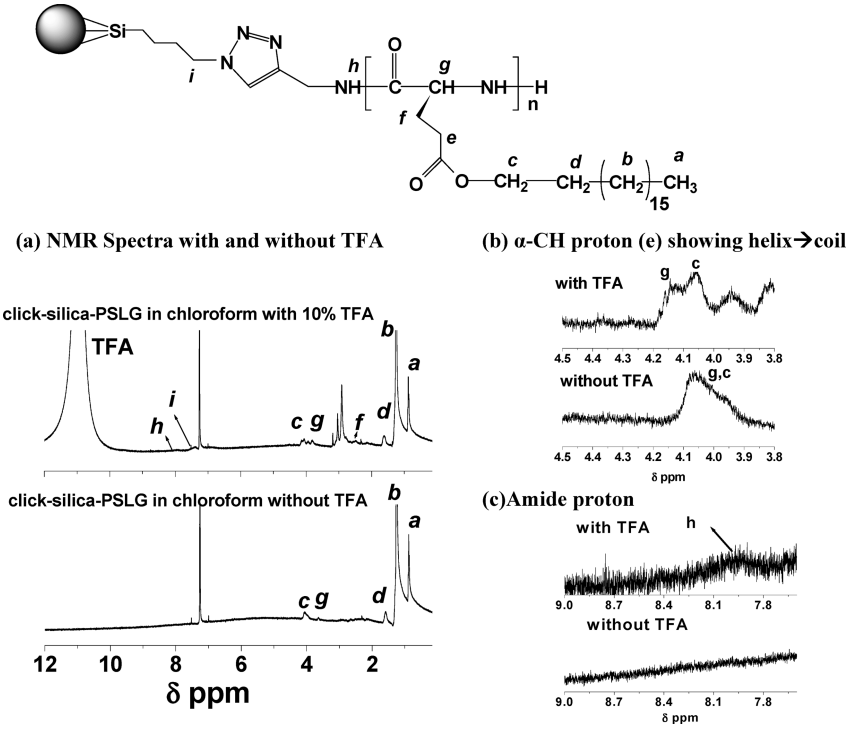


Figure 13. ^{1}H NMR spectra (400 MHz) of colloidal silica-*click*-PSLG in $CDCl_3$ (a) without TFA and with 10% TFA, (b) expanded regions showing the presence and absence of α-CH, and (c) expanded region showing the appearance of −NH protons after the addition of TFA.

as e in the spectrum) of PSLG occurred in the range from 4 to 4.3 ppm, along with $-OCH_2$ protons (as explained earlier for alkyne-PSLG; see Figure 7a). TFA was added to the solution to detect any change in the α-CH proton. It can be seen

(Figure 13b) that by the addition of TFA the α-CH peak started to bifurcate, representing the coexistence of helical and random coiled states. It can be also seen that the broad peak corresponding to NH protons (Figure 13c) has appeared as the helical structure of the polymer is changed to a coiled structure. The appearance of the α-CH peak after the addition of TFA confirmed that the polypeptide had existed in the α-helical form on the nanoparticle surface. Along with the FTIR analysis, this confirmed that the PSLG attached by click chemistry takes the α-helical form.

Conclusion

A new approach based on click chemistry has been developed for grafting α-helical, alkyne-terminated polypeptides onto azido-silica nanoparticles. The hydrophobic poly(γ-stearyl-α-L-glutamate) (alkyne-PSLG) grafted silica particles form stable dispersions in chloroform, THF, and other organic solvents such as toluene. The click precursor, propargylamine, can be used as an initiator for any kind of *N*-carboxyanhydride that can be initiated with a primary amine; therefore, a large variety of such hybrid structures with different polypeptide or block copolypeptide chains on silica particles can be produced. Easy attachment of well-characterized, α-helically oriented polypeptides to silica surfaces using the versatile click chemistry (which prevents side reactions and features good yields) suggests that development of new polypeptide structures on silica surface is possible, and this will enable one to understand the behaviors of different proteins at inorganic interfaces. Other applications of the hybrid particles may include drug delivery, colloidal crystals, and photonic materials.[1]

Acknowledgment. This material is based upon work supported by the National Science Foundation under Grant 0606117 (DMR). We thank Dr. Dan Pu for the MALDI-TOF MS measurement and Prof. Donghui Zhang for helpful comments.

Supporting Information Available: Details of the thermogravimetric analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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