Functionalization of Polymer Microspheres Using Click Chemistry

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We describe a new method to covalently link a wide variety of molecules to the surface of colloidal polymer microspheres using the Cu(I)-catalyzed azide–alkyne reaction, most commonly known under the class of reactions identified by the term click chemistry. The method is generic and readily applied to a spectrum of colloidal particle systems allowing surfaces to be tailored with virtually any desired functionality. To demonstrate this method, polystyrene microspheres were functionalized with two different polyethylene oxide-based polymers, and changes in hydrodynamic radii after functionalization were measured using dynamic light scattering. Control of surface functional groups was demonstrated by fluorescently labeling the colloidal microspheres using the same Cu-catalyzed azide–alkyne cycloaddition reaction.

Introduction

Colloidal microspheres are used in a wide variety of applications, such as coatings, biological assays, and fillers. Additionally, they serve as model systems for the study of self-assembly, glass formation, transport, and a broad range of other phenomena. Surface functionalization is a key issue for colloids. It is most important for colloidal stability because the surface of a microparticle must be compatible with its environment for it to remain dispersed. It is also important in biological assays where proteins, DNA, and other ligands are attached to control how and when colloids bind to various substrates. Finally, attaching dye molecules to colloids is useful for characterization, especially when the particles are functionalized with molecules designed to bind to specific substrates.

Polystyrene microspheres are typically stabilized by ionic surface groups that are the byproduct of charged initiators used during polymerization. Such ionic stabilization is effective in water and other polar solvents when the ionic strength is not too high. To disperse the colloids in solvents with high ionic strength or in nonpolar solvents, the particle surface must be modified, generally by forming a polymer brush on the colloid that provides steric stabilization. A common procedure is simply to add surfactant, often a block copolymer, that physisorbs to the colloid. This approach is convenient and simple but not robust because the surfactant may be displaced when the particles are washed or subjected to ion exchange. Another approach is to temporarily swell particles with a small amount of organic solvent in an aqueous surfactant solution such that the hydrophobic parts of the surfactant become trapped inside the microsphere upon evaporation of the organic, leaving the hydrophilic portions exposed. This method produces "hairy" particles that are stable to washing and ion exchange, but the resulting surfactant layer is again physisorbed and may come off when the particles are dispersed in organic media. Similar concerns apply to fluorescently labeled polystyrene microspheres, widely available commercially, in which the dye is incorporated into the particles by swelling followed by evaporation of an organic solvent. Another common method for the functionalization of polystyrene microspheres involves covalent attachment to amine groups present at the surface. However, cationic amine-functionalized microspheres are already much less stable than standard sulfonated spheres, and their stability decreases even more when the amine groups bind to a dye that generally does not provide the charge required to stabilize the surface. Therefore, it would be useful to have a method for incorporating functional units onto the surface of polystyrene microspheres that does not interfere with charge-stabilizing groups but still allows for covalent bonding to the particle surface.

In this article, we demonstrate how click chemistry can be used as a general method of modifying the surface of micrometer-scale colloidal particles. The method is sufficiently general that it can be used to functionalize colloidal surfaces with polymer chains, dye molecules, and so forth. Whereas the approach is demonstrated using polystyrene spheres, it is sufficiently general that it can readily be adapted to colloids made from other materials. The best-known and most widely

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utilized reaction that fits the click chemistry concept is the copper-catalyzed Huisgen reaction between an azide and an alkyne. This reaction is insensitive to air and moisture, and can be performed both in the presence and the absence of organic solvents, and proceeds orthogonally in the presence of diverse functionality. Since the method was introduced in 2001, click chemistry has found use in the areas of catalysis, materials science, chemistry, biology, and pharmaceuticals research as a way to produce diverse functionality in quantitative yield under mild reaction conditions with only benign byproducts. 

Most recently, this concept has been applied very successfully to the surface modification of glass, films, carbon nanotubes, and, most recently, Au and CdSe nanoparticles as well as metallic, semiconductor, and biological/organic nanoassemblies.

The fundamental idea is to incorporate a robust and orthogonal functional group onto the particle surface that will serve as a link to which almost any molecule can be attached. We modify the traditional technique of emulsifier-free emulsion polymerization of styrene into polystyrene microspheres in order to incorporate an amphiphilic monomer onto the particle surface. The monomer has one part that is compatible with the styrene and polystyrene phases such that it chemically links to the polymer but also contains a hydrophilic functional group that protrudes from the particle surface. It is this functional group, present in sufficient quantity to provide the requisite density of sites, that serves as a link to the functional ligands of our choosing. The particle composition varies only minimally from that of a traditional polystyrene microsphere, such that these colloids may be substituted for commercial spheres in a variety of applications. The recipe may also be extended to more exotic polymer beads, such as carboxyl-modified, cationic, or avidin-coated latexes and may be applied to nonpolystyrene systems such as poly(methyl methacrylate). In this article, the polystyrene microspheres are functionalized with azides, which serve as an effective handle that can be specifically functionalized by the Cu-catalyzed azide–alkyne cycloaddition reaction, which is known for its robustness and compatibility with a range of reaction conditions. This allows a wide selection of moieties, both hydrophilic and hydrophobic, to be attached to the particles, and the facile synthesis of alkyne derivatives in turn significantly adds to the library of functional groups that can be attached to the particles.

**Experimental Section**

**Materials.** Styrene was purchased from Fluka. Divinylbenzene (technical grade, 80% isomers), 4-vinylbenzyl chloride (chloromethyl styrene, VBC, technical grade, ≥90%), macromonomer poly(ethylene glycol) methyl ether methacrylate (1100 Da), potassium persulfate, sodium azide, and N,N-diisopropylmethacrylamide (DIEPA, Hunig’s base) were purchased from Aldrich. Immediately prior to use, vinyl monomers were passed through a column of basic alumina (Fluka) to remove inhibitors, and all other chemicals were used as received without further purification. The acetylene-containing RAFT chain-transfer agent was synthesized according to a published procedure. The Cu(PPh3)3Br catalyst was synthesized by the addition of CuBr2 to a hot methanolic solution of triphenylphosphine. Molecule 7-ethynyl-4-methyl-2H-chromen-2-one (4) was prepared according to Schemel173 for 7-hydroxymethyl-2H-chromen-2-one (1), which was prepared by the reported procedure for solvent-free coumarin synthesis using Pechmann and Knoevenagel condensation reactions. Analytical TLC was performed on commercial Merck plates coated with silica gel GF254 (0.24 mm thick). Silica gel for flash chromatography was Merck Kieselgel 60 (230–400 mesh, ASTM). 1H NMR (200 MHz) measurements were performed on a Bruker AC 200 spectrometer at room temperature. Size-exclusion chromatography (SEC) was carried out at room temperature on a Waters chromatograph connected to a Waters 410 differential refractometer and six Waters styragel columns (five HR-5 mm and one HMMV-20 mm) using tetrahydrofuran (THF) as the eluent (flow rate 1 mL/min). A Waters 410 differential refractometer and a 996 photodiode array detector were employed. Molecular weights were determined relative to linear polystyrene standards. Differential scanning calorimetry (DSC) measurements were performed with a TA Instruments DCS 2920 at a ramp rate of 10 °C/min.

**Synthesis of Trifluoro-methanesulfonic Acid 4-Methyl-2-oxo-2H-chromen-7-yl ester (2).** A mixture of 1 (4.85 g, 27.5 mmol), trifluoromethanesulfonic anhydride (9.69 g, 34.3 mmol), and N,N-diisopropylmethacrylamide (5.3 g, 41.2 mmol) in DCM (60 mL) was stirred at room temperature for 7 h. The reaction mixture was filtered to remove 2 (29.4 mg, 7.61%). 1H NMR (300 MHz, CDCl3): δ 7.70 (d, J = 8.4 Hz, 1 H), 7.28–7.22 (m, 2 H), 6.36 (d, J = 0.9 Hz, 1 H), 2.46 (s, 3 H); 13C NMR (75 MHz, CDCl3) δ 154.4, 151.0, 126.6, 115.6, 117.6, 116.2, 110.8, 19.0. 1H NMR (300 MHz, CDCl3): δ 7.70 (d, J = 8.4 Hz, 1 H), 7.28–7.22 (m, 2 H), 6.36 (d, J = 0.9 Hz, 1 H), 2.46 (s, 3 H); 13C NMR (75 MHz, CDCl3) δ 154.4, 151.0, 126.6, 121.0, 120.3, 117.6, 116.2, 110.8, 19.0.

**Synthesis of 4-Methyl-7-trimethylsilanylethynyl-chromen-2-one (3).** Trifluoromethanesulfonate (2) (2.0 g, 6.5 mmol), tetrakis(triphenylphosphine)-palladium (0.76 g, 0.65 mmol), Cu (0.25 g, 1.4 mmol), and a stirring bar were sealed in a 50 mL round-bottomed flask with a septum. Under the protection of nitrogen trimethylsilylacetylene (1.0 g, 10.5 mmol), N,N-diisopropylmethacrylamide (3.1 g, 24 mmol) and THF (20 mL) were added with a syringe. The reaction mixture was stirred at room temperature under nitrogen for 36 h. After being diluted with EtOAc; the solution was washed with saturated NH4Cl solution, dried over anhydrous Na2SO4, and concentrated on a rotary evaporator. The residue was purified on a silica gel column (hexane/EtOAc = 1:1) providing 3 as a white solid (27.5 g, 76.4%). 1H NMR (300 MHz, CDCl3): δ 7.74 (d, J = 7.8 Hz, 1 H), 7.10–7.06 (m, 2 H); 159.7, 154.4, 151.4, 150.1, 126.6, 121.0, 120.3, 117.6, 116.2, 110.8, 19.0.
Synthesis of 7-Ethynyl-4-methyl-chromen-2-one (4). Anhydrous K$_2$CO$_3$ (2.0 g, 14.5 mmol) was added to the suspension of 3 (1 g, 3.9 mmol) in MeOH (40 mL). The reaction mixture was stirred at room temperature for 20 h. After the solid precipitate was removed, the solvent was stripped off with a rotary evaporator, and the residue was dissolved in water. The pH of the aqueous solution was adjusted to lower than 7 and then extracted with EtOAc (50 mL/C$_2$H$_5$). The combined organic layers were dried over brine and Na$_2$SO$_4$ and concentrated. The residue was purified on a silica gel column (hexane/EtOAc = 4:1) to yield 4 as yellow solid (500 mg, 70%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.50 (d, $J = 7.8$, 1 H), 7.38–7.33 (m, 2 H), 6.26 (d, $J = 1.2$, 1 H), 3.24 (s, 1 H), 2.40 (s, 3 H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 160.5, 153.3, 151.9, 128.0, 125.8, 124.8, 120.6, 120.5, 115.9, 82.3, 80.7, 18.8.

Synthesis of Poly(methyl ether oligo(ethylene glycol) Methacrylate (5). Homopolymerization of the macromonomer poly(ethylene glycol) methyl ether methacrylate proceeded according to Scheme 2. A mixture of RAFT chain-transfer agent (23.2 mg, 0.121 mmol), AIBN (4.9 mg, 29.0 $\mu$L mol), and macromonomer poly(ethylene oxide) methyl ether methacrylate (20.0 g, 18.2 mmol, average $M_n \sim$1100 g/mol) in a minimum volume of dimethylformamide (~10 mL) was degassed by three successive freeze–pump–evacuate cycles, sealed under vacuum, and held at 70°C for 3 h. The viscous crude polymerization mixture was then precipitated three times in 200 mL of ice-cold diethyl ether. The resulting pink polymer was collected via vacuum filtration, yielding 12.4 g of 5 in 62.0% yield. $M_n = 152 000$ g/mol, PDI = 1.23, $T_g = -53$ °C, $T_m = 55$ °C. $^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 4.07 (s, COOC$_2$H$_5$), 3.8–3.4 (m, O(CH$_2$CH$_2$)$_n$OH), 3.37 (s, O(CH$_2$CH$_2$)$_n$CH$_3$), 3.10 (s, 1 H), 1.1–0.8 (CH$_2$C(CH$_3$)$_m$COO).

**Table 1. Recipe for Microsphere Synthesis**

<table>
<thead>
<tr>
<th>species</th>
<th>mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>420</td>
</tr>
<tr>
<td>reactor charge</td>
<td>30</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.079</td>
</tr>
<tr>
<td>oil phase</td>
<td>42.340</td>
</tr>
<tr>
<td>styrene</td>
<td>42.340</td>
</tr>
<tr>
<td>divinylbenzene</td>
<td>0.850</td>
</tr>
<tr>
<td>4-vinylbenzyl chloride</td>
<td>1.810</td>
</tr>
<tr>
<td>ammonium persulfate (APS)</td>
<td>0.800</td>
</tr>
</tbody>
</table>

Polymer Microsphere Synthesis. The quantities of chemicals used in the polymerization reaction are found in Table 1. A 500 mL jacketed reactor was charged with NaCl dissolved in purified (18.2 M $\Omega$ resistance) water. The particles grow until the electrostatic repulsion between particles is sufficient to keep the particles from aggregating in the ionic environment of the reaction vessel. Because of this known dependence of particle size on theionic strength of the reaction vessel, sodium chloride salt was added only to increase the size of the particles and could be omitted or the concentration adjusted to change their final size. The reactor was attached to a circulating bath with temperature set to 70 °C, and the system was stirred mechanically overhead at 200 rpm. The water was purged with nitrogen for 45 min before the addition of the oil phase, and the contents of the reactor were then purged for an additional 30 min to ensure a nitrogen environment. Finally, the initiator was dissolved in the remaining water and added to the reactor. Samples were viewed by optical microscopy at intervals of 20 min beginning 90 min after initiation, and the sample was quenched when there was no visible change in particle size. The total reaction time was 5 h. The particles were then washed three times by centrifugation and redispersed in purified water to remove excess styrene.

Functionalization of Microspheres. The weight percent of spheres in water was calculated for the cleaned latex, and 5 g of solids dispersed in 100 mL of water was added to a 250 mL round-bottomed flask containing a magnetic stir bar. Assuming complete accessibility of chlorine groups on the surface due to the incorporation of 4-vinylbenzyl chloride, the maximum number of Cl functional groups in the sample was determined to be $1.32 \times 10^{-7}$ mol. Sodium azide (0.257 g) was added to the
flask to provide a 3-fold molar excess of N$_3$ to Cl. The flask was fitted with a Vigreux condenser and submerged in an oil bath set to 60 °C on top of a stir plate. The flask was subjected to gentle stirring overnight.

For each click reaction, 5 mL of the azide-functionalized latex solution was added to a 50 mL round-bottom flask and diluted with excess water. Appropriate amounts of the acetylene-functionalized derivative, copper catalyst, and Hunig’s base were added to the latex samples according to Table 2, and each flask was fitted with a magnetic stir bar and a Vigreux condenser. The click reaction was allowed to proceed overnight under gentle stirring at 70 °C. Each latex sample was then cleaned via centrifugation and redispersion in purified water five times before preparing light-scattering samples.

**Physical Characterization.** Fourier transform infrared spectroscopy (FTIR) was performed on a Shimadzu IR Prestige 10. The fluorescence of the dyed sphere was characterized in two ways: by direct observation under a fluorescence microscope and by measuring the fluorescence spectrum using luminescence spectrophotometry. Fluorescence microscopy was performed on a Nikon Eclipse TE300 optical microscope equipped with a high-pressure mercury lamp and a UV-2EC filter. Images were taken with a 100× Plan Apo oil-immersion objective and a Photometrics Coolsnap EZ camera. Fluorescence measurements were made on samples A and D (Table 2) using the SLM Aminco–Aminco Bowman Series 2 luminescence spectrometer and the accompanying AB2 luminescence spectrometer software, version 5.31. The excitation slit size was set to 0.5 nm, and the emission slit size was set to 4 nm. Experiments were performed at both 310 and 300 nm excitation wavelengths. The photomultiplier gain was set to 1100 V.

The sizes of the samples were measured using dynamic light scattering (DLS), and corresponding polydispersities were determined using the method of cumulants.27 Glass sample vials of 4 mL capacity were cleaned using a Micro90 critical cleaning control: no molecule D 0 polymer control: no molecule D 0

<table>
<thead>
<tr>
<th>species</th>
<th>stoichiometric equiv</th>
<th>mass (g)</th>
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</thead>
<tbody>
<tr>
<td>latex-N$_3$</td>
<td>1</td>
<td>0.225</td>
</tr>
<tr>
<td>Cu(PPh$_3$)$_2$Br</td>
<td>0.05</td>
<td>2.276 × 10$^{-3}$</td>
</tr>
<tr>
<td>Hunig’s base</td>
<td>0.05</td>
<td>3.850 × 10$^{-4}$</td>
</tr>
<tr>
<td>acetylene-functionalized derivative</td>
<td>sample 1.2</td>
<td>27-ethyl-4-methyl-chromen-2-one</td>
</tr>
<tr>
<td>acetylene-EGK PEG</td>
<td>B</td>
<td>1.423</td>
</tr>
<tr>
<td>acetylene-EGK branched</td>
<td>C</td>
<td>2.847</td>
</tr>
<tr>
<td>polymer control: no molecule</td>
<td>D</td>
<td>0</td>
</tr>
</tbody>
</table>

where $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, and $\eta$ is the viscosity of water.

**Results**

We first provide a general procedure for the synthesis of unaggregated, charge-stabilized, half-micrometer-sized cross-linked polystyrene spheres containing 4-vinylbenzyl chloride as a comonomer, using emulsifier-free emulsion polymerization in the absence of any organic solvent, buffer, or stabilizer. We then replace the chlorine groups with the azide functionality so that they may participate in subsequent click reactions with alkyne-terminated hydrophilic polymers and an alkyne-terminated molecule that becomes fluorescent when conjugation is extended through the resulting triazole ring. In general, this method of clicking ligands onto modified polystyrene microspheres may be particularly useful for functionalizing colloids with biomolecules or with many other kinds of functionality without interfering with the pre-existing charge or steric stability of the particles.

**Particle Synthesis.** FTIR was utilized as the primary characterization means to determine whether the azide displacement and the resulting azide–alkyne condensation reaction occurred. The organic azide provides a unique absorbance in the IR spectrum at 2260 cm$^{-1}$. Furthermore, the triazole ring that results from the azide–alkyne condensation also exhibits distinct absorptions in the IR. The peak at $\sim$2238 cm$^{-1}$ in trace e of Figure 1 (labeled as 7) is indicative of the click reaction (triazole ring), which shows that the chain covalently bonded to the azide present on the particle surface. The sample also shows the presence of PEG (labeled as 5), demonstrated by the similarity to trace b (labeled as 3). The additional peak in trace e (labeled as 6) is attributed to the existence of the azide, but it cannot be confirmed if the azide is in the form of the triazole ring or if it is a shift from the peak in trace d (labeled as 4).

Samples A and D were viewed under the optical microscope to gauge fluorescence. The unmodified particles (sample D) did not fluoresce, whereas those with 7-ethyl-4-methyl-chromen-2-one after modification (sample A) fluoresced brightly, as shown in Figure 2. The 7-ethyl-4-methyl-chromen-2-one itself has no fluorescence without the triazole ring; fluorescence occurs only after the reaction between an alkyne and an azide forms another ring structure that promotes the resonance required for fluorescence.28 The observed fluorescence thus confirms the attachment of the dye to the colloidal particles.

Fluorescence measurements were then performed to confirm the qualitative observations made using optical-absorbance in the IR spectrum at 2260 cm$^{-1}$. Furthermore, the triazole ring that results from the azide–alkyne condensation also exhibits distinct absorptions in the IR. The peak at $\sim$2238 cm$^{-1}$ in trace e of Figure 1 (labeled as 7) is indicative of the click reaction (triazole ring), which shows that the chain covalently bonded to the azide present on the particle surface. The sample also shows the presence of PEG (labeled as 5), demonstrated by the similarity to trace b (labeled as 3). The additional peak in trace e (labeled as 6) is attributed to the existence of the azide, but it cannot be confirmed if the azide is in the form of the triazole ring or if it is a shift from the peak in trace d (labeled as 4).

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Figure 1. FTIR data for PEG-clicked latex: (a) sodium azide salt; (b) acetylene-functionalized PEG chain; (c) bare microspheres with 4-vinylbenzyl chloride incorporated during polymerization; (d) microspheres after chlorine groups were replaced with azide groups; and (e) microspheres after PEG was clicked to the azide groups. Labeled peaks are attributed to the following: (1 and 2) azonium salts (strong); (3) C–O stretching vibration (strong); (4) azide stretching vibration (strong—very strong); (5) C–O stretching vibration (strong); (6) azide stretching vibration (strong—very strong); and (7) nitro-aromatic azide stretching vibration (strong).

Figure 2. Sample A, viewed under a 100× fluorescence microscope. Dried polystyrene microspheres with 7-ethynyl-4-methyl-2H-chromen-2-one clicked to the surface.

Microscopy. Figure 3 shows a saturated fluorescence peak due to multiple scattering from the particles for both samples for excitation wavelengths at both 310 and 340 nm. A shoulder was observed for the excitation of the dye-clicked particles at 340 nm, but a fully independent peak emerged when the experiment was repeated at 310 nm. Figure 4 reinforces these data by showing the dye-clicked particles (sample A) after subtraction of the data from the unmodified particles (sample F). Both excitation wavelengths show an emission peak at 385 nm.

The particle diameters for the unmodified particles as well as the two types of polymer-clicked particles are shown in Table 3. The particles with molecules attached via click chemistry have larger diameters than the naked particles as a result of the polymer brushes attached to the microsphere surface.

Discussion

We have presented a versatile method for permanently functionalizing colloidal microspheres. The cross-linked polystyrene particles containing 4-vinylbenzyl chloride synthesized for these experiments are charged-stabilized, and the click method allows for the ability to modify them such that they also possess steric stability or fluorescence without affecting the charged groups on the sphere surface. The same can be expected for virtually any desired functionality in that
the basic composition of the particles allows them to be substituted in any situation where traditional polystyrene latex is required. The bare particles themselves, as well as the functionalized particles, remained easily dispersed in water after several washings without the presence of surfactant. Because the particles incorporate cross-linker, they may also be used in organic solvents. As is shown in Table 3, colloids on the order of 500 nm possessed very little polydispersity, consistent with conventional particles synthesized via emulsifier-free emulsion polymerization and less than the polydispersities found in commercially available latexes.

Though polymer latexes containing 4-vinylbenzyl chloride have been synthesized previously by a variety of methods, the method that we illustrate has the advantages of requiring no added surfactants, buffers, polymers, or additional monomers. Moreover, the polymerization occurs in a single step in the presence of potassium persulfate, the most common initiator used for fabricating polystyrene microspheres. The method may be extended to seeded growth polymerization if particles on the order of 1 μm are desired. The sulfate groups on the sphere surface have a $pK_a$ value of 2, allowing the particles to be stable over a larger range of pH values than the cationic functionality that is usually associated with VBC–copolymer latexes. The functionalized latex is very monodisperse and is completely stable in pure surfactant-free water. Despite this stability, the method of synthesizing these particles is limited to situations where the density of required chloromethyl groups is low (less than ~6 mol %) because the presence of the relatively hydrophilic VBC affects the solubility of the monomer phase in water during polymerization.

**Table 3. Effective Diameters of Polymer Microspheres**

<table>
<thead>
<tr>
<th>sample</th>
<th>hydrodynamic diameter (nm)</th>
<th>polydispersity (%)</th>
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<tr>
<td>B</td>
<td>582 ± 13</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>C</td>
<td>593 ± 14</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>D</td>
<td>546 ± 16</td>
<td>4.9 ± 0.1</td>
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</table>

**Figure 3.** Fluorescence data for excitation wavelengths of 310 and 340 nm. (a) Bare particles at 310 nm; (b) dye-clicked particles at 310 nm; (c) bare particles at 340 nm; and (d) dye-clicked particles at 340 nm.

**Figure 4.** Fluorescence data after the subtraction of background due to particles. (a) Difference between bare particles and clicked particles for excitation at 310 nm. (b) Difference between bare particles and clicked particles for excitation at 340 nm.
Conclusions

The technique presented in this article for functionalizing polystyrene latexes using click chemistry may be generalized to other water-soluble polymer colloids, within certain design limitations. The method requires the addition of a comonomer during polymerization that is both compatible with the primary monomer of the colloid and also contains a functional group, such as chlorine, that can be replaced by an azide. In choosing the comonomer, balance must be maintained between hydrophobicity and hydrophilicity: the comonomer must be sufficiently hydrophilic on one end so that it polymerizes at the particle surface, assuring that the functional groups are available in solution. However, the comonomer must be sufficiently hydrophobic such that it does not significantly alter the solubility of the monomer in the surrounding medium, which would in turn affect the mechanism of polymerization. Lastly, the comonomer should be similar in size to the primary monomer so that a layer of it on the surface of the particle does not prohibit the surface charges from stabilizing the colloid.

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