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Facile Synthesis of Multivalent Water-Soluble Organic Nanoparticles via "Surface Clicking" of Alkynylated Surfactant Micelles

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Multivalent interactions occur frequently between biological entities.[1] When strong binding is not achievable with a single receptor−ligand pair, multivalency, or simultaneous binding between multiple receptors and ligands, becomes an effective strategy to enhance the binding. Significant efforts have been devoted in recent years to synthetic multivalent ligands and their interactions with biological hosts.[2] Two of the most widely used scaffolds in multivalency are dendrimers and gold nanoparticles protected with functionalized thiols.

Dubbed as the "click" reaction,[3] the 1,3-dipolar cycloaddition between terminal alkyne and azide has enabled efficient construction of dendrimers,[4] functionalized proteins[5] and carbon nanotubes,[6] foldamers,[7] and numerous other materials. In this Communication, we describe an extremely simple method to prepare water-soluble organic nanoparticles as a new platform for multivalent ligands. A surfactant was designed to afford micelles densely packed with alkynyl groups on the surface. The click reaction was employed to not only cross-link the micelles to form water-soluble nanoparticles but also derivatize the tens of residual alkynyl groups on the nanoparticles in a one-pot reaction. Although cross-linking of surfactant micelles has been reported as early as in the 1970s,[8] the commonly utilized free radical polymerization offers no easy way to functionalize the resulting nanoparticles.

Cationic surfactant **1** was prepared in a few steps from commercially available 4-hydroxybenzaldehyde, 1-bromododecane, and tripropargylamine. With an ammonium headgroup and a long hydrocarbon tail, it forms micelles above 1.4×10^{-4} M in water according to surface-tension measurement (Figure 1S in Supporting Information). The design of the surfactant puts numerous alkynyl groups on the surface of the micelles, which are readily cross-linked by azido derivatives, such as **2a**−**2c**, in the presence of Cu(I) catalyst (Scheme 1). We found that the popular $CuSO_4$−sodium ascorbate combination quickly produced precipitates from a 10 mM micellar solution of **1**, possibly because the divalent sulfate anion complexed with the cationic surfactant and reduced the electrostatic repulsion that kept micelles in solution. Replacement of the $CuSO_4$ with $CuCl_2$, indeed, afforded a nearly transparent solution throughout the cross-linking.

Choice of the cross-linker was important as well. Water-soluble cross-linker **2a** readily afforded surface-cross-linked micelles (SCMs) 8−10 nm in diameter according to dynamic light scattering (DLS) (Figure 2S). Even though DLS also confirmed the formation of SCMs with **2b** (data not shown), much of this cross-linker was observed to be undissolved in the aqueous solution and remained unconsumed even after prolonged reaction time, presumably because its low solubility in water affected

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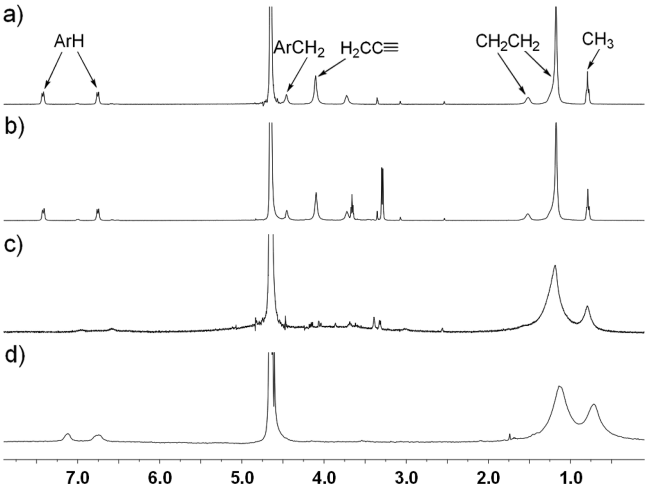
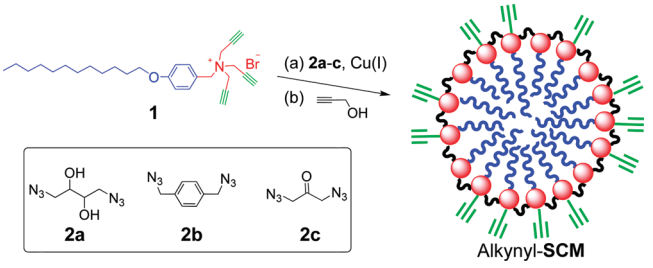


Figure 1. ^{1}H NMR spectra of a 10 mM micellar solution of **1** (a) in D_2O, (b) after addition of 1 equiv of **2a**, (c) after cross-linking, and (d) after dialysis to remove water-soluble impurities.

Scheme 1. Preparation of the Alkynyl-SCM



the cross-linking reaction. We typically perform the reaction with a 1:1 mixture of **1** and **2a** with 2.5 mol % of $CuCl_2$ and 25 mol % of sodium ascorbate at room temperature for 24 h. Propargyl alcohol was added at the end to terminate the click reaction by consuming residual azido groups on the SCMs.

In addition to DLS, formation of SCMs could be monitored by ^{1}H NMR spectroscopy. The proton signals for a 10 mM solution of **1** in D_2O (Figure 1a) were slightly broader than those in $CDCl_3$ (Figure 3S). The peak broadening was an indication for micellization.[9] Addition of **2a** caused no change to the signals of **1** (Figure 1b) but, after 24 h in the presence of Cu(I), broadened the methyl and methylene peaks (δ = 0.8−1.3 ppm) for the dodecyl chain and almost completely suppressed the signals from the protons on the ammonium headgroup (Figure 1c). These effects were not caused by the paramagnetic copper, as extensive dialysis of the sample against water cleaned up the spectrum near δ = 3−4 ppm, but the spectrum looked very similar (Figure 1d).

Cross-linking brought significant changes to the intensity of the different protons in the surfactant. In comparison to the signals prior to cross-linking (Figure 1a,b), the loss of signal intensity followed the order of dodecyl CH_3 < dodecyl CH_2 < aromatic ArH < benzylic CH_2. In other words, the farther the proton is from the cross-linking site, the more of its NMR signal was preserved. This result is expected because the ends of the dodecyl chains should maintain fairly high mobility inside the SCM, making them more visible in NMR spectroscopy than those that are restricted by the cross-linking. Note that the aromatic

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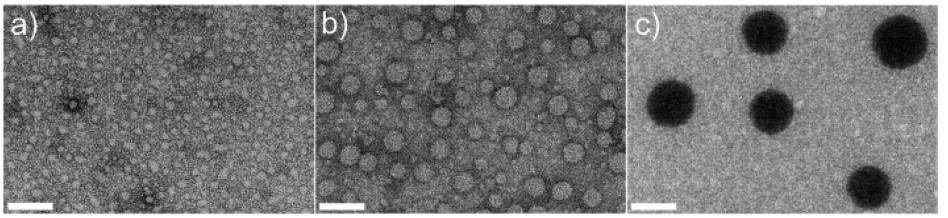
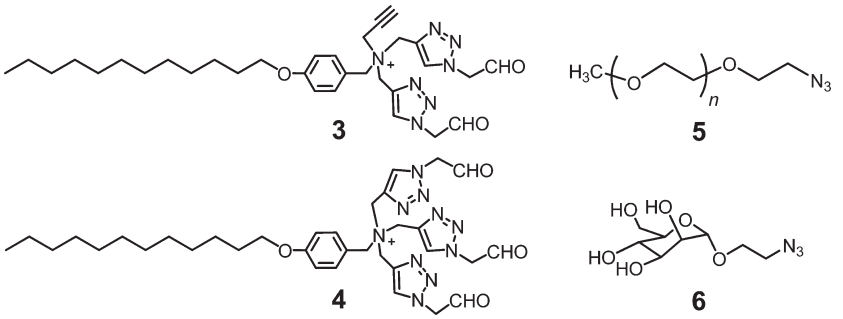


Figure 2. TEM micrographs of alkynyl-, mannose-, and PEG-SCMs from left to right. Scale bar = 50 nm.



peaks of the SCM shifted upfield slightly, presumably as a result of the proximity of the aromatic groups upon cross-linking.

Additional insights on the cross-linking were obtained by cleaving the geminal diol group in the cross-linker. After treatment with an excess of periodic acid, the alkylnyl-SCMs were subjected to ESI-MS analysis. Although many species could be detected, the base peak was ammonium cation **3** (Figure 4S), in line with the 1:1 stoichiometry between **1** and **2a** used in the reaction. A smaller amount of **4** was also found, suggesting that, in some of the surfactants, all three triple bonds underwent the cycloaddition.[10]

The presence of multiple alkynyl groups on the surface of the SCMs makes it extremely easy to functionalize these nanoparticles. After cross-linking, an azido PEG derivative (**5**, MW 2000) was added directly to the alkynyl-SCM solution. Since both cross-linking and postfunctionalization utilized the same click reaction, PEGylation of the SCMs could be catalyzed by the residual catalyst in the solution.[11] Termination by propargyl alcohol is no longer necessary in the postfunctionalization, as the surface of the nanoparticles was fully protected by the hydrophilic polymer. After reaction, excessive **5** and other impurities were removed by dialysis against water. DLS revealed a significant increase in the particle size to ca. 100 nm in diameter (Figure 5S), consistent with the attachment of the PEG chains.

Cleavage of the geminal diol groups enabled us to determine the degree of functionalization in the PEG-SCMs. Integration of the methyl protons from the surfactant and PEG in the cleaved nanoparticles indicated that, on average, one surfactant was functionalized with 0.7−0.8 PEG chains (Figure 6S). The level of functionalization is in line with the number of residual triple bond left on the alkynyl-SCM (resulting from the 1:1 stoichiometry between **1** and **2a**). If the aggregation number of **1** in the micelle is 50,[12] we would have about 35−40 PEG chains on a nanoparticle. Considering the crowdedness that the PEG chains may experience on the surface of an SCM, the level of postfunctionalization is quite remarkable.

We have also decorated the SCMs with a mannose derivative (**6**) for its many interesting biological properties. The resulting particles had a hydrodynamic diameter of 30−40 nm (Figure 7S), larger than the parent alkynyl-SCMs but smaller than the PEG-SCMs. The high density of surface functionalization in these nanoparticles is desirable to multivalent sugar ligands, as the sugar-binding sites in different lectins are separated by 1.3−7.2 nm.[2e] Hawker and Wooley,[13] as well as Liu,[14] recently obtained shell click-cross-linked polymeric micelles successfully, but the density of the functional group on the surface was much lower due to the larger particle size. Because our nanoparticles on average contain one reactive alkyne per surfactant, ligands should be within 1−2 nm when all residual alkynes are functionalized with mannose.[15] A larger distance may be obtained by simply reducing the degree of postfunctionalization.

Transmission electron microscopy (TEM) allowed us to visualize these SCMs directly. The samples were stained with 2% phosphotungstic acid. The parent alkynyl-SCMs give the smallest nanoparticles in the micrograph, averaging about 10 nm in diameter (Figure 2a). The mannose-SCMs are larger, with their size mostly ranging from 15 to 25 nm (Figure 2b). The size increase is reasonable with the surface functionalization. The PEG-SCMs are much larger, showing spherical particles mostly 40−60 nm in diameter (Figure 2c) and some as large as 80 nm (Figure 8S). Interestingly, the PEG-SCMs are positively stained (i.e., particles appearing in dark) by phosphotungstic acid whereas the alkynyl- and mannose-SCMs are negatively stained.[16] For the functionalized mannose- and PEG-SCMs, the particle size determined from TEM is smaller than that from DLS. The result is reasonable as DLS measures the hydrodynamic diameter of fully hydrated nanoparticles in solution whereas TEM measures the stained, dry particles in the collapsed state.

It is possible to introduce additional functional groups through the cross-linker. For example, when azido ketone **2c** was employed as the cross-linker, the nanoparticles gave both carbonyl and triple bond stretches in the FT-IR spectroscopy, whereas the particles prepared with **2a** only showed the triple bond (Figure 9S).

In conclusion, we have created a new platform for multivalent ligands by surface-cross-linking of alkynylated surfactant micelles. The synthesis of the starting materials and the preparation of the nanoparticles are extremely simple. The click chemistry utilized in both cross-linking and postfunctionalization ensures unparalleled functional group compatibility and allows the final functionalized nanoparticles prepared in a one-pot reaction at room temperature in water. Additional functional groups (e.g., ketone) may be introduced through the cross-linker, enabling potential postmodifications orthogonal to the cycloaddition. These features represent significant advantages and cost benefits over other multivalent platforms such as dendrimers and

gold nanoparticles that typically involve multistep synthesis or expensive metal.

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Supporting Information Available: Experimental details for the synthesis, DLS data, ^{1}H NMR and MS spectra, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Additional $CuCl_2$ and sodium ascorbate were still added in some cases for faster reaction.
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