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Ultrathin, Responsive Polymer Click Capsules

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ABSTRACT

We report a general click chemistry approach for the layer-by-layer assembly of ultrathin, polymer films on particles and the subsequent formation of polymer click capsules (CCs). Poly(acrylic acid) copolymers, synthesized with a minor component of either alkyne (PAA-Alk) or azide (PAA-Az) functionality, were alternately assembled on silica particles. The (PAA-Az/PAA-Alk)-coated particles were subsequently functionalized by exploiting the free alkyne click moieties present in the film upon exposure to an azide-modified rhodamine dye. Further, PAA CCs, obtained following removal of the silica particle template, were shown to exhibit pH-responsive behavior. This was demonstrated by reversible size changes of the CCs upon cycling between basic and acidic solutions. Polymer CCs are anticipated to find applications in various fields, including drug delivery and sensing.

The field of click chemistry, initially developed for biomolecule functionalization, has had a significant impact on a number of fields in the past decade,[1] as shown by the rapid growth of click chemistry in materials science in the last 2 years.[2,3] A wide range of advanced materials have been designed using this strategy, including functionalized carbon nanotubes,[4] cross-linked micelles,[5] and dendritic copolymers.[6] Click chemistry refers to a set of covalent reactions that have near quantitative yields under mild conditions. The most well documented example is the Huisgen 1,3-dipolar cycloaddition of azides and alkynes to form 1,2,3-triazoles.[7] The use of this particular click reaction has several significant advantages. First, the reactions are performed under mild conditions and are inert to other chemical moieties, which means click reactions can be performed while maintaining other functionalities for subsequent modification.[7] Second, these click reactions are also highly quantitative, giving a high reaction yield. Consequently, the technique allows the elegant design of diverse materials under mild conditions with varied functionality.

In this work, click chemistry is used as a new approach to produce ultrathin, polymer click capsules (CCs) assembled by the layer-by-layer (LbL) approach (Scheme 1). LbL assembly is a versatile and robust technique for fabricating tailored thin films of different composition.[8−11] The method has predominantly been used to assemble films by using electrostatic[8−11] or hydrogen-bonding[12−14] interactions. In contrast, there have only been limited studies into the use of covalent bonding as a mechanism to assemble LbL thin films.[15−18] Covalently bound films offer the advantage of higher stability due to the formation of a cross-linked polymer network, and are not susceptible to disassembly under varying solution conditions (e.g., salt and pH), as is typically observed for a range of electrostatically coupled and H-bonded films.[14,19,20] Recently, we reported the use of click chemistry to assemble LbL poly(acrylic acid) (PAA) thin films on planar substrates.[21] These click multilayer films were stable over a wide pH range (3−9) and in a range of organic solvents (ethanol, acetone, and dimethylformamide).

Herein, we demonstrate that click chemistry can be used to synthesize ultrathin, multilayered PAA CCs. We show that the PAA CCs can be selectively functionalized using the free click moieties in the multilayers by using a "click"-rhodamine dye, and that the CCs are pH-responsive, reversibly swelling and shrinking by up to 70% when subjected to acid−base pH cycling. The merging of click chemistry and LbL assembly to construct well-defined, multilayered capsules offers a number of important advantages. First, this is a general method that could be readily extended to a wide range of materials, including other polymers, proteins, nanoparticles, nanotubes, micelles, dye molecules, and biological systems. For example, in the case of polymers, the azide and alkyne functionalities can be readily incorporated either by simple copolymerization with functional monomers or by postpolymerization modification of the polymer backbone. Second, materials assembled using click functionality are stabilized via triazole linkages, forming thin films (e.g., capsules) that are extremely stable to hydrolysis, oxidation, or reduction.[1] Third, unlike conventional electro-

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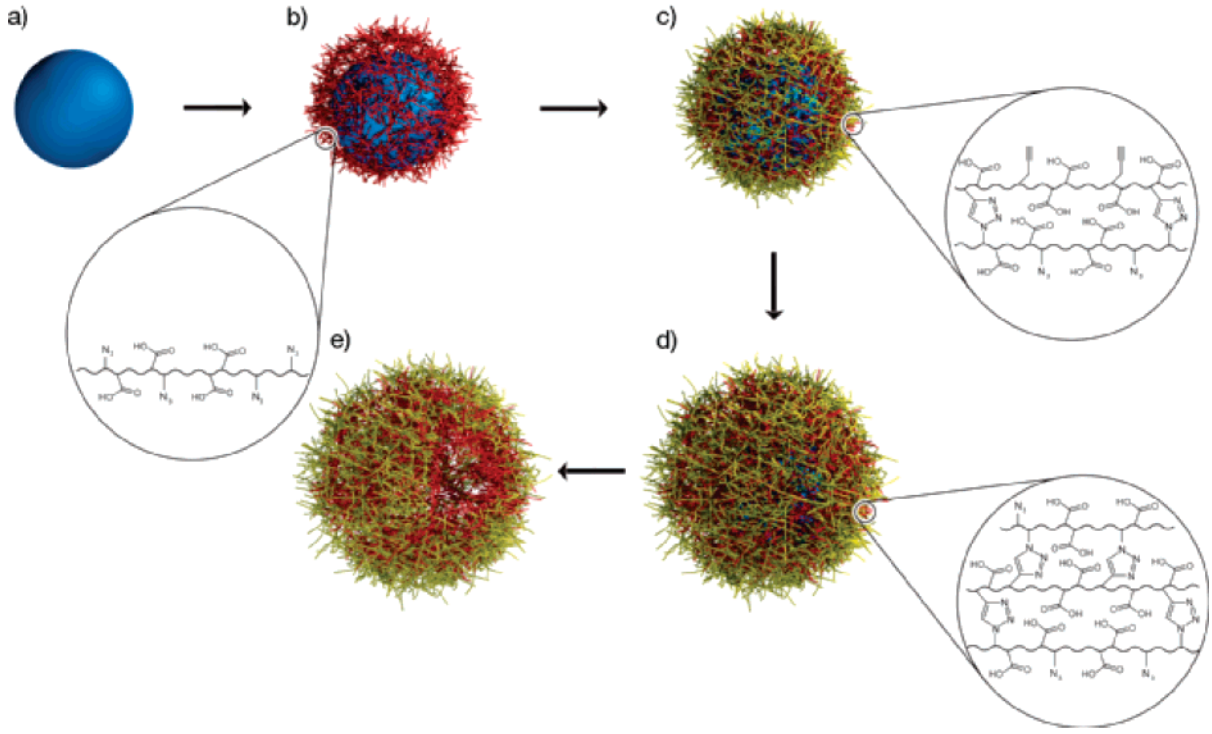
Scheme 1. Assembly of Click PAA Multilayers (PAA-Az/PAA-Alk) on a Colloidal Particle and PAA CC Formation[a]



[a] Key: (a) Template particle. (b) PAA-Az electrostatically adsorbed to the surface. (c) PAA-Alk "clicked" onto the PAA-Az layer. (d) Steps b and c repeated until the desired number of layers is achieved. (e) Removal of the sacrificial core to form click capsules.

statically assembled LbL capsules, which are based on oppositely charged polymer building blocks, CCs can be assembled from either like-charged polyelectrolytes or non-charged materials, the latter being particularly important for biomaterials. Therefore, the technique can be readily used to form single-component capsules. Fourth, CCs prepared by our approach contain a tunable minor cross-linking component (~10%), allowing the majority of the polymer to retain other functionality. Finally, postfunctionalization of CCs via the free click moieties with other materials, including polymers, biomacromolecules, or other functional groups, is possible. Postfunctionalization is particularly useful given that click chemistry is a simple technique performed under mild conditions, with high efficiency in water, making it broadly compatible with biological systems. This would permit biofunctionalization of the CCs for application in areas such as targeted drug delivery, biosensing, and biocatalysis.

Results and Discussion. Poly(acrylic acid) was synthesized with a minor component of either alkyne (PAA-Alk) or azide (PAA-Az) functionality using living radical polymerization (see Supporting Information). NMR characterization showed that the modified PAA contained ~10% of either the alkyne or azide functional groups. The PAA-Alk was also modified with a click-functionalized (Az) rhodamine dye (Rh-Az) to enable multilayer growth to be monitored via fluorescence intensity changes (see Supporing Information for experimental details). LbL assembly on colloids was performed by sequentially exposing ~5 μm poly(ethyleneimine) (PEI)-coated silica particles to PAA-Az and PAA-Alk solutions (0.83 mg mL^{-1}) containing copper sulfate (1.8 mg mL^{-1}) and sodium ascorbate (4.4 mg mL^{-1}) at pH 3.5. The particles were incubated for 15 min in each PAA solution. The particles were then centrifuged and washed three times with Milli-Q water.

The growth of the PAA-Az/PAA-Alk click multilayers was confirmed using flow cytometry (Figure 1). This approach is based on recording the fluorescence intensity of tens of thousands of individual particles after deposition of fluorescently labeled materials, including polymer layers.[22] The increase in fluorescence intensity of the dye (Rh-Az)-functionalized PAA-Alk, and thus the mass of each PAA-Alk layer, was shown to be linear to at least a total of 12 (PAA-Az/PAA-Alk) layers. This suggests linear growth of the click multilayers. The relatively large fluorescence intensity observed for the first PAA-Alk layer (layer 2), compared with subsequent layers, is attributed to electrostatic association of the first PAA-Az layer with the PEI primer layer. These results are in agreement with our earlier work on planar substrates, which also showed linear buildup of the multilayers.[21] Furthermore, fluorescence microscopy confirmed that the polymer multilayer coating on the particles was uniform (Figure 1).

The formation of the PAA-Az/PAA-Alk click multilayers uses only a fraction of the click groups on the polymers due to steric restrictions introduced by the highly coiled conformation of the adsorbed PAA layers at pH 3.5. Consequently, the remaining excess (or free) click groups in the multilayers on the surface of the particles can be used for subsequent functionalization with a range of materials. This could involve small molecules such as dyes or drugs through to

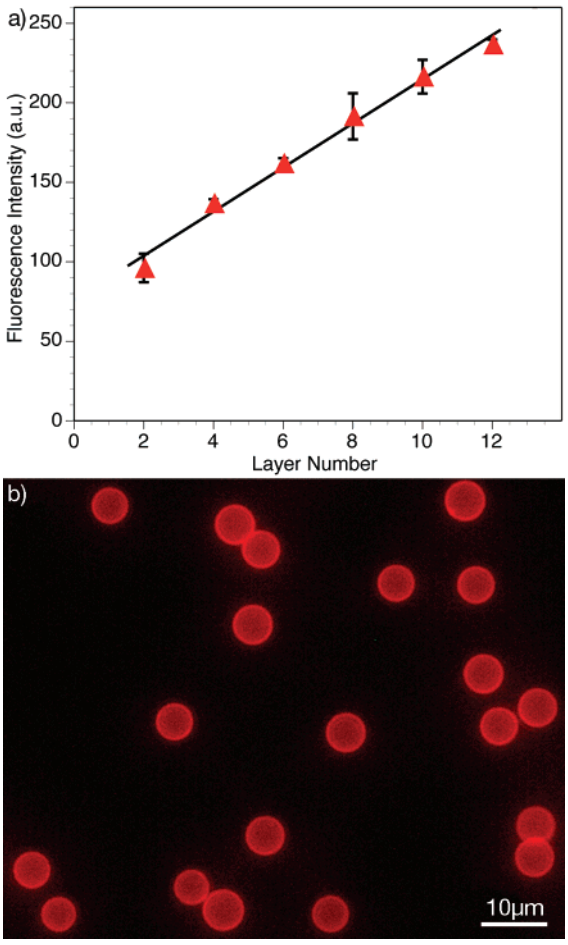


Figure 1. (a) Fluorescence intensity of (PAA-Az/PAA-Alk)-coated silica particles as a function of layer number, as measured by flow cytometry. The measurements were taken after deposition of each PAA-Alk layer, which was fluorescently labeled with rhodamine. (b) Fluorescence microscopy image of silica particles coated with $(PAA\text{-}Az/PAA\text{-}Alk)_6$. PAA-Alk is fluorescently labeled with rhodamine.

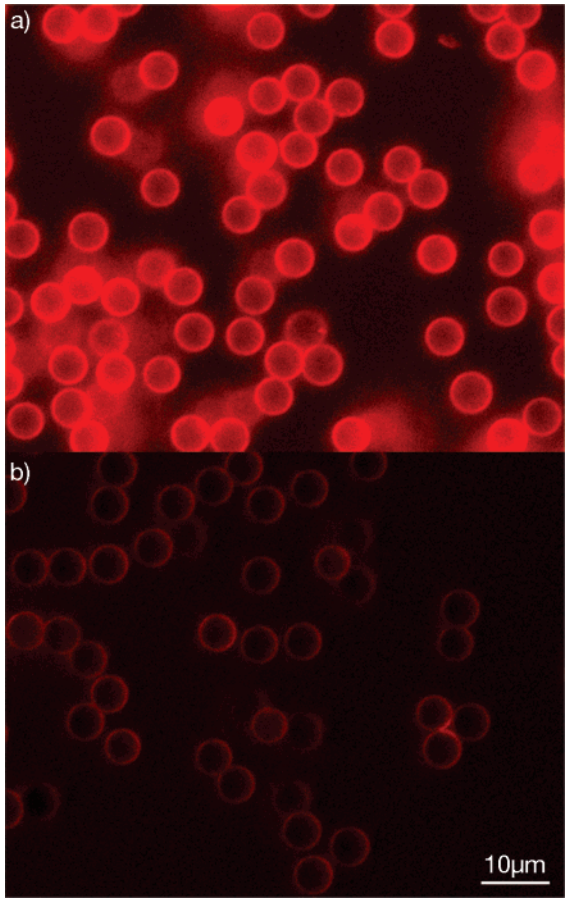


Figure 2. Fluorescence microscopy images of $(PAA\text{-}Az/PAA\text{-}Alk)_3$-coated silica particles functionalized with (a) Rh-Az and (b) nonspecifically adsorbed Rh. The scale bar corresponds to both images.

large molecules such as polymers, peptides, or biomacromolecules. The ability to functionalize the particles was demonstrated by reacting Rh-Az onto the $(PAA\text{-}Az/PAA\text{-}Alk)_3$-coated particles. Both Rh-Az and nonfunctionalized Rh were used to demonstrate the specificity of coupling of Rh-Az to the free Alk click groups in the multilayers. Rh showed some level of nonspecific binding to the $(PAA\text{-}Az/PAA\text{-}Alk)_3$-coated particles (possibly due to the interaction of the Rh and PEI as the first layer on the particles). However, after the particles were subjected to multiple washing steps in both 50/50 v/v dimethyl sulfoxide (DMSO)/water solution (to remove unbound Rh) and acidic water (to remove copper) and finally extensive dialysis, the particles exposed to Rh-Az showed significantly higher fluorescence (a factor of 2.5) than those exposed to unmodified Rh (Figure 2). This indicates that the click-functionalized Rh-Az dye was specifically clicked onto the $(PAA\text{-}Az/PAA\text{-}Alk)_3$ multilayers assembled on the particles.

The click multilayer core−shell particles formed were used to prepare CCs by removal of the sacrificial silica cores with ammonium fluoride-buffered hydrofluoric acid (HF) at pH 5. The resulting PAA CCs were characterized with transmission electron microscopy (TEM) and atomic force microscopy (AFM). After drying, the capsules collapsed and folds were visible from TEM and AFM images (Figure 3). AFM was used to obtain the thickness of the capsule walls by taking a cross-sectional profile of the capsules where they folded only once and then halving the thickness. The wall thickness of the 12-layer $(PAA\text{-}Az/PAA\text{-}Alk)_6$ capsules (including the PEI primer layer) was calculated to be 4.8 nm. This corresponds to less than 0.4 nm per PAA layer. Assembly of the same film on silicon planar substrates under similar conditions to those used in the colloidal assembly yielded a film thickness of 10.3 nm. We ascribe differences in these values to variations in the substrates used and also the different protocols used in preparing films on colloids and planar substrates.[23,24] The formation of capsules was also verified by using differential interference contrast (DIC) microscopy (see Figure S1, Supporting Information). This technique distinguishes materials based on changes in refractive index instead of light absorption, and as such, capsules appear distinctly different to core−shell particles. This confirmed that the solid cores were dissolved and that single-component PAA capsules were prepared.

It is well-known that weak polyacids such as poly(acrylic acid) are pH responsive.[19,25] Consequently, the effect of pH

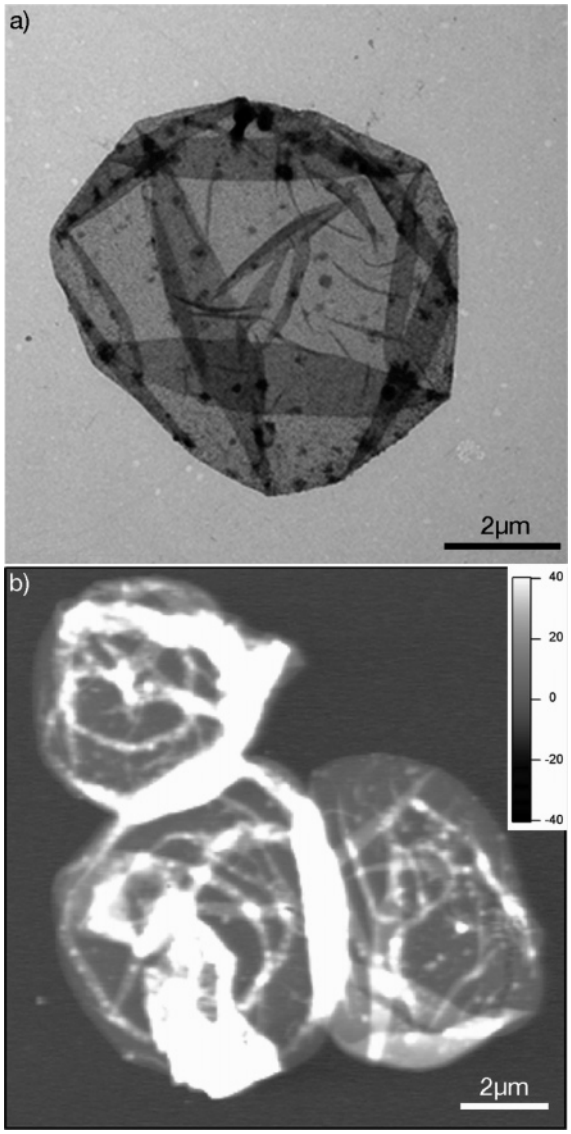


Figure 3. TEM (a) and AFM (b) images of $(PAA\text{-}Az/PAA\text{-}Alk)_6$ click capsules. The thickness of the capsule wall, determined by AFM, is ~5 nm.

on the PAA CCs was investigated. The CCs were alternately incubated in pH 2 and pH 10 solutions, resulting in reversible shrinking and swelling of the capsules, respectively (Figure 4a,b). The capsule diameter oscillated between about 5 and 8 μm in acidic and basic conditions, respectively (Figure 4c). The capsules deformed when swollen under basic conditions (Figure 4b) but reverted to their original spherical shape when exposed to acidic conditions (Figure 4a). The swelling is attributed to ionization of the carboxylic acid groups at higher pH, while the deformation may be explained by the cross-linking between the layers, which causes the capsules to resist greater swelling, leading to buckling/deformation. Such pH-responsive behavior could be exploited to load and concentrate drugs inside the capsules. We expect that further control over the capsule swelling properties can be achieved by altering the amount of azide and alkyne groups in PAA to vary the amount of covalent linkages within the multilayers.

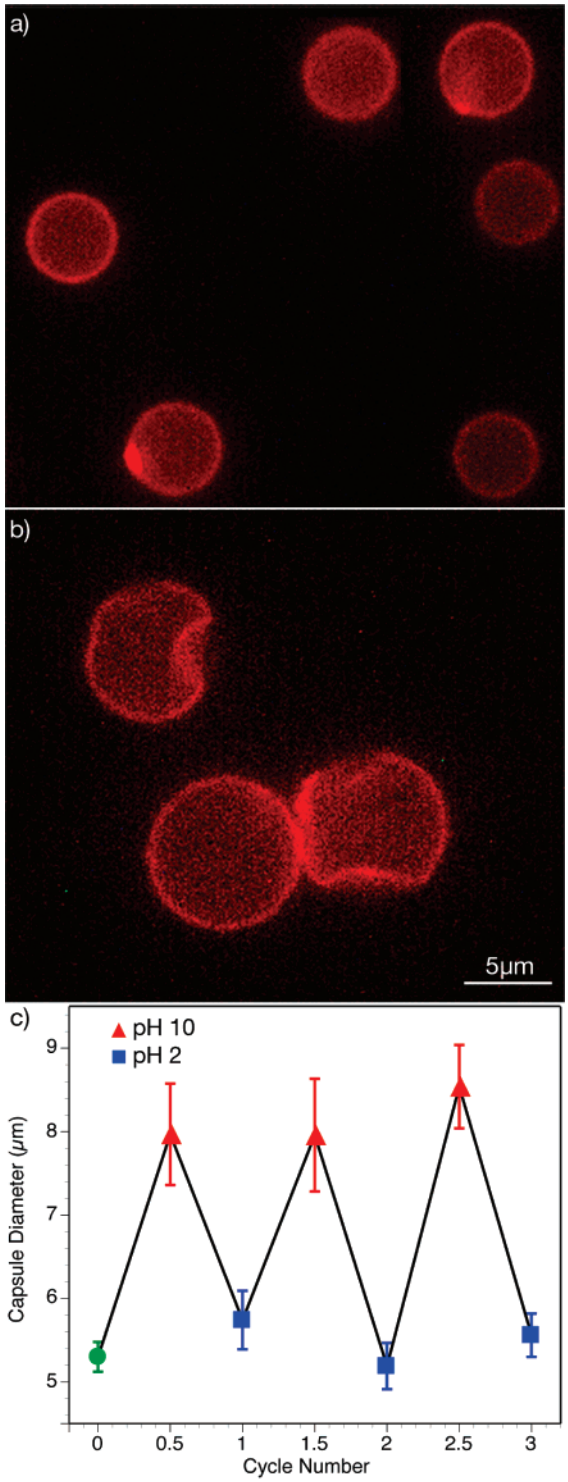


Figure 4. Fluorescence microscopy images of $(PAA\text{-}Az/PAA\text{-}Alk)_6$ click capsules after addition of (a) pH 2 solution and (b) pH 10 buffer. The scale bar corresponds to both images (a) and (b). (c) Reversible pH response of the $(PAA\text{-}Az/PAA\text{-}Alk)_6$ click capsules. Average diameter of the $(PAA\text{-}Az/PAA\text{-}Alk)_6$ click capsules in water (●) and in successive additions pH 10 buffer and pH 2 solution. Size measurements were performed only on the capsules that had not deformed. Data points represent the average diameter of 10 capsules.

Conclusion. In summary, we have reported a new, general click chemistry technique for the covalent LbL assembly of polymer multilayer on particles and the subsequent formation

of one component, pH-responsive, PAA CCs. The click multilayers were readily functionalized through a post-assembly reaction with a "clickable" Rh dye, displaying that the click multilayers serve as a versatile platform for further reactions and functionalization. This is particularly interesting for stabilizing and/or functionalizing colloidal systems (both core−shell and capsules) through specific reactions with various materials. We further demonstrated that the size of the PAA CCs can be reversibly modulated by incubation in acidic or basic solutions. The simple, efficient, and general nature of the click approach, coupled with the stability and responsive properties that are afforded in the materials, and the additional ability to selectively postfunctionalize, provides exciting new opportunities for designing advanced and responsive click capsules for use in a range of therapeutic and diagnostic applications.

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Supporting Information Available: Synthetic details of the monomers and polymers used, experimental details, and instrument specifications. This material is available free of charge via the Internet at http://pubs.acs.org.

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