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ARTICLE

Tuning the Properties of Layer-by-Layer Assembled Poly(acrylic acid) Click Films and Capsules

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ABSTRACT: The combination of click chemistry and layer-by-layer (LbL) assembly provides a useful and convenient means of preparing functional, covalently stabilized films and capsules. Herein, we examine various parameters that affect the buildup of click-LbL assembled multilayers of azide- and alkyne-modified poly(acrylic acid) (PAA_{Az} and PAA_{Alk}, respectively). We demonstrate that film thickness and morphology can be tailored by varying the assembly conditions. The thicknesses of multilayers assembled from PAA_{Az} and PAA_{Alk} [$(PAA_{Az}/PAA_{Alk})_5$] on planar substrates varied from ~70 nm when assembled at pH 2.5 to 10 nm when prepared at pH 4. Increasing the ionic strength of the adsorption solution resulted in an increase in the $(PAA_{Az}/PAA_{Alk})_5$ film thickness, with a maximum of ~30 nm observed for solutions with ionic strengths of 150 mM and greater. A deposition time of 5 min was found to give close to saturated adsorbed layer amounts. Additionally, the influence of the click moieties on multilayer assembly was investigated. By altering the azide content of PAA_{Az} and maintaining the alkyne content of PAA_{Alk} at ~15%, the thicknesses of $(PAA_{Az}/PAA_{Alk})_5$ films were shown to increase exponentially from about 20 nm at 5% azide functionalization of PAA_{Az} to 90 nm at 30% azide functionalization of PAA_{Az}. Furthermore, atomic force microscopy measurements showed distinct morphological changes (i.e., enhanced porosity and/or creases and folds) for $(PAA_{Az}/PAA_{Alk})_5$ films prepared from PAA_{Az} with different azide contents at pH 3.5 when subjected to basic conditions (pH 10). This was attributed to the different cross-linking degree between the multilayers. The current study was extended to the assembly of hollow polymer capsules to determine an optimum range of 10−20% azide functionalization of PAA_{Az} for the assembly of click polymer capsules.

Introduction

Significant attention has been given to multilayered films assembled through bottom-up approaches because they provide a number of advantages for various applications, including drug delivery, microfluidics, optics, sensing, cell culture, and tissue engineering.[1] The sequential buildup of polymers via the layer-by-layer (LbL) method provides an efficient and versatile means of developing functional multilayers. A diverse range of LbL multilayers with tailored structural and physicochemical properties have been assembled, predominantly through electrostatic[2,3] or hydrogen-bonding[4−7] interactions. However, applications such as nanomedicine and nanobiotechnology demand highly robust materials, which can be achieved by covalently cross-linking the multilayers.[8−14] Covalently cross-linked LbL materials can offer a number of advantages over those stabilized using electrostatic or hydrogen-bonding interactions. In particular, they offer increased stability as they are less prone to disassemble under diverse solution conditions such as pH and high salt concentration. Approaches to covalent bonding of multilayer films employ cross-linking via carbodiimide[6,15,16] or glutaraldehyde molecules,[17] disulfide chemistry,[18,19] photochemical[20] and thermal cross-linking techniques.[15]

An alternative approach for the preparation of covalently stabilized multilayer films is based on click chemistry. Click chemistry provides an effective means of covalent coupling through a set of highly specific, 'spring-loaded' reactions performed under mild conditions.[21−24] Recently, we reported the assembly of LbL films using the highly efficient alkyne−azide cycloaddition click reaction.[25] Linear poly(acrylic acid) (PAA) functionalized with alkyne (PAA_{Alk}) or azide (PAA_{Az}) groups was sequentially deposited in the presence of a Cu(I) catalyst to effect cross-linking upon the addition of each PAA layer. The reaction between the alkyne and azide functional groups produces triazole rings that form stable covalent bonds between the individual polymer layers (Scheme 1). These click multilayer films were shown to be stable over a wide pH range (3−9) and maintained their integrity in various organic solvents, including ethanol, acetone, and dimethylformamide.

Combining highly efficient click chemistry with LbL assembly provides a number of distinct advantages to LbL-assembled materials. The method can be applied to a range of components, including polymers, nanoparticles, dye molecules, micelles, and biomolecules such as proteins, peptides or nucleic acids. The relative inertness of alkyne and azide groups allows them to be introduced into components for click-LbL assembly with relative ease. To functionalize polymer building blocks, alkyne and azide groups can be incorporated during the polymerization step or by modification of preprepared polymers. Furthermore, the original properties of the polymer, such as biodegradability or low-fouling properties can be maintained by using only a low percentage of click moieties, which can accordingly be transferred to the properties of the multilayer assembly. Nevertheless, the alkyne or azide groups allow functionality to be obtained on what may otherwise be an inert polymer, such as nonlinear poly(ethylene glycol) (PEG). This has important consequences for the assembly of low-biofouling polymers, such as PEG, into stable, one-component films using mild conditions.[26] Given the

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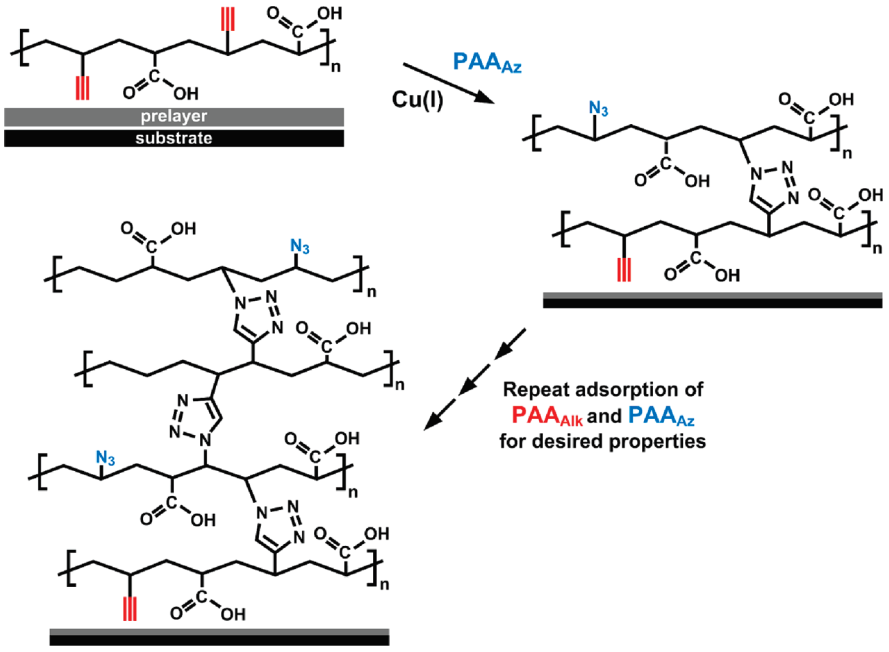
Scheme 1. LbL Buildup of Covalently Stabilized Thin Films of PAA by Click Chemistry[a]



[a] Layers of alkyne- or azide-functionalized PAA (PAA$_{Alk}$ and PAA$_{Az}$, respectively) are sequentially attached to a modified surface through the copper(I)-catalyzed variant of Huisgen's cycloaddition. The polymer structures shown are simplified representations to highlight the assembly process.

modular nature of assembling layers through click chemistry, layers can be incorporated at defined positions within the multilayers without migration of polymers within the film.[27,28] Further, postfunctionalization of click multilayer assemblies through unreacted click moieties allows a number of other materials, such as additional polymers,[29] drugs,[27] and biomolecules,[26] to be included within the films or at the surface.

Several studies have recently demonstrated the advantages of the click-LbL technique, including an investigation into the role of charged Cu(I)−alkyne complexes in film assembly,[30] the use of dendrimers for fine control of film thickness,[31,32] degradable click multilayers,[33] and the assembly of hollow polymer capsules,[27,29,34−36] among others.[37−40] In related work, small molecule click-functionalized cross-linkers have been infiltrated into preformed multilayers to develop a highly customizable approach to generate drug delivery systems.[41,42] In recent work, our group has demonstrated that different layers can be incorporated at defined positions within a multilayer through the modular nature of click-LbL assembly.[27] Alkyne-modified poly(L-glutamic acid) (PGA) was modified with the drug doxorubicin (DOX) and incorporated into the capsule walls at defined layers. The drug loading was finely controlled through the number of layers or by the degree of DOX modification of PGA. Furthermore, variations from the Cu(I)-catalyzed alkyne−azide cycloadditions were introduced into LbL materials using click-type reactions between poly(2-alkenyl azlactone)s and poly(ethylene imine)[43] and thiol−ene chemistry.[44]

Despite these aforementioned studies, a detailed and combined study into the range of parameters that affect the buildup of click multilayers has not been reported. Herein, we investigate the click-LbL assembly of polymer films and capsules and demonstrate control over PAA film properties by adjusting key assembly conditions, such as adsorption time, solution pH, and solution ionic strength. In addition, we assembled PAA$_{Az}$/PAA$_{Alk}$ multilayers using PAA functionalized with various azide contents to investigate its influence on multilayer film thickness and response to film swelling after assembly. This study was extended to polymer capsules to determine a suitable range of polymer functionalization with click groups for the assembly of free-standing LbL capsules. The effect of increased cross-linking on the pH-response of PAA capsules was also observed. The findings from this study can be used in the assembly of covalently cross-linked films and capsules using a variety of polymer types. Therefore, this technique can be used to develop a variety of multilayer films and capsules for potential use as biocompatible coatings, membranes, drug delivery devices, and sensors.

Experimental Section

Materials. Silicon wafers were obtained from MMRC Pty Ltd. (Melbourne, Australia). 5.28 ± 0.25 μm diameter silica particles (50 mg mL^{-1} solids) were purchased from MicroParticles GmbH (Berlin, Germany). High purity water with a resistivity greater than 18 MΩ cm was obtained from an inline Millipore RiOs/Origin system. pH measurements were taken with a Mettler-Toledo MP220 pH meter (Columbus, OH), and the pH values were adjusted with 0.1 M HCl and 0.1 M NaOH. Unless otherwise noted, all materials were purchased from Sigma-Aldrich and used as received.

Polymer Synthesis. *PAA$_{Alk}$.* Poly(acrylic acid) with alkyne functionality (PAA$_{Alk}$) was synthesized as follows. The initial reactants were mixed at an approximate 540:60:1 molar ratio of acrylic acid (90.6×10^{-2} g, 0.013 mol), propargyl acrylate (18.1×10^{-2} g, 1.6×10^{-3} mol), reversible addition chain-transfer (RAFT) agent (butyl phthalimidomethyl trithiocarbonate) (7.6×10^{-4} g, 2.3×10^{-4} mol), and 4 mL of dioxane. Ten wt % azobis(isobutyronitrile) (0.2 mg, 1.2×10^{-3} mmol) relative to the RAFT agent was also added. The solution was purged by bubbling with N_2 (BOC Gases Australia, Sydney, Australia) for 45 min and then stirred at 60 °C in a constant temperature oil bath for 11 h. The product was dialyzed for 24 h to remove excess monomer. ^{1}H NMR (D_2O) ppm: 1.00−1.92 CH_2 (polymer), 2.04−2.58 CH (polymer), 2.70−2.83 triple bond CH (polymer), the final polymer peak is combined with the solvent peak at 4.47−4.84 OCH_2 (polymer). There was also a distinctive peak at 7.71 ppm due to the phthalimdo protons on the RAFT agent. As determined by gel permeation chromatography (GPC), the yellowish polymer had a molecular weight of 53 000 (M_n) with a polydispersity of 1.9 and NMR analysis showed an alkyne component of 15 mol %. The molecular weight

was analyzed using poly(styrene) standards. The larger polydispersity is ascribed to branching of the alkyne functional monomer, which produced a shoulder on the polymer distribution.

PAA_{Az}. For all experiments other than those involving multilayer assembly using PAA_{Az} with different azide contents, PAA_{Az} was synthesized and characterized as described previously.[25] This polymer contained ~16 mol % azide groups by NMR and a molecular weight (M_w) was determined (by GPC) to be 86 000 Da.

As described previously,[29] PAA with various azide contents was synthesized via 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) mediated coupling of acrylic acid with *O*-(2-aminoethyl)-*O*′-(2-azidoethyl)pentaethylene glycol (H_2N-$dPEG_5$-N_3; Quanta Biodesign, Powell, OH). 100 mg of PAA (M_w = 60 000; Polysciences, Warrington, PA) was dissolved in 15 mL of water. 500 mg of DMTMM was added to this solution together with 6, 12, 27, 53, or 74 μL of H_2N-PEG_5-N_3 to produce PAA_{Az} with 5, 10, 20, 30 or 40% azide functionality. The solution was stirred at room temperature for 8 h. After dialysis for 7 days against distilled water, the solution was filtered with a 0.2 μm syringe filter (Pall Corporation, Port Washington, NY) and freeze-dried. The extent of PAA modification was determined by NMR spectroscopy (see Supporting Information).

Planar Substrate Preparation. Silicon wafers were cleaned by sonicating them in a mixture of water and isopropanol (50% v/v) for 20 min and then washing them thoroughly in water. Finally, the slides were heated to 60 °C in a solution containing 5 parts water, 1 part hydrogen peroxide, and 1 part ammonia solution (Merck, Darmstadt, Germany) for 20 min, washed with water, and then dried in a stream of N_2.

Click-LbL Assembly on Planar Substrates. The following describes the standard procedure for the preparation of PAA_{Az}/PAA_{Alk} multilayer films. Silicon wafers were placed in a vial containing PEI solution (1 g L^{-1} in 0.5 M NaCl) for 10 min. Substrates were then sequentially exposed for 15 min to solutions containing PAA_{Az} or PAA_{Alk} (1 g L^{-1}), copper(II) sulfate (1.75 g L^{-1}), and sodium ascorbate (4.4 g L^{-1}) at a constant volume ratio of 3:1:1, respectively. The pH of all solutions was adjusted to pH 3.5 using 0.1 M HCl. Substrates were washed three times in pH 3.5 water for 1 min each and then dried with a stream of N_2. To investigate the fundamental aspects of click-LbL multilayer assembly, specific assembly conditions were varied. The experimental conditions regarding changes to the adsorption solution pH, ionic strength, and adsorption time are described in more detail in Supporting Information. The buildup of PAA multilayers on silicon wafers was monitored by ellipsometry. The film thicknesses determined by ellipsometry do not include the PEI layer. The surface morphology of these films was examined by atomic force microscopy (AFM).

Click-LbL Assembly on Si Particles. *Core−Shell Particle Preparation*. A 100 μL suspension of 5.28 ± 0.25 μm diameter SiO_2 particles (50 mg mL^{-1}) was washed by centrifuging the particles at 800 *g* for 1 min, removing the supernatant and then redispersing the particles in 500 μL of pH 3.5 water. In all, three centrifugation/redispersion cycles were conducted. This procedure was used as the standard washing procedure throughout the LbL assembly process. An equal volume of PEI solution (1 g L^{-1} in 0.5 M NaCl) was added to the particle suspension and left for 10 min with constant shaking. This resulted in a PEI precursor layer on the silica particles. After washing, 500 μL of a pH 3.5 solution of PAA_{Az} (1 g L^{-1}), copper(II) sulfate (1.75 g L^{-1}), and sodium ascorbate (4.4 g L^{-1}) at a constant volume ratio of 3:1:1, respectively, was added with constant shaking for 15 min. After washing, PAA_{Alk} was added using the same process as described for the previous layer to obtain a PAA_{Az}/PAA_{Alk} bilayer. This PAA_{Az}/PAA_{Alk} adsorption procedure was repeated until the desired number of bilayers was obtained. Unless otherwise noted, a total of five bilayers (plus the precursor layer) were assembled onto the silica particles (i.e., $PEI/(PAA_{Az}/PAA_{Alk})_5$).

Preparation of Hollow Capsules. To form hollow capsules, the silica cores were dissolved from the PAA-multilayer-coated silica particles by mixing an equivalent of 1 μL of the coated particle suspension with 2 μL of ammonium fluoride (8 M) buffered hydrofluoric acid (HF) (2M) at pH 5. *Caution! HF is highly toxic and great care must be taken when handling*. Dissolution of the silica particles occurred in less than 1 min. Capsules were spun at 2500 *g* for 12 min to remove the supernatant and washed three times in pH 3.5 water, as discussed earlier. Silica particle dissolution was confirmed by differential interference contrast microscopy.

Capsule Swelling Experiments. pH-induced swelling and shrinkage of capsules were performed by successive additions of 1 μL of HCl at pH 2 and 1 μL of 10 mM sodium carbonate ($NaHCO_3/Na_2CO_3$) buffer solution at pH 10 directly to the capsule solution on microscope slides. Shrinkage or swelling of the capsules occurred within less than 1 min of adding the acidic or basic solution. The size of the capsules is quoted as the average of measurements on 5 to 15 capsules.

Ellipsometry. Measurements were performed on a UVISEL spectroscopic ellipsometer (HORIBA Jobin Yvon S.A.S, Chilly Mazarin, France). Spectroscopic data was acquired between 400 and 800 nm with a 2 nm increment, and unless otherwise stated, thicknesses were extracted with the integrated software by fitting with a classical wavelength dispersion model. No less than three measurements were taken on each sample.

Fluorescence Microscopy. An inverted Olympus IX71 microscope equipped with a DIC slider (U-DICT, Olympus, Tokyo, Japan) with a 40× objective lens (Olympus UPLFL20/0.5NM, W.D. 1.6) was employed to view the capsules. A CCD camera (Cool SNAP fx, Photometrics, Tucson, AZ) was mounted on the left-hand port of the microscope.

Atomic Force Microscopy. AFM images of air-dried PAA click multilayer films on silicon wafers were acquired with an MFP-3D Asylum Research instrument (Santa Barbara, CA). Typical scans were conducted in AC mode with ultrasharp SiN gold-coated cantilevers (NT-MDT; MikroMasch, Tallinn, Estonia) over 50 μm^2. Multilayer thicknesses were determined by scratching the multilayer with a razor blade to expose the substrate and measuring the step height difference.

Results and Discussion

Effect of Adsorption pH on Multilayer Assembly. PAA is an excellent polymer to study the effects of solution pH on the properties of click multilayers due to its well-known pH-dependent conformational changes.[45−47] The assembly of click PAA multilayers was investigated by examining the buildup of five bilayers of PAA_{Az}/PAA_{Alk} on PEI-coated silicon wafers under different pH conditions. Multilayers were assembled using PAA_{Az} (~16 mol % azide) and PAA_{Alk} (~15 mol % alkyne) at pH 2.5, 3.0, 3.5 and 4, and the thicknesses of the resulting films were measured by ellipsometry (Figure 1). Film thicknesses of PAA_{Az}/PAA_{Alk} (excluding the precursor PEI layer) increased with decreasing pH from 11 nm (pH 4) to 73 nm (pH 2.5). Taking into account the PEI/PAA_{Az} prelayers, the average bilayer thickness of 4.2 nm for a PAA_{Az}/PAA_{Alk} bilayer assembled at pH 3.5 is in good agreement with the initial study of click PAA films, which showed an average bilayer thickness of 4.6 nm.[25] Similar thicknesses can also be seen for PAA adsorbed in PAA/PAH multilayers at pH 3.5.[45]

As reported by Rubner and co-workers,[45,46] the two most important parameters for controlling the LbL assembly of weak polyelectrolytes such as PAA and PAH in the pH 2.5−4.5 range are the linear charge densities of the multilayer surface and the adsorbing polymer. When assembling

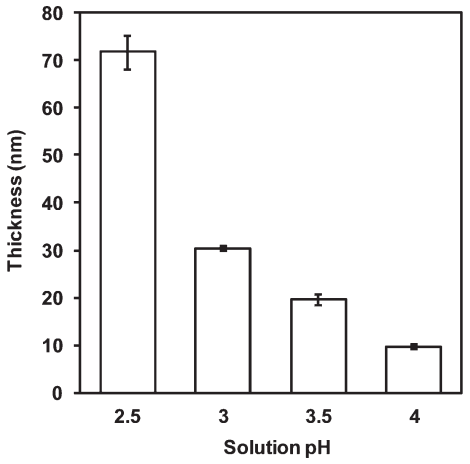


Figure 1. Thicknesses of $(PAA_{Az}/PAA_{Alk})_5$ films assembled onto PEI-coated silicon wafers from pH 2.5, 3, 3.5, or 4 solutions. Multilayers were assembled by adsorbing PAA_{Az} (~16 mol % azide) and PAA_{Alk} (~15 mol % alkyne) for 15 min per layer in the presence of copper(II) sulfate and sodium ascorbate. Thickness values reported exclude the initial PEI primer layer. All thicknesses were obtained by ellipsometry and error bars show the standard deviation of three measurements.

single-component multilayers this linear charge density can essentially be the same on the polymers and may differ only in the type and amount of click functionalization. Nevertheless, a similar trend in film thickness increase for decreasing pH is observed for click-LbL multilayers and PAA/PAH multilayers assembled at pH 2.5−4.5.[45,46] This trend may be attributed to a reduction of intra- and interpolymer repulsion of the click-modified PAA. In the latter case, reducing the charge density of the PAA decreases repulsion between the multilayer surface and the adsorbing polymer, thereby allowing more adsorbing polymer to reach the surface. Furthermore, as generally reported, decreasing the number of carboxylate groups to carboxylic acid groups by decreasing the pH will also result in conformational changes of the PAA from a rod-like chain to an increasingly coiled molecule. This will result in bulky, coiled polymers "clicking" onto the surface, as observed at an adsorption pH of 2.5, where the average PAA_{Az}/PAA_{Alk} bilayer thicknesses was 16 nm. Conversely, a 2 nm bilayer thickness was observed at pH 4. At this pH, the increase in surface charge of preadsorbed PAA and linear charge density of the adsorbing PAA causes fewer polymer molecules to be adsorbed onto the multilayer surface due to electrostatic repulsion. As described later, click-LbL multilayer assembly is ultimately dependent on the number of cross-linking click moieties available, and so any differences in conformation may change the ability of an adsorbing polymer to access functional sites for cross-linking. Nevertheless, adjusting the solution pH of adsorbing click-modified polymers presents a facile way of altering click-LbL film thicknesses when weak polyelectrolytes are employed.

As described in previous studies,[48] in the pH 2.5−4 range, a number of carboxylate (COO^-) groups still exist along the polymer backbone (see also Supporting Information). Therefore, the multilayer surface and the adsorbing polymer both present a net negative charge which would appear to prevent the adsorption of more PAA, regardless of click functionality. This negative charge may be offset by a slight positive charge attributed to a positively charged copper(I)−alkyne complex, as reported by Jierry et al.[30] When present along an alkyne-functionalized polymer chain, a number of these complexes are thought to impart a positive charge on the polymer, which may affect the clicking of a polymer onto the surface. It was observed that a neutral polymer functionalized with alkyne groups and a polyanion with azide groups showed a more favorable buildup than the same neutral polymer with polycations functionalized with azide groups.[30] While these findings may describe a mechanism for click-LbL buildup, it is believed that layer assembly is driven by the high thermodynamic driving force of numerous copper(I)-catalyzed cycloadditions of alkyne and azide groups, as is characteristic of many click reactions.[21,49]

Click-LbL films were further characterized by AFM and their surface roughness was measured. Figure 2 shows AFM images for the $PEI/(PAA_{Az}/PAA_{Alk})_5$ multilayers assembled at the various pH conditions. In agreement with increased layer thicknesses observed in more acidic conditions, multilayer films assembled at lower pH show increased roughness due to increased coiling of the polymer components. This is seen by films assembled at pH 2.5, which exhibit a root-mean-squared (rms) roughness of 21 nm. The roughness values were observed to plateau at higher pH, with rms roughness values of approximately 6−7 nm measured for multilayer films assembled at pH 3.5 or 4.

Effect of Salt on Multilayer Assembly. Salt is well-known for its influence on the thickness of polyelectrolyte multilayers, depending on the polymer components used in assembly and the salt type.[50,51] Added salt is capable of interacting with polymers, altering their solubility or screening electrostatic charges. Figure 3 shows a plot of $PEI/(PAA_{Az}/PAA_{Alk})_5$ multilayer film thicknesses assembled from solutions of different NaCl concentration. Because of the addition of copper(II) sulfate and sodium ascorbate into the dipping solution, salt is already present (7 mM) and so thicknesses were plotted against total ionic strength. Thicker films were observed with increasing ionic strength until 150 mM, where the thickness values plateaued at approximately 33 nm. PAA_{Az}/PAA_{Alk} bilayer thicknesses increased from approximately 4 nm (7 mM) to 7 nm (> 150 mM). Thicker films can be attributed to an increasingly coiled PAA conformation due to screening of charge along the polymer chain and thereby a decrease of intrapolymer repulsion, as reported for PAA/PAH deposition onto particles.[47] Changes in conformation of the click-modified PAA through the addition of salt may also cause alkyne or azide groups present along the chain to be shielded or inaccessible to complementary click functionalities and thereby lead to relatively thinner layers. Further, the addition of salt will likely screen repulsive charges between the multilayer surface and adsorbing polymer. Reducing electrostatic repulsion through the addition of electrolytes in click-LbL buildup has also shown to be important in the assembly of poly(L-lysine) and poly(L-glutamic acid) multilayers.[29]

The plateau of multilayer thickness at 150 mM ionic strength is close to the ~100 mM NaCl maximum ionic strength reported by Dubas and Schlenoff for the buildup of PAA (M_w = 5200 Da) at pH 5 in the assembly of PAA/poly(diallyldimethylammonium chloride) (PDADMA) multilayers.[52] In that study, increasing the adsorption ionic strength beyond this value resulted in a significant decrease in PAA/PDADMA multilayer thickness due to charge compensation by salt ions producing less favorable conditions for multilayer assembly. In contrast, click-LbL multilayers are not limited by the requirement for polyanion/polycation interactions for multilayer assembly and so do not experience a decrease in multilayer thickness with excess salt, as demonstrated in Figure 3. Additionally, unlike the electrostatic assembly of multilayers where ions are released upon the pairing of polyanions and polycations, it is envisaged that

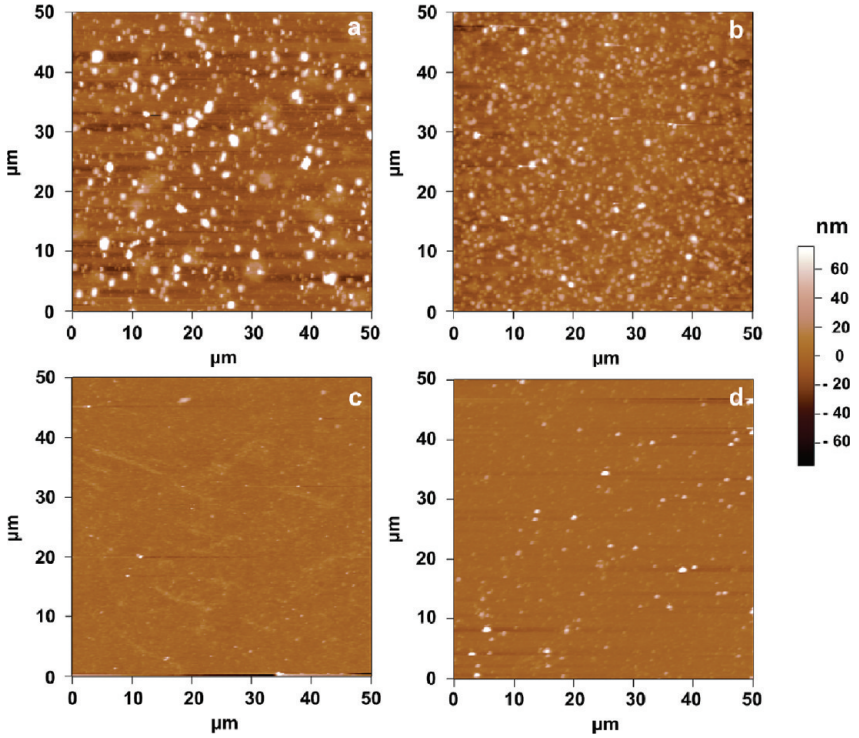


Figure 2. AFM images of air-dried PEI/$(PAA_{Az}/PAA_{Alk})_5$ multilayers assembled onto silicon wafers from pH (a) 2.5, (b) 3.0, (c) 3.5, or (d) 4.0 solutions. Multilayers were assembled by adsorbing PAA_{Az} (~16 mol % azide) and PAA_{Alk} (~15 mol % alkyne) for 15 min per layer without the addition of electrolytes other than copper(II) sulfate and sodium ascorbate for reaction catalysis. Root mean squared roughness values for each of the samples were 21.0, 14.3, 5.7, and 7.2 nm, respectively.

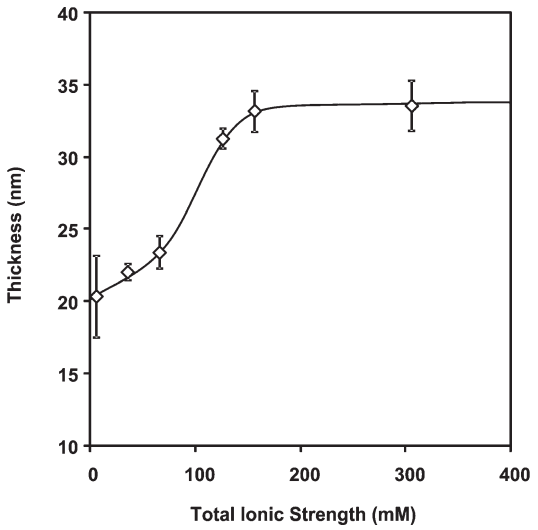


Figure 3. Ellipsometry thickness measurements of PEI/$(PAA_{Az}/PAA_{Alk})_5$ films assembled on silicon wafers from different NaCl concentrations at pH 3.5. The total ionic strength of the polymer dipping solution accounts for the addition of copper(II) (2.2 mM) and sodium ascorbate (4.4 mM) for catalysis of the click reaction. Multilayers were assembled by adsorbing PAA_{Az} (~16 mol % azide) and PAA_{Alk} (~15 mol % alkyne) for 15 min per layer. The error bars show the standard deviation of three measurements.

the counterions remain in the single component click-LbL multilayers in order to compensate for the charge of polymers.

When PAA_{Az}/PAA_{Alk} multilayers were assembled on silica particles (~5 μm diameter), the addition of salt caused irreversible aggregation. This may be primarily attributed to the layering under conditions of further reduced electrostatic repulsion, as similar observations were made for PAA deposited at low ionization degrees when adsorbed during PAA/PAH film formation.[47] As mentioned in that study, the stability of the particles with low charge is favored by minimal salt in solution, as the addition of salt will screen electrostatic repulsion between particles.

Kinetics of Layer Assembly by Click Chemistry. The Cu(I)-catalyzed cycloaddition of alkynes and azides is well suited to the assembly of tailored materials given its high efficiency and specificity.[21,22,49] To investigate the role of the reaction on the kinetics of multilayer assembly, PEI/$(PAA_{Az}/PAA_{Alk})_5$ films were assembled onto silicon wafers under a series of deposition/reaction times varying from 10 s to 10 min. Wafers were placed in a pH 3.5 polymer solution of 0.5 mg mL^{-1} PAA_{Az} (~16 mol % azide) or PAA_{Alk} (~15 mol % alkyne) with Cu(II) and sodium ascorbate and with no stirring applied. The thicknesses of the films formed were measured by ellipsometry (Figure 4). The layer thickness reaches a plateau at around ~30 nm after approximately 5 min, a similar time to that observed for electrostatically assembled polyelectrolyte multilayers.[51] Not hindered by long covalent reaction times, these click-LbL films may be suitable for spray or spin application of films.[53] From Figure 4, together with observations of negligible polymer diffusion between layers of the click cross-linked polymer assemblies,[27] it can be expected that the rate of polymer buildup for a layer is mostly diffusion controlled and occurs in an irreversible manner.

Steric factors, as well as limited freedom of movement available to polymer chains within the multilayer, contribute to not only the rapid plateau observed for polymer adsorption but also the ultrathin nature of click layers. While a buildup of excess charge at a multilayer interface limits the addition of more polymers to the surface of a film assembled by electrostatics, interpenetration of polymer layers by permeation and rearrangement may allow more polymers to be added to the multilayer. In the click-LbL approach,

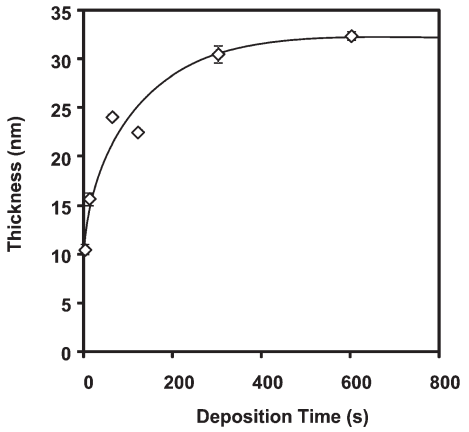


Figure 4. Thicknesses of $(PAA_{Az}/PAA_{Alk})_5$ films assembled onto PEI-coated silicon wafers with varying deposition/reaction times, determined by ellipsometry. Layers of PAA_{Az} (~16 mol % azide) or PAA_{Alk} (~15 mol % alkyne) were deposited from pH 3.5 solution for a specific deposition time. Thicknesses include the measurement of the precursor PEI layer. Error bars show the standard deviation of three measurements.

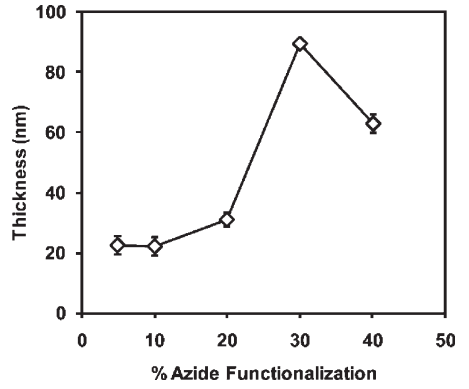


Figure 5. Ellipsometry thicknesses of $PEI/(PAA_{Az}/PAA_{Alk})_5$ films assembled using PAA_{Az} with different amounts of azide functionality. The alkyne functionality of PAA_{Alk} was kept constant at ~15 mol %. Multilayer assembly occurred in pH 3.5 solution and an adsorption time of 15 min was used for the addition of each new layer. The error bars show the standard deviation of three measurements.

however, steric exclusion likely inhibits an incoming click-functionalized polymer from accessing the limited alkyne or azide sites available for cross-linking. During layer buildup, it is postulated that free click moieties exposed at the film surface are rapidly consumed to create triazole linkages. These covalent linkages may limit the extent to which conformational changes and penetration of the adsorbing polymer into the preceding layer occurs, thereby allowing the formation of thin, well-defined layers, but restricting access of incoming polymer to sites of functionality on the film surface. This explanation may also account for the unreacted click groups that have been exploited for functionalization with small molecules in previous studies.[34,37]

Effect of Click Content on Multilayer Assembly. To examine the influence of increased content of click moieties on film and capsule properties, PAA (60 000 M_w) was modified with azide-functional short-chain poly(ethylene glycol) (PEG) molecules by DMTMM coupling to create PAA_{Az} with various amounts of azide functionality (5, 10, 20, 30, and 40 mol %). These polymers were used to assemble five bilayers with the amount of alkyne functionality of PAA_{Alk} kept constant at 15 mol %. Film thicknesses were measured using ellipsometry (Figure 5) and increased exponentially from approximately 22 nm for 5% azide functionality to 89 nm for 30% azide groups within PAA_{Az}. The additional cross-linking points may allow more polymer to be assembled into the multilayer, thereby producing thicker layers. This is in agreement with observations made by Huang and Chang in their preparation of thermoresponsive poly(*N*-isopropylacrylamide) capsules by click-LbL assembly.[36] It was postulated that high degrees of functionalization (40%) may have significantly changed the original properties of PAA so that only a thickness of 63 nm was achieved. For example, a high degree of PEG−azide modification could be expected to reduce PAA coiling due to reduced charge and steric constraints of the PEG chains. It should also be noted that control experiments conducted previously[25] showed that unmodified PAA assembled onto PEI-coated surfaces did not adsorb beyond two layers. This implied that click groups were needed for repeated layer assembly.

The surface roughness of these PAA click multilayers was also measured by AFM, which demonstrated increased roughness with increasing azide functionality of PAA_{Az}. An rms roughness of 5 nm at 5% azide modification and 25 nm at 30% azide functionality were observed. This change in surface roughness is attributed to the ability of an adsorbed polymer to adopt a more energetically favorable conformation. At high degrees of click functionality, an adsorbed polymer could be tethered to a greater number of points on the multilayer surface and may therefore have less freedom of movement to adopt a more favorable conformation, resulting in a rougher surface.

As demonstrated in previous work,[34,39] PAA multilayers cross-linked by click chemistry are stable over a wide pH range and can respond to changes in pH. Because of the ionizable carboxylic acid groups of the polymer backbone, PAA multilayers have been shown to reversibly shrink and swell with acid−base cycling. Numerous studies have reported on the swelling of poly(carboxylate) films, particularly PAA and PMA, stabilized by covalent cross-linking.[7,16,19] Polymer structure, pH, and ionic strength are all known to influence film swelling. To observe morphological changes in multilayer films with increased pH, click PAA multilayers with various amounts of azide functionality were immersed in pH 10 solution for 10 min and dried under a stream of N_2. At high pH, a PAA polymer chain forms a more rod-like conformation as the degree of ionization, and therefore intrapolymer repulsion, increases along the backbone. The click PAA multilayers were studied by AFM before and after immersion in pH 10 solution and are shown in Figure 6. At 5% azide functionality of PAA_{Az}, a number of pores are observed due to the increased polymer repulsion and the degree of cross-linking not being sufficient to maintain the integrity of the polymer film. Fewer pores are observed at higher degrees of functionality. Creases can be observed in all samples, with their sizes increasing with increased azide functionality. These features can be attributed to a greater resistance to film swelling from the increased degree of cross-linking. It is noted that the morphological changes observed in 20, 30, and 40% samples are similar.

Capsule Assembly and Swelling. Click PAA multilayers were also assembled onto silica particles (~5 μm diameter) and postlabeled with azide-functional Alexa Fluor 488. However, multilayers could not be assembled from 30 and 40% azide PAA, as these polymers caused irreversible aggregation during the deposition step. For a given molecular weight of PAA, high amounts of azide modification reduce the amount of ionizable groups along the polymer

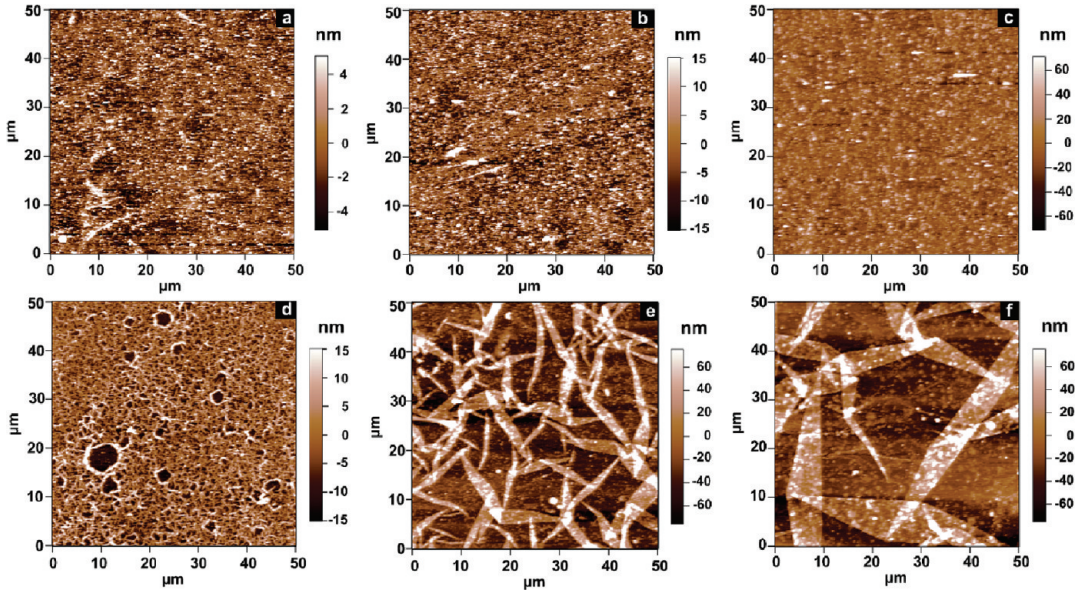


Figure 6. AFM images of PEI/(PAA_{Az}/PAA_{Alk})$_5$ films assembled using PAA_{Az} with different amounts of azide functionality and PAA_{Alk} at constant alkyne functionalization (~15%) before and after incubation in pH 10 solution. Images a−c show films assembled with 5%, 10%, and 20% azide functionality, respectively, at pH 3.5 after N_2 drying with respective surface roughness (rms) values of 4.6, 8.9, and 17.8 nm. Images d−f show the same films assembled with 5%, 10%, and 20% azide functionality after 10 min incubation in pH 10 solution.

backbone, which may reduce the surface charge and subsequent repulsion of particles, resulting in aggregation. After removal of the silica template by ammonium fluoride-buffered HF at pH 5, the remaining samples were analyzed by fluorescence microscopy. No capsules were observed for multilayers assembled from PAA_{Az} with 5% azide functionality, as insufficient functionality was available to provide stable multilayers for three-dimensional structures. However, capsules were observed for those containing PAA_{Az} with 10 and 20% azide functionality, and are shown in Figure 7, parts a and c, respectively. Both fluorescence microscopy and AFM images show debris and capsules with structural deformations for multilayers assembled with 10% azide functionality in comparison with the 20% sample, which shows capsules of regular spherical shape in pH 3.5 solution. The first report of click capsules showed stable capsules using PAA with ~10% alkyne and azide modification.[34] In that report, the azide group was separated from the PAA polymer backbone by a propyl group. However, in this current study, the azide group is separated by five ethylene glycol units connected to the polymer backbone through an amide bond. It is believed that the differences in the click-functional groups attached to the PAA backbone may contribute to the swelling properties of the click capsules. Furthermore, the PAA_{Az} molecular weight (M_n) of 135 400 used in the previous work compared to the 60 000 Da M_n used in the present study may also contribute to differing capsule properties. Nevertheless, the higher amounts of cross-linking allow the capsules to maintain the shape of the original particle template.

The ability of capsules to swell or shrink with changes to environmental conditions is useful for the loading or release of cargo such as dyes, drugs or other therapeutics. Click PAA capsules have shown to reversibly swell up to 70% of their original size with increased pH conditions.[34] To test the effect of increased cross-linking on the pH response of click capsules, fluorescently labeled capsules assembled from PAA_{Az}/PAA_{Az} multilayers of 10 or 20% azide functionality were cycled through pH 2 and pH 10 solutions, and their diameters were measured by fluorescence microscopy.

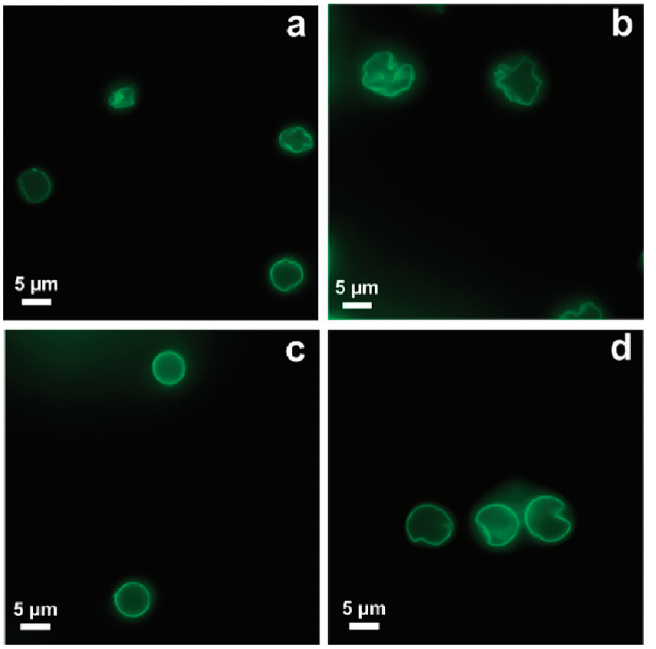


Figure 7. Fluorescence microscopy images of click PAA capsules assembled from PEI/(PAA_{Az}/PAA_{Alk})$_5$ with 10 or 20% azide functional PAA on a sacrificial core of 5 μm-diameter SiO_2 particles. Assembly of PAA_{Az} and PAA_{Alk} layers occurred in pH 3.5 solution with 15 min allowed for the addition of each layer. After multilayer assembly, the particles were labeled with azide functional Alexa Fluor 488 ($AF488_{Az}$) and the core removed by HF treatment. Images show capsules assembled from 10% PAA_{Az} at (a) pH 3.5 and (b) pH 10 and 20% PAA_{Az} at (c) pH 3.5 and (d) pH 10 solutions.

Figure 8 shows the capsule diameter with changing solution pH where both samples demonstrated swelling due to the ionization of carboxylic acid groups characteristic of those observed in click PAA capsules reported earlier.[34] However, the increased cross-linking may provide greater resistance to changes in size and shape, as observed in the 20% azide sample, which showed only slight buckling in

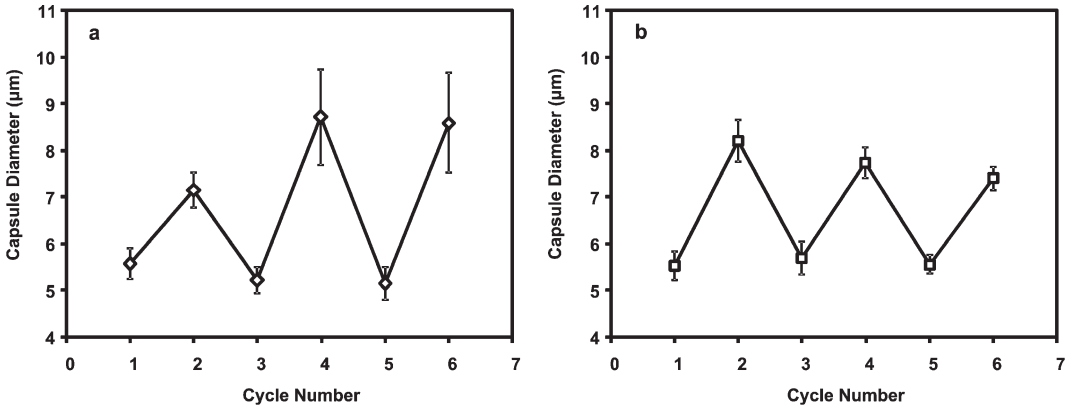


Figure 8. Swelling and shrinking behavior of PEI/$(PAA_{Az}/PAA_{Alk})_5$ capsules assembled with (a) 10% and (b) 20% azide functional PAA. AF488-labeled capsules were cycled through pH 2 and pH 10 solution, and capsule diameters were measured from fluorescence microscopy images. Data were averaged from no fewer than 10 measurements. Error bars show the standard deviation of capsule diameters measured by the largest straight line of capsule diameters.

comparison with the 10% azide sample after the addition of pH 10 solution, as shown in Figure 7, parts b and d, respectively. Because of the nonspherical shapes observed, capsule diameters were measured on the longest axis of the cross-section. In addition, capsules assembled from 10% azide functionality show larger diameters after the second addition of pH 10 solution. It is thought that this phenomenon is due to rearrangement or loss of polymers within the multilayer which is a result of insufficient covalent cross-linking. Nevertheless, the average capsule diameters were similar for both samples: at pH 2 capsule diameters were 5.3 and 5.6 μm for the 10 and 20% azide samples, respectively, while the capsule diameters were 8.1 and 7.8 μm at pH 10, respectively. Although the measured diameters of the capsules are similar, the folds and deformation observed in capsules assembled from the 10% azide functionality suggests a larger surface area after the addition of pH 10 solution.

Conclusion

The combination of click chemistry and LbL assembly affords a number of advantages for the assembly of multilayer thin films and hollow capsules. In this work, we investigated a number of parameters influencing the buildup of films and capsules assembled using this method with building blocks of azide- and alkyne-functional PAA. Observations into the buildup of the click multilayers indicate that the rate of polymer buildup for a layer is mostly diffusion controlled and occurs in an irreversible manner. Steric factors, as well as limited freedom of movement of the cross-linked polymer chains within the multilayer, are likely to contribute to the ultrathin nature of click layers. Control over film thickness and morphology was demonstrated by changing the ionic strength and pH of the assembly conditions. Furthermore, the thickness and swelling properties of films were shown to be controlled through the number of click moieties within the film. An exponential increase in thickness was observed for films made from PAA_{Az} over the azide functionalization range of 5−30%. The pH-responsive properties of the films were also found to depend on the degree of azide functionalization of PAA_{Az} used to assemble the films. Studies on the assembly of free-standing LbL multilayer capsules showed that an optimal polymer functionalization range for stable capsule formation is between 10 and 20%. The results gained from this study can be transferred to the assembly of covalently cross-linked films and capsules using a variety of polymer types for the development of novel devices for optics, drug and therapeutic delivery, sensing, microreactors, and surfaces for tissue engineering.

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Supporting Information Available: NMR data of azide functional PAA, ζ-potential of the buildup of PAA_{Az}/PAA_{Alk} multilayers on silica particles, determination of the degree of ionization of PAA by Fourier transform infrared measurements, and inductively coupled plasma mass spectrometry analysis of copper content in the capsules. This material is available free of charge via the Internet at http://pubs.acs.org.

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