Polymer Coatings Comprised Entirely of Soft and Semi-Permeable Microcapsules

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ABSTRACT: We report the fabrication and characterization of surface coatings that consist entirely of networks of hollow and semi-permeable polymeric microcapsules. These assemblies incorporate many useful functions of individual microcapsules into coatings with unique compartmentalized structures. Iterative immersion of substrates into suspensions of microparticles coated with polymer multilayers presenting either (i) amine or (ii) amine-reactive azlactone functionality resulted in the reactive/covalent layer-by-layer assembly of coatings of colloidal particles. Removal of particle cores yielded compartmentalized coatings consisting of semi-permeable hollow capsules that were physically stable in aqueous media and upon drying and rehydration. Confocal microcopy and SEM revealed these assemblies to have disordered structures with some apparent hints of stratification resulting from the iterative assembly process. Residual azlactone groups in the capsule walls enabled tailoring of interfacial properties and controlled release behaviors by treatment with hydrophobic or hydrophilic amines. Fabrication of hollow-capsule assemblies on sacrificial substrates permitted triggered delamination and subsequent transfer of these coatings to secondary surfaces. The coatings reported here are mixtures of two different kinds of hollow reactive capsules. This iterative approach could be also used to design coatings comprising many different types of capsules with different properties (e.g., different types of encapsulated cargo or degrees of permeability). The structures and properties of these hollow-capsule coatings could thus prove useful in many applied contexts, ranging from catalysis and controlled release to the development of soft-material coatings with tunable interfacial or mechanical properties.

KEYWORDS: Microcapsules; coatings; reactive; layer-by-layer; network; semipermeable

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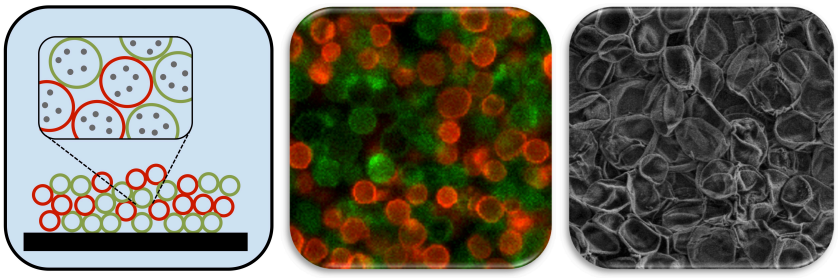

Introduction

Hollow polymer microcapsules are important in a range of fundamental and applied contexts, ranging from tools for basic research to new types of vehicles or containers for drug delivery and catalysis. Many different methods can be used to fabricate polymeric microcapsules, including approaches based on microfluidics,[1-4] phase separation,[5-8] interfacial polymerization,[9-13] and layer-by-layer assembly.[14-19] Of these approaches, layer-by-layer (LbL) assembly, in which sacrificial colloidal particles are iteratively coated with layers of polymer prior to subsequent dissolution of the underlying core, has proven particularly versatile because it enables a broad range of control over capsule structure and function (e.g., capsule size, wall thickness, internal or external functionality, permeability, mechanical properties, encapsulation/release properties, etc.) by judicious choice of polymer-based building blocks and assembly conditions.[14-26] This versatility has led to many exciting potential applications of multilayer capsules.[22,24,27-30] In most cases, polymer microcapsules are designed to be used as free-floating suspended 'containers', or are immobilized individually on surfaces and, in general, are designed to interact with their environments in isolation. In this paper, we report the fabrication of surface coatings consisting of networks of interconnected assemblies of hollow polymer microcapsules; these coatings incorporate several useful properties or functions of individual polymer microcapsules into uniform and conformal polymer coatings with unique, compartmentalized internal structures.

The work reported here was motivated by past work demonstrating that amine-containing polymer multilayer capsules could be immobilized on amine-reactive surfaces.[31] In that work, hollow polymer capsules were fabricated by the reactive layer-by-layer assembly of poly(2-vinyl-4,4-dimethylazlactone) (PVDMA; a polymer containing amine-reactive azlactone

functionality) and poly(ethyleneimine) (PEI; a polymer containing amine functionality) on sacrificial microparticle templates that could subsequently be etched and removed.[31] That reactive approach leads to capsules that can be readily functionalized after fabrication and that are physically stable in a wide variety of harsh environments (including at high ionic strength and at high surfactant concentrations).[31-33] It occurred to us that residual amine functionality on the surfaces of those immobilized capsules could also be used to support the reactive deposition of additional azlactone-functionalized capsules, and that this iterative approach could, potentially, be used to design new types of networks or coatings comprised entirely of hollow polymer capsules.

Here, we report a template-based approach to the design of coatings comprised of hollow, semi-permeable polymeric capsules. Our approach is based on the iterative exposure of surfaces to suspensions of model $CaCO_3$ microparticles coated with nanoscale amine- or azlactone-functionalized polymer multilayers. We demonstrate that subsequent removal of the sacrificial cores in these materials yields compartmentalized networks of interconnected hollow capsules that are physically stable in aqueous environments and able to host cargo for extended time periods. Characterization by confocal microscopy and scanning electron microscopy reveal these capsule assemblies to have relatively disordered structures consistent with an iterative, kinetically trapped assembly process driven by covalent interactions. The azlactone functionality remaining in these coatings allows tailoring of the interfacial properties and controlled release behaviors of these materials by post-fabrication functionalization with amine-containing compounds. Finally, we demonstrate that these novel capsule assemblies can be lifted off of their underlying substrates to produce free-floating films that can be subsequently transferred onto secondary surfaces.

The structures of these hollow-capsule assemblies differ from those of porous materials fabricated by the deposition of polymers on close-packed spherical templates, which typically leads to materials with open-cell structures.[34-36] These materials also differ substantially in structure, form, and scale from previously reported LbL films that contain embedded liposomal structures[37-42] or hydrogel microparticles.[43-45] Those and other related materials[46,47] are also sub-compartmentalized and have been investigated as hosts for agents in a broad range of applied contexts, but have structures and properties that are different from the coatings reported here, which consist entirely of interconnected and hollow microscale polymer capsules. In this latter context, the materials reported here are similar, in some ways, to recently reported 'protocellular' materials and, more broadly, to other micro-compartmentalized structures assembled from aggregates of semi-permeable protein/polymer or lipid vesicles.[48-50] The methods reported here enable the design of capsule assemblies composed of mixtures of multiple different types of capsules with different physical properties or contents, and could thus prove useful in contexts ranging from drug delivery and catalysis to new and general methods for the design of novel and responsive soft material coatings.

Materials and Methods

Materials. 2,2′-Azoisobutyronitrile (AIBN), branched poly(ethyleneimine) (PEI, M_W ~25,000 Da), fluorescein isothiocyanate-dextran (FITC-Dextran, M_W ~2000 kDa), cystamine hydrochloride purum (≥98.0%), acetonitrile, tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), calcein, calcium chloride, sodium carbonate, and acetone were purchased from Sigma-Aldrich (Milwaukee, WI). Cystamine was prepared, as described previously, by deprotonation of cystamine hydrochloride.[51] Tetramethylrhodamine (TMR) cadaverine was purchased from

Setareh Biotech. 6-Aminofluorescein was obtained from Tokyo Chemical Industry (Tokyo, Japan). Ethylenediaminetetraacetic acid trisodium salt hydrate (EDTA), 3-(dimethylamino)propylamine (DMAPA, 99%) and glutathione (GSH, 98%, reduced) was acquired from Acros Organics (New Jersey, USA). Phosphate-buffered saline (PBS) 10x was obtained from Dot Scientific (Burton, MI) and diluted in MilliQ water before use. Methanol was obtained from Macron Fine Chemicals (Center Valley, PA). Test grade n-type silicon wafers were purchased from Silicon Materials, Inc. (Glenshaw, PA). Glass coverslips and Lab-Tek II Chamber slides were obtained from Fisher Scientific (Rochester, NY). 2-Vinyl-4,4-dimethylazlactone (VDMA) was a kind gift from Dr. Steven M. Heilmann (3M Corporation, Minneapolis, MN). Poly(2-vinyl-4,4-dimethylazlactone) (PVDMA, M_W ~31,600; $Đ$ = 7.2) was synthesized by polymerization of VDMA, as reported previously.[52] PVDMA labeled with TMR (1 mol%; referred to as $PVDMA_{TMR}$ from here on) and 6-aminoflourorescein (5 mol%; $PVDMA_{FL}$) was synthesized as described previously.[52] Distilled water was deionized using a Milli-Q system (Millipore, Bedford, MA) to give water having a resistivity of 18.2 MΩ. All materials were used as received without further purification unless otherwise noted.

General Considerations. Fluorescence and bright-field microscopy images were acquired using an Olympus IX71 inverted microscope (Center Valley, PA) using a 4x, 20x, or 60x objective lens. Laser-scanning confocal microscopy (LSCM) was performed using a Leica SP8 3X STED super-resolution confocal microscope and processed using Leica Instruments Software or a Nikon AR1S HD confocal microscope and processed using Nikon Elements Software. Scanning electron microscopy (SEM) images were acquired using methods similar to those described previously.[32] Measurements of solution fluorescence were made using a Tecan Infinite 200 Pro

plate reader.

Coating of Planar Substrates. Glass or silicon substrates were cleaned using methods similar to those described previously.[53] Polymer solutions were prepared in acetone (20 mM; with respect to repeat unit molecular weight). Coatings were deposited layer-by-layer on substrates according to a previously reported procedure[54] to give PEI/PVDMA films ~40 nm thick having a final nominal layer of PVDMA. Redox-degradable coatings were fabricated using a similar procedure reported previously[55] to deposit 45 bilayers of PVDMA and cystamine, yielding films ~200 nm thick and having a final nominal layer of azlactone-containing PVDMA. Films were dried under a stream of air and then either used immediately or under vacuum until use.

Fabrication of Coated Particles. Spherical $CaCO_3$ microparticles with average diameters of ~3-5 μm, either unloaded or loaded with the fluorophores FITC-Dextran (~2000 kDa) or calcein, were fabricated using a method described previously.[56] Loaded particles were prepared by dissolving 1 mg/mL of FITC-Dextran or calcein into a $CaCl_2$ solution. Microparticles were rinsed using acetone prior to coating. PEI, $PVDMA_{FL}$, and $PVDMA_{TMR}$ were dissolved in acetone (20 mM with respect to the polymer repeat unit). Coated particles and hollow capsules were fabricated using methods reported previously,[32] using solutions of $PVDMA_{FL}$ for coated particles terminated with PEI or $PVDMA_{TMR}$ for those terminated with PVDMA. Additional layers were added until the appropriate number of layer pairs (four for PVDMA-terminated particles and four and a half for PEI-terminated particles) were deposited. After fabrication was complete, the coated particles were washed twice using DMSO and then re-suspended in DMSO.

Layer-by-Layer Assembly of Films of Coated Particles. Multilayer assemblies of PEI/PVDMA-coated particles were fabricated in Lab-Tek II chamber slides. Briefly, glass or silicon surfaces coated with PEI/PVDMA films (with PVDMA as the outermost layer) were cut into ~8 mm × 15 mm rectangles to fit into the chambers. Suspensions (5 mg/mL; 600 μL) of PEI/PVDMA$_{FL}$- or PEI/PVDMA$_{TMR}$-coated particles were then added into each chamber. The first layer of coated particles was deposited by submerging the substrates into the suspension of primary amine-containing PEI/PVDMA$_{FL}$-coated particles. The coated particles were allowed to settle onto substrates for 10 min, agitated, and allowed to settle for an additional 5 min to react with the amine-reactive surfaces. The substrates were then removed from the suspension and dipped into a DMSO rinse solution, followed by rinsing in a stream of DMSO, to remove any loosely bound particles from the surface. The second layer of particles was added to the assemblies by submerging the substrates into the suspension of amine-reactive PEI/PVDMA$_{TMR}$-coated particles and allowing the particles to settle on the substrate surface for 10 min, and then agitating and allowing them to settle for an additional 5 min to react with the primary amine-containing particles. DMSO was then used to rinse the substrates, as described above. This process of submerging the substrates in alternating suspensions of PEI/PVDMA$_{FL}$- and PEI/PVDMA$_{TMR}$-coated particles (with a DMSO rinse in between) was repeated until four layers (or some other desired number of layers) of coated particles had been deposited on the surface. The substrates coated with multilayer assemblies of particles were then rinsed with DMSO and methanol and dried under a stream of air. For experiments using materials formed by nonspecific interactions between coated particles, PEI/PVDMA$_{TMR}$-coated particles were treated with 3-dimethylaminopropylamine (DMAPA) in THF (20 mM) for 1 hr prior to assembly,[31] rinsed with THF, and re-suspended in DMSO. Substrates coated with multilayer assemblies of coated

particles were analyzed by fluorescence microscopy and SEM after 1, 2, and 4 layers of deposition. The substrates were dried under vacuum prior to SEM imaging. Cross-sectional SEM images of coated particles were acquired from cross-sections after fracturing the substrates.

Fabrication of Multilayer Assemblies of Hollow Capsules. The $CaCO_3$ cores of the multilayer assemblies of coated particles described above were etched by treatment with a 0.2 M solution of EDTA for 30 min. Substrates coated with the resulting assemblies of hollow capsules were rinsed with water three times and analyzed by fluorescence and confocal microscopy while submerged in water. The samples were then removed from water, dried under vacuum, and characterized in a similar manner as described above.

Lift-off and Transfer of Hollow Capsule Assemblies. Assemblies of polymer-coated microparticles were fabricated as described above on substrates coated with previously reported amine-reactive polymer multilayers that undergo triggered degradation in response to reducing agents.[55] These assemblies were then submerged in a 100 mM solution of EDTA (PBS; pH=7.4) to dissolve the $CaCO_3$ templates. Subsequently, an equal volume of a 10 mM solution of the reducing agent GSH in PBS was added to the EDTA solution (to yield a solution that was 50 mM EDTA, 5 mM GSH) to degrade the sacrificial polymer coating. Degradation of the underlying sacrificial film resulted in a free-floating film of hollow polymer capsules. A secondary substrate (e.g., a glass coverslip) was maneuvered under the free-floating film and the aqueous phase was then removed, first with a pipette and then by blotting any remaining solution with a Kimwipe. Samples were then dried overnight at ambient conditions resulting in a dried film of assembled hollow polymer capsules on the secondary substrate.

Results and Discussion

Fabrication and Characterization of Multilayer Assemblies of Mutually Reactive Particles

Assemblies of mutually reactive polymer capsules could, in principle, be fabricated by the iterative exposure of substrates to suspensions of two different types of chemically reactive hollow polymer capsules. In practice, however, hollow microscale capsules do not sediment readily, and they also often bend or fold upon contact with solid surfaces and, thus, do not pack as efficiently as spheres upon deposition onto a surface. We therefore pursued an alternative approach involving the assembly of polymer-coated microparticles, followed by subsequent removal of their solid cores. This template-based approach has the relative advantage of rapid sedimentation and the potential to control particle packing. In addition, this approach provides opportunities to design coatings consisting of cargo-loaded capsules (e.g., by using cargo-loaded cores). Overall, this template-based approach provides opportunities to access films composed of hollow capsules with internal structures, geometries, and degrees of interconnectedness that would be otherwise difficult or impossible to achieve using a hollow-capsule assembly approach.

We fabricated amine- and azlactone-functionalized colloidal particle building blocks by depositing PEI/PVDMA films on $CaCO_3$ microparticle templates (average diameter ~3-5 μm; See Materials and Methods) using reactive layer-by-layer assembly.[32] $CaCO_3$ cores were selected as colloidal templates because they are readily fabricated, can be used to encapsulate cargo, and can be etched and removed under mild aqueous conditions to facilitate the formation of hollow polymeric capsules.[56,57] Coated particles terminated with outer layers that present either amine-reactive (azlactone), or amine-containing outermost layers were prepared by manipulating the number of cycles used during layer-by-layer assembly. For example, completion of four full

PEI/PVDMA deposition cycles resulted in particles terminated with an outer layer of PVDMA (referred to from hereon as amine-reactive particles; shown schematically in Figure 1A). Likewise, particles coated with four and a half PEI/PVDMA deposition cycles contained an outer layer of amine-containing PEI referred to from hereon as amine-containing particles; shown schematically in Figure 1B). Planar glass or silicon substrates were then exposed iteratively to solutions of these mutually reactive particles. For these initial experiments, the glass or silicon substrates were coated with a thin PEI/PVDMA film presenting amine-reactive azlactone functionality (Figure 1C). We note that the speed and irreversibility of reactions between amine and azlactone functionalities could prevent particles from forming thermodynamically stable, closely-packed hexagonal assemblies (e.g., Figure 1D) typical of colloidal assemblies with reversible, non-covalent interactions,[58,59] likely yielding assemblies with more disorganized and kinetically-trapped structures (e.g., Figure 1E; *vide infra*).

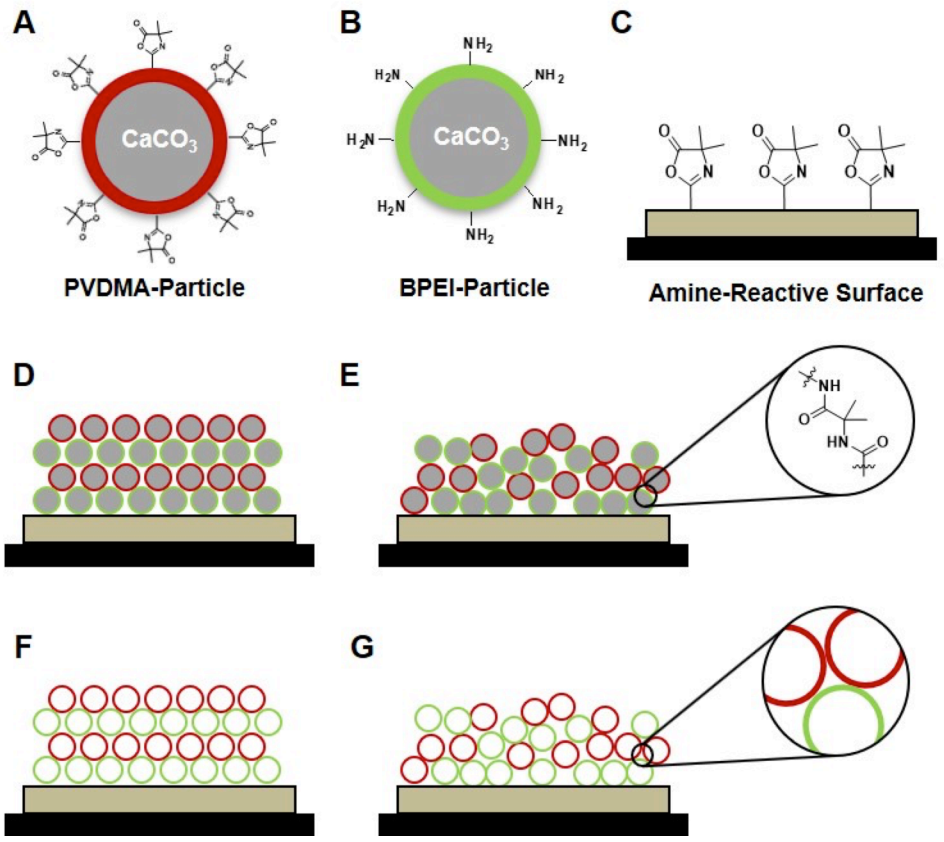

Figure 1: (A-C) Schematic illustrations showing (A) a PVDMA-terminated, PEI/PVDMA multilayer film-coated $CaCO_3$ microparticle (an amine-reactive particle), (B) a BPEI-terminated, PEI/PVDMA multilayer film-coated $CaCO_3$ microparticle (an amine-containing particle), and (C) a PEI/PVDMA base layer-coated (yellow) planar substrate (black) presenting amine-reactive azlactone functionality. (D-G) Schematic illustrations showing two possible arrangements (D,F, ordered; E,G, disordered) in assemblies of amine-containing and amine-reactive particles on amine-reactive surfaces before (D-E) and after (F-G) removal of the sacrificial $CaCO_3$ templates.

To facilitate characterization of coated particles and particle-containing films using fluorescence microscopy, we fabricated amine-containing and amine-reactive particles using fluorescently labeled PVDMA (amine-containing particles were fabricated using 6-aminofluorescein labeled PVDMA ($PVDMA_{FL}$), and amine-reactive particles were fabricated using tetramethylrhodamine cadaverine labeled PVDMA ($PVDMA_{TMR}$); see Materials and Methods and past studies[52]). In the fluorescence microscopy images discussed below, (i) green

fluorescence corresponds to $PVDMA_{FL}$ in amine-containing particles and (ii) red fluorescence arises from $PVDMA_{TMR}$ in amine-reactive particles.

Panels A-F in Figure 2 show fluorescence microscopy images of a silicon substrate after iterative deposition of amine-containing and amine-reactive particles. Figure 2A,B shows an image after the deposition of amine-containing particles. Inspection of these images reveals a

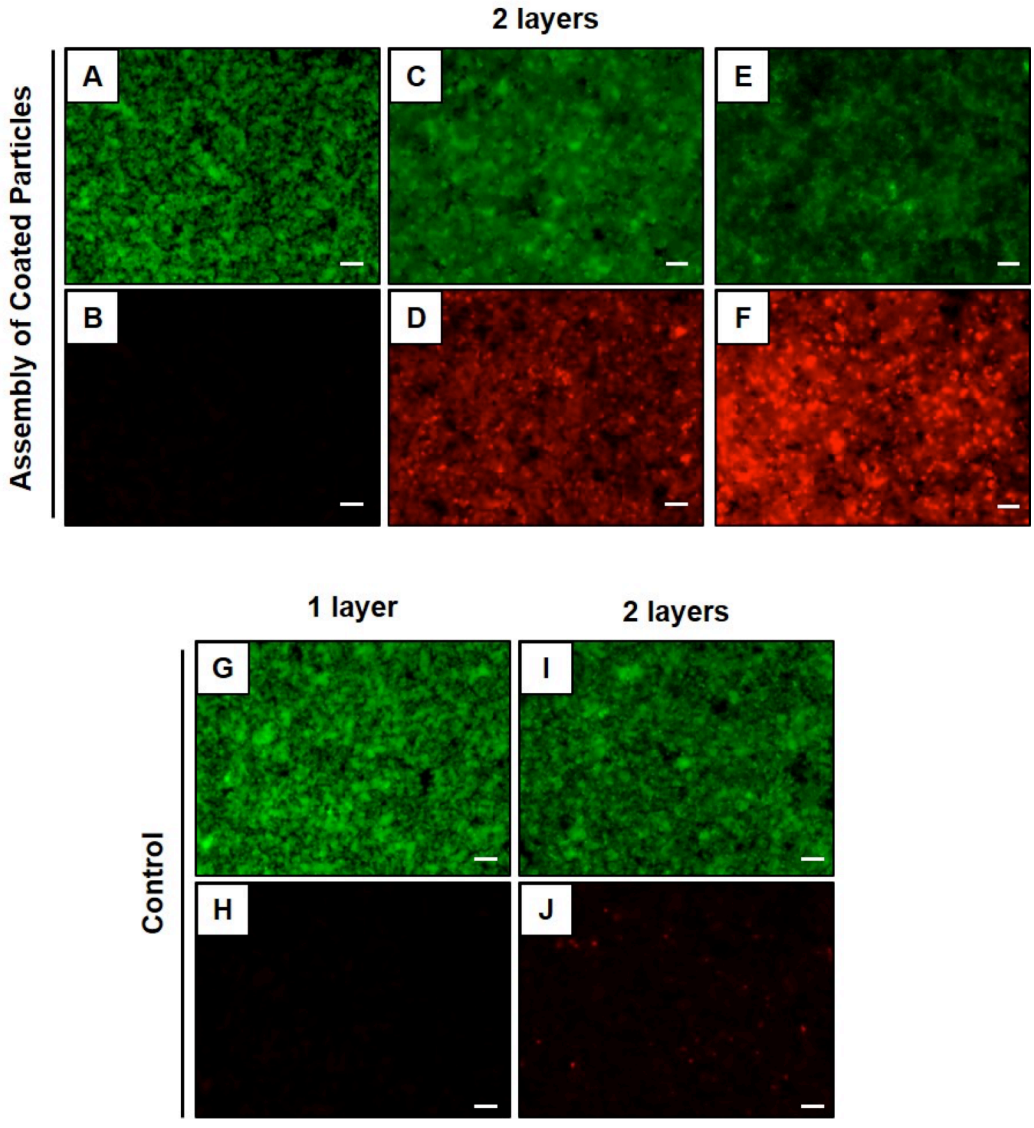

Figure 2: (A-F) Green (A,C,E) and red (B,D,F) fluorescence microscopy images of (A-B) a single layer, (C-D) a two-layer and (E-F) a four-layer assembly of amine-containing and amine-reactive particles on amine-reactive surfaces. (G-J) Green (G,I) and red (H,J) fluorescence microscopy images of a single layer (G,H) of PEI-particles after deposition of DMAPA-treated particles (I,J); see text for an additional discussion of this control experiment. Scale bars are 30 μm.

dense array of surface-immobilized amine-reactive particles, consistent with reactive immobilization on the amine-reactive surface. For convenience, substrates having been exposed to one deposition cycle are from hereon described as having one "layer" of particles, regardless of the specific morphology or degree of surface coverage. Further inspection of this image reveals some void spaces that were not covered with particles, as evidenced by the absence of green fluorescence.

This sample was then exposed to a solution of amine-reactive particles to produce a substrate containing two "layers" of particles (Figure 2C,D). These images reveal a large population of particles with red fluorescence (Figure 2D), and partial obstruction of green fluorescence arising from the underlying amine-containing particles (Figure 2C), consistent with the deposition of amine-reactive particles on top of the amine-containing particles. We then deposited a third layer of amine-containing particles and a fourth layer of amine-reactive particles, yielding an assembly with four nominal layers of coated particles. As shown in Figure 2E,F, these resulting four-layer assemblies appeared to exhibit nearly complete coverage of the surface, with fewer void spaces, suggesting an increase in surface-immobilized particle density as the number of deposition cycles increased.

To provide further insight into the nature of inter-particle interactions driving assembly of these structures, we conducted additional experiments using amine-reactive particles that were first treated with dimethylaminopropylamine (DMAPA), a small-molecule primary amine-containing nucleophile, to consume remaining azlactone groups and decorate the surfaces of these particles with tertiary amine groups.[31] These DMAPA-treated particles were then deposited onto the one-layer particle coating shown in Figure 2G,H. Inspection of Figure 2I,J reveals the resulting surface to be almost completely devoid of red fluorescence associated with DMAPA-

treated particles. When combined, these results are consistent with a mechanism of layer growth that involves reactions between amines and azlactones, and not simple, physical adsorption driven by other interactions.

We next characterized the morphologies and thicknesses of the assemblies above using SEM. Inspection of Figure 3A,C,E reveals the density of particles to increase as the number of layers increased, consistent with the results observed in Figure 2 using fluorescence microscopy. Inspection of cross-sectional images of these materials, shown in Figure 3B,D,F, reveals large

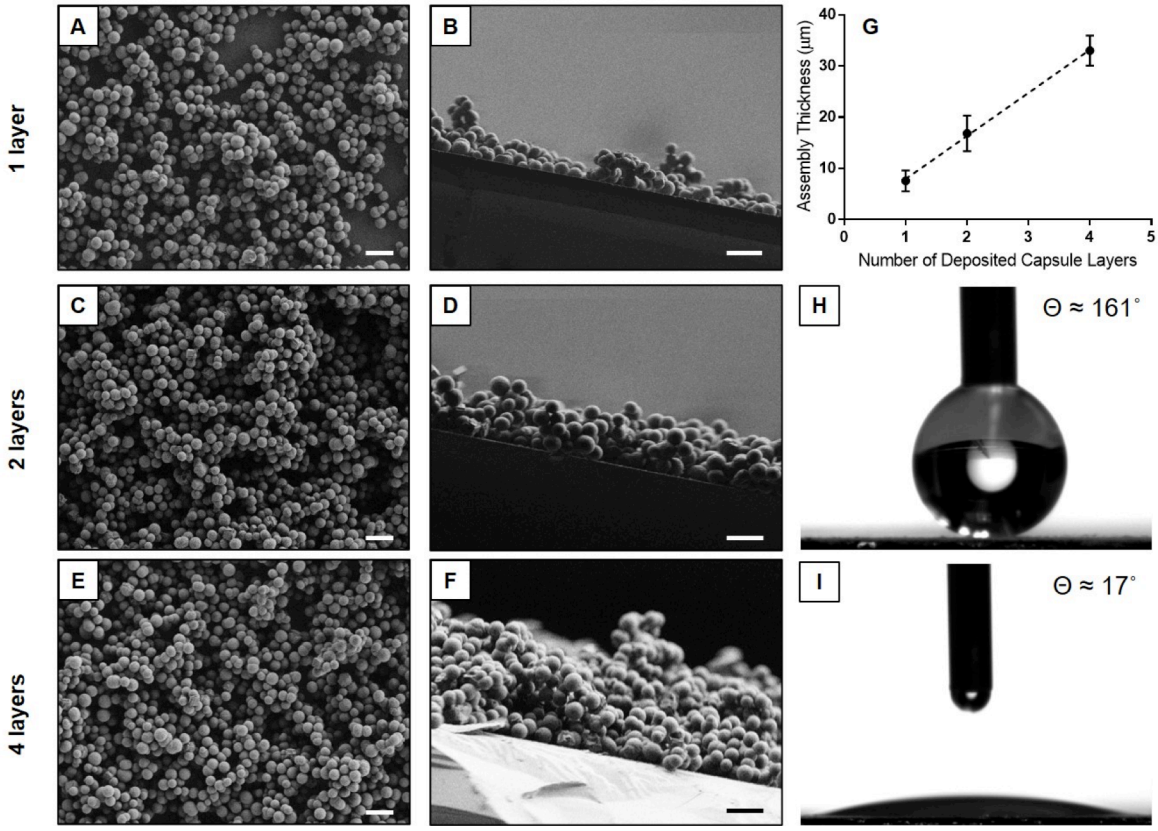

Figure 3: Top down (left column) and corresponding cross-sectional (middle column) SEM images for (A-B) one-layer, (C-D) two-layer, and (E-F) four-layer assemblies of amine-reactive and amine-containing particles on amine-reactive surfaces. (G) Growth profiles of amine-reactive and amine-containing capsule assemblies as measured by SEM. (H,I) Water contact angle analysis of four-layer assemblies of coated particles functionalized with either (H) decylamine or (I) DMAPA. Scale bars are 10 μm.

differences in film thickness. We measured the average thicknesses of the 1, 2, and 4 layer particle coatings to be 7.3 ± 2 μm, 16.2 ± 3.5 μm, and 31.7 ± 2.9 μm respectively (Figure 3G), suggesting approximately linear growth in thicknesses as the number of layers of particles was varied. Each layer of particles deposited appeared to be thicker than the average diameter of a particle, an outcome that likely arises from particle polydispersity, the non-close-packed nature of reactive assembly, and the occasional deposition of larger aggregates of several particles as can be seen in Figure 3. From these images we conclude that the internal structures of these material are more similar to the disordered arrangement of particles illustrated in Figure 1E than the ordered arrangement shown in Figure 1D.

These assemblies of coated particles could be further functionalized chemically by reacting the residual azlactone functionality in the particle coatings with small-molecule primary amines. Figure 3H,I shows water droplets placed on four-layer particle coatings after treatment with decylamine (a hydrophobic amine) and DMAPA (a hydrophilic amine). Inspection of these images reveals significant changes in water contact angles. Figure 3H reveals an advancing contact angle of 161.1 ± 4.7˚ for decylamine-treated films. This contact angle falls within the range generally considered to be "superhydrophobic",[60-62] a result that likely arises from the microscale topography present on the surfaces of these decylamine treated films (the contact angle hysteresis of these films was high (57.2 ± 6.6˚), however, likely due to a lack of corresponding roughness at the nanoscale[61]). Figure 3I reveals a contact angle of 17.4 ± 5.8˚ for DMAPA-treated films, and droplets immediately wetted and soaked into these hydrophilic materials. These results are consistent with functionalization with hydrophobic and hydrophilic functional groups.

Fabrication and Characterization of Multilayer Assemblies of Hollow Capsules

Additional experiments demonstrated that the $CaCO_3$ cores in the particle assemblies above could be etched and removed to form assemblies or networks of hollow polymer capsules. We found treatment of these materials with EDTA for 30 minutes (200 mM; pH =8) at room temperature to be sufficient to remove all $CaCO_3$ cores. The removal of the solid particle cores permitted imaging and characterization of hollow capsule structures using epifluorescence and confocal microscopy. Panels A-B in Figure 4 show images of a four-layer particle assembly after EDTA treatment (images were acquired for assemblies hydrated in water; see Figure S1 for additional images of one- and two-layer assemblies). Inspection of these images reveals the both green and red fluorescence arising from the fluorescently labeled polymers used to fabricate the amine-reactive and amine-containing particle coatings, respectively (and as described in the experiments above). These images contrast to those shown in Figure 2E,F above, in which the

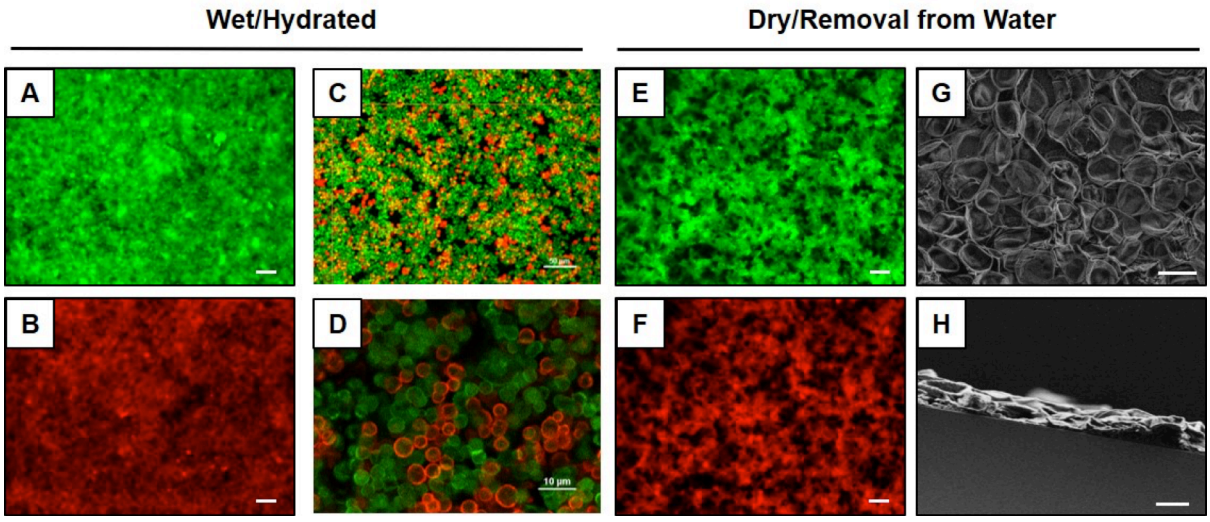

Figure 4: (A-B, E-F) Green (A,E) and red (B,F) fluorescence microscopy images of four-layer assemblies of amine-containing and amine-reactive particles after the removal of the $CaCO_3$ cores. Assemblies (A-B) incubated in water and (E-F) dried under vacuum. (C-D) Top-down confocal microscopy images of hydrated four-layer assemblies at different magnifications. (G-H) Top down (G) and cross-sectional (H) SEM images of the dried four-layer assembly shown in (A-B, E-F). Scale bars are 30 µm (A-B, E-F), 50 µm (C), 10 µm (D) and 4 µm (G-H).

presence of the particle cores obscured the observation of underlying layers of particles. No substantial detachment or disintegration of these assemblies was observed during these experiments, suggesting the general preservation of the overall multilayer assembly after EDTA treatment.

The confocal images shown in Figure 4C,D obtained from a focal plane located near the middle of the z-axis of the coating, reveal circular cross-sections of both green and red fluorescent capsules. These capsules were hollow and generally intact; we did not observe substantial evidence of capsule tearing or rupture by confocal microscopy or interconnected channels or through-pores between adjacent capsules at micrometer length scales. The presence of both green and red capsules in the same focal plane (Figure 4C, D) provides further support for the view that the structures of these assemblies are disordered and more similar to that depicted in Figure 1G, as opposed to perfectly stratified layers of capsules in a close-packed arrangement (Figure 1F). Additional confocal images shown in Figure S2, acquired at different locations along the *z* axis of a four-layer capsule assembly, hint that some degree of stratification in the assemblies could potentially be achieved, but the nature of the covalent assembly and dispersity of the model $CaCO_3$ microparticles used here likely leads to kinetically trapped structures.

The structures of these hollow capsule assemblies differ from those of open-cell structures fabricated in past studies by the deposition of bulk polymer or thin films on assemblies of sacrificial microparticle cores,[34-36] but are similar in some respects to recently reported 'protocellular' structures assembled from larger populations of microscale protein/polymer- or lipid-based vesicles.[48-50] The iterative approach to assembly used here allows for the design of compartmentalized capsule assemblies comprised of mixtures of different types of capsules. The

capsule assemblies described here were fabricated using two kinds of reactive capsules; however, in principle, this approach could be used to design assemblies containing many different capsules with different individual properties (e.g., physicochemical properties, encapsulated cargo, stimuli responsiveness, permeability, etc.). For example, in the context of the azlactone chemistry used here, we have reported other amine-reactive, azlactone-containing capsules that degrade hydrolytically[53] or reductively[55] that could be used to introduce additional stimuli-responsive functionality.

The fluorescence microscopy images in Figure 4E-H show the multilayer assemblies of hollow capsules characterized above after they were removed from water and dried. We observed more open spaces on the surface compared to the hydrated four-layer capsules shown in Figure 4A-D, presumably due to capillary effects resulting from evaporation of water and reductions in capsule volume or size as a result of collapse upon drying. Further evidence of this is seen in SEM images of dried capsules shown in Figure 4G,H. These images reveal flattened hollow capsules with substantial folds, consistent with the morphology of dried multilayer capsules reported in past studies,[13,20,29] suggesting that these assemblies can be dried without substantial tearing or cracking of the individual capsules that comprise them. Additional experiments discussed below demonstrated that these capsule assemblies can be dried, manipulated and rehydrated without substantial changes in overall structure.

The ability to dissolve and etch the cores of these particle assemblies creates opportunities to design hollow capsule assemblies that have the potential to encapsulate and release one or more other agents. We fabricated coated particles using $CaCO_3$ cores containing FITC-Dextran, a model water-soluble, fluorescently-labeled polymer. Figure 5A,B shows a microscopy image of hollow capsule assemblies fabricated by the assembly and subsequent

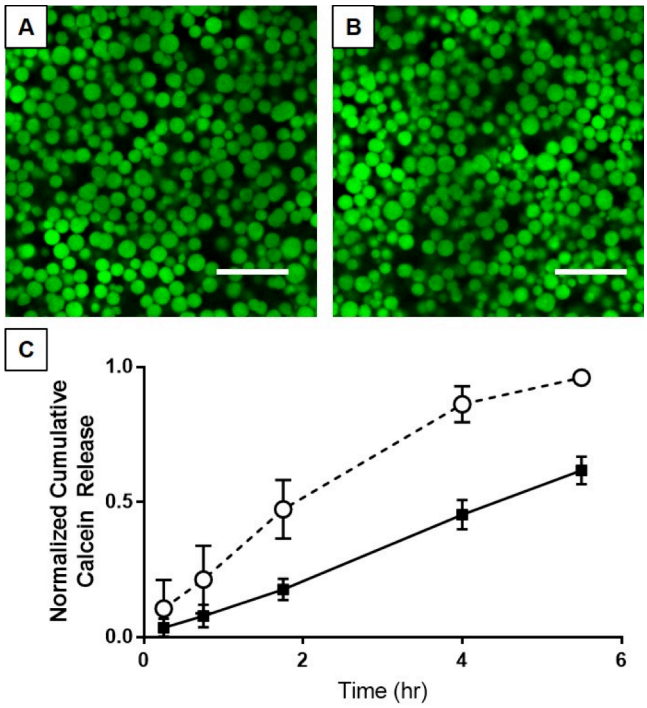

Figure 5: (A,B) Fluorescence confocal micrographs of four-layer assemblies of capsules loaded with 2000 kDa FITC-Dextran 1 hour (A) or 7 days (B) after core dissolution and incubation in PBS. (C) Plot showing the release of calcein from assemblies of coated particles incubated in 31 mM EDTA solutions in PBS with decylamine (filled) and DMAPA (open) surface functionality. The EDTA solutions were replaced at each of the timepoints shown. All data is normalized to the eventual cumulative release of calcein at the 24-hour timepoint, which is not shown here.

etching of film-coated particles containing 2000 kDa FITC-Dextran. We observed high levels of green fluorescence localized within the capsules within an hour of etching the $CaCO_3$ cores (Figure 5A) and after seven days of immersion in PBS (Figure 5B), suggesting that the walls of the capsules in these coatings remained intact and impermeable to this macromolecular cargo for extended periods of time.

In contrast, experiments preformed using particles encapsulating the small-molecule fluorophore calcein resulted in the rapid release of calcein upon etching of the $CaCO_3$ cores. Calcein is a small molecule (MW = 623 g/mol) and would be expected to pass readily through

the walls of the capsules used here. In these experiments, calcein release generally appeared to be controlled by the concentration of EDTA added or the rate at which it infiltrated the assemblies, both of which would impact the rate of $CaCO_3$ dissolution. The strategies described above to tailor the interfacial wetting properties of these materials (Figure 3H-I) could thus be used to control rates of calcein release. Figure 5C shows the release of calcein from six-layer assemblies of coated particles functionalized either with DMAPA (open circles) or decylamine (closed circles) and incubated in ~30 mM EDTA solutions in PBS. These results reveal decylamine-functionalized assemblies to release calcein more slowly over an initial six-hour time period as compared to DMAPA-functionalized assemblies. These results likely reflect differential rates of penetration of the EDTA solutions into these superhydrophobic and hydrophilic films (and, thus, differences in the rates at which the $CaCO_3$ cores dissolve, as discussed above). These differences in release rates are small, in part owing to the low molecular weight nature of calcein used as model fluorescent cargo here. It is likely that the functional properties of these films (e.g., porosity or permeability) could be tailored to tune the release profiles of these capsules over a broader range. In addition, azlactone-functionalized multilayer capsules reported in past studies that degrade hydrolytically[53] or reductively[55] could be used to design films that host and release high molecular weight cargo (e.g., enzymes, polymers, or particles) that are too large to diffuse through the capsule walls (e.g., Figure 5 A,B).

Fabrication of Freestanding Hollow Capsule Assemblies and Transfer to Secondary Substrates

During the course of these studies, we occasionally observed capsule assemblies stored in aqueous environments for prolonged periods to delaminate from underlying substrates and lift off as continuous sheets. Building from these observations, we conducted additional experiments

using hollow multilayer assemblies of capsules fabricated intentionally on sacrificial coatings that can be actively and selectively degraded to promote delamination. As a model, we fabricated hollow multilayer assemblies on thin (~200 nm) polymer films that are reductively degradable (see schematic in Figure 6A).[55] Figure 6B shows a four-layer capsule assembly after treatment with the small-molecule reducing agent glutathione (GSH) and subsequent delamination (the

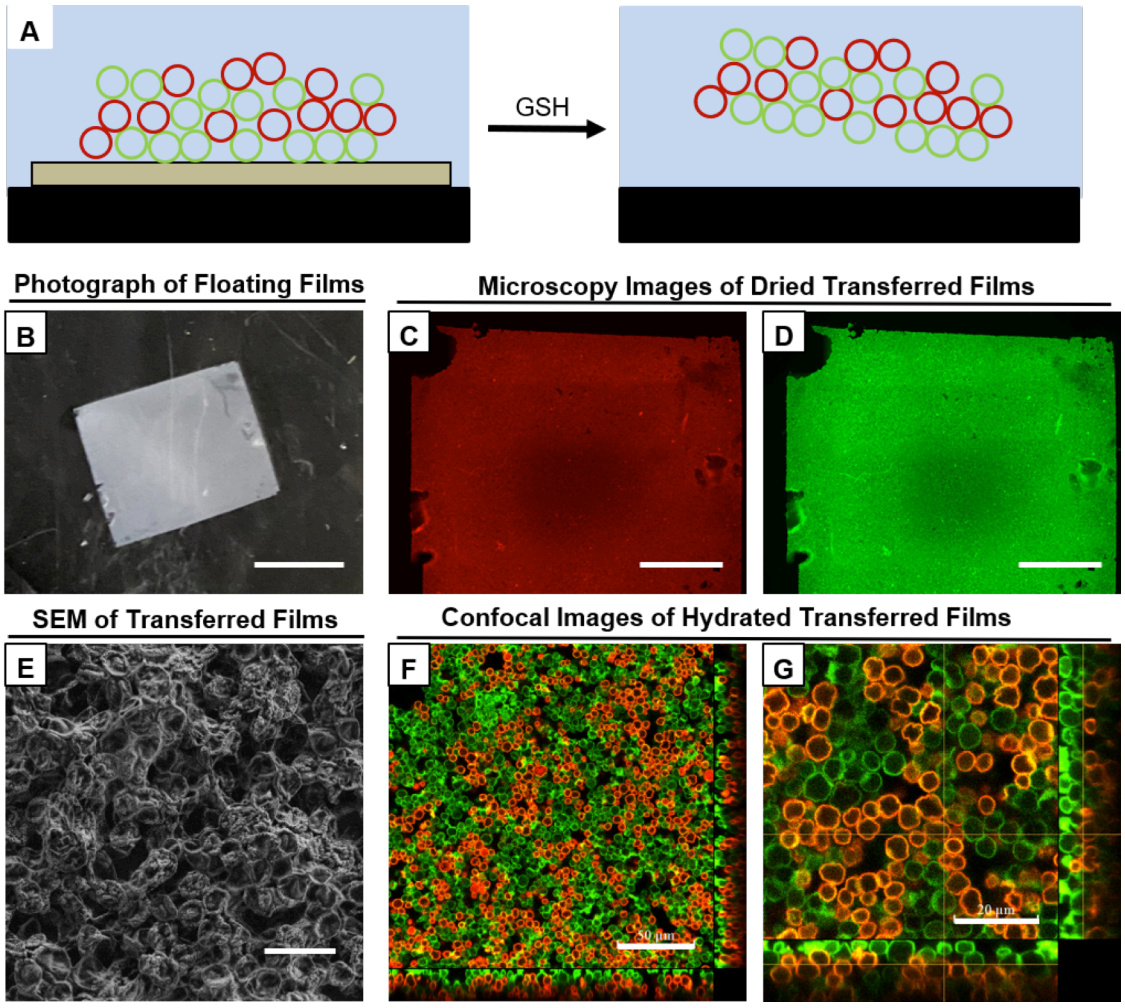

Figure 6: (A) Schematic illustration depicting the delamination of hollow-capsule assembly coatings from a substrate by the dissolution of a sacrificial redox-degradable film (yellow). (B) Digital photograph of a delaminated capsule assembly floating in buffer solution. (C-D) A reconstructed series of low magnification (4x) fluorescence microscopy images showing a dried film transferred onto a glass coverslip in the red (C) and green (D) channels. (E) SEM Image of a dehydrated capsule assembly after transfer to a glass cover slip. (F-G) Confocal microscopy images at various magnifications showing rehydrated capsule assemblies after transfer to a glass cover slip. Scale bars are (B) 5 mm, (C,D) 2.5 mm, (E) 10 μm, (F) 50 μm, and (G) 20 μm.

film here is shown suspended in aqueous media). These free-floating films could be readily transferred onto secondary substrates such as glass slides or other surfaces.

Figure 6 C-D shows red and green fluorescence microscopy images of a capsule assembly transferred to a glass coverslip and then dried, and shows that the film generally remained intact after manipulation and subsequent transfer. Figure 6F,G shows confocal microscopy images of transferred capsule assemblies that were dried and rehydrated, and reveals red and green capsules in a single *xy* confocal slice, similar to those shown in Figure 4C,D and Figure S2 (images were acquired in aqueous solution; the non-spherical shapes of some of these capsules are the result of variability in the shapes of the $CaCO_3$ cores used to fabricate these films and are not a byproduct of the transfer process). Further inspection of the *xz* and *yz* confocal reconstructions on the sides of Panels F and G of Figure 6 show distributions of green and red capsules in these planes that again hint at some degree of stratification that may arise from the iterative approach used to fabricate the coatings. Finally, Figure 6E shows a SEM image of a transferred capsule assembly after drying and removal of water. This image shows that these films can withstand lift-off, manipulation, and transfer to other substrates without formation of substantial tears, pores, or other defects at micrometer length scales.

Summary and Conclusions

We have reported the fabrication and characterization of hollow, semi-permeable polymeric microcapsule networks on surfaces using an iterative LbL approach. Iterative exposure of substrates to suspensions of sacrificial microparticles coated with multilayers containing amine or azlactone functionality, followed by removal of the particle cores, led to coatings consisting of mixtures of hollow polymer capsules. These capsule assemblies had

relatively disordered structures with some degree of potential stratification resulting from the iterative nature of the assembly process, as determined by confocal microscopy and SEM. These results are consistent with a process that leads to kinetically trapped structures rather than the close-packed structures typically exhibited by colloidal assemblies fabricated using weak/reversable interactions. These hollow capsule assemblies are physically stable in aqueous solutions and can be rehydrated and dried without apparent physical damage. Additionally, these semi-permeable capsules can be loaded with small-molecule and macromolecular cargo, suggesting potential applications in catalysis and controlled release. The residual azlactone groups in the colloidal building blocks used to fabricate these assemblies can also be used to tailor chemical properties that further influence the behaviors of these materials (e.g., wetting behaviors and rates of release of encapsulated cargo). Finally, we demonstrated that these assemblies can be delaminated from the substrates on which they are fabricated and subsequently transferred onto other secondary surfaces.

The studies reported here used two mutually reactive colloidal building blocks to design assemblies composed of mixtures of two types of hollow capsules. However, the iterative nature of this process could be used to design assemblies of many different types of building blocks to design hollow-capsule assemblies containing many different types of capsules. This approach could also be used with a range of other mutually reactive functional groups that could provide additional control over the properties of the capsules or the structure and organization of the resulting coatings.[63,64] The ability to define the properties or contents, and potentially the juxtaposition, of many different individual and compartmentalized capsules in a conformal coating could prove useful in a range of different contexts, ranging from drug delivery and

catalysis to the development of new soft material coatings with tunable interfacial and mechanical properties.

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Supporting Information. Additional fluorescence, confocal and scanning electron microscopy images showing characterization of hollow-capsule coatings (PDF). This material is available free of charge via the Internet.

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