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Multicompartment Particle Assemblies for Bioinspired Encapsulated Reactions

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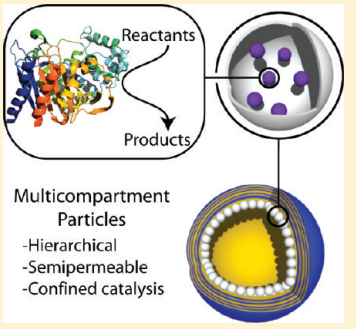
ABSTRACT: In recent years, interest in mimicking the complex hierarchical architectures and functionalities of biological systems and associated spatially confined reactions has led to the design and synthesis of a range of multicompartment particles. Multicompartmentalization allows the incorporation of a range of structures in a single particle, thus allowing optimization of physicochemical properties of the assembly (e.g., permeability, stability, stimuli-response) and various multiple, spatially separated reactions. In this Perspective, we describe particle-based multicompartment systems, detailing the building blocks and assembly approaches employed to produce each subcompartmentalized system. The properties that contribute to the performance of multicompartment systems in diverse applications are also discussed, with a focus on coupled enzymatic reactions in confined volumes. The challenges associated with these assemblies for encapsulated reactions are identified and further directions and plausible developments in the field are suggested.



Engineering micro- and nanoparticle systems has generated significant research interest due to their potential application in diverse fields such as encapsulated catalysis, sensing, and drug delivery.[1] A chief example is the creation of artificial cells, with the aim to mimic metabolic processes.[2] Biological cells have specialized internal subcompartments known as organelles, which are surrounded by a selectively permeable biological membrane that serves to partition the cell interior into numerous subcompartments. The movement of reactive species across these membranes is controlled by specific transmembrane protein channels, allowing precise regulation over the chemical species present within the organelles. Due to this unique compartmentalized structure, biological cells are able to spatially separate and precisely regulate hundreds of enzymatic (cascade) reactions, even in the presence of multiple potentially reactive species in the cell interior. Mimicking biological cells using bottom-up assembly has led to the development of several multicompartment assemblies. Assembling such nature-inspired structures presents a significant challenge; nevertheless, multicompartmentalization is a key requirement for the development of next-generation carriers because these systems combine physicochemical properties that are not readily achievable using single-component assemblies. This can lead to a marked improvement in the performance of such nanoengineered assemblies for confined chemical and biochemical reactions and triggered cargo release.

In this Perspective, we describe a number of multicompartment particles that use liposomes, cubosomes, polymersomes, polymer capsules or colloidosomes as the building blocks for the carrier and/or subcompartments. We discuss the application of multicompartment architectures, which include vesosomes (liposomes-in-liposomes), polymersomes-in-polymersomes, and subcompartmentalized polymer capsules (Figure 1), in the areas of microencapsulated catalysis and the creation of cell mimics.

Multicompartmentalization is a key requirement for the development of next-generation carriers because these systems combine physicochemical properties that are not readily achievable using single-component assemblies.

Cargo encapsulation within these assemblies and the properties that contribute to their performance in diverse applications are discussed, along with potential directions toward the development of artificial cell and cell organelle mimics.

Multicompartment Liposomes. Liposomes are formed via the self-assembly of amphiphilic phospholipids into closed bilayer membrane vesicles under aqueous conditions. Liposomes range from 50 nm to 50 μm in diameter, whereas phospholipid bilayers are typically 3 to 6 nm thick. The presence of hydrophobic and hydrophilic regions within the structure allows the encapsulation of a variety of cargo, including enzymes, oligonucleotides, peptides, proteins, imaging agents, and anticancer drugs.[3] Liposomes have been widely used in applications such as encapsulated catalysis and biosensing, and are intensively studied in different areas of biomimetic chemistry, biomembrane physics, and for

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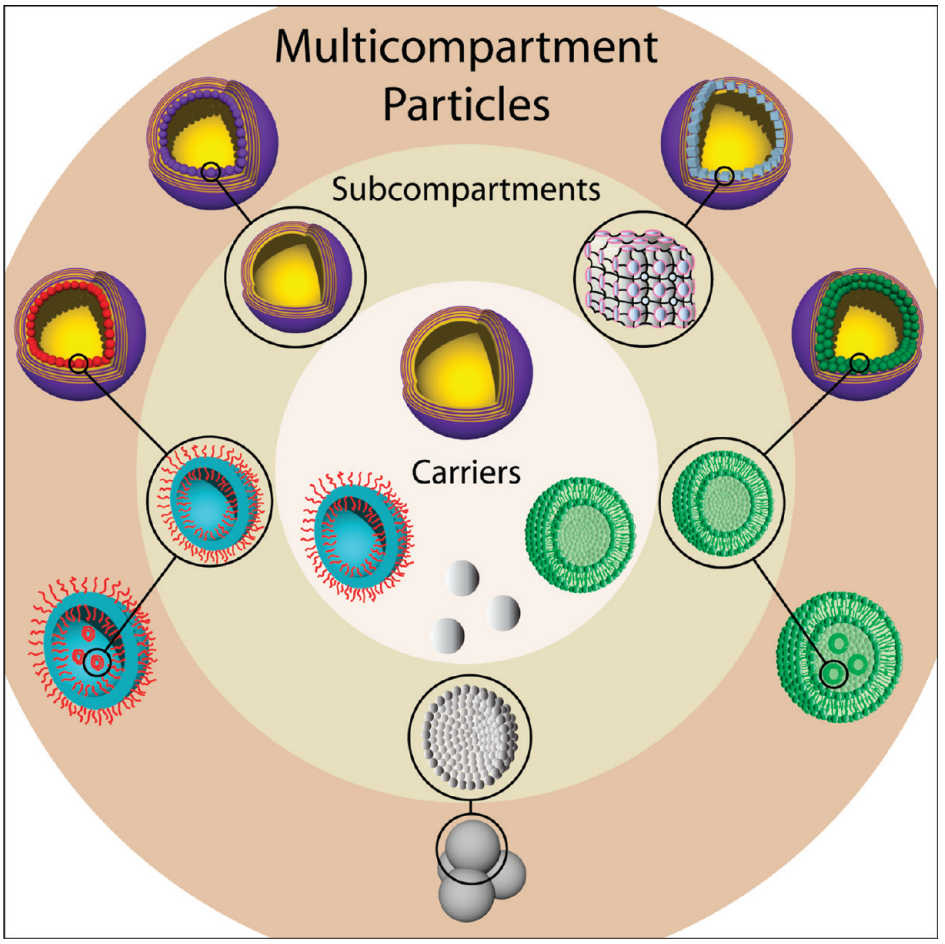

Figure 1. Examples of the carriers (center) and subcompartments (middle ring) used to fabricate multicompartment particles (outer ring). Center circle, clockwise from the top: Polymer capsules, liposomes, colloidal particles, and polymersomes. Middle ring, clockwise from the top right: Cubosomes, liposomes, colloidosomes, polymersomes and polymer capsules. Outer ring, clockwise from the top right: Polymer capsules subcompartmentalized with cubosomes or liposomes; liposomes-in-liposomes (vesosomes); multicompartment colloidosomes; polymersomes-in-polymersomes; and polymer capsules subcompartmentalized with polymersomes or polymer capsules.

artificial cell synthesis.[4] Their physical properties vary depending on the composition and chemistry of the phospholipids (e.g., lipid headgroup charge and saturation of the lipid alkyl chains). Like cellular bilayer membranes, liposomal membranes are impermeable to macromolecules, ionic species, and many small organic molecules, allowing spatial separation of different chemical environments. However, the enhanced permeability of lipid membranes at the gel−liquid phase transition temperature (T_m) can be used as a trigger to release encapsulated cargo.[5] In addition, pH- and light-responsive functionalities have also been incorporated into the lipid bilayer to facilitate triggered cargo release.[6] These properties make liposomes excellent candidates as encapsulated reaction vessels. Liposomal properties have been improved to make them more robust and enhance their performance through the addition of cholesterol to the lipid bilayer membrane; coating their surface with polymers such as poly(ethylene glycol) (PEG) or poly(*N*-vinyl pyrrolidone) (PVPON); and the incorporation of polymerizable lipid amphiphiles into the membrane.[3]

Liposomes permit the encapsulation of enzyme molecules without chemical modification of the enzyme, preserving the enzyme affinity to cofactor and substrate molecules. Several groups have utilized liposomes to conduct biomimetic, confined enzymatic reactions. For example, Luisi and co-workers loaded liposomes with DNA, enzymes, ribosomes, tRNAs, nucleoside triphosphates (NTPs), amino acids, and small molecules, for the confined expression of green fluorescent protein (GFP).[7] Other studies have also shown the confined synthesis of α-hemolysin,[8] β-glucuronidase,[9] and the cascade synthesis of T7-RNA polymerase and GFP,[10] thus demonstrating the flexible nature of liposome nanoreactors. However, because liposome membranes are impermeable to many molecules, fabrication of advanced artificial cell mimics typically requires the incorporation of protein transport channels[8,11] into liposome membranes, to allow the controlled movement of substrate and products across the membrane.

Liposomes-in-liposomes assemblies, termed vesosomes, represent a liposome-based subcompartmentalized system.[12] The assembly of nested bilayer compartments employs the reversible ethanol-induced interdigitation of saturated phosphatidylcholine bilayers. Below T_m, a number of saturated phospholipids form bilayer sheets upon the addition of ethanol. Above T_m, these membrane sheets become flexible, causing them to spontaneously close and form unilamellar vesicles, which can simultaneously encapsulate preformed liposomes in the suspension to form vesosomes. The vesosomes possess several advantages compared to unilamellar liposomes: the interior vesicles can be

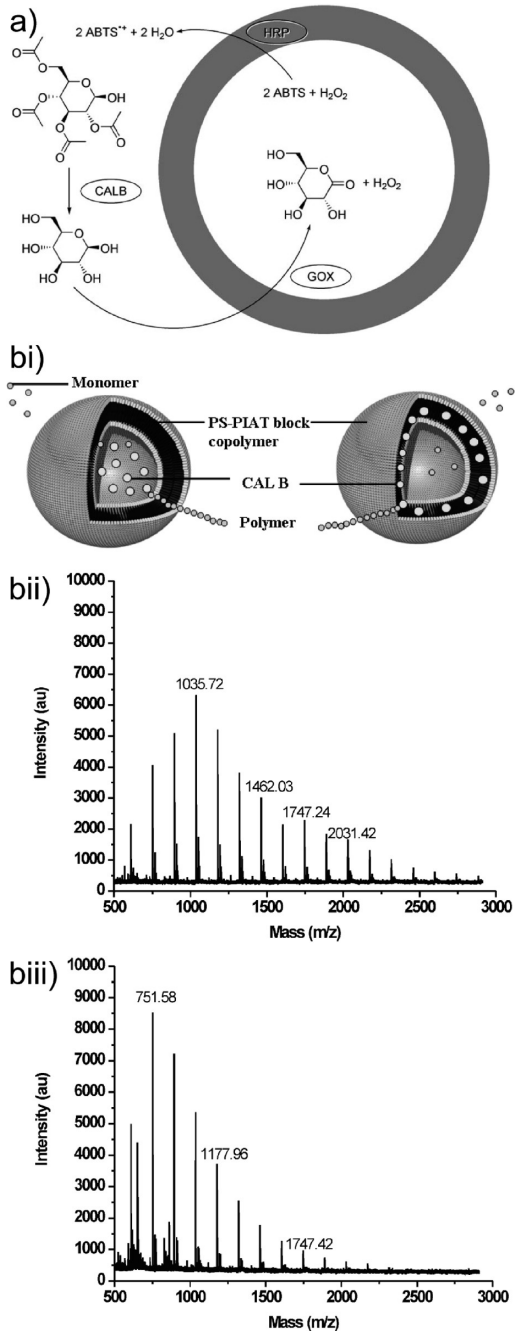

Figure 2. (a) Schematic representation of a three-step cascade reaction catalyzed by enzymes positioned within different polymersome compartments. CALB converts glucose acetate into glucose, which is then used by GOX and HRP for the conversion of ABTS to $ABTS^{\bullet+}$. (b) (i) Schematic representation of catalytic ROP of lactones into polyesters, in which the activity of the enzyme CALB was compared depending on whether it was positioned in the polymersome aqueous lumen or in the core of the membrane. (ii) MALDI-ToF mass spectrum showing the molecular weight distribution of polyesters formed through ROP catalyzed by CALB located in the polymersome lumen. The spectrum was similar to when CALB was free in solution (data not shown). (iii) MALDI-ToF mass spectrum showing the molecular weight distribution of polyesters formed through ROP catalyzed by CALB located in the hydrophobic polymersome membrane core. (a) Adapted from ref 27. Copyright WILEY-VCH Verlag GmbH & Co. KGaA. Reproduced with permission. (b) Adapted from ref 28. Copyright American Chemical Society. Reproduced with permission.

of different composition from each other and from the exterior vesicles; different cargo can be encapsulated within a single carrier in well-defined ratios; and release profiles of encapsulated molecules can be extended over longer times. Zasadzinski and co-workers demonstrated the encapsulation of titania particles, DNA, and a model drug prochlorperazine in vesosomes.[13] The multilamellar structure of vesosomes provides a physical barrier for the internal compartments to direct interaction with degradative enzymes in the external medium such as phospholipases, and consequently extends the retention of the encapsulated cargo by 2 orders of magnitude compared with unilamellar liposomes of the same composition.[14] Vesosomes incorporating different types of liposomes can also be used to conduct sequentially mixed reactions.[15] Encapsulation of dichlorodimethylacridinone phosphate into liposomes consisting of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine/1,2-dimyristoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (T_m = 23 °C) and fluorescein diphosphate into liposomes consisting of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine/1,2-dipalmitoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (T_m = 41 °C) was performed prior to incorporation of both liposome populations into vesosomes. Increasing the temperature above the T_m of each subcompartment caused sequential release of the encapsulated cargo into the outer vesosome compartment, where conversion to fluorescent products by alkaline phosphatase occurred. Such assemblies are gaining increasing interest as they allow the study of enzymatic reactions at the single molecule level,[15] facilitating the observation of previously unobserved enzyme behavior.[16]

Multicompartment Polymersomes. Polymersomes, which are synthetic mimics of liposomes, are formed via the self-assembly of amphiphilic block copolymers in the presence of a solvent that is miscible with only one polymer block.[17] Similar to liposomes, polymersomes can encapsulate and deliver both hydrophilic and hydrophobic cargo.[18,19] However, polymersomes possess greater mechanical stability[20] as a result of hydrophobic polymer chain interdigitation,[21] caused by the higher molecular weights of block copolymers compared with typical phospholipids. Moreover, the enhanced mechanical stability of the polymer membrane compared with its phospholipid counterpart does not compromise its elastic and fluid-like nature.[22] Structures formed from block copolymers can retain encapsulated cargo for long time periods (i.e., at least 3 months)[23] as a consequence of their very slow membrane dynamics and subsequent formation of kinetically stable, locally isolated structures.[24] Furthermore, the synthetic nature of the block copolymers allows the incorporation of diverse stimuli-responsive functionalities, facilitating the design of complex multicompartment assemblies. Because of these desirable properties, a number of research groups have used polymersomes as enzymatic nanoreactors with attention increasingly focused on fabricating multicompartment polymersome assemblies as cellular mimics.

To facilitate the continuous exchange of substrate and product molecules, fabrication of biomimetic polymersome nanoreactors usually requires the introduction of protein transport channels into the hydrophobic membrane,[25] because block copolymer membranes are typically impermeable to most molecules. To utilize the aqueous lumen of poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA–PDMS–PMOXA) polymersomes as a vessel for biomineralization, Meier and co-workers incorporated ion-carrier channels into the impermeable polymersome membrane.[25] These membrane proteins allowed the passage of calcium into the aqueous polymersome lumen for the controlled precipitation of calcium phosphate.

However, the nonpermeable structure of polymersome membranes is also advantageous in many circumstances, as chemical concentration gradients can be produced. Proton concentration gradients are employed in cellular organelles to drive many biochemical reactions. Choi and Montemagno incorporated multiple protein transport channels (proton pumps) into poly(2-ethyl-2-oxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-ethyl-2-oxazoline) polymersomes for the biomimetic production of adenosine triphosphate (ATP).[26] The synthesis was conducted by a sequence of reactions that utilized two different proton pumps, demonstrating the potential of these polymersomes to act as functional cell organelle mimics.

To simplify the assembly of polymersome nanoreactors, van Hest and co-workers pioneered the synthesis of semipermeable polymersomes, which can retain macromolecules such as enzymes, but are permeable to small molecules,[27] thus eliminating the requirement for membrane-bound protein transport channels. These polymersomes comprise a rigid hydrophilic block, poly(L-isocyanoalanine(2-thiophen-3-yl-ethyl)amide) (PIAT), and a mobile hydrophobic block, polystyrene (PS). It was demonstrated that the compartmentalization of three different enzymes within separate polymersome regions could be precisely controlled. The enzymes, *Candida antarctica* lipase B (CALB), glucose oxidase (GOX), and horseradish peroxidase (HRP), were placed outside the polymersome, within the polymersome lumen, and within the polymersome membrane, respectively. These encapsulated enzymes were capable of catalyzing coupled reactions, which resulted in the formation of a lactone from glucose and the subsequent release of hydrogen peroxide within the polymersome lumen for catalytic reaction with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), yielding $ABTS^{\bullet+}$ (Figure 2a). The conversion in this cascade of reactions was 100 times greater than when the enzymes were not encapsulated. Furthermore, following their incorporation into polymersomes, the enzymes showed increased retention (87.5 ± 5%) of their original activity over 1 month compared with the free enzymes in solution, which showed total activity loss in less than 1 week.

PIAT−PS polymersomes have also been used as confined, regulated containers for the enzymatic ring-opening polymerization (ROP) of lactones into polyesters within an aqueous environment (in bulk solution, lactones are readily hydrolyzed by water, inhibiting polymerization).[28] It was envisaged that the confinement of the enzyme CALB within porous PIAT−PS polymersomes would alter the conditions of the ROP reaction, resulting in a change in the position of the equilibrium to favor the formation of polymers/oligomers. The enzymatic ROP of lactones upon positioning CALB in the polymersome aqueous lumen was compared with when it was confined within the hydrophobic membrane (Figure 2bi). When encapsulated in the lumen, the enzymatic activity was comparable to the "free" enzyme in bulk aqueous medium, and MALDI-ToF mass spectra revealed that the molecular weight distributions of the resulting polyesters were similar (the spectrum for when the enzyme was positioned in the lumen is shown in Figure 2bii). The polyesters generated following CALB loading in the polymersome membrane were of lower molecular weight (Figure 2biii), which was attributed to steric hindrance of the enzyme. The limitation of this system, however, was that formation of the polymer product resulted in loss of the polymersome morphological integrity.

In addition to these examples of single-compartment polymersome nanoreactors and artificial cell organelles, more complex structures comprising subcompartmentalized polymersomes have recently been developed. The first example of this was reported by Chiu and co-workers,[29] in which poly(acrylic acid)-*co*-poly(distearin acrylate) P(AA-*co*-DSA) polymersomes (prepared via a double emulsion technique) were developed. These polymersomes allowed the passage of polar molecules, including macromolecules such as hemoglobin, through channels across the polymersome membrane, which were created in response to the pH-sensitive acrylic acid domains changing their ionization state in response to an increase in pH from 5 to 8. While the passage of polar molecules such as calcein was hindered at pH 5, interchain repulsion between PAA chains at basic pH triggered the formation of pores across the membranes and subsequent passage of polar molecules.

In addition to multicompartment polymersomes incorporating responsive transport domains, polymersome assemblies formed using different polymersomes for the carrier and subcompartments have emerged as promising cell and cellular organelle mimics, as they afford increased levels of spatial organization and complexity. Recently, Nallani and co-workers prepared multicompartment polymersomes using nonpermeable PMOXA−PDMS−PMOXA polymersomes encapsulated within porous PIAT−PS polymersomes

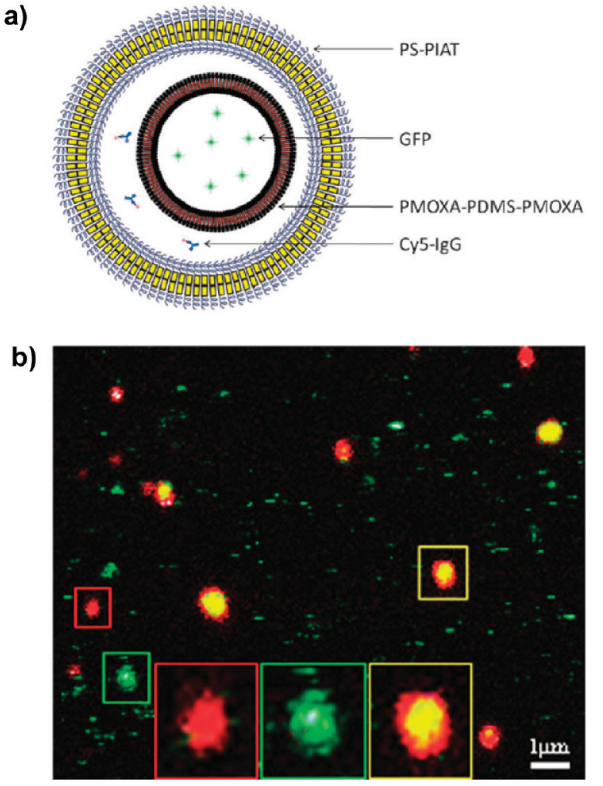

Figure 3. (a) Schematic representation of the multiple polymersome assembly comprising two distinct polymersome compartments, loaded with different fluorescent cargo, without leakage between the compartments. (b) Confocal laser scanning microscopy image of single and multiple polymersome assemblies. Red fluorescence shows Cy5-IgG loaded in PS−PIAT polymersomes while the green fluorescence corresponds to GFP encapsulated in PMOXA−PDMS−PMOXA polymersomes. Colocalization of the red and green signals, resulting in yellow emission, corresponds to the multicomponent polymersomes comprising PS−PIAT polymersomes subcompartmentalized with PMOXA−PDMS−PMOXA polymersomes, each loaded with their respective fluorescent cargo. Adapted from ref 30. Copyright Royal Society of Chemistry. Reproduced with permission.

(Figure 3a).[30] By adding a solution of PIAT−PS in tetrahydrofuran to preformed PMOXA−PDMS−PMOXA polymersomes in aqueous solution, a mixture of single and subcompartmentalized polymersomes was formed. It was demonstrated that a model protein (cyanine-5 conjugated immunoglobulin G (Cy5-IgG)) could be encapsulated within PIAT−PS polymersomes without leakage into the PMOXA−PDMS−PMOXA subcompartments (Figure 3b). This assembly process is also promising for confined catalytic applications, as incorporation of polymersome subcompartments responsive to different stimuli would permit sequential mixing of multiple encapsulated cargo and subsequent reactions.

Polymersome assemblies formed using different polymersomes for the carrier and subcompartments have emerged as promising cell and cellular organelle mimics, as they afford increased levels of spatial organization and complexity.

Subcompartmentalized Polymer Capsules. Multilayer polymer capsules fabricated by the layer-by-layer (LbL) process have emerged as versatile components for the assembly of multicompartment particles due to their desirable physicochemical properties. Colloidally stable polymer capsules of controlled size are produced by the sequential adsorption of interacting polymers, such as polyanions and polycations or hydrogen-bond donating and accepting polymers, onto micro- or nanoparticles followed by template dissolution.[31−36] Polyelectrolyte multilayers are often highly charged and hence typically exhibit high biofouling. Consequently, for biological applications, hydrogen-bonding interactions can be used to drive assembly. This allows the incorporation of neutral, low-fouling polymers such as PEG and PVPON,[34,35] yielding capsules that are responsive over a biologically relevant pH range.[36] The pH sensitive nature of hydrogen-bonding interactions requires covalent cross-linking of the polymer multilayers to produce capsules stable over a wide pH range. This is achieved using bifunctional small molecule cross-linkers[37] or functionalized polymers, such as thiolated poly(methacrylic acid) (PMA_{SH}).[38,39] The cross-linking process is highly versatile and permits physicochemical properties such as permeability[40] and degradation[41] to be tailored by varying cross-linking density.

LbL polymer capsules are typically permeable to small molecules,[42] but show size-dependent permeability to macromolecules,[43] allowing confinement of catalytic enzymes and protection of the cargo against external degradative enzymes. Subcompartmentalization of LbL capsules with nonpermeable structures can improve confinement of small molecules, such as dyes and drugs.[44] Nevertheless, the permeable nature of polymer multilayers is often advantageous in microreactor applications, because it permits the continuous exchange of small substrate and product molecules. Lvov and co-workers confined HRP to the interior[45] and exterior[46] of polyelectrolyte capsules for catalytic phenol polymerization. Hydrogen peroxide (H_2O_2) and the phenol, tyramine, readily permeated the capsule walls, where HRP catalyzed the formation of tyramine-derived polyphenols, which were retained within the capsules. Localization of HRP to the exterior of the capsule resulted in polymerization of tyramine producing a polyphenol layer of controllable thickness upon the polyelectrolyte capsules.

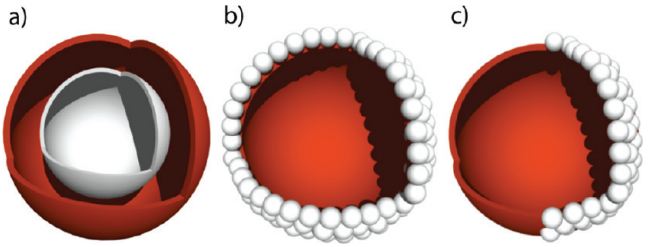

Figure 4. (a) Concentric two-compartment capsule showing inner (white) and outer (red) capsules. Multilayer structure of each capsule not shown for clarity. (b) Pericentric capsule showing a carrier capsule (red) decorated with multiple subcompartments (white). Subcompartments may be of different composition or cargo loading and may be contained within the carrier capsule or adsorbed onto the surface. (c) A Janus capsule formed via anisotropic deposition of subcompartment capsules (white) to the surface of the carrier capsule.

The semipermeable nature of LbL microcapsules was also exploited by Price et al., who reported that by encapsulating DNA and the endonuclease DNase I within the interior of cross-linked PMA capsules, DNA degradation could be triggered through the diffusion of divalent cations (Ca^{2+} and Mg^{2+}) across the capsule walls, which activated the endonuclease at 35 °C.[47] Successful degradation of encapsulated fluorescently labeled DNA was confirmed via disappearance of the fluorescent signal from the capsule interior. This represents an advance toward developing cell mimics in which continuous biochemical reactions can be confined and controlled. Price et al. also utilized polymer capsules for continuous RNA synthesis from an encapsulated DNA template.[48] RNA polymerase permeated the capsule walls and bound to its corresponding promoter sequence on encapsulated double-stranded DNA, triggering RNA synthesis via its transcription. Due to size and charge repulsion effects, the transcribed RNA remained entrapped within the capsules.

An extension of single-compartment capsule microreactors is multicompartment capsule assemblies (Figure 4), which facilitate the segregation of multiple enzymes for spatial control of enzymatic cascade reactions. For example, Kreft et al.[43] assembled concentric two compartment polyelectrolyte capsules containing GOX and peroxidase (POD), confined to the outer and inner compartments, respectively. In the presence of appropriate substrates, GOX and POD catalyze a reaction cascade producing resorufin, which can be detected using fluorescent techniques (Figure 5a). The two compartment capsules were fabricated by sequential coprecipitation of the enzymes with calcium carbonate ($CaCO_3$) followed by LbL assembly onto the particles and core dissolution. Glucose was oxidized by GOX to produce H_2O_2 that diffused through the polymer multilayers into the inner compartment and free solution. Following the addition of amplex red, an increase in red fluorescence, due to production of resorufin, was observed in the inner capsule compartment. Formation of resorufin could not occur in the outer compartment or free solution because of the confinement of POD to the inner compartment. It was proposed that the eventual increase in fluorescence in the outer compartment was due to steady state diffusion of resorufin from the inner compartment. Although

qualitative in character, the biomimetic confinement of a cascade enzymatic reaction was demonstrated.

Using the enzyme−$CaCO_3$ coprecipitation approach, Bäumler et al.[49] fabricated a three-compartment enzyme particle system containing β-glucosidase (βGLU), GOX, and POD confined to outer, middle, and inner compartments, respectively. The start of the reaction cascade (Figure 5a) was detected by increased green fluorescence in the outer compartment, caused by βGLU catalyzed hydrolysis of a nonfluorescent substrate into glucose and fluorescein. Glucose thus triggered the GOX−POD cascade, the end of which was detected by increased red fluorescence confined to the inner compartment. Significantly, particles with barrier compartments of bovine serum albumin (BSA) located between the enzyme compartments showed a retarded reaction rate in comparison to particles without barrier compartments (Figure 5b). This was attributed to the additional diffusion resistance for substrate molecules resulting from the barrier layers. By tailoring barrier thickness, these structures may allow individual kinetic parameters in complex enzyme cascades to be determined.

Multilayer polymer capsules fabricated by the LbL process have emerged as versatile components for the assembly of multicompartment particles due to their desirable physicochemical properties.

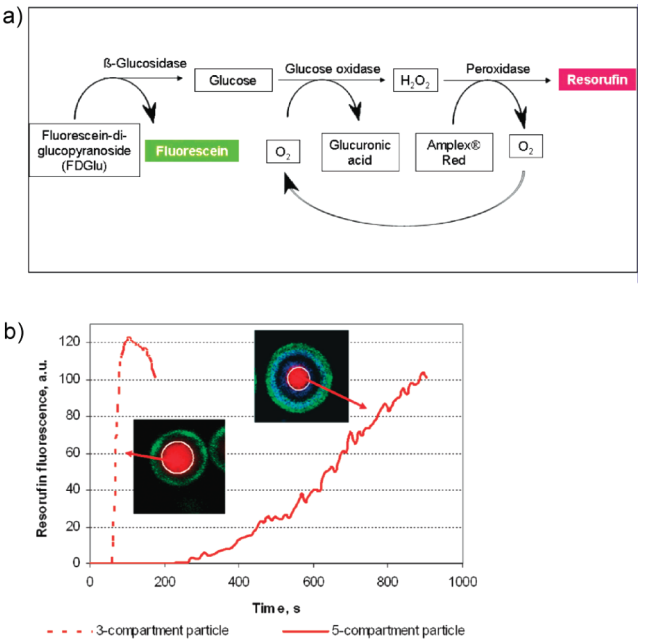

Figure 5. (a) Enzymatic cascade reaction performed in three compartment enzyme particles using fluorogenic substrates to allow monitoring of reaction kinetics. (b) Time-dependent increase in resorufin fluorescence for three compartment three-enzyme particles and five compartment three-enzyme particles, i.e., particles containing BSA barrier compartments between enzyme compartments. Green represents fluorescein located in the outer compartment, and blue represents Alexa Fluor 680-labeled BSA (not enzymatically active). Adapted from ref 49. Copyright American Chemical Society. Reproduced with permission.

Alternatively, pericentric-type multicompartment capsules[50] can be produced by adsorbing small capsules onto template particles followed by additional multilayer assembly on these coated particles. Pericentric assembly facilitates incorporation of a greater number of subcompartments compared with concentric assembly,[50] potentially leading to higher enzyme loading. Using this approach, Kulygin et al. assembled PMA_{SH} capsules containing smaller PMA_{SH} capsule subcompartments.[51] 300 nm-diameter cross-linked PMA_{SH} capsules were first adsorbed onto 3 μm-diameter aminated silica (SiO_2^+) particles, followed by assembly of PMA_{SH} and PVPON multilayers onto the capsule-coated SiO_2^+ particles. Control over the number of subcompartments was achieved by varying the ratio of 300 nm capsules to 3 μm SiO_2^+ particles. Cross-linking of the outer PMA_{SH} multilayers with a bifunctional thiol-reactive cross-linker and subsequent silica dissolution afforded PMA_{SH} carrier capsules subcompartmentalized with smaller PMA_{SH} capsules. Selective degradation of the subcompartments or carrier capsules could be achieved by employing either degradable or nondegradable cross-linkers. Moreover, subcompartments responsive to different stimuli incorporated into the capsules could be selectively degraded without damaging the integrity of the carrier capsules, demonstrating the potential for triggered mixing of macromolecular substrates and enzymes encapsulated within the subcompartments.

Fabrication of capsules with anisotropic composition, or Janus capsules, is attracting increasing interest as they may act as large amphiphiles, allowing the self-assembly of supramolecular aggregate structures.[52,53] To fabricate anisotropic, multicompartment capsules, Delcea et al.[54] first produced small (500 nm-diameter) and large (4.8 μm-diameter) polyelectrolyte capsules. The large capsules were then embedded in a multilayer film of hyaluronic acid (HA) and poly(L-lysine) (PLL) supported on a glass slide. Partial embedding of the large capsules was observed, thus allowing the anisotropic adsorption of small capsules to the surface of the large capsules by electrostatic interactions. Multicompartment Janus capsules were isolated by inversion of the glass slide followed by the addition of sodium hydroxide, causing disassembly of the HA/PLL film without compromising the integrity of the Janus capsules.

Polymer capsules subcompartmentalized with structures other than polymer capsules have also received attention, as particles with a range of complementary physicochemical properties can be produced. A novel subcompartmentalized assembly was recently introduced by exploring the combination of liposomes and polymer capsules to form structures known as capsosomes.[55] The key advantages of liposomes and polymer capsules are maintained by combining these two fundamentally different systems. Polymer capsules serve as colloidally stable scaffolds and allow for fine control over the membrane permeability by varying the type of deposited polymer and number of polymer layers adsorbed. In contrast, liposomes can stably encapsulate small, fragile hydrophobic and hydrophilic cargo, and divide the interior of the capsule into subcompartments. The architecture of the capsosomes therefore allows controlled interaction between the internal and external milieu, and represents a promising platform for constructing synthetic cell mimics.

The creation of capsosomes is based on LbL assembly of liposomes and polymers onto particle templates (Figure 6a).

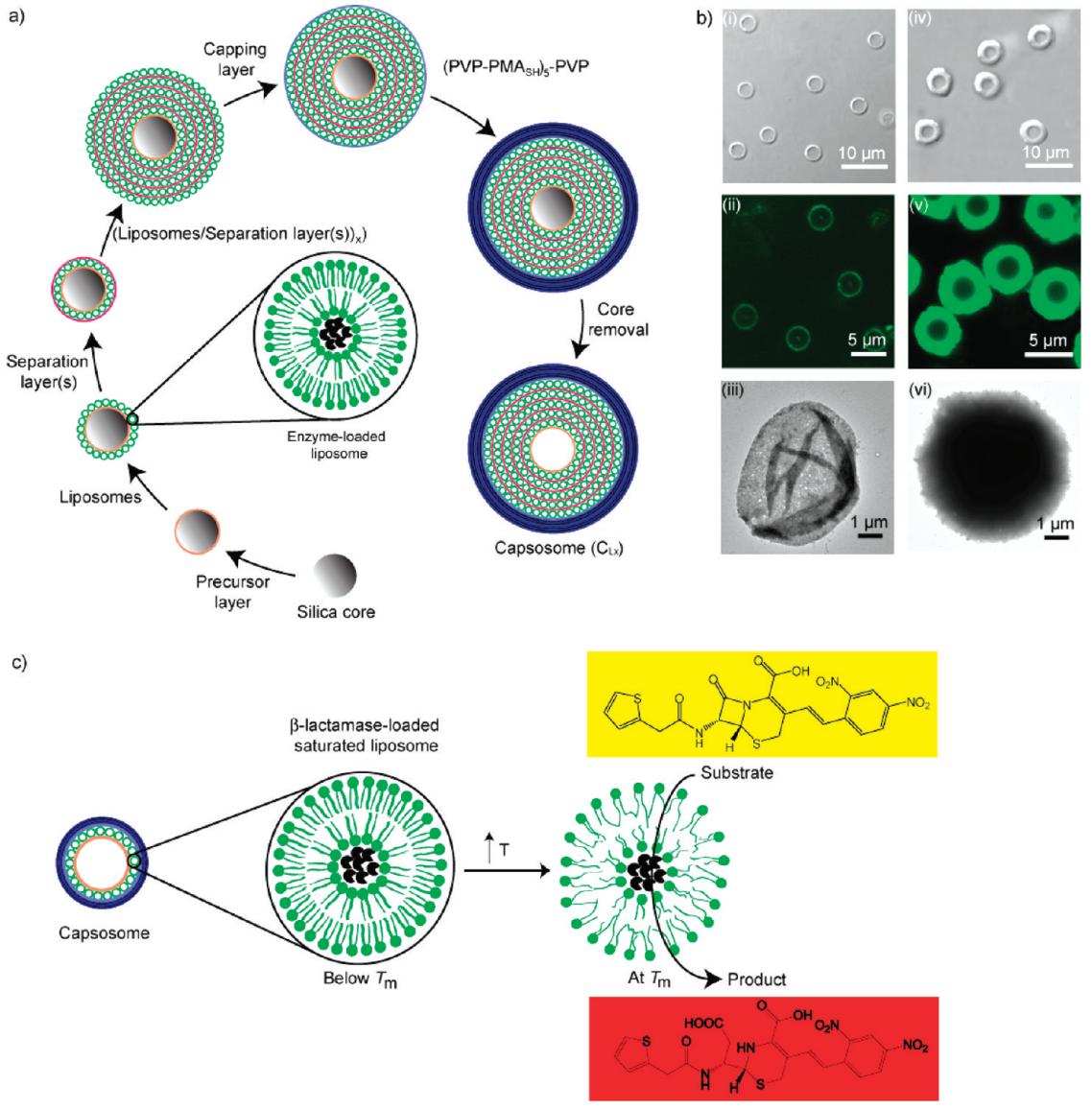

Figure 6. (a) Schematic illustration of capsosome assembly. A silica particle is coated with a polymer precursor layer and enzyme-loaded liposomes, followed by the alternating adsorption of polymer separation layer(s) and liposomes. A polymer capping layer is adsorbed prior to the LbL deposition of interacting polymers, PVPON and PMA_{SH}, to form the membrane of the carrier capsule. Core dissolution results in capsosomes with multiple layers of intact liposomes. (b) Differential interference contrast microscopy (i, iv), confocal laser scanning microscopy (ii, v), and transmission electron microscopy (iii, vi) images showing capsosomes with one (i, ii, iii) or eight (iv, v, vi) liposome deposition steps. (c) Phase transition temperature (T_m) of the liposomes triggers enzymatic catalysis of β-lactamase encapsulated in the liposomal subunits. Adapted from ref 58. Copyright American Chemical Society. Reproduced with permission.

A polymer precursor layer is first adsorbed onto silica particles, followed by deposition of liposome and polymer separation layer(s). The assembly of liposomes and separation layers is repeated until the desired number of liposome layers is achieved. A polymer capping layer is then adsorbed, followed by the deposition of polymer multilayers to form the carrier capsule. Colloidally stable capsosomes are obtained by dissolution of the particle templates. For the first generation of capsosomes, electrostatic interactions were exploited to encapsulate intact liposomes, composed of zwitterionic unsaturated phospholipids, into nondegradable poly(allylamine hydrochloride) (PAH)/poly(styrene sulfonate) (PSS) carrier capsules.[55]

To overcome the potential limitations of electrostatic assembly, cholesterol, a natural membrane constituent, was conjugated to three different polymers: PLL, PMA, and PVPON. These polymers were used to provide stable immobilization of intact zwitterionic or negatively charged unsaturated or saturated liposomes during the assembly.[56,57] The polymer/liposome assembly was then followed by the assembly of PMA_{SH} carrier capsules to form (bio)degradable capsosomes (Figure 6b). Depending on the choice of the deposited polymer precursor and capping layers, the spatial positioning of the liposomal subunits in the capsosomes can be controlled, which yields capsosomes with membrane-associated or "free-floating" subunits.[56,57] The design of capsosomes with membrane-associated subunits allows the assembly of multicompartment capsosomes with up to ca. 160 000 liposomal subunits in a 3 μm-diameter carrier capsule,[58] which permits the encapsulation of a large amount of bioactive molecules.

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Both the long-term stability of the liposomal subunits in capsosomes[56] and the size-dependent retention of the encapsulated cargo molecules[58] have been assessed. In addition, different chemistries to stabilize the membrane of the carrier capsules have been considered.[59] Surface functionalization of the capsosomes was performed with PEG, which hindered the diffusion of phospholipases across the multilayer polymer wall of the carrier capsule.[60] This showed that PEGylation can prolong the stability of the liposomes in the compartmentalized assembly, a crucial feature for the success of continuous enzymatic reactions in chemically and enzymatically diverse biological environments. A significant development achieved with capsosomes relates to triggered encapsulated catalysis. The enzyme β-lactamase was encapsulated in the liposomal subcompartments of the capsosomes permitting chemical[61] and physical[58] stimuli to be used as triggers for the enzymatic hydrolysis of nitrocefin (Figure 6c). Induced catalysis employing the phase transition temperature of the liposomes allows successive rounds of enzymatic reactions in capsosomes,[58] a key function of therapeutic artificial cells, without the loss of colloidal stability of the carriers or loss of functional activity of the enzymes.

The capsosome concept has recently been extended to the incorporation of cubosomes and polymersomes in multilayer polymer capsules. Cubosomes are bicontinuous cubic phase structures, typically around 100−300 nm in size, composed of lipid bilayers that self-assemble to partition hydrophobic and hydrophilic regions into continuous but nonintersecting spaces. Cubosomes have found application as biosensors and delivery vectors,[62] because they are mechanically rigid, structurally stable, and have a larger interfacial surface area per unit volume than liposomes, allowing increased cargo loading compared to liposomes.[63] Driever et al. embedded monoolein and phytantriol cubosomes within PAH/PSS carrier capsules.[44] Small angle X-ray scattering techniques confirmed that the cubosomes remained intact within the PAH/PSS multilayers. Unstabilized cubosomes typically exhibit burst release of encapsulated molecules due to the diffusion from the cubic phase matrix, preventing long-term encapsulation of active molecules. Cubosome incorporation into LbL polymer capsules reduced the burst release of the model small molecule fluorescein from the cubosomes, facilitating the rate of release to be tuned by varying the number of polymer multilayers.

Alternatively, polymer capsules have been subcompartmentalized with pH-responsive polymersomes for controlled DNA release.[64] These polymersomes are comprised of poly(oligoethylene glycol methacrylate)-*block*-poly(2-(diisopropylamino)ethyl methacrylate) (POEGMA-PDPA). LbL capsules incorporating these polymersomes were formed at physiological pH (ca. pH 7.3) by assembling tannic acid with both the polymersomes and PVPON via hydrogen-bonding interactions. The polymersomes were stable at physiological pH but dissociated into single copolymer chains in response to a change in pH to cellular endocytic conditions due to protonation of the PDPA block below its pK_a (6.4). Adjusting the pH back to physiological conditions triggered release of the plasmid DNA payload from the capsules, which was pre-encapsulated within the polymersome subcompartments. This system is of interest as a controlled drug release vector and has the potential to regulate release kinetics through selection of the polymer chemistry.

Multicompartment Colloidosomes. Colloidosomes, capsules with a colloidal particle shell,[65] are produced using Pickering emulsions that are formed via the adsorption of dispersed colloidal particles to the interface of emulsion droplets.[66,67] The emulsions can be oil-in-water (O/W) or water-in-oil (W/O).[65]

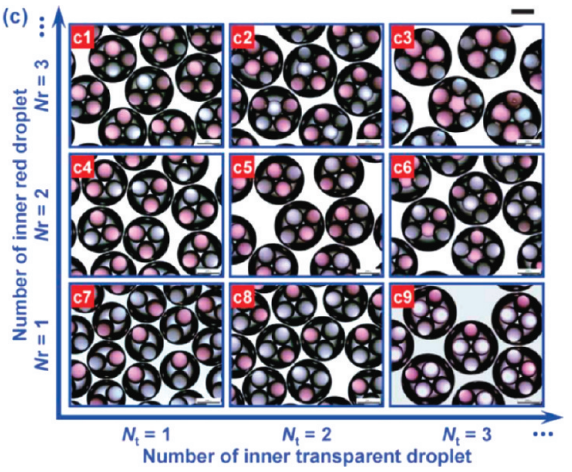

Figure 7. Optical microscopy images of double emulsions (oil-in-water-in-oil, O/W/O) droplets formed via a capillary microfluidic device. The number of red and transparent inner oil droplets can be tuned precisely and independently. Scale bar is 200 μm. Adapted from ref 70. Copyright Royal Society of Chemistry. Reproduced with permission.

As the particles on the droplet interface are approximately close-packed, an array of voids on the colloidosomes is produced.[65] The size of these holes, and hence colloidosome permeability, can be tuned by adjusting the size of the adsorbed colloids. Stabilization of the adsorbed particles of these Pickering emulsions produces robust colloidosomes. For adsorbed polymer nanoparticles, sintering above the polymer glass transition temperature causes fusion of the adsorbed colloidal particles. Colloidosomes containing heat-sensitive cargo can be stabilized electrostatically by the addition of polyelectrolytes,[65] via chemical cross-linking of reactive groups on the particle surface,[68] or polymerization from the surface of the adsorbed particles.[69] Extended stabilization regimes (e.g., longer sintering or polymerization times) can also be used to modulate colloidosome permeability. Once stabilized, the continuous phase of the colloidosome dispersion is optionally replaced by centrifugation into a solution of the desired phase to produce stable micrometer- to millimeter-sized colloidosomes.[65]

Due to the ability to generate monodisperse multiple emulsions with defined numbers of inner droplets (Figure 7),[70,71] microfluidic techniques are increasingly being utilized to produce multicompartment colloidosomes and polymer capsules. Using a capillary microfluidic device, Lee and Weitz produced water-in-oil-in-water (W/O/W) emulsions containing hydrophobic silica nanoparticles dispersed in the oil phase.[72] Evaporation of the solvent resulted in multicompartment colloidosomes. Moreover, by varying the flow rates of the inner water and oil phase in the microfluidic device, the number of colloidosome compartments could be tailored.

Recently, Kim et al.[73] produced multicompartment polymer capsules with arrays of nanoholes at the capsule surface. W/O/W emulsions with tunable numbers of aqueous subcompartments were prepared using a photopolymerizable monomer as the oil phase. Rearrangement of the encapsulated aqueous droplets was observed, minimizing the interfacial energy of the aqueous droplets and deforming the monomer droplet to form a thin film around the inner water droplets. Consequently, hydrophobic SiO_2 nanoparticles dispersed in the monomer phase were embedded in this film, which following photo-crosslinking of the monomer, remained embedded in the polymer shell. Nanohole arrays could be

produced by dissolution of the SiO_2 particles, allowing release of fluorescent macromolecules encapsulated in the inner aqueous compartments. W/O/W emulsions containing two different types of inner water droplets were also prepared, potentially allowing the separation and triggered mixing of two interacting species in one capsule. These systems may be limited to the encapsulation of large macromolecules, as the smallest size of nanoparticles which can be readily synthesized and processed is limited. Although microfluidic methods have not yet been used to produce multicompartment systems capable of triggered release from selected subcompartments and subsequent enzymatic reaction, it is expected that future research will allow this goal to be met.

Future Outlook. In this Perspective, we have outlined and discussed recent advances in the creation of multicompartment assemblies utilized for encapsulated catalysis and as building blocks for synthetic cells. Multidisciplinary efforts to engineer subcompartmentalized particles using liposomes, polymersomes, and polymer capsules have led to astonishing progress; nevertheless, significant challenges remain. A key application of multicompartment particles is the creation of synthetic cells for enzymatic therapies to replace deficient enzyme functionality. It is envisaged that replication of cells and cellular structures will improve enzyme performance, thus facilitating the improved synthesis of bioactive molecules or degradation of waste products. While promising, the multicompartment enzyme systems developed to date are mainly proof-of-concept, achieving functionality only under controlled conditions. Investigations into the performance of multicompartment particles in chemically diverse biological environments, and studies of their interactions with cellular structures are critical to ensure long-term performance of these assemblies in biological systems.

Future research should also focus upon generating improved relationships between chemical functionality and physical structure, to facilitate the rational design of multicompartment systems with desired biological responses. The use of coarse-grained (CG) molecular models of liposomes and polymersomes has recently emerged as a powerful technique to simulate self-assembly and biomimetic behaviors such as vesicle fusion, budding and formation of interfacial domains, with increased interest toward predicting the kinetics of stimuli response in such self-assembled structures.[74] As for liposomes and polymersomes, application of CG models to LbL systems (such as polymer capsules) may improve tailoring of physicochemical properties by varying chemical structure.

Although challenges remain to the use of these multicompartment assemblies in biological applications, the studies highlighted demonstrate the exciting results already achieved using multicompartment enzyme assemblies. We envisage that future research based upon these findings, in addition to the continued study of intracellular reaction mechanisms, will lead to fabrication of robust, multicompartment assemblies approaching the long-term catalytic performance of living cells.

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