Biomimetic Liposome- and Polymersome-Based Multicompartmentalized Assemblies

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ABSTRACT

Liposomes and polymersomes have attracted significant attention and have emerged as versatile materials for therapeutic delivery and in the design of artificial cells and organelle mimics. Through judicious choice of building blocks, these synthetic carriers can be readily engineered with tailored interfacial properties, offering new possibilities for the design of advanced assemblies with specific permeability, stability, stimuli-response, and targeting capabilities. In this Feature Article, we highlight recent studies on biomimetic liposome- and polymersome-based multicompartmentalized assemblies en route toward the development of artificial cells, microreactors, and therapeutic delivery carriers. The strategies employed to

produce these carriers are outlined and properties that contribute to their performance are discussed. Successful applications of these biomimetic assemblies are highlighted, and finally, areas that require additional investigation for future development of these assemblies as next-generation therapeutic systems are outlined.

KEYWORDS: Liposomes, Polymersomes, Biomimetic Assembly, Multicompartment, Artificial Cells, Microreactor, Therapeutic Carrier

Introduction. In recent years, the development of state-of-the-art strategies and techniques to exploit the unique properties and functions of novel materials has opened up new avenues to a wide range of biomedical applications, such as encapsulated catalysis, drug delivery, and diagnostics. Most notably, the field of biomimetics has seen a dramatic escalation in the development of advanced particulate systems, driven largely by the motivation to replicate nature's assemblies from synthetic components. Constructing simplified synthetic cells[1-5] that resemble living cells is an increasingly expanding area because it contributes to an understanding of biological processes and provides new and unique prospects for future therapeutic applications – from self-regulating bioreactors to new biomedical devices.

Advanced particulate systems that mimic the structure of biological cells can be assembled using biomimetic approaches. Biological cells are self-contained living entities that are able to perform complex chemical reactions and multiple, coupled cellular functions within spatially defined environments due to their unique subcompartmentalized structure. Inspired by this architecture, recent research has been focused on the construction of multicompartment particulate systems, which forms the main theme of this Feature Article. Designing hierarchical carriers that mimic the compartmental organization of biological cells not only allows coencapsulation of diverse (bio)molecules that can be spatially confined but

also enables the incorporation of various stimuli for regulating enzymatic cascade reactions and for controlled simultaneous and/or subsequent release of the encapsulated (therapeutic) molecules.

Considerable progress has been achieved in designing functional bioinspired systems. In this Feature Article, we focus on prominent examples of multicompartmentalized assemblies based on liposomes and polymersomes, which are self-assembled vessels made from amphiphilic lipid and block copolymers, respectively. A brief introduction of liposomes and polymersomes is presented. Detailed aspects of liposomes and polymersomes as protocell constructs and as therapeutic delivery carriers can be found in previous reviews.[6-15] We provide an overview of the state-of-the-art and highlight recent advances in the liposome- and polymersome-based multicompartmentalized assemblies; these include liposomes-in-liposomes, polymersomes-in-polymersomes, and subcompartmentalized polymer capsules containing multiple intact liposomes or polymersomes (Figure 1). The assembly approaches employed to produce these bioinspired carriers are discussed, successful applications of these multicompartment assemblies are highlighted, and possible further developments of these assemblies as biomedical platforms are discussed.

Biomimetic Liposome-Based Assemblies. Liposomes are supramolecular assemblies of amphiphilic lipids, which self associate to enclose an aqueous compartment surrounded by a lipid bilayer membrane (Figure 1a). A number of different approaches have been used to assemble these lipid vesicles, including thin-film hydration, solvent injection, reverse-phase evaporation, sonication, and membrane extrusion.[6,16] Once formed, liposomes are typically 20 nm to 10 μm in diameter with the phospholipid bilayer about 4-5 nm thick.[16] Their charge and surface properties can easily be tuned simply by varying the composition of the phospholipids or adding new components to the lipid mixture during the preparation

methods.[6,9] Due to the presence of hydrophobic and hydrophilic domains, a variety of (therapeutic) cargo (e.g., enzymes, nucleic acids, proteins, anti-cancer drugs, peptide vaccines, and imaging agents) can be loaded into this assembly;[6,8,10] hydrophobic drugs are loaded into the liposome membrane while hydrophilic molecules can be encapsulated in the aqueous core. A number of different stimuli have been exploited to trigger the release of the encapsulated cargo from liposomes, including temperature,[17,18] pH,[19] light,[20] redox potential,[21] magnetic fields,[22,23] near IR,[24] and ultrasound,[25] each with its specific advantage depending on the applications.[9]

Zasadzinski and co-workers reported the first multicompartment liposome architecture, termed vesosomes,[26] where small unilamellar liposomes are entrapped within a larger liposome (Figure 1b). Two different approaches were described for the formation of the liposomes-in-liposomes; nevertheless, the key concept is the spontaneous encapsulation of preformed liposomes within a lipid bilayer membrane. The first approach involves the formation of cochleate cylinders,[27] which are derived from the fusion of unilamellar liposomes composed of negatively charged dioleylphosphatidylserine (DOPS) into continuous planar membrane sheets, followed by spiral folding to form cigar-like cylinders in the presence of Ca^{2+}. Preformed liposomes were then added to the dispersion of these cylinders and upon addition of a chelating agent ethylenediaminetetraacetic acid (EDTA), Ca^{2+} was removed and the cylinders unrolled to form a closed membrane while encapsulating the small liposomes (Figure 2).[26,28] The second approach involves the formation of interdigitated lipid bilayer sheets generated from the fusion of liposomes composed of saturated dipalmitoylphosphatidylcholine (DPPC) induced by the addition of ethanol below gel-liquid phase transition temperature (T_m) of the liposomes. When these bilayers are heated at elevated temperature above the T_m, the membrane sheets become less rigid and spontaneously close to form unilamellar lipid vesicles. During the transition from bilayer

sheets to closed vesicles, these interdigitated membranes can simultaneously encapsulate preformed (cargo-loaded) liposomes, thus form a multicompartmentalized liposome assembly.[29] The newly generated vesicles are capable of maintaining their structural integrity, even at temperatures below the T_m.

Vesosome formation does not require any chemical or mechanical processing. The encapsulation efficiency of the small liposomes within the larger vesicles is 60-70%,[28,30] which corresponds to a significant fraction of the total number of liposomes present in the solution. Although these methods facilitate the incorporation of internal liposomal subcompartments that can be made of different lipid compositions, the formation of the external vesicles is limited to certain types of phospholipids. For the first approach, cochleate cylinders can only be generated from certain negative phospholipids such as phosphatidylserine and similarly, the latter approach only applies to certain phospholipids that can form an interdigitated bilayer, e.g., dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine. In addition, the necessity to raise the temperature above T_m of the saturated phospholipids (typically > 45 °C) might potentially denature proteins and other fragile biological molecules, therefore limiting the type of cargo that can be encapsulated in the vesosomes formed via the interdigitation method.

The size of vesosomes can be tuned by the addition of cholesterol into the lipid mixture and by varying the concentrations of ethanol added to the interdigitated lipid sheets.[29] After a series of extrusion cycles, 150 nm-diameter external vesicles containing intact 50 nm liposomes were obtained;[29] this size is within that of drug delivery vehicles used for circulation *in vivo* (i.e., 100-300 nm).[31,32] Freeze-fracture transmission electron microscopy confirmed the stability of the internal vesicles in vesosomes after 1 month of storage at 4 °C.[30]

The potential of vesosomes to act as therapeutic carriers is evidenced by the successful encapsulation of DNA, a model drug prochlorperazine, or antibiotic ciprofloxacin within the compartments as well as the sustained release profile of the encapsulated cargo.[30,33] As the bilayer-within-a-bilayer structure forms a physical barrier to direct interaction between the internal compartments and external environment, vesosomes efficiently protect the internal vesicles from degradation under biomedically relevant conditions, i.e., in the presence of serum or degradative enzymes, such as phospholipases, that can otherwise lyse the vesicular structures and result in premature release of the encapsulated cargo. The additional bilayer membrane therefore delays cargo leakage. In serum, enhanced ciprofloxacin retention was reported, from <10 min in unilamellar liposomes to ~6 h in vesosomes with more than 40% of the encapsulated cargo still retained even after 10 h incubation in serum at 37 °C.[33] Upon exposure to phospholipases, the multicompartmentalized liposome assembly extends cargo retention by two orders of magnitude in comparison to unilamellar liposomes of the same composition.[34] Vesosomes also retain the essential features of conventional unilamellar liposomes; one of which is the possibility to functionalize the surface of the vesicles with inert, biocompatible polymers such as poly(ethylene glycol) (PEG), which remains a gold standard for preparing long-circulating liposomes.[8] Another study in the same laboratory reported that PEG-lipid coating prevents vesosome aggregation in blood and leads to a half-life of ~2 h in mice with similar biodistribution patterns to those of conventional unilamellar liposomes.[33]

Vesosomes therefore possess several advantages compared to unilamellar liposomes because: i) release profiles of encapsulated molecules can be controlled and extended over a longer period of time; ii) drug cocktails (particularly for drug combinations with different physical properties such as charge, polarity, and molecular weight) can be sequestered to avoid complex formation or coprecipitation of the different types of cargo encapsulated

within a single carrier and delivered simultaneously or sequentially – this is expected to significantly improve therapeutic efficacy while minimizing the potential for drug resistance; and iii) the interior vesicles can be prepared from entirely different phospholipid compositions from one another and from the exterior vesicles – affording this system with tunable stability and degradation properties. Given the multicompartment nature of vesosomes, this carrier system has been proven to overcome the major limitation of unilamellar liposomes, i.e., the premature cargo release in physiological environments. Hence, it is envisaged that vesosomes would be a viable alternative for the delivery of weakly basic drugs, which leak too rapidly from liposomes.

In another study, Mishra *et al.* pioneered the use of vesosomes for transcutaneous immunization (TCI), where the model antigen, tetanus toxoid (TTx), was encapsulated within the internal liposomal compartments.[35] It was observed that TCI with vesosomal systems significantly increased the level of anti-TTx antibodies in an *in vivo* model in comparison to topically administered cationic unilamellar liposomes or a free TTx formulation, suggesting a more effective stimulation of the immune response, possibly due to better localization of the internal vesicles along with the encapsulated antigen in a confined environment. These findings suggest the potential of vesosomes for effective topical delivery of vaccines.

Liposome-in-liposome assemblies have also been reported by Bolinger *et al.*, where the formation of the multicompartment assembly is achieved by a lipid film hydration process.[36] A suspension of preformed liposomes was used to hydrate a dried lipid film. Upon hydration, large unilamellar liposomes were formed and simultaneously engulfed the small preformed liposomes. The size of the multicompartment assembly reported here ranges from 1 to 10 μm in diameter with 100 nm-diameter internal vesicles and its application as a microreactor was demonstrated by temperature-triggered consecutive enzymatic reactions. The external vesicles, which are composed of 1,2-dioleyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)]

(DOPG, T_m = -18 °C), encapsulate a model enzyme alkaline phosphatase and two populations of substrate-loaded liposomal subcompartments composed of different lipid compositions: dichlorodimethylacridinone phosphate (DDAO phosphate)-loaded 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine/1,2-dimyristoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] liposomes (DMPC/DMPG, T_m = 23 °C) and fluorescein diphosphate (FDP)-loaded 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine/1,2-dipalmitoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] liposomes (DPPC/DPPG, T_m = 41 °C). Upon increasing the temperature above the T_m of each liposomal subcompartment, substrates were sequentially released into the interior of the external vesicle and converted to fluorescent products by alkaline phosphatase within the same microreactor (Figure 3). By using fluorescence correlation spectroscopy, the enzymatic reactions within the multicompartment assembly can be monitored at a single molecule level.

So far, the aforementioned multicompartmentalized assemblies are based on single-component liposome systems. We recently reported a new class of multicompartment assembly, termed capsosomes,[37,38] which unite two fundamentally different systems – polymer capsules and liposomes (Figure 1c). Polymer capsules are assembled via the layer-by-layer (LbL) technique, i.e., the sequential adsorption of interacting polymers onto sacrificial template particles followed by core removal.[39-44] LbL assembly has proven to be a versatile approach in constructing therapeutic carriers due to the facile control over size, shape, composition, and permeability of the capsule membrane through the choice of the deposited polymer and the number of layers adsorbed. Liposomes, on the other hand, are able to encapsulate small and fragile hydrophobic and hydrophilic cargo. Therefore, by combining these two assemblies, capsosomes present a promising platform toward the design of therapeutic artificial cells because they retain the beneficial properties of both polymer capsules and liposomes – namely the semi-permeable nature and structural stability of the

capsules (resembling cell membranes) as well as the ability of liposomes to entrap diverse cargo and provide confined environment for enzymatic catalysis (resembling cell organelles).

Capsosomes are fabricated via the sequential deposition of polymers and liposomes onto particle templates (Figure 4a). A polymer precursor layer is first adsorbed onto a silica core, followed by the deposition of liposomes to form the initial polymer/liposome layer. This step can be repeated until the desired number of the liposome layers is achieved. A polymer capping layer is then adsorbed, followed by LbL assembly of the polymer carrier capsule and subsequent dissolution of the particle templates to yield colloidally stable (bio)degradable capsosomes.

A unique noncovalent linkage approach was developed using cholesterol – a natural membrane constituent – as the anchoring units between the liposomes and the polymer films. Cholesterol moieties can easily be conjugated to different polymers, i.e., poly(L-lysine) (PLL), poly(methacrylic acid) (PMA), and poly(*N*-vinylpyrrolidone) (PVPON), and the anchoring strategy via these cholesterol-modified polymers enables the stable incorporation of zwitterionic or negatively charged, unsaturated or saturated liposomes, without rupturing or being displaced from the polymer surfaces.[45,46] Surface functionalization of the capsosomes with PEG has been shown to prolong the stability of the liposomes in the compartmentalized assembly and therefore can prevent premature release of cargo at physiological conditions.[47]

A noteworthy feature of capsosomes is the encapsulation of up to ca. 160,000 50 nm-diameter liposomal subunits in a 3 µm-diameter carrier capsule.[48] This amount exceeds what others have reported and to the best of our knowledge is the highest level of subcompartmentalization system described for synthetic cell mimics, allowing the encapsulation of a large amount of (therapeutic) cargo. In addition, the architecture of capsosomes enables control over the number of subcompartments as well as their spatial

positions within the carrier capsules simply by varying the number of deposited liposome layers and by changing the polymer building blocks that sandwich the liposome layer, respectively.[46,48]

The potential of capsosomes as drug delivery vehicles has been demonstrated.[49,50] Pristine capsosomes do not exhibit inherent cytotoxicity, making them potentially suitable as biomedical platform. Small, hydrophobic anti-tumor drugs (thiocoraline or paclitaxel) were encapsulated in the membrane of the liposomal subcompartments and cell studies using colon cancer cells confirmed the functionality of these capsosomes, as evidenced by reduced proliferation when cells were treated with capsosomes (1 μm-diameter) encapsulating the cytotoxic drugs.[49,50] The cargo concentration-dependent viability of cancer cells when exposed to capsosomes containing a different number of drug-loaded liposomes was verified, confirming the control over the amount of therapeutic payload achieved by simply varying the number of deposited liposome layers.[50]

A significant development achieved with capsosomes relates to triggered encapsulated catalysis en route toward enzymatic therapy applications. We recently reported the coencapsulation of glutathione reductase – an enzyme that actively reduces glutathione disulfide (GSSG) into reduced glutathione (GSH, an antioxidant) – and disulfide-linked PMA-KP9 polymer-peptide conjugates into capsosomes (Figure 4a).[51] Induced catalysis of encapsulated glutathione reductase employing the increased membrane permeability at the T_m of the liposomes results in the production of GSH, which subsequently triggers the release of the encapsulated antigenic peptide KP9 from the capsosomes by reducing the disulfide linkages of the conjugates (Figure 4b). Employing T_m of the liposomes allows successive enzymatic catalytic reactions without any loss of the functional activity of the enzymes.[48] These results demonstrate the potential of capsosomes to continuously generate a potent antioxidant at human body temperature while simultaneously releasing small molecule

therapeutics. Future studies will develop these systems into a biomimetic platform that combines both enzymatic therapy and controlled release of therapeutic agents in a single assembly.

The relatively large size of capsosomes (typically > 1 μm-diameter) might limit the use of these carriers as targeted drug delivery vectors. This factor, however, is beneficial in the area of vaccination, as it facilitates the uptake of these vehicles by antigen presenting cells (APCs) via phagocytosis. Upon intracellular processing, these carriers can subsequently release the encapsulated immunogenic peptides and proteins to the APCs. Recent studies have shown that 1 μm-diameter polymer capsules of varied composition and surface chemistry encapsulating antigenic epitopes (KP9 oligopeptides, whole ovalbumin protein, or immunogenic ovalbumin peptides) were effectively internalized by APCs and resulted in *in vitro* and *in vivo* stimulation of T cells.[52-54] The success of these polymer capsules in delivering their therapeutic payload to the APCs lays the foundation to investigate capsosomes as candidate carriers for vaccine delivery.

Biomimetic Polymersome-Based Assemblies. Polymersomes are synthetic mimics of liposomes and composed of amphiphilic block copolymers (Figure 1d). Similar to liposomes, polymersomes comprise an aqueous lumen sequestered from the external aqueous environment by a hydrophobic membrane and therefore can encapsulate both hydrophilic and hydrophobic cargo. They can be stably prepared by a wide range of techniques common to liposomes, such as film rehydration, electroformation, sonication, and extrusion to generate nano- to micron-sized polymer vesicles.[11] However in comparison to liposomes, polymersome membranes are often several times thicker due to the higher molecular weights of block copolymers compared with typical phospholipids. While the membrane thickness of liposomes is typically 4-5 nm, the hydrophobic core thickness of polymersomes can be

engineered to exceed 5 nm by simply varying the copolymer hydrophobic block molecular weight.[55] As a result of the thicker membrane, polymersomes possess better stability and mechanical strength and are less permeable to small water-soluble molecules. The synthetic nature of the block copolymers enables the incorporation of components that allow polymersomes to respond to external stimuli such as pH, oxidative species, and enzymatic degradation.[11]

Similar to vesosomes, multicompartmentalized polymersome assemblies have also recently been developed (Figure 1e). Chiu *et al.* reported the first example of a polymersomes-in-polymersomes assembly prepared by a two-stage double emulsion technique in a water/oil/water system.[56] Copolymers were dissolved in an organic phase (tetrahydrofuran/chloroform – THF/$CHCl_3$), followed by emulsification and evaporation of the organic solvent to form polymer vesicles. These preformed vesicles were subsequently introduced to a water phase of the second stage double emulsion process to form the multicompartment assembly, and range in size from 1 to 15 μm. The size of these vesicles can be tuned by varying the ratio of THF to $CHCl_3$ and by changing the content of the copolymers. In this system, the outer and inner compartments are comprised of poly(acrylic acid)-*co*-poly(distearin acrylate) (poly(AAc-*co*-DSA)) polymersomes equipped with pH-responsive transmembrane channels (Figure 5a), allowing controlled release of encapsulated cargo in response to environmental pH changes; this pH-responsive process is reversible in the range of pH 5 to 8. While these polymersomes are impermeable to small hydrophilic solutes such as calcein at pH 5 (Figure 5bi), a change in the ionization state of the AAc units at pH 8 results in the formation of pores and subsequent transport of the encapsulated cargo across the membranes (Figure 5bii).

Multicompartment polymersomes prepared via a double emulsion technique were also reported by Weitz and co-workers.[57,58] Using a capillary microfluidic device, polymersomes

with multiple internal compartments containing different model encapsulants were produced without the risk of cross-contamination (Figure 6a). The main advantage of this technique is the ability to control the number as well as the size of the compartments in the assembly simply by tuning the flow rates of the phases in the microfluidic channels and by changing the capillary diameter. The size of the multicompartment polymersome assembly obtained via the microfluidic approach ranges from 50 to 200 μm in diameter, containing 2-8 inner compartments. Both the external and internal polymersomes are composed of biocompatible poly(ethylene glycol)-*b*-poly(lactic acid) (PEG-*b*-PLA) diblock copolymers. Controlled release of the encapsulated cargo was achieved by the introduction of mechanical strain or the selective dissociation of the polymersome membranes by hydrolysis. The first approach induced rupturing of the membrane from the outermost to the innermost polymersomes. The second approach, on the other hand, represents a more sophisticated way to program the release of the encapsulated materials. The stability of the external and internal polymersome membrane can be tuned by the incorporation of additional PLA-homopolymer into the hydrophobic region of the bilayer, which results in a different degradation rate of the compartments. Polymersomes with a PLA-homopolymer-free bilayer slowly dissociated in water in 17 days; in contrast, polymersomes with a PLA-homopolymer-loaded bilayer showed enhanced stability for at least 2 months.[58] Hence, depending on the composition of the bilayer membrane, the sequential release of the encapsulated cargo can be tuned from the outermost to the innermost polymersomes or *vice versa* (Figure 6b). It is envisaged that this multicompartmentalized polymersome assembly would be an attractive platform for cosmetic delivery applications as well as for the delivery of multiple growth factors for tissue regeneration.

Further developments in compartmentalized polymer vesicles include multicompartment system based on polymersomes of different compositions for the carrier and the

subcompartments, as reported by Nallani and co-workers.[59] Non-permeable poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA-PDMS-PMOXA) polymersomes prepared by film rehydration method were entrapped within semi-permeable polystyrene-*block*-poly-(L-isocyanoalanine(2-thiophen-3-yl-ethyl)amide) (PS-PIAT) polymersomes. The polymersome-in-polymersome assembly was achieved via direct dissolution method, where PS-PIAT polymers dissolved in THF were added dropwise to a solution containing preformed PMOXA-PDMS-PMOXA polymersomes. The size of this multicompartment assembly ranges from 150 to 200 nm and selective encapsulation of two biomolecules, green fluorescent protein (GFP) and cyanine-5 conjugated immunoglobulin G protein (Cy5-IgG), was demonstrated in the inner and outer compartments, respectively. This multicompartment system is particularly promising as a vector in therapeutic applications and can be designed such that different polymersomes release their encapsulated cargo in response to different chemical or environmental stimuli.

Another recent development in polymersome-in-polymersome assemblies aimed at closely resembling biological cells was reported by Lecommandoux and co-workers.[60,61] Biocompatible poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) (PTMC-*b*-PGA) polymersomes formed via nanoprecipitation were loaded into poly(butadiene)-*b*-poly(ethylene oxide) (PB-*b*-PEO) polymersomes by emulsion-centrifugation. A model cargo, doxorubicin, was encapsulated into the inner polymersomes of the multicompartment assembly and the release profile was compared to single-compartment doxorubicin-loaded PTMC-*b*-PGA polymersomes. As expected, the release rate of doxorubicin from the single-compartment assembly was found to be two times higher than those from the multicompartment assembly due to the additional diffusion barrier. In addition, cargo release was observed at 37 °C and negligible at 20 °C, demonstrating the temperature-responsiveness of the PTMC-*b*-PGA polymersomes. An interesting feature of this system is the incorporation

of highly viscous alginate or dextran solution into the cavity of the ~20 μm-diameter compartmentalized assembly to resemble the cell cytoplasm. The Brownian motion of the inner polymersomes within the cavity of the "gelly" polymersomes was monitored and the viscosity of the dextran system was reported to resemble the viscosity of the red blood cell cytoplasm, i.e., 0.01 Pa·s.[61] This study constitutes further progress toward cell mimicry.

Analogous to capsosomes, LbL-assembled polymer carrier capsules encapsulating pH-responsive polymersome subcompartments has recently been reported (Figure 1f).[62] Poly(oligoethylene glycol methacrylate)-*block*-poly(2-(diisopropylamino)ethyl methacrylate) (POEGMA-PDPA) polymersomes are intact at physiological pH, however, at pH values below the pKa of 6.4, the hydrophobic-to-hydrophilic transition of the PDPA block initiates the dissolution of the block copolymer chains in aqueous media and results in dissociation of the polymersomes into single polymer chains. Lomas *et al.* demonstrated controlled DNA release from this subcompartmentalized system triggered by changes in environmental pH.[62] Plasmid DNA was loaded into the POEGMA-PDPA polymersomes prior to adsorption of these compartments onto polymer-coated particles. The polymer/polymersome assemblies were then followed by the sequential deposition of tannic acid and PVPON via hydrogen bonding interactions to form the membrane of the carrier capsules. In response to pH changes from ca. pH 7.3 (physiological conditions) to pH 5 (cellular endocytic conditions), the polymersomes dissociated and the encapsulated negatively charged DNA was released from the subcompartments, subsequently forming complexes with the positively charged PDPA block. Upon adjusting the pH back to physiological conditions, the electrostatic interactions within the DNA-PDPA complexes were disrupted, triggering release of the DNA payload from the polymer capsules. By the judicious selection of polymer building blocks, this system has the potential to tune cargo release and therefore represents a promising platform as a controlled therapeutic carrier.

Concluding Remarks and Outlook. Synthetic particle systems that mimic the architecture of biological cells are expected to have an impact on next-generation therapeutic concepts. In this Feature Article, we have provided an overview of the state-of-the-art and highlighted recent developments of biomimetic liposome- and polymersome-based multicompartmentalized assemblies and their applications in the design of artificial cells, microreactors, and therapeutic delivery carriers. Early successes in engineering such particulate systems are promising and interest in this field is rapidly growing, as evidenced by the increase in the number of different approaches reported to construct diverse multicompartmentalized assemblies over recent years. Despite tremendous efforts, the studies reported here are mainly proof-of-concept and there are many interesting challenges ahead before the full potential of these biomimetic assemblies as biomedical platforms is realized.

In the area of cell mimicry, the multicompartmentalized assemblies reported to date closely resemble two key features of a cell: (i) the cell membrane – an external compartment controlling passage of molecules in and out of the assembly and (ii) cell organelles – internal compartments providing confined subunits. Further developments of compartmentalized systems with functional artificial organelles (e.g., ribosomes, mitochondria, Golgi apparatus, lysosomes) will contribute to a better understanding of how biological cells function. These multicompartment assemblies can be also utilized for enzymatic therapy applications,[1] for example, to replenish lost cellular enzymatic activities that are essential to degrade waste products and/or to support the synthesis of bioactive molecules; some examples include catalase – an enzyme responsible for the breakdown of damaging hydrogen peroxide (catalase deficiency leads to peroxisomal disorder acatalasia); asparaginase – an enzyme that catalyzes the hydrolysis of L-asparagine and consequently inhibits protein synthesis in tumor cells; or xanthine oxidase – the lack of this enzyme leads to high concentration of xanthine in

blood and causes renal failure. Hence, to further address the complexity of therapeutic cell mimics, it is equally important to demonstrate the versatility of these platforms with medically relevant enzymatic (cascade) systems and to equip them with self-regulating systems to respond to specific stimuli. This is feasible with the unique feature of multicompartment systems, which offer the possibility to incorporate multifunctionality in terms of encapsulation, release, permeability, stability, and stimuli response (e.g., pH, temperature, redox state, ultrasound, etc). Furthermore, the performance of these compartmentalized assemblies in chemically diverse biological environments needs to be investigated to address their biocompatibility and long-term bioactivity. It is expected that the developments of multicompartment assemblies in cell mimicry will concurrently advance their utility as microreactors.

In the area of therapeutic delivery, the nanosized multicompartment systems developed to date offer practical solutions to spatially coencapsulate different types of therapeutics within the same carrier, en route to cascade release for delivery of multiple therapeutics. In addition, the multicompartment architecture is highly beneficial in terms of delivering higher dosages of therapeutics while lowering the amounts of carrier materials (e.g., lipid or polymer building blocks) delivered. As with any drug delivery technology, the challenges of the system's *in vivo* performance need to be addressed; these include surface functionalization of the carriers with low-fouling materials to evade body's natural defense mechanisms against foreign materials and to extend the blood residence time. Furthermore, detailed studies on the interaction between these multicompartment carriers and cells (i.e., cellular internalization pathways and subsequent processing of the multicompartment systems in biological cells) in comparison to single-compartment carriers will provide important insights into intracellular trafficking as well as cargo release mechanisms, and this understanding will promote rational design of intelligent carriers with desired biological responses. Future research is also likely

to direct the multicompartment systems as a tool in theranostics – a merger between therapeutic delivery and diagnostic imaging.[63] Therapeutic molecules and imaging agents can be spatially coencapsulated in different compartments and this system will enable simultaneous imaging and monitoring of drug delivery kinetics and their efficacy in diseased tissue. Given the considerable progress that has been achieved in the development of liposomes and polymersomes in their native forms *in vitro* and *in vivo*, we anticipate that these developments can be readily translated into biomimetic multicompartment systems and deliver significant advances in their utilization as efficient therapeutic vectors in the future.

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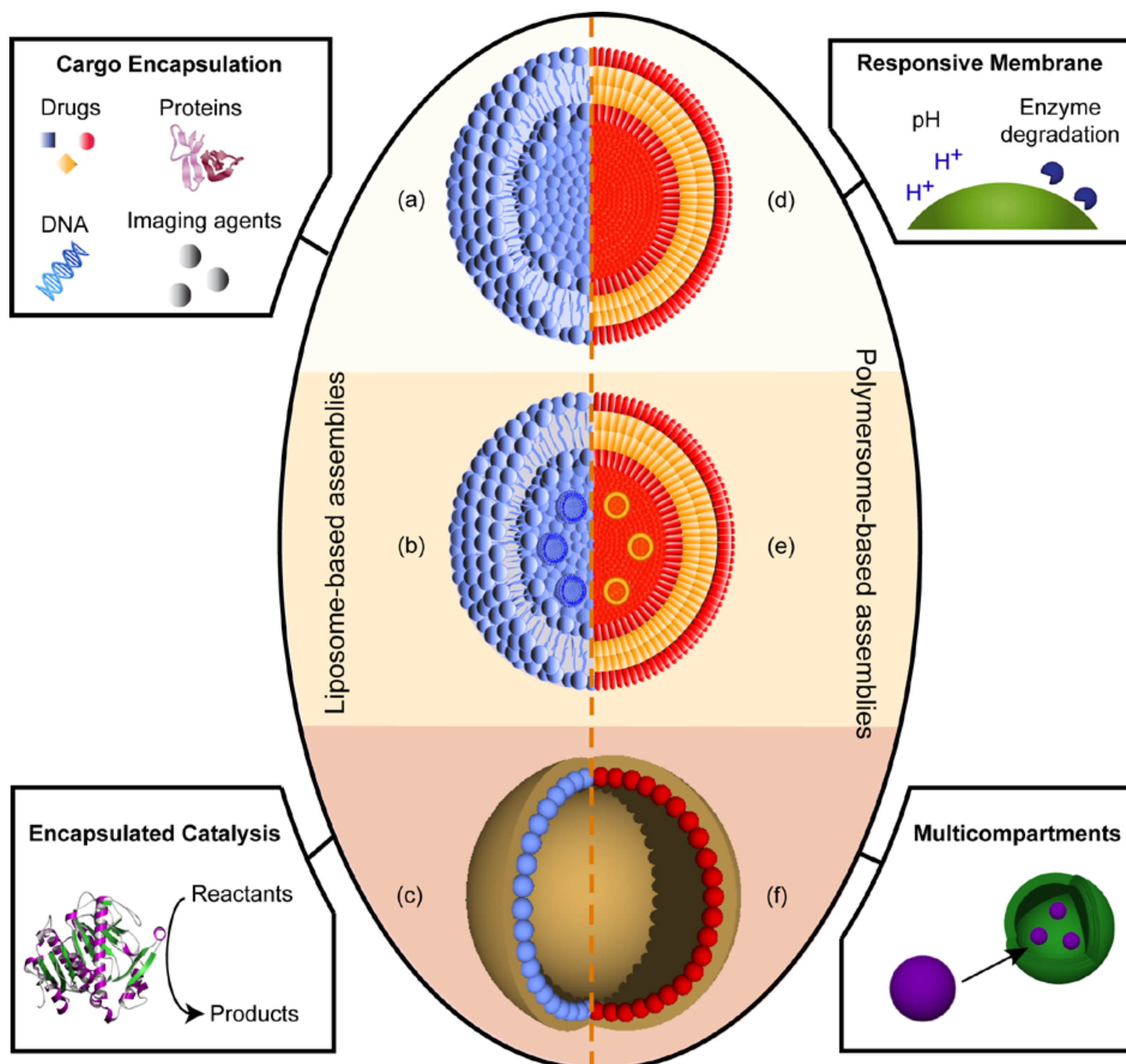

Figure 1. Biomimetic liposome- and polymersome-based assemblies. Liposomes (a) and polymersomes (d) are used as components for the design of multicompartmentalized systems: liposomes-in-liposomes (b), polymersomes-in-polymersomes (e), and subcompartmentalized polymer capsules containing multiple intact liposomes (c) or polymersomes (f). Developing next-generation carriers for the design of therapeutic artificial cells and drug delivery vehicles requires the versatility to encapsulate a range of (therapeutic) cargo, the ability to trigger release of the encapsulated cargo in response to environmental stimuli, the versatility to perform biologically relevant enzymatic (cascade) systems, and multicompartmentalization to spatially coencapsulate different types of (therapeutic) cargo within the same carrier.

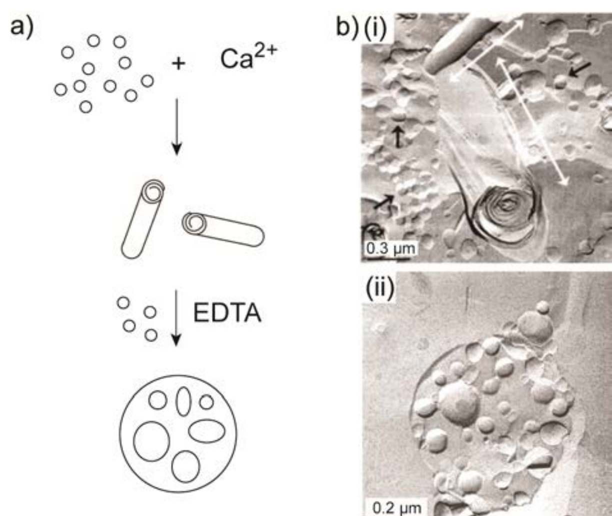

Figure 2. a) Schematic illustration of the assembly of vesosomes from cochleate cylinders. Upon addition of EDTA, the cylinders unroll and to form a closed membrane while simultaneously encapsulating the preformed liposomes. b) Freeze-fracture transmission electron microscopy (TEM) images of intermediate (i) and final (ii) structures of vesosomes. The vesosomes were extruded through a 400 nm pore size filter. Adapted from Ref 28 and 29. Copyright American Chemical Society. Reproduced with permission.

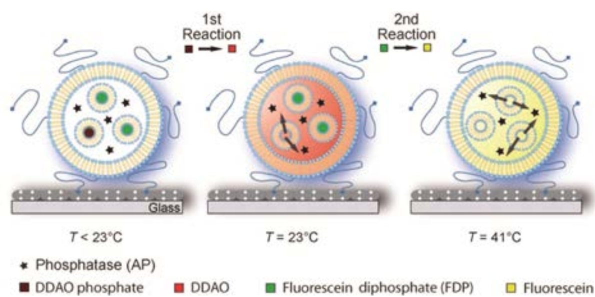

Figure 3. Schematic illustration of the consecutive enzymatic reactions within a liposome-in-liposome assembly. The external vesicle encapsulates a model enzyme alkaline phosphatase (AP) and two populations of substrate-loaded internal vesicles: dichlorodimethylacridinone phosphate (DDAO phosphate)-loaded liposomes with T_m = 23 °C and fluorescein diphosphate (FDP)-loaded liposomes with T_m = 41 °C. Upon increasing the temperature above the T_m of each liposomal subcompartment, substrates are sequentially released into the interior of the external vesicle and converted to fluorescent products by the enzyme. Adapted from Ref 36. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

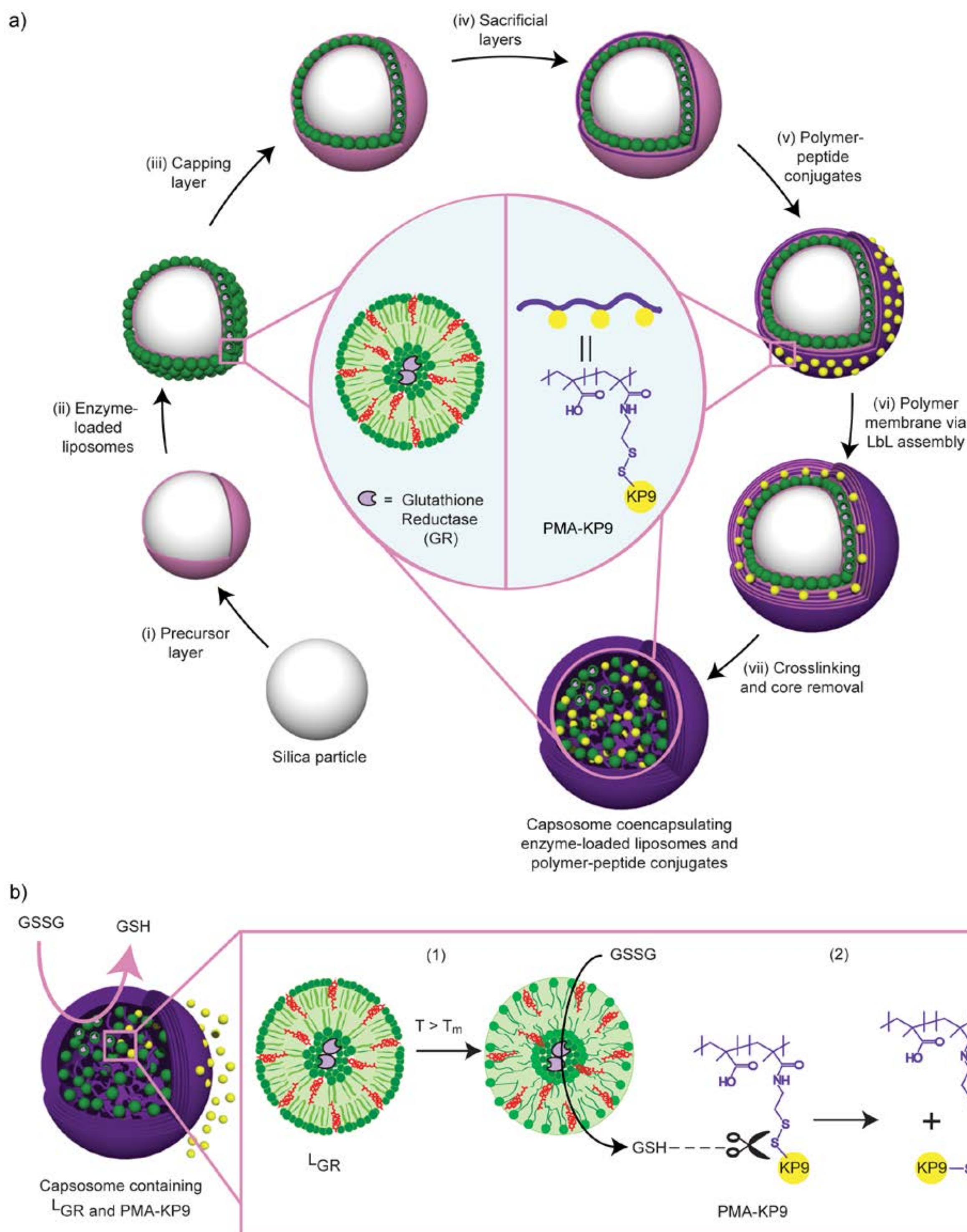

Figure 4. a) Schematic illustration of the assembly of capsosomes coencapsulating glutathione reductase-loaded liposomes and PMA-KP9 polymer-peptide conjugates into the polymer carrier capsules. b) Release of encapsulated KP9 oligopeptides triggered by the catalytic activity of glutathione reductase in capsosomes employing the increased membrane permeability at the T_m of the liposomes. Adapted from Ref 51. Copyright American Chemical Society. Reproduced with permission.

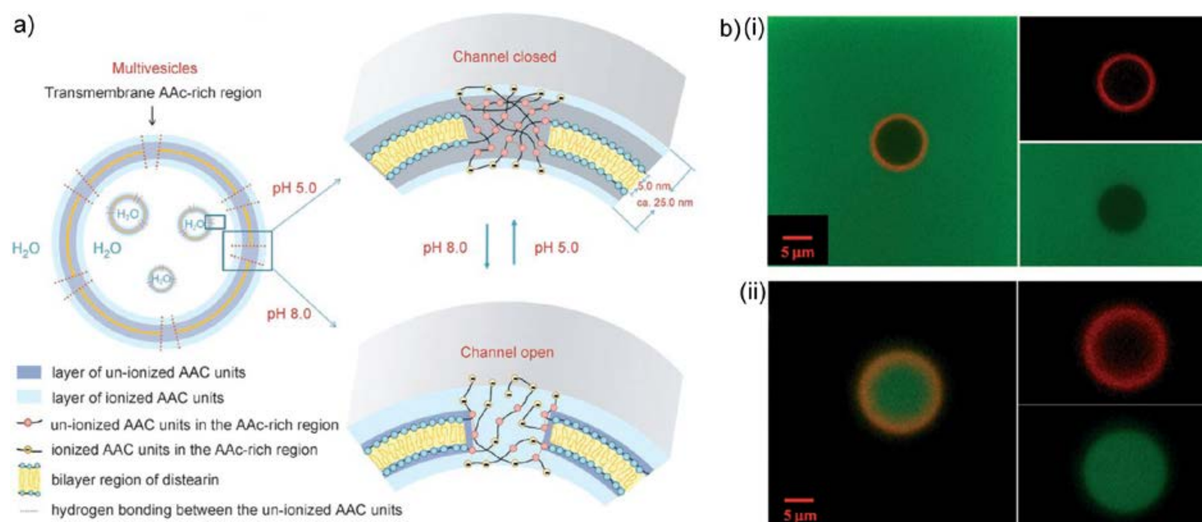

Figure 5. a) Schematic illustration of a multicompartment poly(AAc-*co*-DSA) polymersome assembly equipped with pH-responsive transmembrane channels. The pH-responsive process is reversible in the range of pH 5 to 8. b) Confocal laser scanning microscopy images of Nile-red stained polymersomes with the addition of calcein at pH 5 (polymersomes are impermeable to calcein) (i), and after pH adjustment to 8 (calcein diffuses into polymersomes) and replacement with fresh buffer of pH 5 (calcein is confined within the polymersomes) (ii). Adapted from Ref 56. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

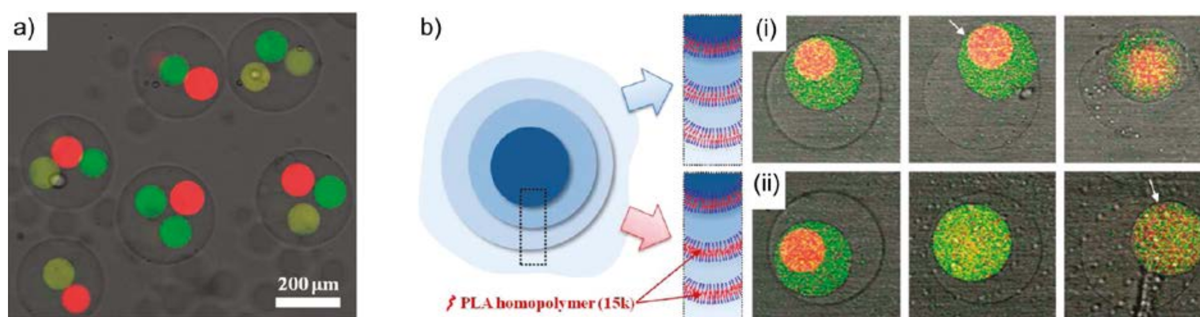

Figure 6. a) Confocal laser scanning microscopy image of polymersomes-in-polymersomes encapsulating sulforhodamine B (red), 8-hydroxyl-1,3,6-pyrenetrisulfonic acid (green), and a mixture of both dyes (yellow) in each internal compartments. b) A series of confocal laser scanning microscopy images showing sequential dissociation of the polymersome membranes from the outermost to the innermost polymersomes (i) and from the innermost to the outermost polymersomes (ii). The selective dissociation of the polymersomes can be controlled by the incorporation of additional PLA-homopolymer into the hydrophobic region of the bilayer, which enhances the stability of the membrane. Adapted from Ref 58. Copyright American Chemical Society. Reproduced with permission.

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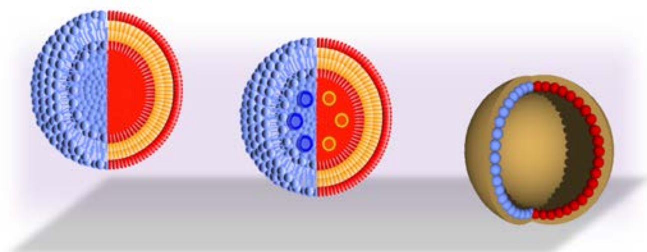
Biomimetic Liposome- and Polymersome-Based Multicompartmentalized Assemblies

Rona Chandrawati and Frank Caruso



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