Synthetic Compartments for Biomedical Applications

Subjects: Polymer Science

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Nano- and micrometer-sized compartments composed of synthetic polymers are designed to mimic spatial and temporal divisions found in nature. Self-assembly of polymers into compartments such as polymersomes, giant unilamellar vesicles (GUVs), layer-by-layer (LbL) capsules, capsosomes, or polyion complex vesicles (PICsomes) allows for the separation of defined environments from the exterior. These compartments can be further engineered through the incorporation of (bio)molecules within the lumen or into the membrane, while the membrane can be decorated with functional moieties to produce catalytic compartments with defined structures and functions. Nanometer-sized compartments are used for imaging, theranostic, and therapeutic applications as a more mechanically stable alternative to liposomes, and through the encapsulation of catalytic molecules, i.e., enzymes, catalytic compartments can localize and act in vivo. On the micrometer scale, such biohybrid systems are used to encapsulate model proteins and form multicompartmentalized structures through the combination of multiple compartments, reaching closer to the creation of artificial organelles and cells.

Keywords: polymers ; compartments ; biomedical

1. Introduction

Mimicking the structure and function of materials found in nature is a well-known strategy for developing materials with structures and functions of interest. Vesicles are nature's simplest compartments (e.g., organelles inside cells) and have existed since the first cells. While cell membranes are based on phospholipids, as they are natural amphiphiles, vesicle-forming molecules can also be synthetic. Copolymers are alternative amphiphiles that self-assemble into macromolecular assemblies [1][2]. Block copolymers, containing hydrophilic and hydrophobic regions that can be arranged in different repeating orders, have properties that can be controlled through chemical modification to support a desired application [3]. Based on the number of blocks used, amphiphilic polymers are referred to as di- or triblock copolymers, AB or ABA, respectively, with A representing the hydrophilic and B the hydrophobic block. Polyelectrolytes, polymers containing ionic or ionizable groups, also have the ability to self-assemble into synthetic compartments such as layer-by-layer (LbL) capsules or PICsomes [4]. Various molecular properties of the polymer blocks such as polydispersity, charge, block ratio and length, and molecular weight are essential to induce the supramolecular assemblies formed thereof. The addition of biomolecules changes the properties of the polymer membranes, the integration of phospholipids can change membrane mechanical properties [5][6][7], and membrane proteins can alter membrane permeability [8] and even provide the desired functionality via their intrinsic bioactivity [9][10].

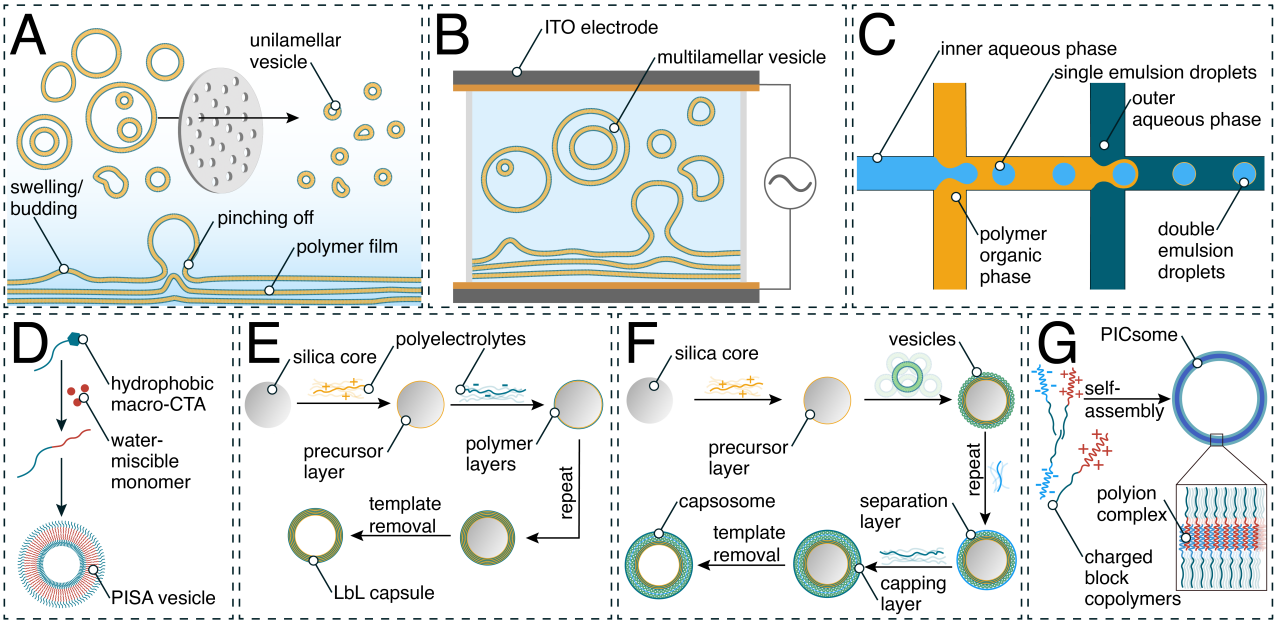
2. Generation of Synthetic Compartments

A hollow cavity surrounded by a synthetic barrier (membrane, layers of polymers) represents the common architecture that forms single compartments. When more complex structures are the aim, there are two different approaches: (i) encapsulation of small nanocompartments in GUVs to generate compartments-in-compartments and (ii) zipping together compartments to generate clusters or networks [11][12][13][14].

Self-assembly of amphiphilic block copolymers in aqueous solutions can result in several types of nano- or micrometer-sized structures such as micelles, tubes, or vesicles (polymersomes and GUVs) [15][16]. Self-assembly is driven by noncovalent interactions such as the hydrophobic effect or electrostatic interactions [17]. A list of polymers commonly utilized to form compartments with a hollow-sphere architecture is presented in **Table 1**.

Here the researchers present the most established self-assembly methods for nano- and micro-sized polymer vesicles (Scheme 1) and briefly describe how layer-by-layer assembly is used to generate capsules and capsosomes. In methods such as solvent switch, the block copolymer is dissolved in a water-miscible organic solvent. This is followed by the dropwise addition of an aqueous buffer to slowly replace the organic phase [18][19]. Contrarily, the cosolvent method is based on the dropwise addition of a copolymer solution to an aqueous buffer phase, which induces the self-assembly

process of copolymers [20]. One drawback of these methods is the residual presence of organic solvent in the final solution, which is undesirable for biologically relevant applications. The film rehydration method (Scheme 1A) takes place in a more biocompatible manner, as the organic solution of the copolymer is completely dried, forming a film. The thin copolymer film is subsequently rehydrated with an aqueous solution, inducing the self-assembly process and resulting in supramolecular architectures (micelles, polymersomes, worm-like assemblies). When the hydrophilic–hydrophobic ratio *f* of the copolymers is in the range of 35 ± 10% [21], vesicles are the favored supramolecular assembly, even if the copolymers have a rather high polydispersity index (PDI) [13][22]. Film rehydration is well suited for loading polymersomes with sensitive molecules, such as enzymes and proteins, during the self-assembly process [23]. However, as this process is based on statistic loading of the desired molecules present in the rehydration solution, the encapsulation efficiency is highly dependent on their solubility and usually ranges between 5 and 20% for encapsulation of a single type of high-molecular-weight molecule, such as proteins and enzymes, and decreases even further for coencapsulation of two types of proteins [24][25][26].



Scheme 1. Schematic representation of selected production methods of synthetic compartments: (**A**) film rehydration and subsequent extrusion of block copolymers; (**B**) electroformation; (**C**) double emulsion formation with a microfluidic setup; (**D**) polymerization-induced self-assembly (PISA); (**E**) layer-by-layer (LbL) assembly; (**F**) assembly of capsosomes; and (**G**) PICsomes.

When the aim is giant unilamellar vesicles, methods used for polymersome generation are joined by other methods, including electroformation. The electroformation technique (Scheme 1B) is based on the spontaneous swelling of a dried block copolymer film that has been deposited on two electrodes of indium tin oxide (ITO)-coated glass or platinum and the consequent formation of GUVs in the presence of an aqueous solution stimulated by an electric field [13][27]. This method has a high yield of assemblies with high levels of unilamellarity [28] but is limited by a broad size dispersity and can be used only for uncharged amphiphiles to avoid electrostatic interactions affecting the self-assembly process [29]. Emulsion centrifugation is a method for the formation of GUVs wherein a water-in-oil emulsion suspension is transferred into a water phase by centrifugation [30]. The single emulsions cross a polymer monolayer at the water/oil interface and are coated by a second monolayer, resulting in a bilayered GUV. Microfluidic technology represents a step forward that has been recently used for high-throughput GUV formation with a narrow size distribution based on microdevice channel sizes and junction design [30][31][32]. Polymer-stabilized water–oil–water (w/o/w) double emulsions are used to form GUVs. An aqueous solution is enclosed in a layer of organic phase, consisting of the amphiphilic block copolymer dissolved in a volatile organic solvent (Scheme 1C). A flow of outer aqueous solution then pinches off the double emulsion droplets. Double emulsions can be created with a multitude of different microfluidic designs, such as glass capillaries [32][33] or molded microchannels in varying layouts [34][35][36]. Subsequent evaporation of the volatile organic solvent leads to the formation of a GUV with a polymer membrane. An extremely high encapsulation efficiency (99%) can be obtained by including in the inner flow the molecules (enzymes, proteins) desired to be encapsulated within the generated GUVs. The molecules planned to be entrapped in the membrane (biopores, membrane proteins) are also included in the aqueous flow at this stage [35]. Despite the high encapsulation efficiency and monodispersity, there are still limitations to using this method for GUV formation, such as its complexity and the necessity of specialized equipment.

An entirely different approach is that of polymerization-induced self-assembly (PISA), a technique that directly produces nanoassemblies during the block copolymer synthesis (Scheme 1D) [37][38][39]. PISA's principle is based on the chain extension of a soluble precursor polymer block in a suitable solvent with the simultaneous use of a second polymerizing monomer, resulting in the formation of an insoluble second block, yielding polymersomes [37][38][39]. The advantage of the method is its efficiency, as it combines synthesis with self-assembly and is characterized by monodispersity. On the other hand, this method is limited by decreased colloidal stability in the presence of ionic surfactants. Furthermore, PISA can only be applied for a small selection of monomers [40].

Polymer capsules are frequently formed through LbL deposition (Scheme 1E), a technique that involves the controlled adsorption of polymer layers on a sacrificial colloidal particle, alternating between oppositely charged materials, i.e., one layer of negatively charged polymer is followed by one of positively charged polymer [41][42][43]. Once the appropriate number of layers has been applied, the system is submerged in a solution designed to either dissolve the template or simply detach the vesicle membrane, allowing one to separate out the freshly formed vesicles. LbL capsule formation is simple yet versatile in terms of compartment size and components, including their functionality possibilities [44][45]. However, this method is limited by its dependence on the sacrificial template. Formed through a similar technique, capsosomes are capsules of which the membranes are composed of smaller vesicles. Their fabrication involves the deposition of an initial number of polymer layers onto a colloidal particle of specified size. Next, one or multiple layers of liposomes are attached, segregated by a polymeric separation layer, followed by a final deposition of several protective polymer layers (Scheme 1F) [46][47]. Assemblies of a different type, PICsomes, are the vesicular form of polyion complex (PIC) particles and are self-assembled through electrostatic interactions [19][22]. PICsomes are composed of oppositely charged block copolymers (Scheme 1G), and their membranes are semipermeable, especially to hydrophilic solutes. A major advantage of PICsomes is their facile formation—they naturally self-assemble through the mixing of their charged components [48]. One disadvantage of PICsomes is that their components typically need to be combined at equal concentrations to result in a charge-neutral vesicle, though recent experimentation has shown that charge-balance-sensitive materials can be formed by varying this ratio [49].

Table 1. Polymers used for biomedical applications.

Polymer	Method of Self-Assembly	Characteristics
Carbohydrate-*b*-PPG	Direct hydration method [50]	Forms capsosomes, inherently permeable to low-molecular-weight compounds
Chitosan	Sonication-assisted mixing (capsules) [51], LbL [52]	Biocompatible, natural polymer
CTAB	LbL [52]	Surfactant, forms micelles in the absence of another polymer
PA/DEX	LbL [53]	Biocompatible polysaccharide (anionic)
P(OEGMA300-*grad*-HPMA)	PISA [39]	Biocompatible assembly, monomers and a macromolecular precursor need to be: (i) solvophilic and (ii) compatible with each other
PA/PLA	LbL [53]	Biocompatible cationic polyelectrolyte
PAA	LbL [54]	Anionic polyelectrolyte
PAH	LbL [55][56]	Cationic polyelectrolyte
PAMAM	Mixing (PICsomes) [57]	Dendrimer (branched structure)

Polymer	Method of Self-Assembly	Characteristics
P(Asp-AP)	Mixing (PICsomes) [58][59][60]	Anionic polyelectrolyte, forms PICsomes, cannot form vesicles on its own
PATK	Mixing (PICsomes) [61]	Cationic polyelectrolyte
PBd-*b*-PEG	Double emulsion microfluidics [33]	Biocompatible
PBd–*b*-PEO	Emulsion centrifugation [30], Electroformation [62], Film rehydration [63]	Pure or as hybrid (with POPC) polymersomes for membrane protein insertion, assembly of asymmetric polymer/lipid (POPC) hybrid membranes
PBO-*b*-PG	Microfluidic double emulsion, solvent switch [64]	Biocompatible
PCL-*b*-P[Glu-stat-(Glu-ADA)]	Solvent switch [65]	Biodegradable, bone-targeting
PCL-*b*-PTrp-*b*-P(Lys-statPhe)	Solvent switch [66]	Biocompatible, biodegradable, antibacterial
PDMS-*b*-heparin	Film rehydration [67]	Forms polymersomes in combination with PMOXA-*b*-PDMS-*b*-PMOXA, forms micelles by itself
PDMS-*g*-PEO	Electroformation, Film rehydration [68]	Pure or as hybrid (with PC) polymersomes and GUVs for membrane protein insertion
PEG-*b*-PCL	Electroformation [69], film rehydration [70]	Multidomain membrane formation with lipids (DPPC)
PEG-P(CLgTMC)	Direct hydration method [71]	Biodegradable, intrinsic fluorescence
PEG-*b*-P(CPTKMA-*co*-PEMA)	Solvent exchange method [72]	Biocompatible, conjugated with campthothecin
PEG-GPLGVRG-PCL-PGPMA	Film hydration method [73]	Biocompatible, MMP-cleavable peptide and CPP-mimicking polymer
PEG-*b*-PHPMA	PISA [74]	Highly hydrated membrane, size-selective transport of molecules
PEG-*b*-PIC	Solvent exchange [75]	Biocompatible, iodine-rich for SPECT/CT and radioisotope therapy
PEG-*b*-PLA	Film rehydration [70], double emulsion microfluidics [33]	Forms polymersomes with and without lipid mixing, biodegradable

Polymer	Method of Self-Assembly	Characteristics
PEG-*b*-polypeptide	Mixing (PICsomes) [76]	pH-responsive, biocompatible
PEG-*b*-PAsp	Mixing (PICsomes) [58][60]	Linear polymer, forms PICsomes, micelles or hydrogels, biocompatible
PEG-*b*-PS	Solvent switch method [77][78]	Biocompatible, formation of stomatocytes, rigid assemblies
PEI-*b*-PDLLA	Microfluidic double emulsion [79]	Biocompatible, cationic assemblies, can form polymer stomatocytes
PEO-*b*-PBO	Film rehydration [80]	Forms asymmetric polymersomes
PEO-*b*-PCL	Emulsification-induced assembly [81]	Low interfacial tension solvent or SDS is needed to control the assembly
PEO-*b*-PCL-*b*-PMOXA	Film rehydration [82]	Rehydration at 62 °C due to the semi crystalline nature of the PCL block
PEO-*b*-P(CMA-stat-DEA-stat-GEMA)	Solvent exchange method [83]	Biocompatible, CMA photocrosslinking stabilization
PEO-*b*-PEHOx-*b*-PEtOz	Solvent switch, film rehydration [22]	Asymmetric membrane, can be used for directed protein insertion
PEO-*b*-PPO-*b*-PEO (Pluronics L121)	Double emulsion microfluidics [33]	Assembly via DNA linkage
PiB-*b*-PEG	Freeze–thaw extrusion [84]	Biocompatible, high chemical and thermal stability
PLys	Mixing (PICsomes) [85]	Cationic polyelectrolyte
PMA	LbL [56]	Labor-intensive LbL assembly
PMOXA-*b*-PDMS	Film rehydration [86][87], microfluidic double emulsion [35]	Formation of nano- and micro-sized vesicles in biocompatible, aqueous conditions, various channels and proteins can be inserted
PMOXA-*b*-PDMS-*b*-PMOXA	Film rehydration [67][88]	Formation of nano and micro-sized vesicles in biocompatible, aqueous conditions, various channels and proteins can be inserted
PMPC-*b*-PDPA	Film rehydration [80][89]	Formation of (asymmetric) polymersomes, can be electroporated

Polymer	Method of Self-Assembly	Characteristics
POEGMA-*b*-P(ST-co-VBA)	PISA [37]	Biocompatible assembly, monomers and a macromolecular precursor need to be: (i) solvophilic and (ii) compatible with each other
Poly(dopamine)	LbL [90]	Simplified LbL capsule formation
PS-*b*-PEO	Emulsification [91]	High capacity of ammonia capture in bile salt-containing buffer
PSMA-PBzMA	PISA [38]	Biocompatible assembly, monomers and a macromolecular precursor need to be: (i) solvophilic and (ii) compatible with each other
PSS-*b*-PEO-*b*-PSS	Mixing (PICsomes) [57]	Forms PICsomes with loops within the membrane when combined with poly(amidoamine) dendrimers
PVP	LbL [92]	Work-intensive LbL assembly

3. Requirements for Compartments to Be Used in Biomedical Applications

For any biologically relevant material, the transition from the research laboratory to a clinical setting is a complex procedure. This is particularly true when the final application is in the field of medicine, pharmaceutical production, or personal care [93][94][95]. In the case of compartments, there is a complex set of requirements, first for the copolymers used to generate the compartments and then for the compartments themselves. Therefore, for an amphiphilic block copolymer or a polyelectrolyte to be used in biomedical applications some crucial aspects need to be considered: (i) the polymers should be able to self-assemble in aqueous solutions [15][19][82]; (ii) they need to be nontoxic and biocompatible [96]; (iii) they need to have the appropriate physical characteristics (i.e., flexibility, membrane thickness) [97] in order to facilitate the functional insertion, encapsulation, and reconstitution of biomolecules [98][99]; (iv) control over their surface charge is crucial for the attachment of biomolecules or further in vivo applications [100]; (v) they need to maintain stability under physiological conditions [101][102] (i.e., in high temperature or salt concentration, their functionality must remain, and the availability of the encapsulated biomolecules must be sustained); and (vi) of particular import for therapeutic compartments is their ability to undergo endosomal escape so the therapeutic agent is not hindered by cells' defense mechanisms and can arrive at the pathogenic site [103][104][105]. Besides these, one of the most limiting factors is the fate of copolymers in the body. Therefore, their biodegradability still remains a real challenge for various copolymers that fulfill the complex list of factors aforementioned.

Second, the compartments planned to be used in medical applications should fulfill their own set of requirements. While polymersomes, capsules, and capsosomes should preserve their integrity and act as catalytic compartments, they must also allow the molecular flow of substrates and products. Their membrane can be rendered permeable by biopores such as ion channels [98][106], peptide pores [26][107], DNA nanopores [89], and natural membrane proteins [102]. While many examples of functional protein reconstitution in polymeric membranes have been shown, the increased hydrophobic mismatch of polymeric membranes as compared with lipidic ones limits the choice of membrane protein. High membrane fluidity is essential for protein incorporation, as it allows integrating proteins into membranes that are several times thicker than the protein itself and helps the protein to overcome the size mismatch between its own hydrophobic region and the hydrophobic region of the polymer membrane [97]. Furthermore, the surface charge plays a crucial role in the in vivo functionality, biodistribution, and cellular uptake of the systems [108][109]. Specifically, positively charged compartments have been found to be better uptaken by cells and exhibit improved biodistribution [110][111][112], while negatively charged ones are usually less toxic and better at specific tissue targeting [112][113][114].

The surface functionalization of polymersomes, capsules, and capsosomes opens up many possibilities for the assembly of complex soft compartments. However, the attachment of functional groups changes the overall charge and might

induce interactions with the environment or trigger aggregation, increasing the intrinsic toxicity. Additionally, the formation of clusters of compartments increases the size of the final assembly. This limits the possible applications mainly to the intercellular matrix [115][116]. Overall, use in medical applications requires both the polymer properties (e.g., toxicity, biocompatibility) and those of the compartment (e.g., membrane composition, functionalization, size, charge) to be carefully tailored.

4. Applications of Compartments in the Biomedical Field

Numerous studies have investigated the benefits and limitations of nanometer-sized compartments for their use in imaging, therapeutics, and theranostics. As a first step, in vitro studies have explored the activity and cytotoxicity of the systems for these applications, while in vivo studies have highlighted their suitability and investigate in-depth parameters for clinical translation. Meanwhile, micrometer-sized vesicles have facilitated the modeling of cells, a crucial step in bottom-up synthetic biology aiming to bring further insights to real-life processes. An overview of biomolecules that have been used within compartments or in their membranes is presented in **Table 2**.

Table 2. Biomolecules and their applications in compartments.

Biomolecule	Polymer	Location in Assembly	Application/Function
Actin	PMOXA-*b*-PDMS-*b*-PMOXA [67]	Encapsulated (in GUVs)	Polymerization to form a cytoskeleton
ATP synthase	PDMS-*g*-PEO, PBd-*b*-PEO [68][117]	Incorporated within membrane (GUVs)	ATP generation
Bacteriorhodopsin	PDMS-*g*-PEO, PBd- *b*-PEO [117]	Incorporated within membrane (GUVs)	Pumping protons across membrane
Catalase	PEG-*b*-PS [78], PAH and DEX [55]	Encapsulated with the stomata of polymer stomatocytes and LbL capsules	Conversion of hydrogen peroxide to oxygen and water for self-propelled movement
Cholesterol–DNA	PEO-*b*-PPO-*b*-PEO (Pluronics L121), PBd-*b*-PEG, PLA-*b*-PEG [33]	Incorporated within membrane (GUVs)	Clustering of polymersomes
Cytochrome bo_3 ubiquinol oxidase (Cyt bo_3)	PBd–PEO:POPC hybrid [118], PDMS-*g*-PEO and PDMS-*g*-PEO/PC hybrid [68][119]	Incorporated within membrane (polymersomes, GUVs)	Pumping protons across membrane
DNA nanopore NP-3c	PMPC-*b*-PDPA [89]	Incorporated within membrane (GUVs)	Pore formation for cross-membrane diffusion
Dopa decarboxylase (DDC)	PMOXA-*b*-PDMS [120]	Encapsulated (in polymersomes)	Production of dopamine
Erythrosine B (and its ester derivatives)	F127 Pluronic (mixed with DPPC lipids) [121]	Incorporated within membrane (polymersomes)	Photodynamic therapy

Biomolecule	Polymer	Location in Assembly	Application/Function
Glucose oxidase (Gox)	PMOXA-*b*-PDMS [35][87], PEG-*b*-P(CPTKMA-*co*-PEMA) [72], PATK and PEG-*b*-Pasp [61]	Encapsulated (in GUVs, polymersomes, and PICsomes)	Catalysis of glucose oxidation to hydrogen peroxide and D-glucono-δ-lactone
Gramicidin	PMOXA-*b*-PDMS [35], PMOXA-*b*-PDMS-*b*-PMOXA [67]	Incorporated within membrane	Membrane permeabilization towards ions
Horseradish peroxidase (HRP)	PMOXA-*b*-PDMS [35], PMOXA-*b*-PDMS-*b*-PMOXA [86][101], carbohydrate-*b*-PPG [50]	Encapsulated (in GUVs, polymersomes, capsosomes)	Catalysis of oxidation of organic substrates by hydrogen peroxide
Icosane	PAA and PAH (LbL) [54]	Encapsulated (in capsules)	Acting as a phase change material for thermal energy storage
Inducible nitric oxide synthase (iNOS)	PMOXA-*b*-PDMS-*b*-PMOXA [122]	Encapsulated (in polymersomes)	Oxidation of l-arginine to l-citrulline and nitric oxide (NO)
Ionomycin	PMOXA-*b*-PDMS-*b*-PMOXA [67]	Incorporated within membrane	Membrane permeabilization towards ions
Laccase	PMOXA-*b*-PDMS [123]	Encapsulated (in polymersomes)	Oxidation of phenolic and nonphenolic compounds
Lactoperoxidase (LPO)	PMOXA-*b*-PDMS [87]	Encapsulated (in polymersomes)	Oxidation of Amplex red using hydrogen peroxide
L-asparaginase	PMPC-*b*-PDPA and PEO-*b*-PBO [80], PEG-*b*-Pasp and P(Asp-AP) [60], PEG-*b*-PHPMA [74]	Encapsulated (in polymersomes, PICsomes)	Catalysis of L-asparagine to l-aspartic acid and ammonia
Lipase	PMOXA-*b*-PDMS-*b*-PMOXA [67]	Encapsulated (in polymersomes)	Catalysis of the hydrolysis of fats
Luciferase	PMOXA-*b*-PDMS [124]	Encapsulated (in polymersomes)	Bioluminescence
Melittin	PMOXA-*b*-PDMS [87][107][125]	Incorporated within membrane (polymersomes, GUVs)	Pore formation for cross-membrane diffusion
Methionine γ-lyase (MGL)	PEG-P(Asp) and PLys [59][85]	Encapsulated (in PICsomes)	Cancer therapy

Biomolecule	Polymer	Location in Assembly	Application/Function
Outer membrane protein F from *E. coli* (OmpF)	PMOXA-*b*-PDMS [35], PMOXA-*b*-PDMS-*b*-PMOXA [86][101]	Incorporated within membrane (GUVs, polymersomes)	Pore formation for cross-membrane diffusion
Penicillin acylase	PMOXA-*b*-PDMS-*b*-PMOXA [126]	Encapsulated (in polymersomes)	Production of antibiotic cephalexin
Rnase H	PEG-*b*-polypeptide (with single-stranded oligonucleotides) [76]	Encapsulated (in PICsomes)	Gene knockout therapy
β-galactosidase	PMOXA-*b*-PDMS [35] carbohydrate-*b*-PPG [50]	Encapsulated (in GUVs, capsosomes)	Catalysis of the hydrolysis of β-galactosides into monosaccharides
β-glucuronidase	PMOXA-*b*-PDMS [125]	Encapsulated (in polymersomes)	Cleavage of the glucuronide moiety from glucuronide-conjugates
Soluble guanylyl cyclase (sGC)	PMOXA-*b*-PDMS-*b*-PMOXA [122]	Encapsulated (in polymersomes)	Production of cyclic 3,5-guanosine monophosphate (cGMP)
Trypsin	PMPC-*b*-PDPA [89]	Encapsulated (in polymersomes)	Hydrolyzation of proteins
Tyrosinase	PMOXA-*b*-PDMS [127]	Encapsulated (in polymersomes)	Oxidation of L-DOPA
Urate oxidase (UOX)	PMOXA-*b*-PDMS-*b*-PMOXA [12]	Encapsulated (in polymersomes)	Production of hydrogen peroxide for a cascade reaction

References

1. Palivan, C.G.; Goers, R.; Najer, A.; Zhang, X.; Car, A.; Meier, W. Bioinspired polymer vesicles and membranes for biological and medical applications. Chem. Soc. Rev. 2016, 45, 377–411.
2. Matoori, S.; Leroux, J.C. Twenty-five years of polymersomes: Lost in translation? Mater. Horiz. 2020, 7, 1297–1309.
3. Feng, H.; Lu, X.; Wang, W.; Kang, N.-G.; Mays, J.W. Block copolymers: Synthesis, self-assembly, and applications. Polymers 2017, 9, 494.
4. Kim, K.T.; Meeuwissen, S.A.; Nolte, R.J.; van Hest, J.C. Smart nanocontainers and nanoreactors. Nanoscale 2010, 2, 844–858.
5. Go, Y.K.; Leal, C. Polymer–lipid hybrid materials. Chem. Rev. 2021, 121, 13996–14030.
6. Thoma, J.; Belegrinou, S.; Rossbach, P.; Grzelakowski, M.; Kita-Tokarczyk, K.; Meier, W. Membrane protein distribution in composite polymer—Lipid thin films. Chem. Commun. 2012, 48, 8811–8813.
7. Fauquignon, M.; Ibarboure, E.; Meins, J.-F. Membrane reinforcement in giant hybrid polymer lipid vesicles achieved by controlling the polymer architecture. Soft Matter 2021, 17, 83–89.

8. Gilbert, R.J.; Serra, M.D.; Froelich, C.J.; Wallace, M.I.; Anderluh, G. Membrane pore formation at protein—Lipid interfaces. Trends Biochem. Sci. 2014, 39, 510–516.
9. Hu, Z.; Ho, J.C.; Nallani, M. Synthetic (polymer) biology (membrane): Functionalization of polymer scaffolds for membrane proteins. Curr. Opin. Biotechnol. 2017, 46, 51–56.
10. Hindley, J.W.; Law, R.V.; Ces, O. Membrane functionalization in artificial cell engineering. SN Appl. Sci. 2020, 2, 593.
11. Goers, R.; Thoma, J.; Ritzmann, N.; Di Silvestro, A.; Alter, C.; Gunkel-Grabole, G.; Fotiadis, D.; Müller, D.J.; Meier, W. Optimized reconstitution of membrane proteins into synthetic membranes. Commun. Chem. 2018, 1, 35.
12. Belluati, A.; Craciun, I.; Liu, J.; Palivan, C.G. Nanoscale enzymatic compartments in tandem support cascade reactions in vitro. Biomacromolecules 2018, 19, 4023–4033.
13. Rideau, E.; Wurm, F.R.; Landfester, K. Self-assembly of giant unilamellar vesicles by film hydration methodologies. Adv. Biosyst. 2019, 3, 1800324.
14. Cabukusta, B.; Neefjes, J. Mechanisms of lysosomal positioning and movement. Traffic 2018, 19, 761–769.
15. Blanazs, A.; Armes, S.P.; Ryan, A.J. Self-assembled block copolymer aggregates: From micelles to vesicles and their biological applications. Macromol. Rapid Commun. 2009, 30, 267–277.
16. Discher, D.E. Polymer vesicles. Science 2002, 297, 967–973.
17. Antonietti, M.; Förster, S. Vesicles and liposomes: A self-assembly principle beyond lipids. Adv. Mater. 2003, 15, 1323–1333.
18. Daubian, D.; Gaitzsch, J.; Meier, W. Synthesis and complex self-assembly of amphiphilic block copolymers with a branched hydrophobic Poly(2-Oxazoline) into multicompartment micelles, pseudo-vesicles and Yolk/Shell nanoparticles. Polym. Chem. 2020, 11, 1237–1248.
19. Wehr, R.; Gaitzsch, J.; Daubian, D.; Fodor, C.; Meier, W. Deepening the insight into poly(butylene oxide)-Block-poly(Glycidol) synthesis and self-assemblies: Micelles, worms and vesicles. RSC Adv. 2020, 10, 22701–22711.
20. Li, B.-Y.; Li, Y.-C.; Lu, Z.-Y. The important role of cosolvent in the amphiphilic diblock copolymer self-assembly process. Polymer 2019, 171, 1–7.
21. Balasubramanian, V.; Herranz-Blanco, B.; Almeida, P.V.; Hirvonen, J.; Santos, H.A. Multifaceted polymersome platforms: Spanning from self-assembly to drug delivery and protocells. Prog. Polym. Sci. 2016, 60, 51–85.
22. Daubian, D.; Fillion, A.; Gaitzsch, J.; Meier, W. One-pot synthesis of an amphiphilic ABC triblock copolymer PEO- b -PEHOx- b -PEtOz and its self-assembly into nanoscopic asymmetric polymersomes. Macromolecules 2020, 53, 11040–11050.
23. Parnell, A.J.; Tzokova, N.; Topham, P.D.; Adams, D.J.; Adams, S.; Fernyhough, C.M.; Ryan, A.J.; Jones, R.A. The efficiency of encapsulation within surface rehydrated polymersomes. Faraday Discuss. 2009, 143, 29–46.
24. Belluati, A.; Craciun, I.; Meyer, C.E.; Rigo, S.; Palivan, C.G. Enzymatic reactions in polymeric compartments: Nanotechnology meets nature. Curr. Opin. Biotechnol. 2019, 60, 53–62.
25. Pachioni-Vasconcelos, J.D.; Apolinário, A.C.; Lopes, A.M.; Pessoa, A., Jr.; Barbosa, L.R.; Rangel-Yagui, C.D. Compartmentalization of therapeutic proteins into semi-crystalline PEG-PCL polymersomes. Soft Mater. 2021, 19, 222–230.
26. Lomora, M.; Garni, M.; Itel, F.; Tanner, P.; Spulber, M.; Palivan, C.G. Polymersomes with engineered ion selective permeability as stimuli-responsive nanocompartments with preserved architecture. Biomaterials 2015, 53, 406–414.
27. Ibarboure, E.; Fauquignon, M.; Le Meins, J.-F. Obtention of giant unilamellar hybrid vesicles by electroformation and measurement of their mechanical properties by micropipette aspiration. J. Vis. Exp. 2020, 155, 60199.
28. Walde, P.; Cosentino, K.; Engel, H.; Stano, P. Giant vesicles: Preparations and applications. ChemBioChem 2010, 11, 848–865.
29. Garni, M.; Wehr, R.; Avsar, S.Y.; John, C.; Palivan, C.; Meier, W. Polymer membranes as templates for bio-applications ranging from artificial cells to active surfaces. Eur. Polym. J. 2019, 112, 346–364.
30. Peyret, A.; Ibarboure, E.; Le Meins, J.-F.; Lecommandoux, S. Asymmetric hybrid polymer—Lipid giant vesicles as cell membrane mimics. Adv. Sci. 2018, 5, 1700453.
31. dos Santos, E.C.; Angelini, A.; Hürlimann, D.; Meier, W.; Palivan, C.G. Giant polymer compartments for confined reactions. Chemistry 2020, 2, 470–489.
32. Shum, H.C.; Kim, J.-W.; Weitz, D.A. Microfluidic fabrication of monodisperse biocompatible and biodegradable polymersomes with controlled permeability. J. Am. Chem. Soc. 2008, 130, 9543–9549.

33. Luo, R.; Göpfrich, K.; Platzman, I.; Spatz, J.P. DNA-based assembly of multi-compartment polymersome networks. Adv. Funct. Mater. 2020, 30, 2003480.

34. Deshpande, S.; Caspi, Y.; Meijering, A.E.; Dekker, C. Octanol-assisted liposome assembly on chip. Nat. Commun. 2016, 7, 1–9.

35. dos Santos, E.C.; Belluati, A.; Necula, D.; Scherrer, D.; Meyer, C.E.; Wehr, R.P.; Lörtscher, E.; Palivan, C.G.; Meier, W. Combinatorial strategy for studying biochemical pathways in double emulsion templated cell-sized compartments. Adv. Mater. 2020, 32, 2004804.

36. Weiss, M.; Frohnmayer, J.P.; Benk, L.T.; Haller, B.; Janiesch, J.W.; Heitkamp, T.; Börsch, M.; Lira, R.B.; Dimova, R.; Lipowsky, R.; et al. Sequential bottom-up assembly of mechanically stabilized synthetic cells by microfluidics. Nat. Mater. 2018, 17, 89–95.

37. Phan, H.; Taresco, V.; Penelle, J.; Couturaud, B. Polymerisation-induced self-assembly (PISA) as a straightforward formulation strategy for stimuli-responsive drug delivery systems and biomaterials: Recent advances. Biomater. Sci. 2021, 9, 38–50.

38. Dorsman, I.R.; Derry, M.J.; Cunningham, V.J.; Brown, S.L.; Williams, C.N.; Armes, S.P. Tuning the vesicle-to-worm transition for thermoresponsive block copolymer vesicles prepared via polymerisation-induced self-assembly. Polym. Chem. 2021, 12, 1224–1235.

39. Xu, S.; Corrigan, N.; Boyer, C. Forced gradient copolymerisation: A simplified approach for polymerisation-induced self-assembly. Polym. Chem. 2021, 12, 57–68.

40. Canning, S.L.; Smith, G.N.; Armes, S.P. A critical appraisal of RAFT-mediated polymerization-induced self-assembly. Macromolecules 2016, 49, 1985–2001.

41. Donath, E.; Sukhorukov, G.B.; Caruso, F.; Davis, S.A.; Möhwald, H. Novel hollow polymer shells by colloid-templated assembly of polyelectrolytes. Angew. Chem. Int. Ed. 1998, 37, 2201–2205.

42. Eivazi, A.; Medronho, B.; Lindman, B.; Norgren, M. On the development of all-cellulose capsules by vesicle-templated layer-by-layer assembly. Polymers 2021, 13, 589.

43. Elizarova, I.S.; Luckham, P.F. Layer-by-layer adsorption: Factors affecting the choice of substrates and polymers. Adv. Colloid Interface Sci. 2018, 262, 1–20.

44. Kurapati, R.; Groth, T.W.; Raichur, A.M. Recent developments in layer-by-layer technique for drug delivery applications. ACS Appl. Bio Mater. 2019, 2, 5512–5527.

45. Campbell, J.; Vikulina, A.S. Layer-by-layer assemblies of biopolymers: Build-Up, mechanical stability and molecular dynamics. Polymers 2020, 12, 1949.

46. Francesch, M.B. Capsosomes: The revolutionary enzyme carriers. Eur. Pharm. Rev. 2019, 24, 34–36.

47. Städler, B.; Chandrawati, R.; Goldie, K.; Caruso, F. Capsosomes: Subcompartmentalizing polyelectrolyte capsules using liposomes. Langmuir 2009, 25, 6725–6732.

48. Sun, J.; Li, Z. Polyion complexes via electrostatic interaction of oppositely charged block copolymers. Macromolecules 2020, 53, 8737–8740.

49. Liu, Y.; Maruyama, T.; Kc, B.; Mori, T.; Katayama, Y.; Kishimura, A. Inducible dynamic behavior of polyion complex vesicles by disrupting charge balance. Chem. Lett. 2021, 50, 1034–1037.

50. Nishimura, T.; Sasaki, Y.; Akiyoshi, K. Biotransporting self-assembled nanofactories using polymer vesicles with molecular permeability for enzyme prodrug cancer therapy. Adv. Mater. 2017, 29, 1702406.

51. Odrobińska, J.; Gumieniczek-Chłopek, E.; Szuwarzyński, M.; Radziszewska, A.; Fiejdasz, S.; Strączek, T.; Kapusta, C.; Zapotoczny, S. Magnetically navigated core—Shell polymer capsules as nanoreactors loadable at the oil/water interface. ACS Appl. Mater. Interfaces 2019, 11, 10905–10913.

52. Cai, H.; Wang, P.; Zhang, D. PH-responsive linkages-enabled layer-by-layer assembled antibacterial and antiadhesive multilayer films with polyelectrolyte nanocapsules as biocide delivery vehicles. J. Drug Deliv. Sci. Technol. 2019, 54, 101251.

53. Svenskaya, Y.; Garello, F.; Lengert, E.; Kozlova, A.; Verkhovskii, R.; Bitonto, V.; Ruggiero, M.R.; German, S.; Gorin, D.; Terreno, E. Biodegradable polyelectrolyte/magnetite capsules for mr imaging and magnetic targeting of tumors. Nanotheranostics 2021, 5, 362–377.

54. Seitz, S.; Ajiro, H. Self-assembling weak polyelectrolytes for the layer-by-layer encapsulation of paraffin-type phase change material icosane. Sol. Energy Mater. Sol. Cells 2019, 190, 57–64.

55. Larrañaga, A.; Isa, I.L.; Patil, V.; Thamboo, S.; Lomora, M.; Fernández-Yague, M.A.; Sarasua, J.-R.; Palivan, C.G.; Pandit, A. Antioxidant functionalized polymer capsules to prevent oxidative stress. Acta Biomater. 2018, 67, 21–31.

56. Sharma, V.; Vijay, J.; Ganesh, M.R.; Sundaramurthy, A. Multilayer capsules encapsulating nimbin and doxorubicin for cancer chemo-photothermal therapy. Int. J. Pharm. 2020, 582, 119350.

57. Huang, J.; Li, C.; Gao, Y.; Cai, Y.; Guo, X.; Cohen Stuart, M.A.; Wang, J. Dendrimer-based polyion complex vesicles: Loops make loose. Macromol. Rapid Commun. 2022, 43, e2100594.

58. Hori, M.; Cabral, H.; Toh, K.; Kishimura, A.; Kataoka, K. Robust polyion complex vesicles (PICsomes) under physiological conditions reinforced by multiple hydrogen bond formation derived by guanidinium groups. Biomacromolecules 2018, 19, 4113–4121.

59. Koval, V.; Morozova, E.; Revtovich, S.; Lyfenko, A.; Chobanian, A.; Timofeeva, V.; Solovieva, A.; Anufrieva, N.; Kulikova, V.; Demidkina, T. Characteristics and stability assessment of therapeutic methionine γ-Lyase-loaded polyionic vesicles. ACS Omega 2022, 7, 959–967.

60. Sueyoshi, D.; Anraku, Y.; Komatsu, T.; Urano, Y.; Kataoka, K. Enzyme-loaded polyion complex vesicles as in vivo nanoreactors working sustainably under the blood circulation: Characterization and functional evaluation. Biomacromolecules 2017, 18, 1189–1196.

61. Li, J.; Anraku, Y.; Kataoka, K. Self-boosting catalytic nanoreactors integrated with triggerable crosslinking membrane networks for initiation of immunogenic cell death by pyroptosis. Angew. Chem. Int. Ed. 2020, 59, 13526–13530.

62. Seneviratne, R.; Catania, R.; Rappolt, M.; Jeuken, L.J.; Beales, P.A. Membrane mixing and dynamics in hybrid POPC/poly(1,2-butadiene-Block-ethylene oxide) (PBd-b-PEO) lipid/block co-polymer giant vesicles. Soft Matter 2022, 18, 1294–1301.

63. Peyret, A.; Ibarboure, E.; Pippa, N.; Lecommandoux, S. Liposomes in polymersomes: Multicompartment System with temperature-triggered release. Langmuir 2017, 33, 7079–7085.

64. Wehr, R.; dos Santos, E.C.; Muthwill, M.S.; Chimisso, V.; Gaitzsch, J.; Meier, W. Fully amorphous atactic and isotactic block copolymers and their self-assembly into nano- and microscopic vesicles. Polym. Chem. 2021, 12, 5377–5389.

65. Zhou, X.; Cornel, E.J.; Fan, Z.; He, S.; Du, J. Bone-targeting polymer vesicles for effective therapy of osteoporosis. Nano Lett. 2021, 21, 7998–8007.

66. Yang, Y.-Y.; Chen, L.-S.; Sun, M.; Wang, C.-Y.; Fan, Z.; Du, J.-Z. Biodegradable polypeptide-based vesicles with intrinsic blue fluorescence for antibacterial visualization. Chin. J. Polym. Sci. 2021, 39, 1412–1420.

67. Belluati, A.; Thamboo, S.; Najer, A.; Maffeis, V.; von Planta, C.; Craciun, I.; Palivan, C.G.; Meier, W. Multicompartment polymer vesicles with artificial organelles for signal-triggered cascade reactions including cytoskeleton formation. Adv. Funct. Mater. 2020, 30, 2002949.

68. Marušič, N.; Otrin, L.; Zhao, Z.; Lira, R.B.; Kyrilis, F.L.; Hamdi, F.; Kastritis, P.L.; Vidaković-Koch, T.; Ivanov, I.; Sundmacher, K.; et al. Constructing artificial respiratory chain in polymer compartments: Insights into the interplay between Bo3 oxidase and the membrane. Proc. Natl. Acad. Sci. USA 2020, 117, 15006–15017.

69. Go, Y.K.; Kambar, N.; Leal, C. Hybrid unilamellar vesicles of phospholipids and block copolymers with crystalline domains. Polymers 2020, 12, 1232.

70. Khan, S.; McCabe, J.; Hill, K.; Beales, P.A. Biodegradable hybrid block copolymer—Lipid vesicles as potential drug delivery systems. J. Colloid Interface Sci. 2020, 562, 418–428.

71. Cao, S.; Xia, Y.; Shao, J.; Guo, B.; Dong, Y.; Pijpers, I.A.; Zhong, Z.; Meng, F.; Abdelmohsen, L.K.; Williams, D.S.; et al. Biodegradable polymersomes with structure inherent fluorescence and targeting capacity for enhanced photo-dynamic therapy. Angew. Chem. Int. Ed. 2021, 60, 17629–17637.

72. Ke, W.; Li, J.; Mohammed, F.; Wang, Y.; Tou, K.; Liu, X.; Wen, P.; Kinoh, H.; Anraku, Y.; Chen, H.; et al. Therapeutic polymersome nanoreactors with tumor-specific activable cascade reactions for cooperative cancer therapy. ACS Nano 2019, 13, 2357–2369.

73. Li, J.; Ge, Z.; Toh, K.; Liu, X.; Dirisala, A.; Ke, W.; Wen, P.; Zhou, H.; Wang, Z.; Xiao, S.; et al. Enzymatically transformable polymersome-based nanotherapeutics to eliminate minimal relapsable cancer. Adv. Mater. 2021, 33, 2105254.

74. Blackman, L.D.; Varlas, S.; Arno, M.C.; Houston, Z.H.; Fletcher, N.L.; Thurecht, K.J.; Hasan, M.; Gibson, M.I.; O'Reilly, R.K. Confinement of therapeutic enzymes in selectively permeable polymer vesicles by polymerization-induced self-assembly (pisa) reduces antibody binding and proteolytic susceptibility. ACS Cent. Sci. 2018, 4, 718–723.

75. Cao, J.; Wei, Y.; Zhang, Y.; Wang, G.; Ji, X.; Zhong, Z. Iodine-rich polymersomes enable versatile SPECT/CT imaging and potent radioisotope therapy for tumor in vivo. ACS Appl. Mater. Interfaces 2019, 11, 18953–18959.

76. Kim, B.S.; Naito, M.; Chaya, H.; Hori, M.; Hayashi, K.; Min, H.S.; Yi, Y.; Kim, H.J.; Nagata, T.; Anraku, Y.; et al. Noncovalent stabilization of vesicular polyion complexes with chemically modified/single-stranded oligonucleotides and

peg-b-guanidinylated polypeptides for intracavity encapsulation of effector enzymes aimed at cooperative gene knockdown. Biomacromolecules 2020, 21, 4365–4376.

77. Choi, H.; Lee, G.-H.; Kim, K.S.; Hahn, S.K. Light-guided nanomotor systems for autonomous photothermal cancer therapy. ACS Appl. Mater. Interfaces 2018, 10, 2338–2346.
78. Sun, J.; Mathesh, M.; Li, W.; Wilson, D.A. Enzyme-powered nanomotors with controlled size for biomedical applications. ACS Nano 2019, 13, 10191–10200.
79. Pijpers, I.A.; Cao, S.; Llopis-Lorente, A.; Zhu, J.; Song, S.; Joosten, R.R.; Meng, F.; Friedrich, H.; Williams, D.S.; Sánchez, S.; et al. Hybrid biodegradable nanomotors through compartmentalized synthesis. Nano Lett. 2020, 20, 4472–4480.
80. Bueno, C.Z.; Apolinário, A.C.; Duro-Castano, A.; Poma, A.; Pessoa, A.; Rangel-Yagui, C.O.; Battaglia, G. L-asparaginase encapsulation into asymmetric permeable polymersomes. ACS Macro Lett. 2020, 9, 1471–1477.
81. Jin, S.-M.; Jeon, J.; Park, M.-K.; Kim, G.H.; Lee, E. Multicompartment vesicles formation by emulsification-induced assembly of poly(ethylene oxide)-block-poly(ε-Caprolactone) and their dual-loading capability. Macromol. Rapid Commun. 2018, 39, 1700545.
82. Konishcheva, E.V.; Daubian, D.; Rigo, S.; Meier, W.P. Probing membrane asymmetry of abc polymersomes. Chem. Commun. 2019, 55, 1148–1151.
83. Xiao, Y.; Sun, H.; Du, J. Sugar-breathing glycopolymersomes for regulating glucose level. J. Am. Chem. Soc. 2017, 139, 7640–7647.
84. Askes, S.H.; Pomp, W.; Hopkins, S.L.; Kros, A.; Wu, S.; Schmidt, T.; Bonnet, S. Imaging upconverting polymersomes in cancer cells: Biocompatible antioxidants brighten triplet-triplet annihilation upconversion. Small Weinh. Bergstr. Ger. 2016, 12, 5579–5590.
85. Kulikova, V.V.; Morozova, E.A.; Anufrieva, N.V.; Koval, V.S.; Lyfenko, A.D.; Lesnova, E.I.; Kushch, A.A.; Revtovich, S.V.; Demidkina, T.V. Kinetic and pharmacokinetic characteristics of therapeutic methinonine γ-Lyase encapsulated in polyion complex vesicles. Biochimie 2022, 194, 13–18.
86. Garni, M.; Einfalt, T.; Goers, R.; Palivan, C.G.; Meier, W. Live follow-up of enzymatic reactions inside the cavities of synthetic giant unilamellar vesicles equipped with membrane proteins mimicking cell architecture. ACS Synth. Biol. 2018, 7, 2116–2125.
87. Maffeis, V.; Belluati, A.; Craciun, I.; Wu, D.; Novak, S.; Schoenenberger, C.-A.; Palivan, C.G. Clustering of catalytic nanocompartments for enhancing an extracellular non-native cascade reaction. Chem. Sci. 2021, 12, 12274–12285.
88. Thamboo, S.; Najer, A.; Belluati, A.; von Planta, C.; Wu, D.; Craciun, I.; Meier, W.; Palivan, C.G. Mimicking cellular signaling pathways within synthetic multicompartment vesicles with triggered enzyme activity and induced ion channel recruitment. Adv. Funct. Mater. 2019, 29, 1904267.
89. Messager, L.; Burns, J.R.; Kim, J.; Cecchin, D.; Hindley, J.; Pyne, A.L.; Gaitzsch, J.; Battaglia, G.; Howorka, S. Biomimetic hybrid nanocontainers with selective permeability. Angew. Chem. Int. Ed. 2016, 55, 11106–11109.
90. Hosta-Rigau, L.; York-Duran, M.J.; Zhang, Y.; Goldie, K.N.; Städler, B. Confined Multiple enzymatic (cascade) reactions within poly(dopamine)-based capsosomes. ACS Appl. Mater. Interfaces 2014, 6, 12771–12779.
91. Matoori, S.; Bao, Y.; Schmidt, A.; Fischer, E.J.; Ochoa-Sanchez, R.; Tremblay, M.; Oliveira, M.M.; Rose, C.F.; Leroux, J.-C. An investigation of PS-b-PEO polymersomes for the oral treatment and diagnosis of hyperammonemia. Small 2019, 15, 1902347.
92. Kulygin, O.; Price, A.D.; Chong, S.-F.; Städler, B.; Zelikin, A.N.; Caruso, F. Subcompartmentalized polymer hydrogel capsules with selectively degradable carriers and subunits. Small 2010, 6, 1558–1564.
93. Arun, Y.; Ghosh, R.; Domb, A.J. Biodegradable hydrophobic injectable polymers for drug delivery and regenerative medicine. Adv. Funct. Mater. 2021, 31, 2010284.
94. Yorulmaz Avsar, S.; Kyropoulou, M.; Di Leone, S.; Schoenenberger, C.-A.; Meier, W.P.; Palivan, C.G. Biomolecules turn self-assembling amphiphilic block co-polymer platforms into biomimetic interfaces. Front. Chem. 2019, 6, 645.
95. De Leo, V.; Milano, F.; Agostiano, A.; Catucci, L. Recent advancements in polymer/liposome assembly for drug delivery: From surface modifications to hybrid vesicles. Polymers 2021, 13, 1027.
96. Rideau, E.; Dimova, R.; Schwille, P.; Wurm, F.R.; Landfester, K. Liposomes and polymersomes: A comparative review towards cell mimicking. Chem. Soc. Rev. 2018, 47, 8572–8610.
97. Itel, F.; Najer, A.; Palivan, C.G.; Meier, W. Dynamics of membrane proteins within synthetic polymer membranes with large hydrophobic mismatch. Nano Lett. 2015, 15, 3871–3878.

98. Lomora, M.; Itel, F.; Dinu, I.A.; Palivan, C.G. Selective ion-permeable membranes by insertion of biopores into polymersomes. Phys. Chem. Chem. Phys. 2015, 17, 15538–15546.

99. Garni, M.; Thamboo, S.; Schoenenberger, C.-A.; Palivan, C.G. Biopores/membrane proteins in synthetic polymer membranes. Biochim. Biophys. Acta BBA Biomembr. 2017, 1859, 619–638.

100. Draghici, C.; Kowal, J.; Darjan, A.; Meier, W.; Palivan, C.G. “Active surfaces” formed by immobilization of enzymes on solid-supported polymer membranes. Langmuir 2014, 30, 11660–11669.

101. Einfalt, T.; Witzigmann, D.; Edlinger, C.; Sieber, S.; Goers, R.; Najer, A.; Spulber, M.; Onaca-Fischer, O.; Huwyler, J.; Palivan, C.G. Biomimetic artificial organelles with in vitro and in vivo activity triggered by reduction in microenvironment. Nat. Commun. 2018, 9, 1127.

102. Edlinger, C.; Einfalt, T.; Spulber, M.; Car, A.; Meier, W.; Palivan, C.G. Biomimetic strategy to reversibly trigger functionality of catalytic nanocompartments by the insertion of PH-responsive biovalves. Nano Lett. 2017, 17, 5790–5798.

103. Sun, X.; Liu, C.; Liu, D.; Li, P.; Zhang, N. Novel biomimetic vectors with endosomal-escape agent enhancing gene transfection efficiency. Int. J. Pharm. 2012, 425, 62–72.

104. Rehman, Z.U.; Hoekstra, D.; Zuhorn, I.S. Mechanism of polyplex- and lipoplex-mediated delivery of nucleic acids: Real-time visualization of transient membrane destabilization without endosomal lysis. ACS Nano 2013, 7, 3767–3777.

105. Wong, A.S.; Mann, S.K.; Czuba, E.; Sahut, A.; Liu, H.; Suekama, T.C.; Bickerton, T.; Johnston, A.P.; Such, G.K. Self-assembling dual component nanoparticles with endosomal escape capability. Soft Matter 2015, 11, 2993–3002.

106. Gaur, D.; Dubey, N.C.; Tripathi, B.P. Biocatalytic self-assembled synthetic vesicles and coacervates: From single compartment to artificial cells. Adv. Colloid Interface Sci. 2022, 299, 102566.

107. Belluati, A.; Mikhalevich, V.; Yorulmaz Avsar, S.; Daubian, D.; Craciun, I.; Chami, M.; Meier, W.P.; Palivan, C.G. How do the properties of amphiphilic polymer membranes influence the functional insertion of peptide pores? Biomacromolecules 2020, 21, 701–715.

108. Xing, X.; Ma, W.; Zhao, X.; Wang, J.; Yao, L.; Jiang, X.; Wu, Z. Interaction between surface charge-modified gold nanoparticles and phospholipid membranes. Langmuir 2018, 34, 12583–12589.

109. Fröhlich, E. The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles. Int. J. Nanomed. 2012, 5577.

110. Hasan, N.; Cao, J.; Lee, J.; Hlaing, S.P.; Oshi, M.A.; Naeem, M.; Ki, M.-H.; Lee, B.L.; Jung, Y.; Yoo, J.-W. Bacteria-targeted clindamycin loaded polymeric nanoparticles: Effect of surface charge on nanoparticle adhesion to MRSA, antibacterial activity, and wound healing. Pharmaceutics 2019, 11, 236.

111. Jeon, S.; Clavadetscher, J.; Lee, D.-K.; Chankeshwara, S.; Bradley, M.; Cho, W.-S. Surface charge-dependent cellular uptake of polystyrene nanoparticles. Nanomaterials 2018, 8, 1028.

112. Du, X.-J.; Wang, J.-L.; Iqbal, S.; Li, H.-J.; Cao, Z.-T.; Wang, Y.-C.; Du, J.-Z.; Wang, J. The effect of surface charge on oral absorption of polymeric nanoparticles. Biomater. Sci. 2018, 6, 642–650.

113. Kohli, A.K.; Alpar, H.O. Potential use of nanoparticles for transcutaneous vaccine delivery: Effect of particle size and charge. Int. J. Pharm. 2004, 275, 13–17.

114. El Badawy, A.M.; Silva, R.G.; Morris, B.; Scheckel, K.G.; Suidan, M.T.; Tolaymat, T.M. Surface charge-dependent toxicity of silver nanoparticles. Environ. Sci. Technol. 2011, 45, 283–287.

115. Deprey, K.; Becker, L.; Kritzer, J.; Plückthun, A. Trapped! A critical evaluation of methods for measuring total cellular uptake versus cytosolic localization. Bioconjug. Chem. 2019, 30, 1006–1027.

116. Sharma, A.R.; Kundu, S.K.; Nam, J.-S.; Sharma, G.; Priya Doss, C.G.; Lee, S.-S.; Chakraborty, C. Next generation delivery system for proteins and genes of therapeutic purpose: Why and how? BioMed Res. Int. 2014, 2014, e327950.

117. Kleineberg, C.; Wölfer, C.; Abbasnia, A.; Pischel, D.; Bednarz, C.; Ivanov, I.; Heitkamp, T.; Börsch, M.; Sundmacher, K.; Vidaković-Koch, T. Light-driven ATP regeneration in diblock/grafted hybrid vesicles. ChemBioChem 2020, 21, 2149–2160.

118. Khan, S.; Li, M.; Muench, S.P.; Jeuken, L.J.C.; Beales, P.A. Durable proteo-hybrid vesicles for the extended functional lifetime of membrane proteins in bionanotechnology. Chem. Commun. 2016, 52, 11020–11023.

119. Otrin, L.; Witkowska, A.; Marušič, N.; Zhao, Z.; Lira, R.B.; Kyrilis, F.L.; Hamdi, F.; Ivanov, I.; Lipowsky, R.; Kastritis, P.L.; et al. En route to dynamic life processes by SNARE-mediated fusion of polymer and hybrid membranes. Nat. Commun. 2021, 12, 4972.

120. Artificial Melanogenesis by Confining Melanin/Polydopamine Production inside Polymersomes-Meyer-2021-Macromolecular Bioscience-Wiley Online Library. Available online:

https://onlinelibrary.wiley.com/doi/full/10.1002/mabi.202100249 (accessed on 27 April 2022).

121. de Freitas, C.F.; Calori, I.R.; da Silva, A.C.P.; de Castro, L.V.; Sato, F.; Pellosi, D.S.; Tessaro, A.L.; Caetano, W.; Hioka, N. PEG-coated vesicles from pluronic/lipid mixtures for the carrying of photoactive erythrosine derivatives. Colloids Surf. B Biointerfaces 2019, 175, 530–544.
122. Belluati, A.; Craciun, I.; Palivan, C.G. Bioactive catalytic nanocompartments integrated into cell physiology and their amplification of a native signaling cascade. ACS Nano 2020, 14, 12101–12112.
123. Liu, J.; Craciun, I.; Belluati, A.; Wu, D.; Sieber, S.; Einfalt, T.; Witzigmann, D.; Chami, M.; Huwyler, J.; Palivan, C.G. DNA-directed arrangement of soft synthetic compartments and their behavior in vitro and in vivo. Nanoscale 2020, 12, 9786–9799.
124. Catalytic Polymersomes to Produce Strong and Long-Lasting Bioluminescence—Nanoscale (RSC Publishing). Available online: https://pubs.rsc.org/en/content/articlelanding/2021/NR/D0NR07178A#!divAbstract (accessed on 27 April 2022).
125. Korpidou, M.; Maffeis, V.; Dinu, I.A.; Schoenenberger, C.-A.; Meier, W.P.; Palivan, C.G. Inverting glucuronidation of hymecromone in situ by catalytic nanocompartments. J. Mater. Chem. B 2022.
126. Langowska, K.; Palivan, C.G.; Meier, W. Polymer nanoreactors shown to produce and release antibiotics locally. Chem. Commun. 2012, 49, 128–130.
127. Meyer, C.E.; Schoenenberger, C.-A.; Wehr, R.P.; Wu, D.; Palivan, C.G. Artificial melanogenesis by confining melanin/polydopamine production inside polymersomes. Macromol. Biosci. 2021, 21, 2100249.
