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# Giant Polymer Compartments for Confined Reactions

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**Abstract:** In nature, various specific reactions only occur in spatially controlled environments. Cell compartment and subcompartments act as the support required to preserve the bio-specificity and functionality of the biological content, by affording absolute segregation. Inspired by this natural perfect behavior, bottom-up approaches are on focus to develop artificial cell-like structures, crucial for understanding relevant bioprocesses and interactions or to produce tailored solutions in the field of therapeutics and diagnostics. In this review, we discuss the benefits of constructing polymer-based single and multicompartments (capsules and giant unilamellar vesicles (GUVs)), equipped with biomolecules as to mimic cells. In this respect, we outline key examples of how such structures have been designed from scratch, namely, starting from the application-oriented selection and synthesis of the amphiphilic block copolymer. We then present the state-of-the-art techniques for assembling the supramolecular structure while permitting the encapsulation of active compounds and the incorporation of peptides/membrane proteins, essential to support in situ reactions, e.g., to replicate intracellular signaling cascades. Finally, we briefly discuss important features that these compartments offer and how they could be applied to engineer the next generation of microreactors, therapeutic solutions, and cell models.

**Keywords:** artificial cells; biomimicry; polymer GUVs; polymer capsules; single compartments; multicompartments

## 1. Introduction

Compartmentalization produces a remarkably efficient organization of membranes and biomolecules that is essential to cope with the complexity of metabolic reactions in cells, and whose stability and functions are vital [1]. Inspired by natural biocompartments, significant efforts have been made to produce compartments that mimic cells and organelles, either in terms of their membrane properties or of the reactivity of encapsulated biomolecules [2]. Micrometer-sized vesicles, namely giant unilamellar vesicles, GUVs for short, are preferably used in this context, since their size and architecture can mimic cells, such as to extract information regarding reactions in a bio-relevant confined space. In addition, they allow for real time visualization of the membrane structure (providing information regarding membrane fluidity and integrity), and of biochemical reactions and enzymatic crowding effects that occur within a controlled and simplified environment, yet still preserving defined characteristics of cells. Lipid based compartments are straightforward systems for mimicking a cell/organelle membrane, nevertheless, their mechanical instability and the presence of membrane defects are limiting factors. One elegant way of introducing robustness to compartments, and at the same time of expanding new membrane properties, is the use of compartments made of copolymers. With the progress in polymer chemistry, numerous

amphiphilic block copolymers have been synthesized with a variety of compositions, block ratios and functions [3]. Due to a greater chemical versatility compared to lipids, block copolymers increase the opportunities to achieve desired self-assembled morphologies made of membranes with tailored properties and excellent biocompatibility. The architecture of such compartments—whether they are micro- or nano-sized—offers three different regions: the inner cavity for encapsulating hydrophilic molecules, the membrane for insertion of hydrophobic molecules, and the external membrane surface, for attachment of specific molecules [4,5] and eventually, for immobilization onto external functional surfaces [6,7]. From a topological point of view, nano-sized compartments, such as small layer-by-layer (LbL) capsules [8], polymersomes [9] or liposomes [10], can be designed as to mimic organelles, the cellular subcompartments. They have been used as nano-scale catalytic compartments, serving as to produce various desired compounds, as artificial peroxisomes [11], acting in tandem to support cascade reactions or as subcompartments inside GUVs to allow development of multicompartment systems [12]. When nanocompartments are encapsulated inside polymer giants in combination with active compounds, they are able to communicate among them to allow reactions, similarly to intracellular organization [13]. Permeability of membranes (either in a single or in multicompartments) favors molecular transport (enzyme substrates and products) and can be achieved in various ways, resulting from the chemical nature of the copolymer [14], by insertion of peptides [15,16] and membrane proteins [17,18]. A schematic of the most common compositions that polymer single and multicompartments can attain is presented in Figure 1.

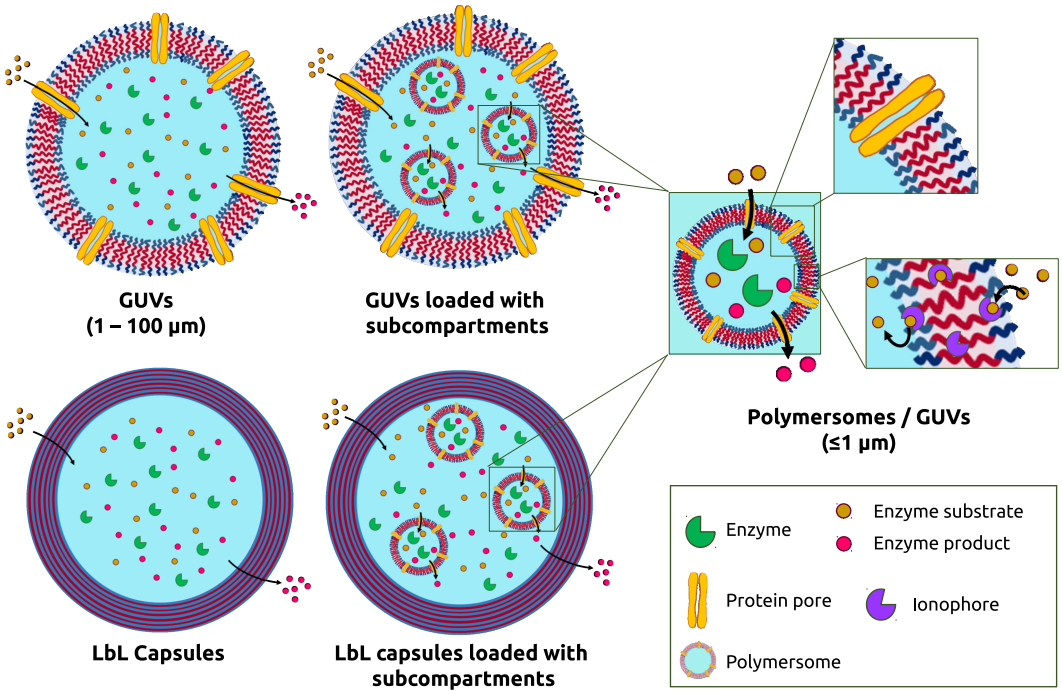



**Figure 1.** Schematic representation of the different types of polymer compartments (polymer giant unilamellar vesicles (GUVs) and layer-by-layer (LbL) capsules), showing their diversity in terms of size, arrangements and the different types of biomolecules, including their possible locations within the assemblies.

This review presents micrometer-sized compartments either as single or as multicompartment reaction space, as powerful tools for biomimicry, lowering the degree of complexity to enable studies on targeted processes. We first introduce the synthesis of amphiphilic block copolymers through the various known polymerization techniques as building blocks of such compartments and indicate the conditions and properties required to support in situ reactions. Different methods for the preparation of these vesicular structures and their combination with active compounds (e.g., enzymes

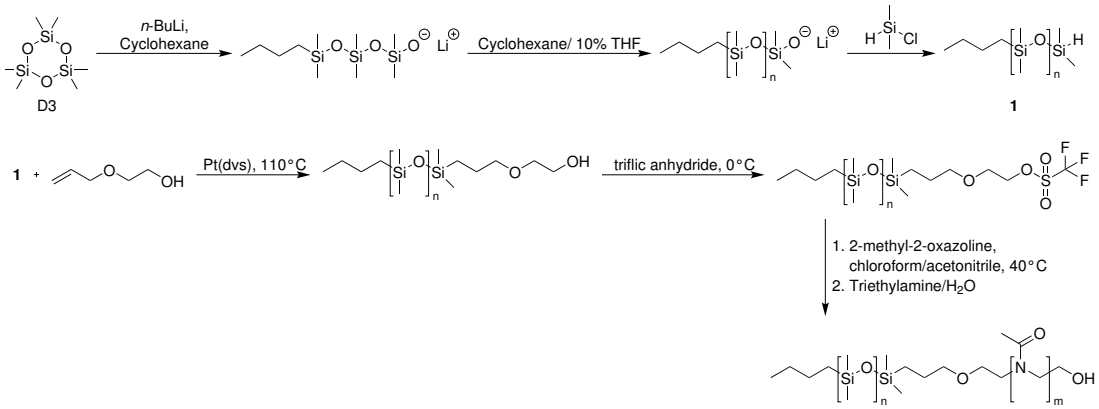
and peptides/membrane proteins) are presented together with the crucial points ensuring an efficient compartmentalization for desired applications. Permeabilization methods will not be described in this review, as they were already discussed extensively elsewhere [19,20]. We rather explore the biomimetic approach of these compartments equipped with peptides/membrane proteins to render them permeable for molecular flow and containing active compounds/subcompartments. Reactions inside confined spaces at microscale allow studying and better understanding of natural mechanisms of such reactions and their role inside them to support applications in various domains, as sensing, therapeutics and catalysis.

## 2. Polymers as Building Blocks of Micrometer-Sized Compartments

The progress in polymer chemistry gave access to a variety of polymers with tailored properties and excellent biocompatibility, thus serving to select specific components, where the precise role of each leads to a well-controlled system. Two or more chemically different polymeric domains, covalently bound together are defined as block copolymers. More specifically, amphiphilic block copolymers are composed of both hydrophilic and hydrophobic blocks, often named as diblocks (AB), triblocks (ABA or ABC) or multiblocks (ABCBA, ABCD, etc.). According to the required properties, amphiphilic block copolymers are built/designed by combining specific types of hydrophilic and hydrophobic blocks.

### *2.1. Amphiphilic Block Copolymers as Building Blocks for Generation of GUVs*

In order to prepare amphiphilic block copolymers, controlled polymerization techniques are commonly used: Atom transfer radical polymerization (ATRP) [21], reversible addition fragmentation chain transfer (RAFT) [22,23], ionic polymerization and combinations thereof [3,24]. Typically, sequential chain extension can be used, in which a first block is polymerized using the aforementioned techniques, forming the so-called macro-initiator. Immediate addition of a second monomer leads to chain-extension, yielding a diblock copolymer. This approach allows the adjustment of each block length by terminating the corresponding chain extension according to the desired degree of polymerization. Tri- or multiblock copolymers can be obtained analogously either by sequential chain extension (asymmetric ABA, ABC, ABCD, etc.) or by a bifunctional initiator (symmetric ABA, ABCBA, etc.). Monomer conversion as well as the living character of the polymer chain are fundamental parameters to be considered among each chain extension. In ionic polymerizations, high monomer conversions can easily be reached by maintaining narrow polydispersity [25]. For example, poly(ethylene oxide)-*block*-polybutadiene (PEO-*b*-PBD) has been successfully synthesized by anionic polymerization in a two steps sequential monomer addition [26–28]. Prior to the addition of the second monomer, modifications are required as for poly(ethylene oxide)-*block*-poly(ethyl ethylene) (PEO-*b*-PEE), in which a PBD precursor is first hydrogenated to yield the PEE macroinitiator. Subsequently, ethylene oxide is polymerized to obtain the diblock copolymer [29]. In another study, poly(acrylic acid)-*block*-polybutadiene (PAA-*b*-PBD) was prepared by sequentional addition of butadiene and *tert*-butylacrylate, followed by hydrolysis to its acid form [28]. The combination of different polymerization techniques is another possibility. Namely, the preparation of poly(dimethyl sulfoxide) (PDMS) by anionic polymerization was followed by activation and cationic ring-opening polymerization of 2-methyl-2-oxazoline (MOXA) monomers to obtain poly(dimethyl sulfoxide)-*block*-poly(2-methyl-2-oxazoline) (PDMS-*b*-PMOXA) diblock copolymers (Scheme 1) [30].




**Scheme 1.** Synthesis route of PDMS-*b*-PMOXA combining both types of ionic polymerizations. Reprinted with permission from [30]. Copyright © 2014 American Chemical Society.

Ionic polymerization techniques are limited due to their high sensitivity to impurities. Hence, the solvent choice is important and the preparation of each reactant has to be handled very carefully to reach the desired purity grade. Controlled radical polymerization techniques (CRP), such as ATRP or RAFT, have recently been developed and have provided interesting and more versatile alternatives for the production of block copolymers. Both techniques require an initiator and the polymerization is governed by an equilibrium between an active species and a dormant one. The latter is constantly re-initiated in order to form the active species responsible for propagation through the addition of monomers. With these techniques, it is usually recommended not to exceed monomer conversions of 90%, above which the probability of termination is higher, risking to form dead chains unable to continue chain-extension [22]. As an example, poly(ethylene oxide)-*block*-poly(4″-acryloyloxybutyl 2,5-bis(4′-butyloxybenzoyloxy)benzoate) (PEO-*b*-PA444) was obtained from PEO modified to a macroinitiator for ATRP on which PA444 has been polymerized [31]. By using RAFT, a diblock copolymer poly(pentafluorophenyl acrylate)-*block*-poly(*n*-butyl acrylate) (PFPA-*b*-P*n*BA) was firstly prepared using the appropriate chain transfer agent (CTA), followed by modification to yield the amphiphilic glycopolymer PN*β*GluEAM-*b*-PBA [32]. More recently, polymerization induced self-assembly (PISA) allowed the preparation of poly(ethylene oxide)-*block*-poly(2-hydroxypropyl methacrylate) (PEO-*b*-PHPMA). In a suitable solvent, a solution of monomer feeds the growing chain on the PEO macroinitiator, producing an amphiphile that gradually self-assembles into structures, while polymerization is ongoing and leading to turbidity in the medium (Figure 2) [33].

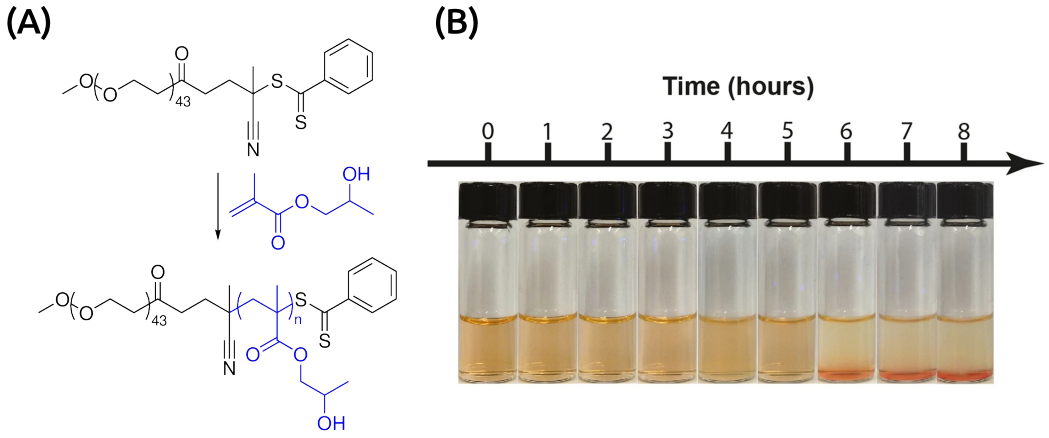



**Figure 2.** (**A**) RAFT polymerization of 2-hydroxypropyl methacrylate controlled by a PEO macroinitiator (**B**) Reaction mixture throughout the PISA polymerization process. Adapted with permission from [33]. Copyright © 2017 Springer Nature.

Although, the possibility of combining synthetic approaches broadens the library of accessible polymers, chemists still need to work hard on the quantitative attachment of the re-initation site for the next polymerization, which is highly recommended to prevent purification difficulties. To circumvent this problem, two or more homopolymers can be connected together using coupling reactions such as Diels-Alder, copper-catalyzed azide-alkyne cycloaddition (CuAAC) or thiol-ene click chemistry, thus offering an increased number of possibilities. To illustrate, PAA-*b*-PBD has been prepared by combining poly(*tert*-butyl acrylate) (P*t*BA) and PBD homopolymers, both synthesized beforehand. A hydrolysis step leads to the final diblock [34]. Poly(dimethylsiloxane)-*block*-poly(ethylene oxide) (PDMS-*b*-PEO) diblock copolymers were synthesized using ring-opening polymerization of hexamethylcyclotrisiloxane to obtain PDMS-$N_3$ and further coupling with PEO-Alkyne chains via click chemistry [35]. However, some reactive conditions can require high temperatures or metal catalysts, which might not be suitable for biomedical applications [36,37]. Moreover, complete end-group functionalization and equimolar ratios of both homopolymers are required, preventing the challenging removal of unreacted homopolymers. Increasing the number of blocks introduces more challenges, especially in re-initiation, purification and finding suitable solvent for all the blocks.

The self-assembly of amphiphilic block copolymers in solution leads to the formation of many different assemblies including spherical, cylindrical, gyroidal and lamellar structures [38]. These assemblies are directly influenced by intrinsic molecular parameters of the amphiphilic block copolymers and the conditions in which the self-assembly process takes place (concentration of the copolymer, presence of solvents, temperature, etc). In this respect, the hydrophilic to the total mass ratio ($f$) calculated as the ratio of the molar mass of the hydrophilic block to the total molar mass of the copolymer is an important parameter, which governs the resulting supramolecular assembly. Vesicular structures are typically obtained for $f$ values ranging from 0.20 to 0.40. Another molecular parameter influencing the self-assembly into different assemblies is the packing parameter ($p = v/a_0 l_c$; $v$ = volume of the hydrophobic part, $a_0$ = contact area of the head group, $l_c$ = length of the hydrophobic part) that describes the degree of curvature from the membrane. For low packing parameter values ($0 < p < 0.5$), the curvature gradually decreases from high to medium, resulting in the formation of spherical or cylindrical micelles, respectively. For higher values ($0.5 < p < 1$), the curvature of the membrane is considerably low, which is more favorable for vesicular structures. The dispersity, D, of the copolymer is affecting the size distribution of the formed vesicles: a narrow dispersity typically leads to uniform-sized polymersomes, whilst on the opposite, a more polydisperse population of vesicles is obtained [39–41].

### *2.2. Polymers as Building Blocks for Generation of Polymer Capsules*

There are a few works that produced polymer capsules via methods originating from the LbL deposition, e.g., single-step polymer adsorption, surface polymerization and ultrasonic assembly [42]. However the vast majority have employed purely the LbL assembly technique [43], where different polymer segments are alternately deposited and adsorbed. These layers are typically formed by homopolymers. The wide range of polymers provides capsules with a variety of walls, as a result of adjusting important parameters, such as composition, permeability and surface functionality of the capsules [44]. Nevertheless, such polymers must have functional groups capable of providing electrostatic interactions or hydrogen bonds. For electrostatic interactions, polyelectrolytes having anionic or cationic groups in their side chains are used, poly(styrene sulfonate (PSS) or poly(allylamine hydrochloride) (PAH), respectively [45,46]. In the case of polymers forming hydrogen bonds, the side chains are composed of functional groups called "hydrogen-bond receptors", which have at least one lone pair (carbonyl, ether, hydroxyl, amino, imino, and nitrile groups), like polyvinylpyrrolidone (PVP), or "hydrogen-bond donors" represented by the presence of a hydrogen atom covalently bound to a more electronegative atom (hydroxyl, amino, and imino groups), like poly(methacrylic acid) (PMAA). These interactions are fundamental for the formation and maintenance of the layers during the LbL deposition.

## 3. Technologies for Engineering Polymer Single and Multicompartments in Combination with Biomolecules

Important features of supramolecular assemblies, designed as functional single or multicompartments to accommodate active compounds, are highly dependent on the preparation methods. Aiming at obtaining the desired structures with optimized characteristics as, size and size distribution, membrane composition and specific functionalities, biomolecular content inside cavities, etc., appropriate procedures need to be selected [47].

### *3.1. Polymer GUVs*

A wide selection of methods to generate polymer vesicular structures are available; ranging from the fairly established bulk techniques, as electroformation and film rehydration, to more automated and high-throughput ones as microfluidcs, currently still underused in the domain of cell mimicry.

#### 3.1.1. Bulk Techniques

**Electroformation**

Electroformation, the most common method to obtain GUVs, involves the swelling of the amphiphilic polymer film in the presence of an electric field. The dry copolymer film is deposited on conductive indium tin oxide (ITO) coated glass slides and is subjected to an alternating sin-wave electric current while it is rehydrated in aqueous solution. The former contains the desired biomolecules to be encapsulated or incorporated inside the GUVs core or membrane, respectively. The electric field induces a periodic electroosmotic movement of the water in between the individual bilayer lamellae in the film, causing the vesicle detachment from the substrate surface [47] as represented in Figure 3B. This method was successfully employed in many different occasions [14,16,48,49]. In particular, Itel et al. [14] formed giants consisting of diblocks (PMOXA-*b*-PDMS) or triblocks (PMOXA-*b*-PDMS-*b*-PMOXA), yielding membranes with thicknesses ranging from 5–30 nm, which can represent 2–10 times that of the phospholipids. Albeit this feature can contribute to a hydrophobic mismatch between membrane thickness and the size of the proteins of more than 5 times, (PMOXA-*b*-PDMS) offered enough flexibility and fluidity to facilitate the membrane protein insertion [50]. To enable reactions, Lomora et al. [51] produced GUVs of different poly(2-methyloxazoline)-*b*-poly(dimethylsiloxane)-*b*-poly(2-methyloxazoline) ($PMOXA_x$-$PDMS_y$-$PMOXA_x$) triblock copolymers. These were equipped with a peptide (Gramicidine, gA) for inducing a selective monovalent ion permeability. Another example was the formation of GUVs with PEO-12 dimethicone, in which permeability was induced by the addition of calcimycin, an ionophore that enabled the transport of $Ca^{2+}$ selectively, serving for the in situ mineralization of calcium carbonate [52]. Nevertheless, this method is not recommended for charged amphiphilic copolymers due to electrostatic interactions, which might affect the self-assembly process [40].

**Film-Rehydration**

A more suitable technique to circumvent the problem of electrostatic interactions is the direct rehydration of a thin polymer film to form the GUVs. For example, this method succeeded in forming GUVs made of a mixture of $PMOXA_5$-*b*-$PDMS_{58}$-*b*-$PMOXA_5$ and the negatively charged $PDMS_{65}$-*b*-heparin copolymers as a mimic for heparan sulfate, known to be exposed on the plasma membrane of most cell types [12]. In the film rehydration method, the block copolymers are first dissolved in an appropriate organic solvent, followed by evaporation either with a stream of nitrogen or by applying vacuum in a rotary evaporator. Rehydration takes place by pouring aqueous solution to the dried film, resulting in the detachment of the GUVs from the substrate surface, (Figure 3A). In general, the desired hydrophilic biomolecular content is encapsulated into the GUVs cavity by mixing it to the rehydration buffer solution. Whereas, as shown by Belluati et al. [15], the hydrophobic ion channels can be inserted in different steps of the hydration processes, e.g.: (i) blended and co-dried

with the copolymer film, (ii) added to the rehydration buffer or (iii) added to the pre-formed GUVs suspension (*ex post*). Moreover, aiming at obtaining functional compartments and to follow a reaction in situ, Garni et al. [18] simultaneously encapsulated a model enzyme horseradish peroxidase (HRP) inside the polymer GUVs and inserted a channel porin, Outer membrane protein F double mutant (OmpF-M), by adding these biomolecules to the rehydration buffer during the formation process. Self-assembly process of GUVs by film-rehydration and electroformation does not produce GUVs with homogeneous sizes, instead a mixture of GUVs in the size range of 1 to 40 μm is formed. In case smaller sizes and narrower size distribution are required, the polymer giants suspension can be subjected to additional processes [53], as freeze-thawing, sonication or extrusion through a polycarbonate membrane [16,51]. Dialysis and size exclusion chromatography are alternative steps to obtaining a relatively monodisperse population.

**Solvent Switch/Exchange**

With the solvent switch method, the supramolecular assembly is induced by adding water drop-wise into a dissolved and molecularly dispersed polymer organic solution, thus, gradually exchanging the organic solvent with water. The turbid solution that is formed is immediately quenched by being poured slowly into an excess of water under continuous stirring. Finally, the organic solvent is removed from the solution via dialysis [54]; an important step especially when envisaging biomimetic applications [55]. However, due to the possible denaturation and degradation caused by traces of organic solvents, such a method may be incompatible with sensitive molecules, limiting their use in biomedical applications [40]. As it has been demonstrated by Daubian et al. [56], depending on the chemical nature of the amphiphile, the solvent switch method may perform faster than the film-rehydration, especially when many metastable phases of the block copolymer can be formed, leading mostly to less aggregates. [57]. With this technique, GUVs are assembled via nucleation and growth of unimers [58,59]. Due to the solvent exchange, the great number of unimers formed deplete the unimers in solution reaching rapidly phase equilibrium, and thus are prevented to grow to larger sizes. GUVs produced hence are the smallest (≈1 μm), and can be tuned to form polymersomes on the nanoscale [57].

3.1.2. Microfluidics

**Double Emulsion Method**

Microfluidic techniques allow for the production of defined polymer stabilized water-oil-water (w/o/w) double emulsions, which are used as templates for generating GUVs. Double emulsion formation proceeds when the inner aqueous phase, containing the biological solution is enveloped by the organic phase, typically consisting of the diblock copolymer dissolved in a volatile and water-immiscible solvent, which breaks up into double emulsions, due to shear caused by the external aqueous phase (Figure 3B) [60–62]. These GUVs have narrow size distributions, with mean sizes ranging from 10–100 μm, which are highly dependent on the microdevice channel sizes and junctions (where generally droplets are formed) [63,64]. To form GUVs from double emulsions, the amphiphile chains are brought together by evaporating the volatile solvent, forming the bilayer membrane. While the complete removal of the organic phase might not be trivial and implies a limitation, this method allows for efficient encapsulation of large amounts of water soluble biomolecules [64]. Thus, its employment is vastly recommended when high-efficiency encapsulation is required, e.g., for loading enzymes and pore-forming proteins within GUVs for mimicking cells. Despite essential contribution on the development of such compartments has been made, there exists only one example where biological machinery (i.e., an aqueous mixture containing *E. coli* ribosomal extract and MreB DNA plasmid) was encapsulated into semi-permeable poly(ethylene oxide)-*block*-poly(lactic acid) (PEO-*b*-PLA) GUVs for carrying out protein expression [65].

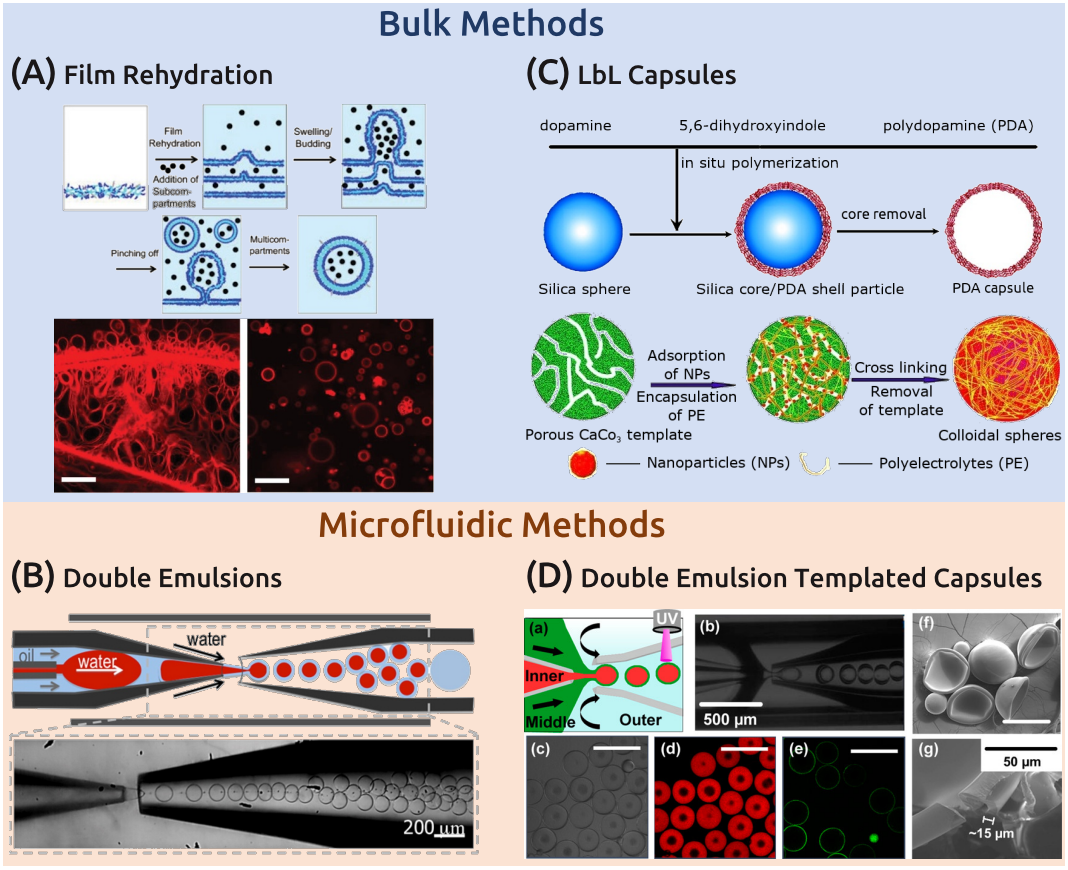



**Figure 3.** Engineering strategies for constructing polymeric single and multi-compartments, capsules and polymer-based giant unilamellar vesicles (GUVs). (**A**) Mechanism of polymer GUV detachment from the substrate surface by the film-rehydration method. Adapted with permission from Thamboo et al. [12]. Copyright © 2019 Wiley-VCH. (**B**) Double emulsion droplets formed in a microfluidic capillary device, which serve as templates for producing GUVs. Adapted with permission from do Nascimento et al. [64]. Copyright © 2016 American Chemical Society. (**C**) Polymer microcapsules produced via layer-by-layer (LbL) deposition onto hard colloidal sacrificial templates. Mechanism using Silica particles, adapted with permission from Yan et al. [66]. Copyright © 2012 Wiley-VCH. Mechanism using $CaCO_3$ particles, adapted with permission from Postma et al. [67]. Copyright © 2009 American Chemical Society. (**D**) Polymer microcapsules produced via double emulsion technique, followed by UV polymerization. Adapted with permission from Xie et al. [68]. Copyright © 2017 American Chemical Society.

### *3.2. Polymer Capsules*

Differently from GUVs, polymer capsules require the employment of either a soft or a hard template. They have operated as delivery vehicles, since they also allow for the selective diffusion of reagents/reaction products; yet their use as microreactors for mimicking cells has been limited.

#### Layer-by-layer Microcapsules

Fabrication of polymer microcapsules involves multiple synthetic steps and compositional complexity for the particular application. The LbL technique requires the use of a colloidal particle as a sacrificial template, which plays a pivotal role, since it determines the capsule size and shape, and most importantly the biomolecular encapsulation method. Soft sacrificial templates have been employed, including the commercial ones: poly(methyl methacrylate) (PMMA) and polystyrene (PS), however they do not allow for the pre-loading of the active components, hampering the microcapsules application for therapeutics, due to low reproducibility of the diffusion process involved in the post-loading method [69]. Instead, when employing hard sacrificial templates, e.g.,

calcium carbonate [66] or silica [67], encapsulation of enzymes and sensitive dyes is reached via their concurrent precipitation with the template, ensuring a high loading efficiency [70,71]. Decomposition of these templates is then induced for the creation of the inner cavity loaded with the specific biomolecule (Figure 3C). With respect to the outer shell, two polymers interacting by electrostatic forces or hydrogen bonding are deposited alternately on the template before it is dissolved to obtain the hollow sphere. Typically, PVP and PMAA which interact via hydrogen bonding at pH values below the pKa of PMAA are used for this technique. Using PMAA, the stability of these capsules can be extended to physiologically relevant pH by crosslinking. The resulting pure PMAA hydrogel capsules are biodegradable, nontoxic, semipermeable and thus well suited for biomedical applications. More recent studies replace the labour intense LbL assembly of PMAA/PVP capsules by polydopamine shells that are deposited on the template in a single step [72].

**Double Emulsion Templated Microcapsules**

Opposite to the conventional fabrication methods, where multiple laborious synthetic steps must be satisfied, microfluidics offers an alternative technique for a rapid, with low polydispersity and highly reproducible production of polymer microcapsules. To this aim, double emulsion droplets, formed following the same procedure aforementioned, serve as non-sacrificial templates [73]. For generating polymer microcapsules, flowing droplets are subjected to UV irradiation and thus continuously and rapidly polymerize (Figure 3D). Here, the oil phase contains a photocurable polymer and a photoinitiatior dissolved in a water miscible organic solvent [69]. For biomedical applications, poly(ethylene glycol) diacrylate (PEGDA) microcapsules of around 15 μm were produced and allowed for the diffusion of molecules as large as heparin labeled with Fluorescein isothiocyanate (FITC) ($M_W \approx 10$ kD) [68]. These results demonstrate the biosensing ability and the promising versatility of microfluidics for the preparation of microreactors.

### *3.3. Building Multicompartments*

Multicompartments are considered as an advance towards functional models for eukaryotic cells and their cellular organelles, which are able to perform multiple, chemically incompatible, enzymatic reactions simultaneously by separating them in subcompartments. Multicompartment vesicles were pioneered when the so called vesosomes (liposomes encapsulated inside larger liposomes) were first developed [74]. This process was promptly transferred to synthetic polymeric assemblies, such as polymeric vesicles or LbL capsules, resulting in all conceivable combinations. Multicompartments consist mainly of bigger outer compartments that can be loaded with different kinds of subcompartments, as subsequently detailed.

#### 3.3.1. Loading Polymeric GUVS with Subcompartments

The encapsulation of subcompartments as, small polymersomes, micelles or liposomes, but also nanoparticles, is usually attained during the polymer GUV self-assembly. Each subcompartment can be previously equipped with biomolecules and/or the biomolecules can be encapsulated together with the mixture of empty subcompartments. For example, GUVs of polystyrene-*b*-poly(L-isocyanoalanine (2-thiophen-3-yl-ethyl) amide) (PS-*b*-PIAT) were prepared by the solvent switch method, using as aqueous phase, a mixture of the cyanine-5 conjugated immunoglobulin G proteins (Cy5-IgG) and a suspension of smaller polymersomes made of PMOXA-*b*-PDMS-*b*-PMOXA, previously generated by film rehydration and equipped with green fluorescent protein (GFP) [75]. Co-localized red and green fluorescence emission measurements were used to compute that only 45% of the supramolecular assemblies resulted in multicompartments. Marguet et al. [76] also demonstrated the generation of polymer multicompartments based on the emulsion-centrifugation method. The inner polymersomes were formed by nanoprecipitation of poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) (PTMC-*b*-PGA), and subsequently loaded in GUVs made of polybutadiene-*b*-poly(ethylene oxide) (PBD-*b*-PEO) by emulsion–centrifugation. By using such technique, yet for formation of giant

liposomes, the loading efficiency reached up to 98% [49]. Regardless of the method used, the obtained structures will always consist of a combination of single and multicompartments. Double emulsion microfluidics has also been used to form multicompartments made of PEO-*b*-PLA diblock-copolymers for both the inner and the outer membranes [77]. Despite promised control over the number of the inner polymersomes by solely adjusting the flow rates, no loading efficiency was reported.

#### 3.3.2. Layer-by-Layer Multicompartments

LbL multicompartments are constructed with either one smaller LbL capsule as single subcompartment (shell-in-shell structure) [78] or thousands of subcompartments that are deposited onto the template during the preparation of the micron-sized outer capsule. In this regard, the subcompartments may comprise small LbL capsules, polymersomes [9] or liposomes [10], with the former being used for the majority of the LbL multicompartments. The LbL deposition offers the control over the spatial positioning of the subcompartments. Depending on the polymers used for the precursor or separation layer, they either stay attached to the inner walls of the LbL capsule or become "free-floating" after template removal [79]. If only one hemisphere of the template is exposed to the subunit deposition, Janus type multicompartments can also be prepared by the LbL approach [80]. As for single compartments, it is possible to encapsulate the biological content inside the subunits or the lumen of the main compartment, in addition, it can be also found within or outside of the membranes. Replacement of the liposomal subcompartments with polymersomes offers the possibility to address challenges, as prolonged stability of the subcompartments to sustain activity of the encapsulated enzyme. However, examples for polymersome subunits in LbL capsules remain scarce [9].

## 4. Vesicular Compartments for In Situ Reactions

Biomimicry offers strategies for the creation of vesicular compartments with incorporated peptides/membrane proteins and encapsulated active compounds providing an approach for various applications. Polymeric compartments with encapsulated cargo have been employed in imaging, sensing, therapeutics, as artificial cells, etc. So far, such compartments were almost solely assembled by film rehydration, electroformation, and LbL.

### *4.1. Reactions inside Single Compartments*

#### 4.1.1. GUVs

Reconstruction of biological structures and processes can be achieved with a bottom-up approach using GUVs. Encapsulation of enzymes inside the cavities of GUVs is an emerging way to fabricate artificial environments that mimic the complexity of cells by introducing similar functionalities. The resulting GUVs serve as platforms to visualize biological processes in real time, contributing to our understanding of human cells, which in turn promotes new developments of biomedical applications [81,82]. Since the permeability of polymeric GUVs is essential for in situ reactions, one biomimicry approach is to equip them with peptides/membrane proteins to allow molecular transport through the membrane. Up to now, there are only few examples of polymeric GUVs with incorporated membrane proteins/peptides and they are primarily based on PMOXA-*b*-PDMS-*b*-PMOXA triblock copolymer membranes. For example, the permeability of GUVs, to selectively transport $Ca^{2+}$ ions, was attained by inserting several ionophores: calcimycin [52], Lasalocid A, and *N*,*N*-dicyclohexyl-*N*′,*N*″-dioctadecyl-3-oxapentane-1,5-diamide [83]. Furthermore, Gramicidine (gA) allowed $Na^+$ and $K^+$ ions to specifically pass the membranes of GUVs [16]. The hydrophobic mismatch of pore length and membrane represented a barrier to membranes thicker than 12.1 nm, whereas thinner membranes facilitated successful gA insertion. The bee venom melittin was inserted into various PMOXA-*b*-PDMS-*b*-PMOXA membranes. The insertion process and the resulting functionality of the peptide have been related to the membrane curvature [15]. Besides, the

membrane protein OmpF was successfully reconstituted in membranes of GUVs allowing an enzymatic reaction inside the cavity, which was monitored in real time with a confocal microscope (Figure 4) [18].

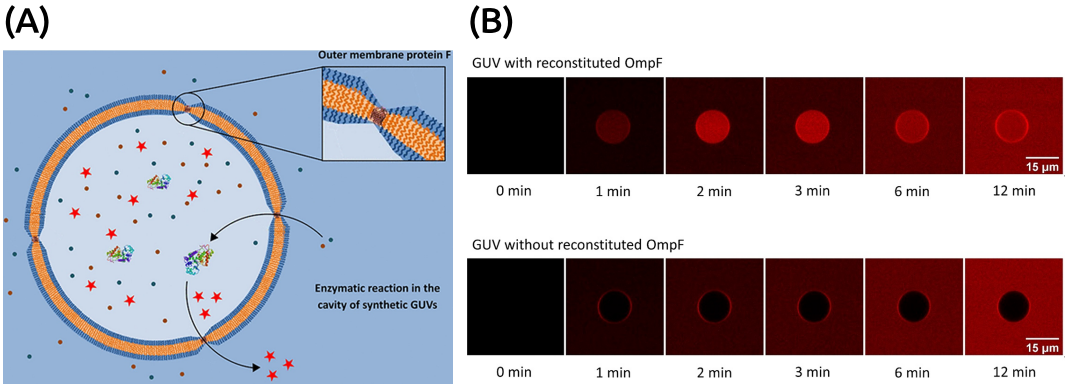



**Figure 4.** Reaction inside single polymer GUVs. (**A**) Schematic representation of a polymeric GUV equipped with the membrane protein OmpF. Substrates and products diffuse through the membrane, thus enabling an enzymatic reaction. (**B**) Fluorescence micrographs of a single GUV recorded at several time points after addition of the substrates showing the difference of GUVs with and without reconstituted OmpF. Adapted with permission from Garni et al. [18]. Copyright © 2018 American Chemical Society.

#### 4.1.2. Layer-by-Layer Microcapsules

LbL capsules with an encapsulated enzyme offer various possibilities in sensing and imaging [84]. The preparation of $(PSS/PAH)_4/PSS$ shell structures, the co-encapsulation of urease and the pH sensitive fluorophore enabled the quantification of urea on a single capsule level [70]. Continuing with this approach Kazakova et al. [71] managed to encapsulate lactate oxidase, peroxidase, or glucose oxidase, with respective sensitive dyes to detect lactate, oxygen, and glucose levels [71]. In another $(PSS/PAH)_4$ system the detection of oxaloacetic acid with NADH as cofactor was possible. Thus, the efficacy of an enzyme fluorophore coupled system was demonstrated (Figure 5) [85]. Magnetic polydopamine capsules enhanced the activity after reusing and the long-term stability of the encapsulated Candida Rugosa Lipase compared to the free enzyme [86]. Reuse is a key factor for potential application in industry. Moreover, further attempts are required to validate the performance of these systems in vitro and in vivo. Another application of LbL capsules is therapeutics: e.g., microcapsules with encapsulated L-Asparaginase in poly-L-arginine and dextran sulfate layers were tested in vitro on leukemic cell lines resulting in a decreased proliferation [87]. LbL enables convenient encapsulation of enzymes in one single particle. However, their semi-permeable membrane allowing unspecific transport of small molecules is rather a deficiency, that needs to be overcome for future applications.

### *4.2. Reactions inside Multicompartmentalized Structures*

#### 4.2.1. GUV Multicompartments

In biological cells, evolution has developed the system of subcompartmentalization (cellular organelles) within individual cells in order to allow specific reactions to take place in a spatially defined manner. This is an efficient solution, as many reactions (e.g., protein lysis, electron transport) require very specific conditions (e.g., low pH, proton gradient) to occur. Careful application of biomimicry principles allows integral cell mimics as combining nano- and microstructures with biomolecules. In this respect, biomolecule equipped polymersomes have previously been shown to be functional as artificial organelles both in vitro and in vivo where they supported the natural cellular metabolism, and have even been shown to function "on demand" in a life-like manner [11,88]. Artificial cell mimics have been designed by constructing synthetic multicompartmentalized systems. The most significant

examples were found when using a larger polymer-based GUV loaded either with smaller nano-sized liposomes or smaller nano-sized polymersomes [89]. Synthetic GUVs based on ($PBD_{46}$-*b*-$PEO_{30}$) loaded with hydrophilic dyes, liposomes (DPPC), and polymersomes ($PBD_{23}$-*b*-$PEO_{14}$) allowed fast, selectively triggered release due to a light-induced increase in osmotic pressure, resulting in rupture of the GUVs [90]. Examples of polymeric GUVs acting as artificial cell-mimics are still scarce, but more complex multicompartmentalized GUVs exist. Namely, where enzymes and membrane protein equipped polymersomes coexisted in the GUVs inner cavity [12]. By applying the principle of multicompartmentalization, an artificial cell mimic is created with subcompartmentalized polymersomes acting as artificial organelles. This allows cascade reactions to occur successively due to the segregation of enzymes in different subcompartments. The proximity among subcompartments provided by larger GUVs, facilitates the diffusion of reagents and reaction products, while confining the enzymes to their individual subcompartments. PBD-*b*-PEO polymer GUVs can mimic structural and functional eukaryotic cells by encapsulating enzyme-filled intrinsically semi-permeable PS-*b*-PIAT polymer nanoreactors together with free enzymes and substrates to fulfill a three-enzyme cascade reaction inside the multicompartmentalized structures [13]. Although this study represents an important step towards artificial cells, it only reports the fluorescent product of the reaction, without providing detailed information about localization of the enzymes. In addition, such examples lack the complexity of cells because they are mainly developed with only few functional elements and by using buffer medium. For the creation of an artificial cell mimic by multicompartmentalization, there are requirements still not fulfilled. The selective permeabilization of every membrane of the involved compartments, which allows for a higher control of the diffusion of substrates and products across the membranes and a more complex medium mimicking the cytoplasm represent advancements not yet provided. Systems addressing this question are multicompartmentalized GUVs with stimuli-responsive and non-responsive subcompartments (Figure 6). With an external signaling molecule passively diffusing through the GUV's membrane, inducing the disassembly of the stimuli-responsive nanoparticle and the release of the entrapped cargo (peptides or enzyme substrates). These molecules allowed a selective ion flux through the GUV's membrane or an enzymatic reaction inside the GUV [12].

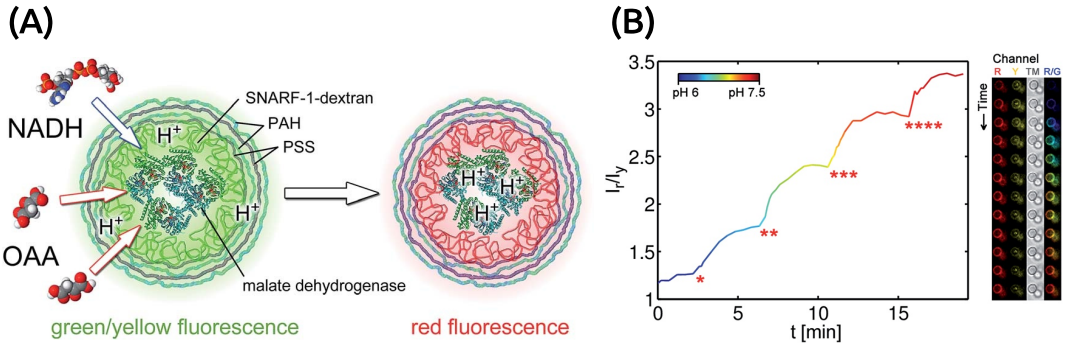



**Figure 5.** Reaction inside single polymer microcapsules. (**A**) Schematic illustration of an oxaloacetic acid (OAA) or nicotinamide adenine dinucleotide (NADH) sensing microcapsule. The encapsulated pH-sensitive fluorescent dye ( seminaphtharhodafluor (SNARF-1)-dextran) responds to a decrease in local proton concentration caused by the enzymatic reaction. (**B**) Reaction kinetics demonstrating NADH as the limiting factor. The first dose of substrate is added at (*) and then added gradually, after the plateau was reached, from (**)-(****). The corresponding micrographs on the right hand side represent the reaction of one capsule (red (R), yellow (Y), transmission (TM), and the false-colored ratio $I_r/I_y$ (R/G)). Adapted from Harimech et al. [85] under the terms of CC BY 3.0.

More and more experimental successes in combination with vesicle engineering techniques are leading into a new era of complexity in artificial cells. These have attracted increasing attention as substituents for living cells. Polymer GUVs and polymersomes offer an ideal platform to engineer cell mimics, allowing reactions to take place in compartmentalized spaces. Meanwhile, they remain

stable for longer periods compared to their lipid-based counterparts. With the beforehand mentioned functionalization with membrane proteins and peptides, a life-like functionalization of membranes is approved.

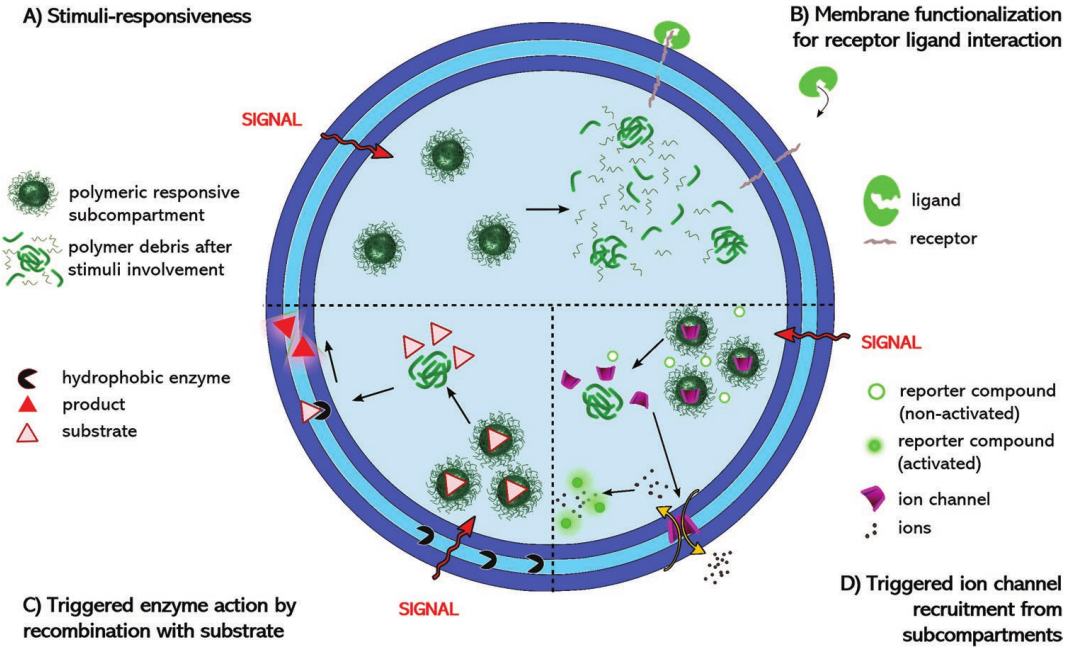



**Figure 6.** Multicompartmentalized GUV with reduction sensitive and ion channel recruiting modular subcompartments (**A**–**D**). Dithiothreitol was used as a triggering signal (red arrow). Reprinted With permission from Thamboo et al. [12]. Copyright © 2019 Wiley-VCH.

#### 4.2.2. Layer-by-Layer Polymer Capsules as Multicompartments

Multicompartmentalization allows for separation in preparation and modularity in formulation to increase also functionality in theranostics [91,92]. LbL assembled capsules prepared of polyvinylpyrrolidone (PVP) and thiolated poly(methacrylic acid) (PMAA) containing smaller crosslinked capsules showed possibilities in catalysis and drug delivery [8]. PVP and tannic acid (TA) LbL capsules filled with POEGMA$_{26}$-*b*-PDPA$_{50}$ polymersomes loaded with pDNA could release the cargo in response to pH changes [9]. Microfluidics have been improving the development of such attractive microreactor systems with increased complexity and modularity. Encapsulation of glucose oxidase (GOx) conjugated on quantum dots or on gold nanorods (NR) into PEO-based microreactors acting as glucose biosensor, while amine functionalized NRs were employed as heparin sensors. GOx oxidized glucose to gluconic acid and $H_2O_2$ leading to fluorescence quenching by the quantum dots providing a sensitivity in the range of glucose sensors for diabetes diagnostics [68]. However, most capsules only work in defined pH-ranges and do not resist enormous local pH-changes, which would be necessary for in vivo applications [84].

## 5. Conclusions and Outlook

Biological systems as cells are highly compartmentalized across several length scales. Their precise features, as biomolecule compartmentalization through attachment to membranes and cytoskeleton scaffolds, lateral organization on membrane rafts, as well as compartmentalization in membrane-bound or protein-based organelles, are current subject of study. Understanding their underlying mechanisms opens exciting avenues in many application fields, notably in material science, biotechnology and medicine. As we have seen, efforts at achieving this are accelerating and synthetic approaches to mimic increasingly intricate biological structures are being developed.

This review demonstrates the relevance of polymer-based systems with special focus on polymer GUVs and capsules, for addressing the challenge of eukaryotic cell biomimicry. We only reviewed examples of supramolecular structures, whose membrane is equipped with peptides and membrane proteins since they genuinely represent bioinspired catalytic compartments where the membranes are mimicking biomembranes.

The progress in polymer chemistry gave access to a variety of polymers with tailored properties as biodegradability and non-toxicity that leads to enhanced structural properties and with the significant fluidity, necessary to cope with the insertion of peptides and membrane proteins. Due to their large variety of chemical composition and functionalization, a greater versatility for further improvement of their properties concerning the desired application can be achieved, regarding stability, loading efficiency, intervesicular interaction, and selective permeability for specific substrates. Besides the polymer, the selection of an appropriate preparation method for engineering the supramolecular structures in single and multicompartments to conform biochemical reactions, is detrimental. Although electroformation and film rehydration are the most commonly used techniques, both of them do not produce homogeneous sizes of GUVs. Alternatively, microfluidics became a serious candidate, showing great potential to generate polymer GUVs with narrow size distribution and controlled biomolecular content at high-throughput.

GUVs loaded with enzymes inside their cavities and equipped with peptides/membrane proteins acting as "gates" for the diffusion of molecules through the synthetic membrane, constitute complex reaction spaces. Careful application of biomimicry principles allows integral cell mimics as combining nano- and microstructures with biomolecules. The inner compartments (the organelle mimics) that are loaded in the outer one (the cell membrane mimic) do not necessarily need to encapsulate the same content, where the various contents can even be incompatible or act synergistically. As a result, combinatory drug delivery becomes possible in one single vector. Such systems serve multifold purposes. Besides the aforementioned design of an artificial cell, allowing systematic studies of biological phenomena in simplified environments, they are explored as (compartmentalized) microreactors, where segregation of the catalytic steps in separated compartments allows distinct chemical environment (e.g., different pH, redox states, presence of cofactors, etc.) to couple enzymatic reaction steps that would otherwise inhibit one another. Because of the GUV cell-size, real time imaging of the fluorescence activity of model enzymes can be monitored and used for enhancing diagnosis capabilities. Lastly, polymer capsules have been aimed for use in therapeutic applications, by encapsulating hydrophilic molecules in the aqueous core for enzyme therapy and controlled drug release. In addition, such structures can improve the therapeutic index of drugs by influencing drug absorption and metabolism, and can extent drug half-life as well as reduce toxicity. Although an effort at outlining a roadmap for the field had been made, there will surely be many new developments that will take this research area to unforeseen directions. From a material perspective, the advanced control in polymer synthesis and self-assembly that is available nowadays would certainly bring a real breakthrough in this cell biomimicry field. Hence, it is expected a growing interest in such biomimetic approaches to soon offer many new opportunities in drug delivery, cell-sized reactors, biosensors and imaging for therapeutics, which will offer better communication and interaction with living systems.

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## List of Abbreviation

| | |
|---|---|
| ATRP | Atom transfer radical polymerization |
| CRP | Controlled radical polymerization |
| CTA | Chain transfer agent |
| CuAAC | Copper-catalyzed azide-alkyne cycloaddition |
| Cy5-IgG | Cyanine-5 conjugated immunoglobulin G proteins |
| DNA | Deoxyribonucleic acid |
| DPPC | Dipalmitoylphosphatidylcholine |
| *E. coli* | *Escherichia coli* |
| FITC | Fluorescein isothiocyanate |
| gA | Gramicidine |
| GFP | Green fluorescent protein |
| GOx | Glucose oxidase |
| GUV | Giant unilamellar vesicle |
| HRP | Horseradish peroxidase |
| ITO | Indium tin oxide |
| LbL | Layer-by-layer |
| MOXA | 2-methyl-2-oxazoline |
| NAD | Nicotinamide adenine dinucleotide |
| NADH | Nicotinamide adenine dinucleotide (reduced) |
| *n*-BuLi | *n*-butyllithium |
| NR | Nanorod |
| OAA | Oxaloacetic acid |
| OmpF | Outer membrane protein F |
| OmpF-M | Outer membrane protein double mutant |
| PA444 | Poly(4″-acryloyloxybutyl 2,5-bis(4′-butyloxybenzoyloxy)benzoate) |
| PAA | Poly(acrylic acid) |
| PAH | Poly(allylamine hydrochloride) |
| PBD | Polybutadiene |
| PDA | Polydopamine |
| PDEAEMA | Poly(2-(diethylamino)ethyl methacrylate) |
| PDMS | Poly(dimethyl sulfoxide) |
| PDPA | Poly(2-(diisopropylamino)-ethyl methacrylate) |
| PEE | Poly(ethyl ethylene) |
| PEG/PEO | Poly(ethylene glycol)/poly(ethylene oxide) |
| PEGDA | Poly(ethylene glycol) diacrylate |
| PFPA | Poly(pentafluorophenyl acrylate) |
| PGA | Poly(L-glutamic acid) |
| PHPMA | Poly(2-hydroxypropyl methacrylate) |
| PIAT | Poly(L-isocyanoalanine(2-thiophen-3-yl-ethyl)amide) |
| PISA | Polymerization induced self-assembly |

| | |
|---|---|
| PLA | Poly(lactic acid) |
| PMA | Poly(methyl acrylate) |
| PMAA | Poly(methacrylic acid) |
| PMOXA | Poly(2-methyl-2-oxazoline) |
| P*n*BA | Poly(*n*-butyl acrylate) |
| PNIPAM | Poly(*N*-isopropylacrylamide) |
| POEGMA | Poly(oligo(ethylene glycol) methacrylate) |
| PPS | Poly(propylene sulfide) |
| PS | Polystyrene |
| PSBA | Poly(styrene boronic acid) |
| PSS | Poly(styrene sulfonate) |
| P*t*BA | Poly(*tert*-butyl acrylate) |
| PTMC | Poly(trimethylene carbonate) |
| PVP | Polyvinylpyrrolidone |
| RAFT | Reversible addition fragmentation chain transfer |
| SNARF-1 | Seminaphtharhodafluor |
| TA | Tannic acid |
| THF | Tetrahydrofuran |

## References


1. Chen, A.H.; Silver, P.A. Designing biological compartmentalization. *Trends Cell Biol.* **2012**, *22*, 662–670. [CrossRef]
2. Palivan, C.G.; Fischer-Onaca, O.; Delcea, M.; Itel, F.; Meier, W. Protein–polymer nanoreactors for medical applications. *Chem. Soc. Rev.* **2012**, *41*, 2800–2823. [CrossRef]
3. Feng, H.; Lu, X.; Wang, W.; Kang, N.G.; Mays, J.W. Block Copolymers: Synthesis, Self-Assembly, and Applications. *Polymers* **2017**, *9*, 494. [CrossRef]
4. Antonietti, M.; Förster, S. Vesicles and Liposomes: A Self-Assembly Principle Beyond Lipids. *Adv. Mater.* **2003**, *15*, 1323–1333. [CrossRef]
5. Messager, L.; Gaitzsch, J.; Chierico, L.; Battaglia, G. Novel aspects of encapsulation and delivery using polymersomes. *Curr. Opin. Pharmacol.* **2014**, *18*, 104–111. [CrossRef] [PubMed]
6. Rigo, S.; Gunkel-Grabole, G.; Meier, W.; Palivan, C.G. Surfaces with Dual Functionality through Specific Coimmobilization of Self-Assembled Polymeric Nanostructures. *Langmuir* **2019**, *35*, 4557–4565, doi:10.1021/acs.langmuir.8b02812. [CrossRef] [PubMed]
7. Wu, D.; Rigo, S.; Di Leone, S.; Belluati, A.; Constable, E.C.; Housecroft, C.E.; Palivan, C.G. Brushing the surface: Cascade reactions between immobilized nanoreactors. *Nanoscale* **2020**, *12*, 1551–1562. [CrossRef] [PubMed]
8. Kulygin, O.; Price, A.D.; Chong, S.F.; Staedler, B.; Zelikin, A.N.; Caruso, F. Subcompartmentalized Polymer Hydrogel Capsules withSelectively Degradable Carriers and Subunits. *Small* **2010**, *6*, 1558–1564. [CrossRef] [PubMed]
9. Lomas, H.; Johnston, A.P.R.; Such, G.K.; Zhu, Z.; Liang, K.; Koeverden, M.P.V.; Alongkornchotikul, S.; Caruso, F. Polymersome-Loaded Capsules for Controlled Release of DNA. *Small* **2011**, *7*, 2109–2119. [CrossRef]
10. Stadler, B.; Chandrawati, R.; Price, A.D.; Chong, S.F.; Breheney, K.; Postma, A.; Connal, L.A.; Zelikin, A.N.; Caruso, F. A Microreactor with Thousands of Subcompartments: Enzyme-Loaded Liposomes within Polymer Capsules. *Angew. Chem.* **2009**, *48*, 4359 –4362. [CrossRef]
11. Tanner, P.; Balasubramanian, V.; Palivan, C.G. Aiding nature's organelles: Artificial peroxisomes play their role. *Nano Lett.* **2013**, *13*, 2875–2883. [CrossRef]
12. 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. [CrossRef]
13. Peters, R.J.R.W.; Marguet, M.; Marais, S.; Fraaije, M.W.; van Hest, J.C.M.; Lecommandoux, S. Cascade Reactions in Multicompartmentalized Polymersomes. *Angew. Chem. Int. Ed.* **2014**, *53*, 146–150. [CrossRef] [PubMed]

14. Itel, F.; Chami, M.; Najer, A.; Lorcher, S.; Wu, D.L.; Dinu, I.A.; Meier, W. Molecular Organization and Dynamics in Polymersome Membranes: A Lateral Diffusion Study. *Macromolecules* **2014**, *47*, 7588–7596. [CrossRef]
15. 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. [CrossRef] [PubMed]
16. 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. [CrossRef]
17. Baumann, P.; Spulber, M.; Fischer, O.; Car, A.; Meier, W. Investigation of Horseradish Peroxidase Kinetics in an "Organelle-Like" Environment. *Small* **2017**, *13*, 1603943. [CrossRef]
18. 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. [CrossRef]
19. 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. [CrossRef]
20. 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. [CrossRef]
21. Ribelli, T.G.; Lorandi, F.; Fantin, M.; Matyjaszewski, K. Atom Transfer Radical Polymerization: Billion Times More Active Catalysts and New Initiation Systems. *Macromol. Rapid Commun.* **2019**, *40*, 1800616. [CrossRef] [PubMed]
22. Perrier, S. 50th Anniversary Perspective: RAFT Polymerization—A User Guide. *Macromolecules* **2017**, *50*, 7433–7447. [CrossRef]
23. Keddie, D.J. A guide to the synthesis of block copolymers using reversible-addition fragmentation chain transfer (RAFT) polymerization. *Chem. Soc. Rev.* **2014**, *43*, 496–505. [CrossRef] [PubMed]
24. Hadjichristidis, N.; Pitsikalis, M.; Iatrou, H. Synthesis of Block Copolymers. In *Block Copolymers I*; Abetz, V., Ed.; Springer: Berlin/Heidelberg, Germany, 2005; pp. 1–124.
25. Herzberger, J.; Niederer, K.; Pohlit, H.; Seiwert, J.; Worm, M.; Wurm, F.R.; Frey, H. Polymerization of Ethylene Oxide, Propylene Oxide, and Other Alkylene Oxides: Synthesis, Novel Polymer Architectures, and Bioconjugation. *Chem. Rev.* **2016**, *116*, 2170–2243. [CrossRef] [PubMed]
26. Förster, S.; Krämer, E. Synthesis of PB-PEO and PI-PEO Block Copolymers with Alkyllithium Initiators and the Phosphazene Base t-BuP4. *Macromolecules* **1999**, *32*, 2783–2785. [CrossRef]
27. Nuss, H.; Chevallard, C.; Guenoun, P.; Malloggi, F. Microfluidic trap-and-release system for lab-on-a-chip-based studies on giant vesicles. *Lab Chip* **2012**, *12*, 5257–5261. [CrossRef] [PubMed]
28. Christian, D.A.; Tian, A.; Ellenbroek, W.G.; Levental, I.; Rajagopal, K.; Janmey, P.A.; Liu, A.J.; Baumgart, T.; Discher, D.E. Spotted vesicles, striped micelles and Janus assemblies induced by ligand binding. *Nat. Mater.* **2009**, *8*, 843–849. [CrossRef]
29. Hillmyer, M.A.; Bates, F.S. Synthesis and Characterization of Model Polyalkane-Poly(ethylene oxide) Block Copolymers. *Macromolecules* **1996**, *29*, 6994–7002. [CrossRef]
30. Wu, D.; Spulber, M.; Itel, F.; Chami, M.; Pfohl, T.; Palivan, C.G.; Meier, W. Effect of Molecular Parameters on the Architecture and Membrane Properties of 3D Assemblies of Amphiphilic Copolymers. *Macromolecules* **2014**, *47*, 5060–5069. [CrossRef]
31. Mabrouk, E.; Cuvelier, D.; Pontani, L.L.; Xu, B.; Lévy, D.; Keller, P.; Brochard-Wyart, F.; Nassoy, P.; Li, M.H. Formation and material properties of giant liquid crystal polymersomes. *Soft Matter* **2009**, *5*, 1870–1878. [CrossRef]
32. Kubilis, A.; Abdulkarim, A.; Eissa, A.M.; Cameron, N.R. Giant Polymersome Protocells Dock with Virus Particle Mimics via Multivalent Glycan-Lectin Interactions. *Sci. Rep.* **2016**, *6*, 32414. [CrossRef] [PubMed]
33. Albertsen, A.N.; Szymański, J.K.; Pérez-Mercader, J. Emergent Properties of Giant Vesicles Formed by a Polymerization-Induced Self-Assembly (PISA) Reaction. *Sci. Rep.* **2017**, *7*, 41534. [CrossRef] [PubMed]
34. Meeuwissen, S.A.; Bruekers, S.M.C.; Chen, Y.; Pochan, D.J.; van Hest, J.C.M. Spontaneous shape changes in polymersomes via polymer/polymer segregation. *Polym. Chem.* **2014**, *5*, 489–501. [CrossRef]

35. Fauquignon, M.; Ibarboure, E.; Carlotti, S.; Brûlet, A.; Schmutz, M.; Le Meins, J.F. Large and Giant Unilamellar Vesicle(s) Obtained by Self-Assembly of Poly(dimethylsiloxane)-b-poly(ethylene oxide) Diblock Copolymers, Membrane Properties and Preliminary Investigation of Their Ability to Form Hybrid Polymer/Lipid Vesicles. *Polymers* **2019**, *11*, 2013. [CrossRef]
36. Agrahari, V.; Agrahari, V. Advances and applications of block-copolymer-based nanoformulations. *Drug Discov. Today* **2018**, *23*, 1139–1151. [CrossRef]
37. Qi, Y.; Li, B.; Wang, Y.; Huang, Y. Synthesis and sequence-controlled self-assembly of amphiphilic triblock copolymers based on functional poly(ethylene glycol). *Polym. Chem.* **2017**, *8*, 6964–6971. [CrossRef]
38. Discher, D.E.; Ahmed, F. POLYMERSOMES. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 323–341. [CrossRef]
39. 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. [CrossRef]
40. 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. [CrossRef]
41. Mai, Y.; Eisenberg, A. Self-assembly of block copolymers. *Chem. Soc. Rev.* **2012**, *41*, 5969–5985. [CrossRef]
42. Cui, J.; Van Koeverden, M.P.; Müllner, M.; Kempe, K.; Caruso, F. Emerging methods for the fabrication of polymer capsules. *Adv. Colloid Interface Sci.* **2014**, *207*, 14–31. [CrossRef] [PubMed]
43. Zhang, X.; Xu, Y.; Zhang, X.; Wu, H.; Shen, J.; Chen, R.; Xiong, Y.; Li, J.; Guo, S. Progress on the layer-by-layer assembly of multilayered polymer composites: Strategy, structural control and applications. *Prog. Polym. Sci.* **2019**, *89*, 76–107. [CrossRef]
44. Becker, A.L.; Johnston, A.P.R.; Caruso, F. Layer-By-Layer-Assembled Capsules and Films for Therapeutic Delivery. *Small* **2010**, *6*. [CrossRef] [PubMed]
45. Sukhorukov, G.B.; Antipov, A.A.; Voigt, A.; Donath, E.; Möhwald, H. pH-Controlled Macromolecule Encapsulation in and Release from Polyelectrolyte Multilayer Nanocapsules. *Macromol. Rapid Commun.* **2001**, *22*, 44–46. [CrossRef]
46. Georgieva, R.; Moya, S.; Hin, M.; Mitlöhner, R.; Donath, E.; Kiesewetter, H.; Möhwald, H.; Bäumler, H. Permeation of Macromolecules into Polyelectrolyte Microcapsules. *Biomacromolecules* **2002**, *3*, 517–524. [CrossRef] [PubMed]
47. Walde, P.; Cosentino, K.; Engel, H.; Stano, P. Giant vesicles: Preparations and applications. *Chembiochem* **2010**, *11*, 848–65. [CrossRef]
48. Lim, S.; de Hoog, H.P.; Parikh, A.; Nallani, M.; Liedberg, B. Hybrid, Nanoscale Phospholipid/Block Copolymer Vesicles. *Polymers* **2013**, *5*, 1102–1114. [CrossRef]
49. Pautot, S.; Frisken, B.J.; Weitz, D.A. Production of Unilamellar Vesicles Using an Inverted Emulsion. *Langmuir* **2003**, *19*, 2870–2879. [CrossRef]
50. 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. [CrossRef]
51. 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. [CrossRef]
52. Picker, A.; Nuss, H.; Guenoun, P.; Chevallard, C. Polymer vesicles as microreactors for bioinspired calcium carbonate precipitation. *Langmuir* **2011**, *27*, 3213–3218. [CrossRef] [PubMed]
53. Kita-Tokarczyk, K.; Grumelard, J.; Haefele, T.; Meier, W. Block copolymer vesicles—Using concepts from polymer chemistry to mimic biomembranes. *Polymer* **2005**, *46*, 3540–3563. [CrossRef]
54. Fetsch, C.; Gaitzsch, J.; Messager, L.; Battaglia, G.; Luxenhofer, R. Self-Assembly of Amphiphilic Block Copolypeptoids – Micelles, Worms and Polymersomes. *Sci. Rep.* **2016**, *6*, 33491. [CrossRef] [PubMed]
55. Yildiz, M.E.; Prud'homme, R.K.; Robb, I.; Adamson, D.H. Formation and characterization of polymersomes made by a solvent injection method. *Polym. Adv. Technol.* **2007**, *18*, 427–432. [CrossRef]
56. 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. [CrossRef]
57. Dionzou, M.; Morère, A.; Roux, C.; Lonetti, B.; Marty, J.D.; Mingotaud, C.; Joseph, P.; Goudounèche, D.; Payré, B.; Léonetti, M.; Mingotaud, A.F. Comparison of methods for the fabrication and the characterization of polymer self-assemblies: What are the important parameters? *Soft Matter* **2016**, *12*, 2166–2176. [CrossRef]
58. Pearson, R.T.; Warren, N.J.; Lewis, A.L.; Armes, S.P.; Battaglia, G. Effect of pH and Temperature on PMPC–PDPA Copolymer Self-Assembly. *Macromolecules* **2013**, *46*, 1400–1407. [CrossRef]

59. Fernyhough, C.; Ryana, A.J.; Battaglia, G. pH controlled assembly of a polybutadiene–poly(methacrylic acid) copolymer in water: Packing considerations and kinetic limitations. *Soft Matter* **2009**, *5*, 1674–1682. [CrossRef]
60. Lorenceau, E.; Utada, A.S.; Link, D.R.; Cristobal, G.; Joanicot, M.; Weitz, D.A. Generation of Polymerosomes from Double-Emulsions. *Langmuir* **2005**, *21*, 9183–9186. [CrossRef]
61. Shum, H.C.; Lee, D.; Yoon, I.; Kodger, T.; Weitz, D.A. Double Emulsion Templated Monodisperse Phospholipid Vesicles. *Langmuir* **2008**, *24*, 7651–7653. [CrossRef]
62. 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. [CrossRef] [PubMed]
63. Deshpande, S.; Caspi, Y.; Meijering, A.E.C.; Dekker, C. Octanol-assisted liposome assembly on chip. *Nat. Commun.* **2016**, *7*, 1–9. [CrossRef] [PubMed]
64. Do Nascimento, D.F.; Arriaga, L.R.; Eggersdorfer, M.; Ziblat, R.; Marques, M.d.F.V.; Reynaud, F.; Koehler, S.A.; Weitz, D.A. Microfluidic Fabrication of Pluronic Vesicles with Controlled Permeability. *Langmuir* **2016**, *32*, 5350–5355. [CrossRef] [PubMed]
65. Martino, C.; Kim, S.; Horsfall, L.; Abbaspourrad, A.; Rosser, S.J.; and. David A. Weitz, J.C. Protein Expression, Aggregation, and Triggered Release from Polymersomes as Artificial Cell-like Structures. *Angew. Chem.* **2012**, *51*, 6416–6420. [CrossRef] [PubMed]
66. Yan, X.; Li, J.; Möhwald, H. Templating Assembly of Multifunctional Hybrid Colloidal Spheres. *Adv. Mater.* **2012**, *24*, 2663–2667. [CrossRef]
67. Postma, A.; Yan, Y.; Wang, Y.; Zelikin, A.N.; Tjipto, E.; Caruso, F. Self-Polymerization of Dopamine as a Versatile and Robust Technique to Prepare Polymer Capsules. *Chem. Mater.* **2009**, *21*, 3042–3044. [CrossRef]
68. Xie, X.; Zhang, W.; Abbaspourrad, A.; Ahn, J.; Bader, A.; Bose, S.; Vegas, A.; Lin, J.; Tao, J.; Hang, T.; et al. Microfluidic Fabrication of Colloidal Nanomaterials-Encapsulated Microcapsules for Biomolecular Sensing. *Nano Lett.* **2017**, *17*, 2015–2020. [CrossRef]
69. Larrañaga, A.; Lomora, M.; Sarasua, J.; Palivan, C.; Pandit, A. Polymer capsules as micro-/nanoreactors for therapeutic applications: Current strategies to control membrane permeability. *Prog. Mater. Sci.* **2017**, *90*, 325–357. [CrossRef]
70. Kazakova, L.I.; Shabarchina, L.I.; Sukhorukov, G.B. Co-encapsulation of enzyme and sensitive dye as a tool for fabrication of microcapsule based sensor for urea measuring. *Phys. Chem. Chem. Phys.* **2011**, *13*, 11110–11117. [CrossRef]
71. Kazakova, L.I.; Shabarchina, L.I.; Anastasova, S.; Pavlov, A.M.; Vadgama, P.; Skirtach, A.G.; Sukhorukov, G.B. Chemosensors and biosensors based on polyelectrolyte microcapsules containing fluorescent dyes and enzymes. *Anal. Bioanal. Chem.* **2013**, *405*, 1559–1568. [CrossRef]
72. 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. [CrossRef]
73. Chen, H.; Man, J.; Li, Z.; Li, J. Microfluidic Generation of High-Viscosity Droplets by Surface-Controlled Breakup of Segment Flow. *ACS Appl. Mater. Interfaces* **2017**, *9*, 21059–21064. [CrossRef] [PubMed]
74. Kisak, E.T.; Coldren, B.; Zasadzinski, J.A. Nanocompartments Enclosing Vesicles, Colloids, and Macromolecules via Interdigitated Lipid Bilayers. *Langmuir* **2002**, *18*, 284–288. [CrossRef]
75. Fu, Z.; Ochsner, M.A.; de Hoog, H.P.M.; Tomczak, N.; Nallani, M. Multicompartmentalized polymersomes for selective encapsulation of biomacromolecules. *Chem. Commun.* **2011**, *47*, 2862–2864. [CrossRef] [PubMed]
76. Marguet, M.; Edembe, L.; Lecommandoux, S. Polymersomes in Polymersomes: Multiple Loading and Permeability Control. *Angew. Chem. Int. Ed.* **2012**, *51*, 1173–1176. [CrossRef] [PubMed]
77. Kim, S.H.; Shum, H.C.; Kim, J.W.; Cho, J.C.; Weitz, D.A. Multiple Polymersomes for Programmed Release of Multiple Components. *J. Am. Chem. Soc.* **2011**, *133*, 15165–15171. [CrossRef] [PubMed]
78. Kreft, O.; Prevot, M.; Möhwald, H.; Sukhorukov, G.B. Shell-in-Shell Microcapsules: A Novel Tool for Integrated, Spatially Confined Enzymatic Reactions. *Angew. Chem.* **2007**, *46*, 5605–5608. [CrossRef]
79. Hosta-Rigau, L.; Chung, S.F.; Postma, A.; Chandrawati, R.; Caruso, B.S.F. Capsosomes with "Free-Floating" Liposomal Subcompartments. *Angew. Chem.* **2011**, *23*, 4082–4087. [CrossRef]
80. Schattling, P.; Dreier, C.; Städler, B. Janus subcompartmentalized microreactors. *Soft Matter* **2015**, *11*, 5327–5335. [CrossRef]

81. Küchler, A.; Yoshimoto, M.; Luginbühl, S.; Mavelli, F.; Walde, P. Enzymatic reactions in confined environments. *Nat. Nanotechnol.* **2016**, *11*, 409. [CrossRef]
82. Jeong, S.; Nguyen, H.T.; Kim, C.H.; Ly, M.N.; Shin, K. Toward Artificial Cells: Novel Advances in Energy Conversion and Cellular Motility. *Adv. Funct. Mater.* **2020**, *30*, 1907182. [CrossRef]
83. Sauer, M.; Haefele, T.; Graff, A.; Nardin, C.; Meier, W. Ion-carrier controlled precipitation of calcium phosphate in giant ABA triblock copolymer vesicles. *Chem. Commun.* **2001**, 2452–2453. [CrossRef] [PubMed]
84. Zhao, S.; Caruso, F.; Dähne, L.; Decher, G.; De Geest, B.G.; Fan, J.; Feliu, N.; Gogotsi, Y.; Hammond, P.T.; Hersam, M.C.; et al. The Future of Layer-by-layer Assembly: A Tribute to ACS Nano Associate Editor Helmuth Möhwald. *ACS Nano* **2019**, *13*, 6151–6169. [CrossRef] [PubMed]
85. Harimech, P.K.; Hartmann, R.; Rejman, J.; del Pino, P.; Rivera-Gil, P.; Parak, W.J. Encapsulated enzymes with integrated fluorescence-control of enzymatic activity. *J. Mater. Chem. B* **2015**, *3*, 2801–2807. [CrossRef] [PubMed]
86. Hou, C.; Wang, Y.; Zhu, H.; Zhou, L. Formulation of robust organic–inorganic hybrid magnetic microcapsules through hard-template mediated method for efficient enzyme immobilization. *J. Mater. Chem. B* **2015**, *3*, 2883–2891. [CrossRef] [PubMed]
87. Karamitros, C.S.; Yashchenok, A.M.; Möhwald, H.; Skirtach, A.G.; Konrad, M. Preserving Catalytic Activity and Enhancing Biochemical Stability of the Therapeutic Enzyme Asparaginase by Biocompatible Multilayered Polyelectrolyte Microcapsules. *Biomacromolecules* **2013**, *14*, 4398–4406. [CrossRef]
88. 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. [CrossRef]
89. Marguet, M.; Bonduelle, C.; Lecommandoux, S. Multicompartmentalized polymeric systems: Towards biomimetic cellular structure and function. *Chem. Soc. Rev.* **2013**, *42*, 512–529. [CrossRef]
90. Peyret, A.; Ibarboure, E.; Tron, A.; Beauté, L.; Rust, R.; Sandre, O.; McClenaghan, N.D.; Lecommandoux, S. Polymersome Popping by Light-Induced Osmotic Shock under Temporal, Spatial, and Spectral Control. *Angew. Chem. Int. Ed.* **2017**, *56*, 1566–1570. [CrossRef]
91. Delcea, M.; Yashchenok, A.; Videnova, K.; Kreft, O.; Möhwald, H.; Skirtach, A.G. Multicompartmental micro-and nanocapsules: Hierarchy and applications in biosciences. *Macromol. Biosci.* **2010**, *10*, 465–474. [CrossRef]
92. Xiong, R.; Soenen, S.J.; Braeckmans, K.; Skirtach, A.G. Towards theranostic multicompartment microcapsules: In-situ diagnostics and laser-induced treatment. *Theranostics* **2013**, *3*, 141. [CrossRef] [PubMed]