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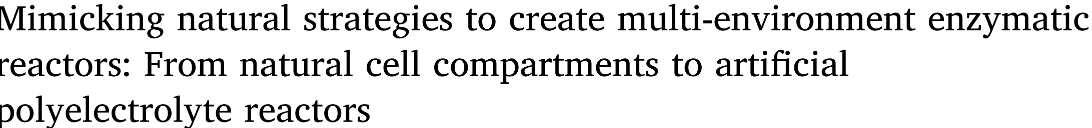

Mimicking natural strategies to create multi-environment enzymatic reactors: From natural cell compartments to artificial polyelectrolyte reactors

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ABSTRACT

Engineering microenvironments for sequential enzymatic reactions has attracted specific interest within different fields of research as an effective strategy to improve the catalytic performance of enzymes. While in industry most enzymatic reactions occur in a single compartment carrier, living cells are however able to conduct multiple reactions simultaneously within confined sub-compartments, or organelles. Engineering multi-compartments with regulated environments and transformation properties enhances enzyme activity and stability and thus increases the overall yield of final products. In this review, we discuss current and potential methods to fabricate artificial cells for sequential enzymatic reactions, which are inspired by mechanisms and metabolic pathways developed by living cells. We aim to advance the understanding of living cell complexity and its compartmentalization and present solutions to mimic these processes in vitro. Particular attention has been given to layer-by-layer assembly of polyelectrolytes for developing multi-compartments. We hope this review paves the way for the next steps toward engineering of smart artificial multi-compartments with adoptive stimuli-responsive properties, mimicking living cells to improve catalytic properties and efficiency of the enzymes and enhance their stability.

1. Introduction

Living cells are highly complex systems in which natural multi-step metabolic reactions occur simultaneously with unsurpassed efficiency and specificity (Winkel, 2004). Multi-enzymatic reactions in living organisms are essential for an efficient metabolic process. Compartmentalization and substrate channeling are two key mechanisms developed by living organisms to organize and control metabolic pathways (Abernathy et al., 2017; de Lorenzo et al., 2015; Milani et al., 2003; Raushel et al., 2003; Schmitt and An, 2017; Sweetlove and Fernie, 2018).

On a cellular scale, compartmentalization of cells that contain multiple sequential enzymes provides an inclusive strategy for improving the metabolic pathways and controlling reaction kinetic rates. Establishing local concentration gradients and reducing unwanted side reactions and toxic intermediates can be achieved by encapsulating enzymatic reactions within subcellular compartments. Many examples of cell compartmentalization can be found in nature (Karsenti, 2008). For example, eukaryotic cells, have several subcellular compartments, organelles, which encapsulate enzymes in different parts of the cell (Chen and Silver, 2012). On a molecular level, direct interactions between enzymes that catalyze sequential reactions, or between enzymes and scaffold proteins, enable substrate channeling of intermediate products from the active site of one enzyme to the next enzyme without diffusion throughout the rest of the cell. Many examples of substrate channeling exist in living plant cells, such as tricarboxylic acid (TCA) cycle, the Calvin cycle and tryptophan synthase (Winkel, 2004; Conrado et al., 2008).

There is a long and distinguished history of studies on substrate channeling and cell compartmentalization in living cells, starting as early as 1930 (Winkel et al., 2004). Enzymatic compartments have been extensively studied experimentally, and artificial cells such as polymer capsules and phospholipid vesicles have been integrated with encapsulation via the layer-by-layer (LbL) assembly approach. Currently, the

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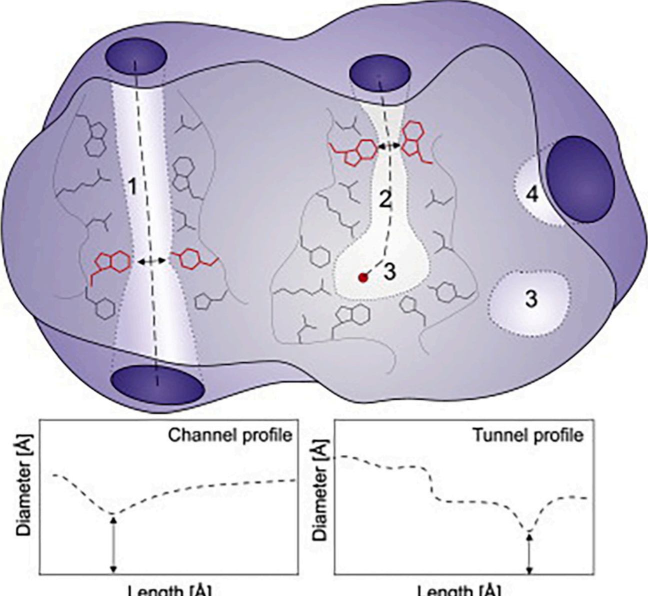

Fig. 1. Schematic representation of: enzyme channel (1), enzyme tunnel (2), buried cavities (3) and surface groove(4). Adapted from Brezovsky et al., 2013 with permission from Elsevier.

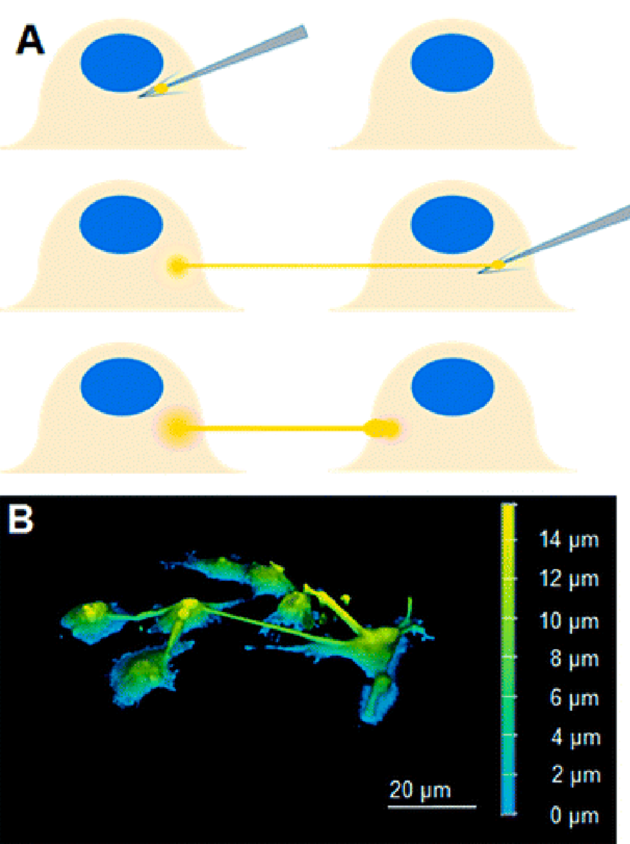

Fig. 2. (A) Scheme of lipid nanotubes tunnels-cell network (LNT-cell network) that mimic natural protein channels made of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine lipid and (B) 3D reconstituted confocal laser scanning microscopy image of lissamine Rhodamine B PE stained LNT–cell network Adapted from Kozintsev and Sugihara, 2017, Published by The Royal Society of Chemistry.

focus of using artificial cells is for biomedical purposes and drug delivery systems (Deng et al., 2017; Giannakopoulou et al., 2020; Hui Chong et al., 2021). However, these multi-enzyme reaction pathways can also be used for many other industrial applications. Undeniably, there is still a need for quantitative understanding of the fundamental design principles (Hinzpeter et al., 2017). Here, the aim is to understand the nature and importance of metabolic pathways to identify possible means to simplify the mechanism and incorporate it into an artificial cell unit for sequential enzymatic reactions at industrial scales. To understand how metabolic pathways work in cells, it is essential to know how enzymes are compartmentalized within the cell, as well as how they are linked together and controlled by the metabolism.

In the following sections we elaborate the key features that control the metabolic pathways of living cells. We demonstrate and discuss two main mechanisms of enzyme-based metabolic pathways, namely substrate channeling and compartmentalization, to understand their functions. Next, we discuss current technologies for creating artificial compartments followed by a discussion on the selection of materials and conditions to explain in more detail how to design and synthesize such compartments and optimize their performance to achieve high reactions efficiencies. Finally, our aim is to highlight the direction in the field with selected examples to understand the processes by which future opportunities to create smart cell mimetic units may emerge.

2. Mechanisms of enzymatic reaction sequences in living cells

2.1. Substrate channeling

Substrate channeling is one of the strategies to control metabolic pathways in living cells. Substrate channeling is the direct link between different enzymes based on molecular linkers or specific interactions that form molecular channels for intermediate transfer. Thus, the intermediates of metabolic pathways can pass directly from the active site of one enzyme to the next enzyme without diffusing throughout the bulk in the cell (Shi et al., 2018). Substrate channeling has been proposed to have a variety of benefits for developing and controlling pathway reactions. Indeed, in multi-enzyme complexes, substrate channeling attains high local intermediate substrate concentrations near the reaction centers of the appropriate enzyme. This mechanism increases reaction rates and shortens transition time, regulates competition between different pathways for common metabolites, protects unstable intermediates from degradation, and inhibits toxic reactions between intermediates and cellular components (Obata, 2020; Raushel et al., 2003; Wheeldon et al., 2016). The distance between enzymes has a significant effect on the rate of reactions via substrate channeling in sequential enzymatic reactions (Hwang and Lee, 2019). In general, the length of diffusion path between enzyme active sites should not surpass 1 nm. At distances above 1 nm, channeling requires molecular interactions or molecular tunneling to connect the enzyme active sites or compartments to each other (Conrado et al., 2008; Wheeldon et al., 2016). However, effective channeling supported by either molecular interactions or tunneling can be achieved with inter-enzyme distances up to 10 nm, if the electrostatic interactions between the intermediate and electrostatic highway through the active sites elevate diffusion, or if protein tunnels connect consecutive active sites of enzymes (Deng et al., 2017). In the following sections protein tunneling and electrostatic highways are extensively illustrated using recent advances and several examples of substrate channeling in vitro.

2.1.1. Protein channels and protein tunnels

Testing on 4306 enzyme structures revealed that more than 64% of enzyme structures employ protein channels and tunnels to allow transfer of substrates and products between enzyme active sites and govern the exchange rates of ligands, ions, and water solvent (Brezovsky et al., 2018) (Fig. 1).

Enzymes can have more than one tunnel and/or channel. A typical

enzyme channel has a length of 20 Å and is made of different amino acids, such as histidine (His), arginine (Arg), tryptophan (Trp), isoleucine (Ile), proline (Pro), leucine (Leu), glycine (Gly) and tyrosine (Tyr) (Pravda et al., 2014). Examples of enzymes with direct channeling are imidazole glycerol phosphate synthase, glutamate synthase, and asparagine synthase, which have hydrophobic protein channel with a length of 25 Å, 30 Å, and 19 Å, respectively (Raushel et al., 2003). Channel walls mostly contain aromatic, charged, and polar amino acids (His, Tyr, Trp, and Arg), while nonpolar, aliphatic amino acids (Pro, Gly, Ile, and Leu) are less confined in channel walls and are more involved in the establishment of the hydrophobic enzyme core, which is vital to maintain a protein fold (Huang et al., 2001). A physico-chemical study of enzymes also shows that two factors have particularly important roles in the functionality of tunnels and channels: the hydrophilicity and the polarity. The hydrophilicity of channels varies and depends mainly on the hydropathy of the amino acids that are incorporated in the channel walls. Similarly, channel polarity depends on the polarity of the amino acids present in its walls, as both highly polar amino acids and low polar amino acids are found in enzyme channels (Pravda et al., 2014). For instance, tryptophan offers a well-defined illustration of the physical interactions between sequential enzymes associated with protein channeling. Tryptophan synthase exists as an $(\alpha\beta)_2$ complex. The two β subunits are tightly linked in the center of the molecule and the two α subunits are located on opposite sides of the ββ subunits. The active sites of the α/β pairs are connected by a 2.5 nm long hydrophobic tunnel in which the intermediate is entirely sequestered from the bulk (Dunn et al., 1990; Miles et al., 1999; Pan et al., 1997.

An example of an artificial structure that mimics natural protein tunnels for direct intercellular transfer of intermediates is lipid nanotube tunnels, which were first reported in 2004 (Rustom et al., 2004). In this case, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) lipid, which is a zwitterionic lipid, was used to create self-assembled lipid nanotubes (LNT) by introducing a microtip coated with the lipid blocks into a cell membrane and dragging it towards a second cell to form a lipid nanotube connecting the two cells (Kozintsev and Sugihara, 2017). The DOPE nanotubes formed a LNT-cell network that permitted direct intercellular transfer of components between cells (Fig. 2). Additionally, these lipid blocks can adsorb onto both glass and plastic surfaces coated by cationic polyelectrolytes, such as polyethylenimine (PEI) and poly-L-lysine (PLL). This characteristic makes lipid blocks a good candidate for a wide range of applications in bio-nanofabrication and sensing, or as a communication tool for many types of proteins and cells in-vivo or in-vitro, while they also can be used as a template for solid structure fabrication (Gözen and Jesorka, 2015; Karlsson et al., 2004).

2.1.2. Electrostatic channeling

Electrostatic channeling is a mechanism of substrate transport in which electrostatic potential plays a key role in transferring substrates between enzymes, specifically in bioenergetic metabolic pathways. Via this mechanism, charged intermediate substrates diffuse through an electrostatic field generated by an oppositely charged path. Electrostatic channeling is basically to confine the diffusive movement of the substrate between the enzymes and avoid substrate diffusion into the bulk. In contrast to physical channels, electrostatic channels are formed only by electrostatic potential, not by physical confinement. Therefore, there is a likelihood that the substrate can escape into the bulk solution, and it is thus crucial that the charge distribution along the intermediate pathway through the enzyme active site favors transport of the substrate (Eun et al., 2014). Although electrostatic channeling is an effective substrate transport mechanism, there are limited known examples of this type of substrate transport in living cells. However, kinetic experiments and molecular simulations have proven the existence of electrostatic channeling that accelerates multi-enzyme reactions (Shi et al., 2018; Wheeldon et al., 2016). So far, the study of natural electrostatic channeling has focused on the tricarboxylic acid cycle and the thymidylate cycle. Study of the surface electrostatic potential of the tricarboxylic acid cycle metabolon structure has revealed the formation of electrostatic channeling upon protein–protein association. Analysis of the surface electrostatic potential of malate dehydrogenase (MDH), citrate synthase (CS), and aconitase (ACON) enzymes showed that rearrangement of surface charge patterns upon protein–protein association led to the formation of a continuous positively charged zone across their interface (Shatalin et al., 1999.). Free CS is slightly negative, while MDH and ACON carry more positive charges at pH 7.8 (the pH of a mitochondrial matrix). This electrostatic pattern accordingly enables direct transport of negatively charged intermediate substrates (oxaloacetate). In the thymidylate cycle, kinetic study of the bifunctional thymidylate synthase–dihydrofolate reductase (TS–DHFR) enzyme provides solid indication of ionic strength-dependent channeling of DHFR, including a 45 Å distance between the TS and DHFR active sites. In this case, a positively charged spot on the surface of the protein structure bridges the two active sites (Wu and Minteer, 2015). Molecular simulations also demonstrate that the opposite charge between the negatively charged dihydrofolate intermediate molecules and positively charged protein structure results in restricted diffusion and promotes substrate channeling between the active sites. While there is kinetic evidence of channeling, the complete structure of electrostatic tunneling has not been defined, and evidence is lacking to conclusively determine a mechanism of channeling. Nevertheless, it might be concluded, that involvement of different types of amino acids in living cells indicates that the metabolon pathway is well-adjusted as a cooperative effect of electrostatic and hydrophobic interactions (Wheeldon et al., 2016).

Efficient transport of substrates via electrostatic channeling in living cells leads to the design and development of multienzymatic systems that work effectively due to the artificial electrostatic channeling for substrate transfer between enzymes. Since charged amino acids on the surface of enzymes generate an electrostatic field, transfer of the enzyme's substrate to the active site is facilitated. Therefore, there is a possibility to design intermolecular interactions surrounding an enzyme's active site and thereby provide a suitable enzyme microenvironment that regulates the enzymatic reaction kinetics and enhances the efficiency of the multienzymatic system (Barbosa et al., 2013, 2015). For instance, in a study by Gao et al. (2015) aldo-keto reductase (AdhD) and horseradish peroxidase (HRP) were intentionally conjugated to short DNA sequences by coupling of a lysine residue of the protein with a 5′-thiol group of the DNA using a maleimide-based coupling reagent (Sulfo-SMCC). In this case, the negatively charged phosphate backbone of DNA provides the presence of a negative electrostatic field in the vicinity of the enzyme that ensures electrostatic interactions between DNA and positively charged substrates (Wilson and Szostak, 1999). Furthermore, DNA-aptamers provide small molecule binding sites that can introduce favorable molecular interactions with substrates in the vicinity of the enzymes. Thus, DNA molecules deposited on the surface of an enzyme can lead to increased effective substrate molarity with a positive effect on the reaction kinetics. Similar strategies include grafting of charged polymers, such as poly(quaternary ammonium), to the surface of an enzyme to tune the substrate specificity and inhibitor binding (Murata et al., 2014), and immobilization of α-chymotrypsin (ChT) using amino-acid-functionalized gold clusters with controlled surface charge to modulate its affinity towards cationic, neutral, and anionic substrates (You et al., 2006).

2.2. Compartmentalization

Compartmentalization is the second metabolic strategy to control enzymatic reactions. In cells, each enzyme is confined in an internal subcellular compartment, an organelle, that controls the reactivity of the enzyme by isolation (Alberts et al., 2002; Ricard, 1999). Organelles create different chemical environments via semipermeable membranes, which sustain the enzyme activity in the environment and retain enzymes and vital components inside the compartment while exchanging nutrients and allowing the intermediate substrate to diffuse through the

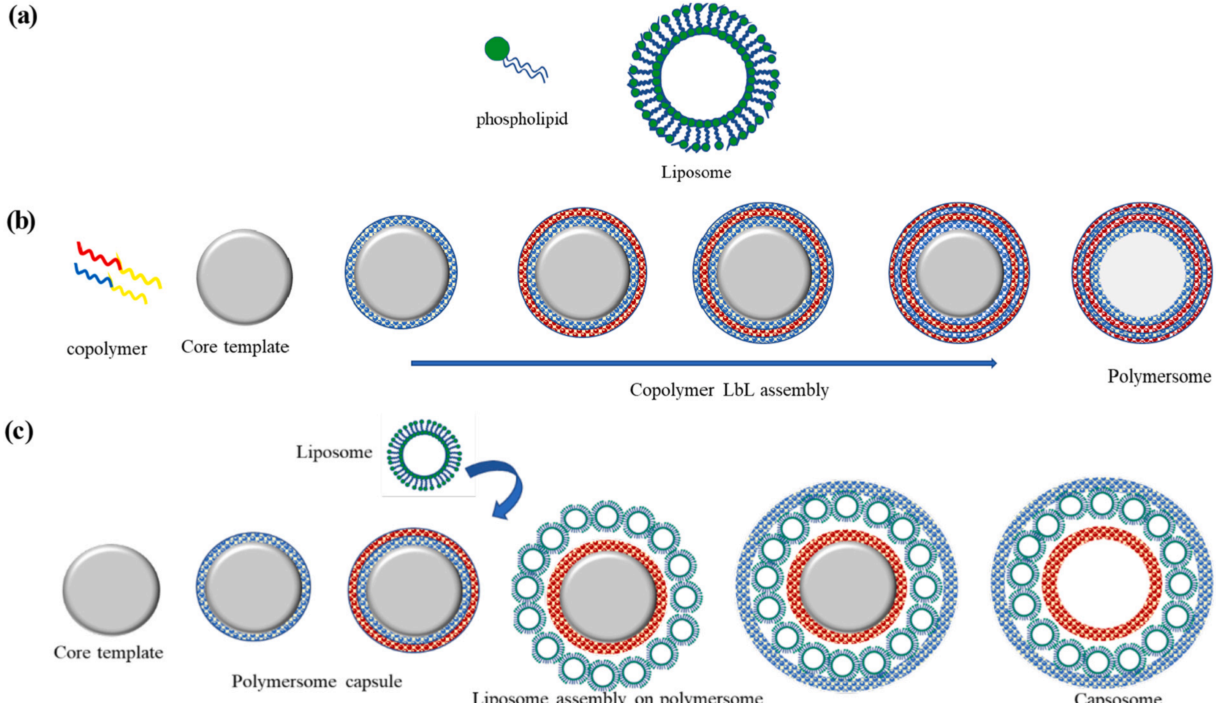

Fig. 3. Schematic representation of steps for creating different types of compartments: (a) liposome formed by self-assembly of phospholipids, (b) polymersome, multilayer assembly of copolymers on the removable core template, (c) capsosome formation via a combination of copolymer self-assembly and deposition of liposome microcapsules sandwiched between the polymer layers.

membrane. Isolation of enzymes minimizes challenges such as toxic pathway intermediates and competing metabolic reactions in multi-enzyme sequential reactions. It is therefore easier for the multi-enzyme reactions to progress in an onward path and thus enzyme activity increases because of the micro-environment effects and cascade kinetics. Living cells, namely eukaryotic and prokaryotic cells, carefully control these processes through a semipermeable lipid membrane (Ricard, 1999). Peroxisome, for example, is a small membrane-bound organelle compartment in eukaryotic cells. This compartment encapsulates catalase which is an enzyme that breaks down hydrogen peroxide (Chen and Silver, 2012). The action of this enzyme is of particular interest as hydrogen peroxide acts as a toxic intermediate in the breakdown of organic substrates in oxidative reactions in living cells. On the other hand, lysosomes are small organelles that contain digestive enzymes, such as hydrolases that break down proteins, carbohydrates and fats, and provide the cell with simple nutrients and other critical components. However, if the lysosome did not sequester these enzymes from the rest of the cell, the enzymes could also degrade components of the cell, which would be fatal (Lawrence and Zoncu, 2019).

Carboxysomes, by contrast, are organelle-like intracellular microcompartments present in prokaryotic cells, built by polyhedral protein shells containing ribulose-1,5-bisphosphate carboxylase/oxygenase and carbonic anhydrase in its core. These organelles are responsible for carbon assimilation and are found in some carbon dioxide binding bacteria that perform photosynthesis or chemosynthesis (Rae et al., 2013). Intracellular compartmentalization of cooperating enzymes is a strategy frequently used by cells. Thus, the first step for the design of cellular mimics is to achieve enzymatic reactions in individual, separated compartments, in a similar way to how they occur in the organelles of a cell to carry out specific metabolic functions (Liu et al., 2017).

3. Sequential reactions in artificial cells

Inspired by nature, researchers are striving to engineer encapsulated metabolic pathways to improve the yield of pharmaceutical products. Recently, some designs have incorporated multiple compartments to mimic the natural structure of living cells, including lipid vesicles (liposomes), polymeric vesicles (polymersomes), polymersome-in-polymersome architectures, and capsosomes (hybrid vesicles or liposomes within layer-by-layer (LbL) polymeric capsules) (Shi et al., 2018) (Fig. 3). In the following section we present and discuss each encapsulation approach in more detail with appropriate examples.

3.1. Liposome and liposome-in-liposome architectures

Phospholipid liposomes are spherically shaped vesicles formed through self-assembly of amphiphilic molecules (Fig. 3(a)), such as phospholipids or long chain fatty acids based on simple physio-chemical forces. Liposome vesicles are formed as a bilayer lipid derived from hydration of phospholipid, amphiphilic molecules in aqueous medium. Liposomes have been used in medicine and pharmacology for diagnostic or therapeutic applications for a long time. Liposomes are well suited for encapsulating active molecules, such us enzymes. However, liposomes are thermally and mechanically unstable under physical constraints, such as the extreme dilution required for food, medical and pharmaceutical applications (Chang and Yeh, 2012). Liposomes with modified surfaces have therefore been developed using several molecules, such as glycolipids or sialic acid, to enhance their stability. For instance, in a study by Ge et al. (2003a, 2003b) negatively charged liposomes from L-α-dimyristoylphosphatidic acid (DMPA) were coated by a single layer of cationic poly-allylamine hydrochloride (PAH) polyelectrolyte to improve the stability of the liposomes. It was shown that coating of liposome surfaces with polyelectrolyte enhanced the liposome stability to ethanol and that it would be resistant to degradation in the presence of surfactants, such as sodium dodecyl sulfate.

3.2. Polymersome and polymersome-in-polymersome architectures

Studies on liposomes and their properties were an inspiration for polymer chemists to start working on compartmentalization of

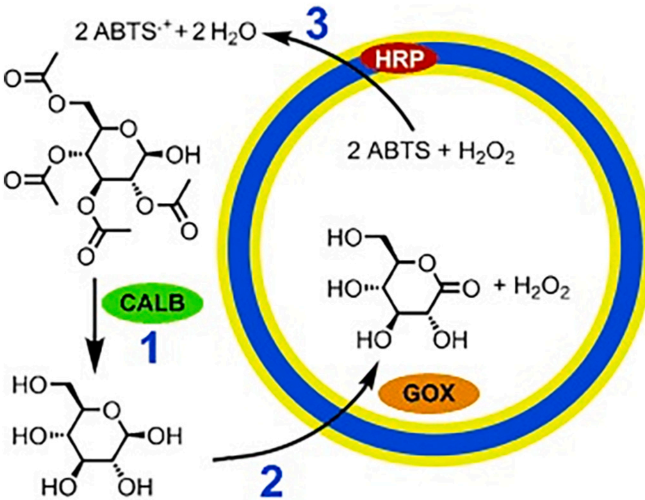

Fig. 4. Schematic representation of multi-step enzymatic reactions in polymersomes: conversion of glucose acetate to ABTS radical cation catalyzed by GOX, HRP and CALB. Adapted from reference Vriezema et al., 2007, with permission from John Wiley and Sons.

enzymatic reactions with polymer-based materials. Extensive studies have been done on microcapsules known as polymersomes where the bilayer membrane is composed of amphiphilic block *co*-polymers (Fig. 3 (b)). Block co-polymers are polymeric structures that are composed of at least two or more homopolymer subunits linked by covalent bonds. A block polymer is identified as a part of a complex macromolecule that shows at least one feature which is not present in the adjacent portions. Diblock co-polymers have two distinct blocks, whereas triblock co-polymers have three and so on. It should be added that proper homopolymer subunits linkage may require an additional, intermediate non-repeating subunit, known as a junction block (McNaught and Wilkinson, 1996). The robustness of polymersomes arises from the molecular characteristics of the hydrophobic segment of the block co-polymers. Polystyrene$_{40}$-b-poly(l-isocyanoalanine(2-thiophen-3-yl-ethyl) amide)$_{50}$ diblock co-polymer (PS–PIAT) is a well-defined block co-polymer that has been used for the fabrication of highly stable polymersomes to encapsulate a variety of guests (Vriezema et al., 2003, 2004). These polymersomes have a sufficiently porous structure which allows diffusion of small molecules, such as substrates, across their membranes, while large molecules, such as enzymes, are trapped inside. PS-PIAT polymersomes may potentially be used as microreactors for industrial applications. Van Hest's group has conducted pioneering work in compartmentalization using block co-polymers. In a representative study, PS-PIAT diblock co-polymer was employed to form polymersomes with porous structures to perform one-pot multiple enzyme reactions (Vriezema et al., 2007). They entrapped glucose oxidase (GOX) within the water pool of the PS-PIAT polymersome. Horseradish peroxidase (HRP) was associated by lyophilization procedure developed for this purpose with the membrane because it is a hydrophilic enzyme and expected to be positioned in the hydrophilic domains of the polymersome membrane. Lastly, *Candida antarctica* lipase B (CALB) was added to the bulk solution together with the substrate (1,2,3,4-tetra-*O*-acetyl-b-glucopyranose, GAc4) (Fig. 4). In the enzyme-decorated polymersome, GAc4 was converted to glucose by CALB, which was subsequently oxidized by GOX to gluconolactone. The hydrogen peroxide produced by GOX in the second step acts as a co-substrate for HRP that catalyzes the oxidation of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to an ABTS radical cation. In the study, the total enzyme activity and the initial rate of substrate conversion by the enzyme-filled polymersomes were compared with the initial conversion rates of the free enzymes dispersed in solution. The study confirmed that the encapsulated GOX had 100-fold higher total enzyme activity but a lower substrate conversion rate than its free counterpart. However, it should be mentioned that assay reactions with free and encapsulated enzymes were carried out at different enzyme concentrations. Axthelm et al. (2008) developed a polymersome nanoreactor comprised of poly (2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA-b-PDMS-b-PMOXA). The nanoreactor was used as an antioxidant nanoreactor by encapsulating Cu,Zn superoxide dismutase to detoxify superoxide radicals. In another study, Langowska et al. (2013) designed a PMOXA-b-PDMS-b-PMOXA polymersome nanoreactor for the local production and release of antibiotics by encapsulation of penicillin acylase which converted 7-amino-desacetoxycephalosporanic acid and phenyl-glycine methyl ester into cephalexin to inhibit bacterial growth.

Besides selection of the block *co*-polymers and/or liposomes for polymersomes and capsosomes formation, that strongly affect the stability and structure of the formed structures, another important factor is the selection of a suitable core template. The core template determines the size and shape of the capsules. Organic templates like poly-lactic acid (PLA) and poly-styrene (PS) (Shenoy et al., 2003) and inorganic templates such as carbonate cores and silica particles (Itoh et al., 2004), which are characterized by well-defined structure and shape can be used for the fabrication of capsules. Organic templates with a wide range of sizes and surface functionalities (mainly carboxyl, hydroxyl, sulfate, or amine groups) can be engineered to facilitate the subsequent deposition of polymer layers. However, the presence of osmotic pressure and organic solvents during template removal may cause the decomposition of the membrane, which is a significant drawback. Inorganic templates like $CaCO_3$ and SiO_2 have high solubility in aqueous solution and form low-molecular-weight ionic dissolution products that can easily leave the capsule interior, thus completely removing the template and avoiding osmotic stress. Carbonate templates have high porosity, which allows the adsorption of a large number of active molecules into the template. Moreover, these templates dissolve under very mild conditions e.g. in ethylenediaminetetraacetic acid (EDTA) solution. However, carbonate template aggregation and instability are issues resulting from the high polydispersity of such templates. By contrast, silica particles are very smooth, have a spherical shape and their size and porosity can be controlled (Caruso and Schüler, 2000; Delcea et al., 2011; Itoh et al., 2004). In general, $CaCO_3$ and silica are the most common templates used for polyelectrolyte capsules in biomedical and biotechnological applications because they are biocompatible, easy to produce, have a high surface area and a high loading capacity (Parakhonskiy et al., 2014; Torchilin, 2014; Yu et al., 2005).

3.3. Capsosome (liposome-in-polymersome) architectures

Capsosomes are liposome sub-compartments combined into a polymer capsule, which capture the advantages of two fundamentally different systems. The polymer capsule provides structural stability while the permeable polymer membranes of the liposomes allow control over the diffusion of molecules between liposome compartments. Liposomes resemble the cell's organelles and provide subunits that allow the following enzymatic reactions to occur within a confined area (Hosta-Rigau et al., 2013). The assembly of capsosomes involves deposition of a polymer layer onto a sacrificial layer, followed by the alternating assembly of liposomes and polymer separation layers. During assembly, large amounts of liposome sub-compartments can be incorporated in one capsule and give an incredible loading efficiency while keeping all loaded molecules separated and allowing sequential and parallel enzymatic reactions to occur simultaneously (York-Duran et al., 2017). Thus cell-mimicking, liposome-based capsosomes could have countless applications in the fields of medicine, pharmaceuticals and biotechnology.

There are several established procedures for creating micro-compartments that can facilitate reactions while exchanging reagents and products with the external environment. Formation of capsule-in-capsule compartment systems with polymer materials has been the

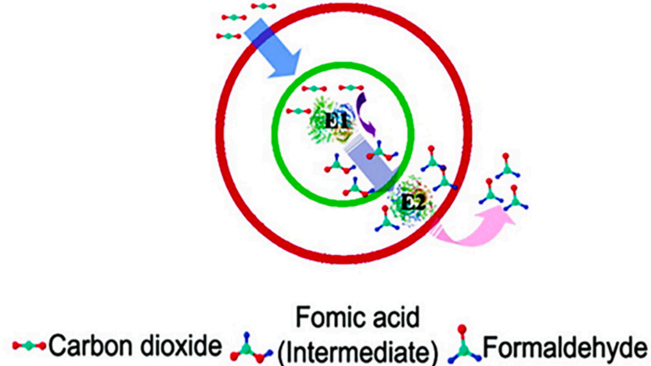

Fig. 5. Schematic representation of multistep enzymatic conversion of CO_2 to formaldehyde in a capsule-in-capsule compartment. Adopted from Shi et al., 2011, with permission from American Chemical Society.

simplest way used to synthesize artificial cells with multi-compartment structures, where multi-enzymes are wrapped in different guest capsules and distributed in a host capsule. A robust multi-compartment system can be constructed through LbL self-assembly by sequential deposition of polyelectrolyte layers on the surface of a calcium carbonate template (Hwang and Lee, 2019). An example of a capsule-in-capsule structure was reported by Shi et al. (2011) and concerns the shell-in-shell microcapsule for the enzymatic conversion of CO_2 to formaldehyde. In this microcapsule, a CO_2 substrate was transferred through three compartments (outer membrane, inter-membrane space, and inner membrane) to be converted to formic acid (Fig. 5). Formate dehydrogenase (FateDH) was used as the first enzyme to convert CO_2 to formic acid, and formaldehyde dehydrogenase (FaldDH) was the second enzyme which converted formic acid into formaldehyde. Consequently, formic acid should be transferred from the first compartment and be converted into formaldehyde in the inner one. The amount of formaldehyde produced by the encapsulated enzymes was considerably greater than what was achieved by using free enzymes (95% and 50%, respectively). The results suggest that the enzymes' encapsulation positively influenced the overall reaction rate, likely due to the physical proximity between enzymes and reduced substrate transfer path between the active sites of the biocatalysts. In this case a capsule-in-capsule system was used in order to provide separate and suitable microenvironments for both enzymes, as they show optimal catalytic activity at different pH and temperature conditions. Furthermore, use of intercompartment membranes at proper porosity ensure transport of substrate and intermediates. In other studies, it has been reported that microcapsules facilitated the rapid mass transfer via ideal localization of substrates and intermediates of the enzymatic cascades within short distances (Peters et al., 2014; Vriezema et al., 2007).

Materials to construct multi-enzyme compartment systems are complex and broad, including polyelectrolytes (e.g. PAH and polystyrene sulfonic acid (PSS)), carbohydrate polymers (e.g. alginate), and *block-co*-polymers (e.g. polystyrene-*block*-polyisocyanoalanine(2-thiophene-3-yl-ethyl)amide (PS-b-PIAT) and polystyrene-*block*-poly (ethylene oxide) (PB-b-PEO)). The materials show different affinity towards different enzymes, which offers a myriad of options for creating multi-compartment systems for specific enzymes. However, in each case, a particular attention should be paid to the selection of an appropriate material that provides a suitable microenvironment for the enzymes in the compartments and ensure high enzyme activity and stability.

4. Polyelectrolyte microcapsules

Polyelectrolyte materials are interesting materials for researchers in the bioengineering field due to their ability to provide charge that can support efficient transfer of substrates/products between the active sites of enzymes, enhances binding of the biomolecules and can improve catalytic activity. Polyelectrolytes as biodegradable, biocompatible, and non-toxic materials for the construction of compartments have become an extremely interesting subject for pharmaceutical, biomedical and biotechnological research (Miles et al., 1999; Muthukumar, 2017). Polyelectrolytes are classified into two categories, polycations and polyanions, based on their charges. Poly-acrylic acid (PAAC), PAH and poly-methacrylic acid (PMA) are some of the most widely studied polyelectrolytes (Burke and Barrett, 2003; Peyratout and Dähne, 2004). Other studies include capsules made of PAA/poly-vinyl alcohol (PVA) (Yun and Kim, 2010), chondroitin sulfate/poly-L-lysine (PLL) (Zhao and Li, 2008), as well as weakly charged poly-ethylenoxide (PEO) and poly-*N*-vinylpyrrolidone (PVPON) (Gun and Routh, 2013; Sukhishvili and Granick, 2000). Table 1 summarizes potential polyelectrolytes for capsule formation and their physical and chemical properties. Polyelectrolytes can also be categorized according to whether they are strongly or weakly charged. Changing the solution pH or ionic strength can change the charge of weak polyelectrolytes. The acid dissociation constant, pK_a, of polymers provides information about the degree of ionization of the electrolyte. Since changes in the pH of polyelectrolyte solutions will affect the thickness of bilayers, pK_a is a key factor in polyelectrolyte selection in the LbL assembly process. For example, fully ionized polyelectrolytes like PAA at pH 10, or PAH at pH lower than 6, will form the thinnest film since they adhere more strongly to the oppositely charged substrate onto which they are deposited (PAA has a pK_a of 6.5 which means that PAA is fully ionized at pH 10 and completely deionized at pH 4) (Goicoechea et al., 2008). Furthermore, polyelectrolyte capsules can be simply functionalized during LbL assembly by incorporation of biocompatible poly-ions, proteins (Caruso and Möhwald, 1999), DNA (Vinogradova et al., 2005), lipids (Moya et al., 2000), multivalent ions, and charged nanoparticles (NPs) into their walls (Bédard et al., 2008). However, there are also some limitations in the application of polyelectrolytes for LbL microcapsules formation, among which the most important are their solubility, instability in organic solvents and negative effect of charged groups onto enzyme structure and its stability. Nonetheless, the formation of LbL microcapsules using various polyelectrolytes should be considered a very promising strategy to construct one-pot multienzymatic structures for conducting sequential enzyme reactions.

4.1. *Designing polyelectrolyte microcapsules via LbL assembly*

The layer-by-layer (LbL) assembly approach is the most common way of preparing microcapsules. In this procedure, layers of oppositely charged polyelectrolytes are deposited on core templates, after which the cores are dissolved to obtain empty polyelectrolyte capsules, showing similarities with the formation of polymersomes (Fig. 3(b)). Additionally, core materials used for the production of LbL microcapsules show similarities in features and type with core substances used for formation of polymersomes and capsosomes. The empty core of the capsules can be filled with the molecules of interest by the responses of polymers incorporated in the shell and by switching between the open and closed states of the polyelectrolyte capsules by changing temperature, pH, or light (Peyratout and Dähne, 2004). The encapsulated molecules can later be released by adjusting the physico-chemical properties of the polymers and manipulating the shell permeability. However, encapsulated molecules, such as enzymes, can also be retained in the core of the LbL capsules in order to provide protection against negative effects of the external reaction microenvironment and improve their stability. The main driving forces for LbL assembly of polyelectrolytes are electrostatic interactions and entropy gain from releasing counterions upon complexation; however, both hydrogen binding and covalent binding can also be involved (Ariga et al., 2007; Fu and Schlenoff, 2016; Scheepers et al., 2021). The electrostatically bound assemblies grant flexibility and high sensitivity towards pH, ionic strength, and polarity.

Table 1
Potential polyelectrolytes for capsule formation and their physical properties.

Poly-electrolyte	Charge	Functional group	pK_a	Structure
Poly-L- lysine (PLL)	cationic	–NH_2 –NH	Weak polycation with pK_{aH} of primary and secondary amine group around 10 (Kienle et al., 2020)	
Poly-allylamine (PAA)	cationic	–NH_2	Weak polycation with pK_{aH} of primary amine group around 9 (Goicoechea et al., 2008)	
Poly-L-arginine (PLA)	cationic	–NH	Weak polycation with pK_{aH} of guanadine group around 12.5 (Waqas et al., 2017)	
Poly-ethyleneimine (PEI)	cationic	–NH	Weak polycation with pK_{aH} of secondary amine group around 7–10 (Curtis et al., 2016)	
Poly-methacrylic acid (PMA)	anionic	–COOH	Weak polycation with pK_a of carboxyl group around 4.5 (Ibarra-Montaño et al., 2015)	
Poly-styrene sulfonic acid (PSS)	anionic	–SO_3^-	Strong polyanion with pK_a of sulfonic acid group around 1 (Lewis et al., 2011)	
Poly-acrylic acid (PAAC)	anionic	–COOH	Weak polyanion with pK_a of carboxyl group around 4.5–5 (Wisniewska et al., 2014)	
Poly-galacturonic acid (PGA)	anionic	–COOH	Strong polyanion with pK_a of carboxyl group around 3.2–3.5 (Souffriau et al., 2012)	
Alginic acid (LAA)	anionic	–COOH	Strong polyanion with pK_a of carboxyl group around 3.5–3.7 (Haug et al., 1974)	
Carboxymethyl cellulose (CMC)	anionic	–COOH	Weak polyanion with pK_a of carboxyl group around 3.6–4.5 (Dogsa et al., 2014)	

For instance, glucose oxidase (GOX) and horseradish peroxidase (HRP) combined with polyelectrolytes were deposited on polystyrene (PS) particles for sequential enzymatic catalysis using the LbL self-assembly method. GOX with negative charge at pH 6.0–8.2 was deposited consecutively with the polycations PEI or PAH, and HRP was compiled with anionic PSS at pH values of 5.2–6.8 (making HRP positively charged) as the intermediate layer. This study showed that high surface area enzyme multilayer-coated particles could be successfully employed without compromising the activity of the enzymes immobilized on the particle surfaces. However, mixing the enzymes with polymers in solution drastically reduced their activity (by up to 70%) due to negative effects of the polyelectrolyte on the enzyme structure (Caruso and Schüler, 2000). Besides formation of microcapsules, the LbL technique may also be used for immobilization and/or *co*-immobilization of enzymes between layers polyelectrolytes (Sigurdardóttir et al., 2018). This approach was used to immobilize urease (URE) and trypsin (TRY) on the surface of a sulfonated polysulfone (PSSf) ultrafiltration membrane in order to prepare multifunctional biocatalytic membranes with self-cleaning and catalytic ability. URE and TRY were sequentially immobilized via the LbL assembly method using PEI and alginic acid (LAA) as cationic and anionic polyelectrolytes, respectively. Higher activity was reported when the enzymes directly contacted the feed solution, whereas higher stability was observed when the enzymes were in the sandwich layer due their protection against negative effect of harsh reaction conditions (Yurekli, 2020).

4.2. Controlling the properties of polyelectrolyte multilayer capsules

Several parameters affect the properties of polyelectrolyte multilayer capsules and their applicability in biomedicine and biotechnology. Permeability may be tuned, notably, by the type of polyelectrolytes used and number of layers assembled, as well as by adjusting other parameters during assembly, including pH, ionic strength, and the concentration of materials employed to form the multilayer capsules (Ji et al., 2020; Sigurdardóttir et al., 2020). The transfer of molecules through the capsule shell is based on diffusion driven by the concentration gradient between the capsule interior and the external medium, and follows the Fickian diffusion mechanism (Larrañaga et al., 2017). The easiest and most efficient strategy for tuning the permeability of enzymes through the membrane in microreactors is to change the capsule shell thickness by increasing or decreasing the number of deposited polyelectrolytes. For instance, increasing the number of bilayers from one to three significantly improved the retention of enzymes within the polymer capsules, as demonstrated by De Temmerman et al. (2011). The increased retention was particularly pronounced for high molecular weight enzymes, such as immunoglobulin G, where the encapsulation efficiency in dextran sulfate/poly-L-arginine-based microcapsules was 58% for one layer, and over 70% for three layers of polyelectrolytes. Moreover, adjusting the acidity of the surrounding solution enhances the control of polyelectrolyte charges. When the pH is altered relative to the pK_a of the polyelectrolytes, protonation/deprotonation of charged groups takes place, which leads to stronger repulsion with subsequent swelling and increased permeability of the capsules. On the other hand, deprotonation decreases the interactions of polymers, causes shrinking, and thus lowers the permeability (Delcea et al., 2011).

The ionic strength of the polyelectrolyte deposition solution is an additional factor affecting the permeability of the multilayer capsules (Gao et al., 2003). Salt ions present in the polyelectrolyte solution partially screen the charges of the polyelectrolyte - a balance controlled by the concentrations of the respective compounds - thus affecting the permeability of capsules by changing the electrostatic attractions inside the multilayers or introducing defects or cavities in the multilayer network. Weak electrostatic attractions result in a more flexible structure or in pore formation (Cuomo et al., 2015). Use of salts can avoid the dissolution of the polyelectrolyte layers when operating at high values of pH as the ionized hydrogen bonds can stay closer to each other. Moreover, comprehensive studies on a wide range of salt concentration (from 5 to 500 mM) in solution show that ionic strength affects the amplitude of electrostatic forces as well as strongly affect capsule size and wall thickness (Gao et al., 2015).

The stability of polyelectrolyte multilayer capsules is another important factor determining applicability of the LbL microcapsules. Stability strongly depends on the pH of the medium. It has been reported, that capsules composed of weak polyelectrolytes (pK_a in the range of 2–10), such like PAH, PAA and PMA, have low stability. In this case, the charge density decreases at pH values close to the pK_a, making them less charged and consequently, the stability and mechanical resistance of multilayers decreases due to weaker interactions between the layers (Sharma and Sundaramurthy, 2020). However, higher stability can be obtained using PAA/PVA, PAA/PMA and PLL and their combination in the acidic pH range. A study on microcapsules composed of PAH and PMA revealed that the microcapsule shell is pH-responsive in both acidic and basic solutions and could be stable over a wide range of pH (2.5–11.5) while swelling between pH 2.6–2.7 (Sharma and Sundaramurthy, 2020). Further, electrostatic stabilization and hydrogen bonding between uncharged carboxylic acids, amino functions and hydroxyl groups could also contribute to enhancing the stability of capsules (Cuomo et al., 2015; Liu et al., 2017; Mauser et al., 2006). Thus, the design of polyelectrolyte multilayer capsules, demands consideration of all of the abovementioned parameters to develop stable capsules for efficient encapsulation of biomolecules and other compounds.

4.3. Stimuli-responsive polymers

The incorporation of artificial organelles into living cells was a breakthrough with regard to controlling enzymatic reactions. Although several researchers have attempted to encapsulate enzymes within carrier vehicles, only a few have reported active artificial organelles. With regard to the latter, stimuli-responsive block *co*-polymers are used to mimic living cells which are sensitive to change in their environments. Stimuli-responsive block co-polymers are smart polymersomes that disassemble into their constituent block co-polymers by responding to changes in the environment. Variation in pH influences the interactions of the polyelectrolytes. On the other hand, PAH/PSS has been used to build multilayer capsules, where PAH changes with pH. The PAH/PSS capsules opened at pH < 6 and released the encapsulated molecules. Liu et al. (2017) developed pH-sensitive functionalized polymersomes (AdaPsome) as artificial organelles and loaded them into biomimetic cells with temperature- and pH-responsive functionalized surfaces. The authors performed enzymatic reactions using GOX as the first enzyme, which converted D-glucose into D-gluconolactone and H_2O_2 as a co-substrate and myoglobin (Myo) as a second enzyme, which catalyzed the reaction between the intermediate substrate H_2O_2 and guaiacol. The result showed that release of intermediates could be controlled by changing the temperature (25–45 °C) at pH adjusted to 6. However, the enzymatic reaction had very low rates at pH 8, which suggested that the artificial organelles were in their closed state at pH 8 and opened at pH 6 to initialize the enzymatic reactions. It should be underlined, that in the developed system, the two enzymes, GOX and Myo, were located in different compartments of the reaction system, therefore efficient diffusion of H_2O_2 was essential. Enzyme positioning in the separate cells was an intentional act, as both biomolecules are sensitive to pH changes and might easily be deactivated at conditions different from their respective optimal conditions. Further, Myo is also vulnerable to high H_2O_2 concentration, which, besides being enzyme co-substrate, might also act as its inhibitor. Therefore, controlled release of reaction intermediates and control of H_2O_2 supply results in high reaction efficiency and enzyme stability. This study and other research on stimuli-responsive compartments have successfully simulated living cell functionality by incorporating stimuli-responsive polymersomes in their systems. Recent work represents encouraging progress towards wider applications of artificial compartments, however further study in this

Table 2
Changes in optimum pH of polyelectrplytes immobilized and free enzymes.

Enzyme	Optimum pH		Carrier	Ref.
	Immobilized	Soluble		
Alcohol dehydrogenase (ADH)	8	7	PAH	Jia et al. (2018)
ATP deaminase	3	5	DEAE-cellulose	Chung et al. (1968)
Nitroreductase	10	6	PLL	Kienle et al. (2020)
Nitroreductase	7	6	PDADMAC	
Nitroreductase	8	6	PAMPS	
α-Chymotrypsin	8	9.5	PASAC	Kurinomaru et al. (2014)
α-Chymotrypsin	8	9	PAA	
Cytochrome C	6	8	PMA	Zhang et al. (2017)
Lysozyme	7	5.5	PAH	Wang et al. (2005)

area is still of the highest importance to meet drawbacks and limitations of current solutions.

5. Enzyme immobilization using polyelectrolyte compartments

Polyelectrolytes are attractive compounds for enzyme immobilization due to their ionic nature and the ease with which the thickness of layers, the loading of enzymes and distribution of multiple enzymes on each layer can be controlled. Consequently, also enzyme activity and stability can be controlled and improved, as compared to the use of classic support materials. Different factors affect enzyme activity in polyelectrolyte microcapsules, such as concentration of enzymes, temperature, pH, and ionic interaction between enzymes and polyelectrolytes. Depositing enzyme and polyelectrolytes of opposite charge has been found to improve enzyme activity by reducing enzyme aggregation on the surface and decreasing substrate transport barriers in the material (Kayitmazer et al., 2013; Kienle et al., 2020). Charged enzymes bind to polyelectrolytes with opposite charges, and such binding simultaneously inhibits electrostatic attraction among enzymes and prevents enzyme aggregation in the bulk medium. Moreover, stable enzyme binding might be achieved by the creation of strong ionic interactions, which simultaneously lead to high activity recovery due to limited disruption of enzyme structure. Recently, it has been observed that aggregation of such enzymes as glyceraldhyde-3-phosphate dehydrogenase, lactate dehydrogenase, and aspartate aminotransferase could be prevented in the presence of both polyanions and polycations (Shalova et al., 2005, 2007). Enzyme loading, its elution, as well as substrates/products transport are significantly affected by variation of the ionic strength of polyelectrolytes (Ji et al., 2020). Furthermore, ionic interactions between the enzyme and oppositely charged polyelectrolytes contribute significantly in stabilizing the enzyme structure. However, the number of ionic interactions depends on the pH of solution due to variations in the enzyme charge and its isoelectric point (IEP). Moreover, enzymes are sensitive to changes in pH and temperature below or above the optimum conditions, which leads to significant decrease of their activity outside the optimal conditions due to destabilization of their structures and denaturation (Sigurdardóttir et al., 2019; Zdarta et al., 2019; Zdarta et al., 2021). Therefore, it is of particular interest to carefully select polyelectrolytes for formulation of compartments not only in terms of their IEP and charge but also by considering their pH and temperature stability in order to obtain biocatalytic systems at high activity retention.

5.1. pH and activity of enzymes

There is often a significant difference in the pH range over which an enzyme is stable when the enzyme is entrapped in a polyelectrolyte compartment compared to its free form in solution (Table 2). Immobilizing the enzyme on polyelectrolytes usually shift its optimal pH. For instance, the optimum pH of various enzymes, including trypsin, chymotrypsin, papain and subtilopeptidase A, was shifted towards more alkaline pH conditions upon their attachment to polyanionic carriers, and moved towards more acidic pH values when they were bound to polycationic carriers, compared to the native enzymes (Zhang et al., 2017). For instance, various proteases immobilized on cyanogen halide activated agarose surprisingly showed a narrower and more alkaline pH active profile than the free enzyme. The optimum pH of proteases was shifted from a neutral towards alkaline pH by 1 to 3 units when these enzymes were immobilized on polyanionic carriers with ionic strength of 0.01 (Jasim et al., 1987). This change could be the result of redistribution of charged particles (H^+ ions) between the polyelectrolyte carrier and the bulk solution (i.e. localized increase in the hydrogen ion concentration at the surface of the polyelectrolytes). Likewise, the optimum pH of chymotrypsin shifted from 8 to 9.5 when it was conjugated with anionic poly(acrylic acid) and form 8 to 9 after binding to cationic poly(allylamine) (PAA). These effects were observed at low ionic strength and disappeared at salt concentrations above 0.5 M (Kurinomaru et al., 2014; Zhang and Hess, 2020).

Using different polyelectrolytes can effectively control the active pH range of enzymes. Negatively charged nitroreductase (NfsB) with an estimated charge of −10 at pH 8 was embedded by depositing alternating layers of the enzyme (as a negative layer) and polycation (as a positive layer) onto porous silica particles. A combination of the weak polycation, poly(L-lysine), the strong polycation, poly(diallyldimethylammonium chloride) (PDADMAC) and the strong polyanion, poly(2- acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS) was employed to study the effect of polyelectrolytes on pH and stability of immobilized enzyme. The stability and optimal pH of enzyme were significantly affected by ionic interactions between NfsB and the polycation. Immobilization with the weak polycation, PLL, increased the optimal pH and the activity of immobilized NfsB more than immobilization with the strong polycation, PDADMAC. Moreover, the activity of NfsB entrapped in polyelectrolytes was enhanced by increasing its charge through changing the pH (in this case, increasing the pH to make the NfsB more negatively charged) compared to the enzyme in solution. However, the absolute activity of immobilized enzyme decreased after deposition within polyelectrolyte layers, as compared to free biocatalysts, indicating the necessity to precisely control the charge and strength of the polyelectrolytes in order to achieve the highest possible activity retention (Kienle et al., 2020). Another study on a reaction that was catalyzed by immobilized cytochrome C (Cyt C) and D-amino acid oxidase (DAAO) showed that coupling negatively charged poly(methacrylic acid) with Cyt C could effectively shift the pH at which Cyt C was active towards neutral and alkaline conditions. This effect enabled both enzymes to work ten times more efficiently at pH 8 than the mixture of free enzymes in solution where Cyt C is more active at acidic pH, while DAAO is more active at alkaline pH (Zhang et al., 2017). To mimic a complex natural system, silica nanoreactors accommodating two artificial enzymes (gold nanoparticles and hemin) were coated by polyelectrolyte multilayers to serve as a permeable membrane to control the flow of substrates and products. It was found that at physiological pH, when hemin was deposited onto the polyelectrolyte membrane, it showed remarkable peroxidase-like activity and could easily be recycled - up to several times. It was found, that depending on the pH and enzyme positioning, novel nanoreactors might be constructed to achieve compartmentalization of nanozymes and applying artificial cascade catalytic systems (Huang et al., 2016).

5.2. Optimum temperature of enzymes in compartments

Similarly to the effect of temperature on chemical reaction rates, increase in temperature can also lead to an increase in enzyme reaction rates. However, enzymes are highly sensitive to temperature, and high temperature may cause activity loss and denaturation of enzymes.

Maintaining the enzyme environment at the optimum temperature is therefore essential. A proper immobilization protocol usually results in significant improvement of thermal resistance and stability of the immobilized biocatalyst as compared to free enzymes (Mateo et al., 2007; Garcia-Galan et al., 2011; Rodrigues et al., 2013). The use of polyelectrolyte compartments gives an interesting opportunity to construct systems with temperature conditions locally matched to the individual enzyme that results simultaneously in high reaction efficiency and high enzyme stability. Additionally, temperature increases in the vicinity of the enzymes can enhance and control the activity of enzymes. In this context, new strategies for locally increasing the temperature of the enzyme microenvironment were developed. *Aeropyrum pernix* glucokinase was embedded on gold nanorods (AuNR, 33 nm × 10.4 nm) through the thiol–gold interaction and then encapsulated the obtained enzyme-AuNR complexes in Ca-alginate beads. Then they introduced irradiation with 11 W cm^{-2} of 800 nm near-infrared light. The temperature inside the beads increased by 20 °C, while the bulk solution temperature increased only by 2 °C after irradiation. As a result, the activity of the enzyme complex increased by 60% under continuous irradiation (Blankschien et al., 2013). Platinum nanoparticles are also used to control the temperature on encapsulated enzymes. The enzymes (including glucoamylase, glucose oxidase, catalase and proteinase K) fixed with Pt-NPs demonstrated a two-fold activity enhancement after exposure to pulsed near-infrared irradiation of 0.2 W cm^{-2} for 10 min while the bulk temperature in the solutions remained stable (Wang et al., 2017a, 2017b).

5.3. Effect of ionic strength on enzyme loading

In multilayer deposition of enzymes and polyelectrolytes, the balance between intramolecular electrostatic repulsion within polyelectrolytes and the electrostatic attraction between polyelectrolytes and enzymes drives the formation of the multilayer structure of immobilized enzyme-polyelectrolytes capsules affecting also enzyme stability and its activity. Polyelectrolytes generally have a homogeneous charge distribution and adaptable conformation, while proteins (enzymes) have heterogeneous spatial charge distribution (van der Straeten et al., 2018). Higher enzyme density will be found when the enzyme charge increases (Ji et al., 2020). Thus, a high enzyme amount will be immobilized under conditions where an appropriate polyelectrolyte coexists with sufficiently attractive polyelectrolyte-enzyme electrostatic interactions. However, under conditions where there is a lack of charge overcompensation of the enzyme layer, or where there is repulsion between identical charges of the enzyme and the polyelectrolytes, multilayer growth becomes impossible (van der Straeten et al., 2018). Studies on the influence of the salt composition of a medium on the functional properties of enzymes in contact with polyelectrolytes can help to control the reaction processes in compartments. The chemical attraction of enzymes to the polyelectrolytes depends on the existence of mono and divalent ions in solution. Divalent counterions in solution cause neutralization of more polyelectrolyte chains and reduce the electrostatic repulsion within the polyelectrolytes. Besides, the ionic strength of the enzyme and polyelectrolyte solutions might also affect hydrophobic interactions between the enzyme and polyelectrolytes, since there are still many hydrophobic sites on the polyelectrolytes due to the structure of the main carbon chain as well as the presence of benzene rings in some polyelectrolytes. In general, at a higher salt concentration (more charge screening), more hydrophobic interactions are formed that affect both the enzyme activity and its elution after immobilization. A study of the multilayer deposition of oppositely charged polyelectrolytes (PSS, PAH) in the presence of Na^{+} and Mg^{2+} with the same ionic strength showed that the amount of polyelectrolyte deposited on the core template was higher in the presence of divalent salt than in the presence of monovalent salt, hence the former resulted in the formation of a thicker membrane film (Elżbieciak-Wodka et al., 2017). In addition, mono and divalent cations affect not only the affinity of enzymes to polyelectrolytes but also their catalytic activity (Tikhonenko et al., 2009). A study of the effects of inorganic mono and divalent salts on the interactions between a cationic polyelectrolyte, PAH, and the oligomeric enzyme, urease, showed that high salt concentrations contributed to decrease electrostatic interactions, in agreement with the correlation between Debye radius and the solution ionic strength. Furthermore, in Na_2SO_4 and $(NH_4)_2SO_4$ solutions, the enzyme was considerably activated at low salt concentrations ranging from 0.6 to 0.8 mM (Dressick et al., 2012). The electron transfer behavior between a polyelectrolyte (polyallylamine derivatized with an osmium complex) and a redox enzyme glucose oxidase (GOX) was also studied by forming compartments in the presence of phosphate ions at various ionic strengths. In the presence of phosphate, the polyelectrolyte adopted a globular structure in solution, which led to better deposition behavior and showed an improved electron transfer through the film. Consequently better electrochemical performance of the produced system was achieved. Further, the results revealed a remarkable change in the amount of enzyme adsorbed in the presence of phosphate in solution. The rise in the adsorbed GOX could be explained by the fact that low ionic strength caused more electrostatic interactions between negatively charged GOX and positively charged polyallylamine osmium complex at the surface of the polyelectrolyte film. The interactions between polyelectrolyte and enzyme improved through phosphate interactions with the basic amino acid residues of GOX (Zappi et al., 2019).

The interaction protein-polyelectrolyte is also affected by the electrolyte concentration as shown for systems as diverse as lysozyme/chondroitin sulfate, lysozyme/hyaluronic acid, beta-lactoglobulin (BLG)/sodium polystyrene sulfonate, and bovine serum albumin (BSA)/polyacid. Interestingly, all systems display maximum complex formation in the range of ionic strength $5 < I < 30$ mM (Hattori et al., 2000; Moss et al., 1997; Seyrek et al., 2003). It should also be added that enzyme loading, and thus the activity of the produced system, would also be affected by the molecular weight of the polyelectrolyte used. For instance, different laccase loading and activity was observed when the enzymes was immobilized on membranes that had been modified with polyethyleneimine with different molecular weights (Andreeva et al., 2018).

6. Alternative approaches for enzyme compartmentalization

6.1. DNA supported enzyme immobilization and compartmentalization

Recently, more and more attention has been directed towards DNA-based nanotechnology for enzyme immobilization and compartmentalization of multienzymatic structures. The rapid development of DNA nanotechnology for use in cascade reactions is driven by the possibility of designing DNA structures with desired functions and properties (Zhang and Seelig, 2011). Enzyme binding using DNA can occur via adsorption, covalent bonding, complementary base-pairing, or via DNA-directed immobilization, where both the biomolecules and support are coupled with complementary DNA moieties, which then allows immobilization through DNA conjugation. Further DNA techniques are based on the formation of well-designed DNA scaffolds for biomolecules deposition, low-molecular-mass DNA structures for efficient enzyme binding (Rashid and Yusof, 2017), and enzyme self-assembly in the presence of DNA structures (Wang et al., 2017a, 2017b). Irrespectively of the DNA-based approach used, the greatest advantage of this technique is that it offers site-specific enzyme co-localization and orientation due to the flexibility of the DNA structures In multi-enzyme systems, the enzymes are close enough to each other to synergistically catalyze the sequential reactions and the close proximity of the biomolecules facilitates substrate channeling (Spivey and Ovadi, 1999; Zhang, 2011). Stability issues concerning the effect of process conditions, storage time and reuse of the enzymes were found to be significantly improved upon immobilization using DNA.

Developing novel methods for the production of cell-free

multienzyme biocatalytic systems is a milestone of synthetic biology. A three-enzyme sequential cascade consisting of amylase, maltase, and glucokinase was obtained by site-specifically immobilizing the enzymes using DNA on a self-assembled DNA origami triangle. The catalytic efficiency of the cascade reaction was significantly improved due to substrate channeling and increased enzyme stability, as well as a co-localized enzyme deposition through the DNA surface affinity. This approach could potentially allow for custom synthesis of complex multienzymatic systems that cannot be performed by conventional organic chemistry approaches (Klein et al., 2019). Controlling the enzyme ratio is a crucial factor for improving enzymatic activity in multienzymatic cascade reactions. Magnetic nanoparticles functionalized with different dopamine derivatives were used as support for the co-immobilization of HRP and GOX through DNA-directed immobilization. The DNA-directed immobilization enabled to precisely control the enzyme ratio by adjusting the quantity of the different functional groups on the support surface. GOX/HRP ration of 2:1 was found to be the optimal ratio at which the specificity constant and catalytic rate constant of both enzymes were doubled, as compared to free enzymes, indicating improvement of enzyme efficiency upon DNA-directed immobilization. The increase was explained by the co-localization of the involved enzymes and promotion of substrate channeling (Yang et al., 2017). Recently, it was reported that the stability and activity of immobilized multienzyme systems suffered from non-specific enzyme adsorption on the surface of carriers due to hydrophobic and/or electrostatic interactions. To overcome these limitations, zwitterion-functionalized magnetic particles with amino, phosphonate, and thiol functional groups were produced for the co-immobilization of GOX and HRP by DNA-directed immobilization. The zwitterionic surface of the magnetic particles suppressed the non-specific adsorption of enzymes, leading to excellent enzymatic activity, stability, and reusability of the co-localized enzymes (Song et al., 2018). An alternative approach was based on cross-linking HRP and GOX using a rationally designed DNA scaffold network, followed by encapsulation of the assembled multi-enzymatic system into zeolite imidazolate framework-8 (ZIF-8). The well-tailored proximity and co-localization of the GOX and HRP on the DNA scaffold network significantly improved the kinetic parameters of the enzymes and their activity, leading to an increase in the overall catalytic efficiency. DNA-based multienzymatic systems can efficiently suppress enzyme leaching from the support resulting in improved enzyme storage stability and reusability (Song et al., 2019).

6.2. Metal organic frameworks (MOFs)

Metal organic frameworks (MOFs) are classified as porous hybrid inorganic-organic materials with structure and architecture similar to zeolites, but with significantly higher flexibility. The 3D structures of these materials, which are assembled in various spatial conformations, are formed by coordinated bonds between metal ions and multidentate ligands/linkers, usually of organic origin (Furukawa et al., 2013). A number of metal ions have been used recently, including transition metals, p-block elements, alkaline earth metals, and actinides, the selection of which strongly affects the stability of the produced MOF and its applications. Additionally, the coordination number of the metal ion determines the shape and geometry of the resulting MOF architecture (Dey et al., 2014). On the other hand, carboxylates, particularly anionic carboxylates with high metal ion affinity, as well as amines, nitrates, phosphates, and sulfonates are frequently used organic ligands. These compounds vary in shape and structure, which both influence the architecture and properties of the produced framework (Burnett et al., 2012). Although many various categories of MOFs have been exploited, typical MOFs are characterized by tailorable properties, ultrahigh porosity and large surface area, high thermal, chemical and mechanical stability as well the presence of numerous diverse functional groups in their structure. The above-mentioned features, along with tunable porosity and open structure, raised attention to apply MOFs as suitable matrices for enzyme assembly and immobilization. MOFs lead to improvement of reaction efficiency, e.g. by allowing high enzyme loading and proper enzyme orientation within the matrix, enhanced accessibility to the enzymes' active site, and by supporting substrate channeling between immobilized enzymes (Mehta et al., 2016). Recently, a series of Zr-based MOFs with interconnected hierarchical mesoporous channels at various diameters were produced and used for the immobilization of lactate dehydrogenase for cofactor regeneration. The obtained support offered water stability, high enzyme-loading capacity and reduced diffusional limitations in transport of substrate and product due its open and interconnected structure. It was demonstrated that enzymes immobilized in the large pores of the MOF were accessible to nicotinamide adenine dinucleotide (coenzymes) allowing for in situ cofactor regeneration. Surprisingly, due to the controlled morphology and effective substrate channeling inside the MOF, the MOF captured enzymes (the cell-free biosynthetic catalytic cascade approach) showed higher activity than their free counterparts (Li et al., 2018). In another study, HKUST-1 MOF was enriched by magnetic nanoparticles and used as a scaffold for spatial co-immobilization of GOX and HRP. Due to the structural properties of the MOF network, spatial co-immobilization and positional assembly of both enzymes was achieved by positioning the GOX via an LbL strategy between MOF layer, as horseradish peroxidase was deposited on the HKUST-1 outer shell. The proper enzyme location resulted in superior kinetics of the co-immobilized GOX, as the Michaelis-Menten kinetic parameters were improved compared to the free enzyme. Further, the produced conjugates exhibited remarkable-y operational stability (Chen et al., 2017). These examples clearly show that by using MOFs, a versatile approach to spatially organized multi-enzyme systems with well-defined nanostructures and improved overall catalytic efficiency may be obtained. To facilitate proper enzyme orientation, amino-acid-boosted one-pot embedding was used to co-immobilize HRP and GOX into the frame of Zn-based microporous MOFs with an ultrahigh loading efficiency. Due to the formation of prenucleation clusters around the biomolecules via a metallothionein-like self-assembly, the enzymes are properly oriented and maintain their native conformation. Encapsulation within porous MOF structures results in highly active biocatalytic systems with improved stability at harsh reaction conditions and over storage time. It was thus demonstrated that noninvasive, protein surface charge-independent co-encapsulation within MOF frameworks should be considered as a versatile strategy for fabrication of multienzymatic systems for application in biomedicine, catalysis, and biosensing (Chen et al., 2019). MOFs might also be a base for the formation and assembly of artificial enzymes. To mimic compartmentalization and substrate-channeling of natural enzymes, a MOF-based nanoenzyme was obtained by encapsulating a natural GOX enzyme within a copper 1,4-benzenedicarboxylate (CuBDC) MOF via a one-step biomimetic mineralization process at room temperature. Due to a convenient orientation of the peroxidase-like active sites in CuBDC, the compartmentalization and substrate-channeling were achieved in the integrated nanozyme resulting in high reaction efficiency - the integrated nanozyme displayed a 12.5-fold enhancement of activity for glucose detection as compared with the cascade reaction system using free enzymes. Further, the biosensor exhibited good linearity, low detection limit and improved stability, reusability, and selectivity, as compared to the free system (Cheng et al., 2020).

6.3. Inorganic materials

Inorganic based compounds, both of single and hybrid origin, are attractive materials for enzyme loading and compartmentalization, mainly due to the high stability of inorganic materials at harsh process conditions, high mechanical resistance and inertness to most classic solvents. These features also provide improved stability of the deposited enzyme and enhance its reusability due to biomolecules protection against inactivation by process conditions and stabilization of entire

enzyme structure (Jesionowski et al., 2014). However, various factors might affect enzyme activity and process efficiency in inorganic structures, including enzyme concentration and orientation, enzyme interactions with the inorganic materials, and the permeability of the external shell of the structure and porous structure of the material Various types of inorganic architectures can be used for enzyme assemblies, such as core-shell structures, micro- and mesoporous materials, as well as capsules and hollow spheres (Sirisha et al., 2016). Similarly, various configurations of enzyme deposition may be applied. For instance, a whole multienzymatic system might be placed inside the support to facilitate substrate transport. On the other hand, a part of the enzymes might be placed inside or on the external side of the surrounding membrane. Further, in some instances, biomolecules might act as a free enzyme in the environment around the capsule in order to provide suitable environment for all of the enzymes involved in the cascade (Jiang et al., 2009). For instance, a bio-inspired strategy called bioadhesion-assisted bio-inspired mineralization was applied to construct multienzyme systems based on titania nanoparticles. In this case, formate dehydrogenase was entrapped inside the titania particles during their formation followed by in situ surface functionalization with DOPA to covalently attached formaldehyde dehydrogenase onto the surface of the particles and provide process conditions suitable for both enzymes. The as-constructed spatially separated multienzyme system showed significantly enhanced formaldehyde yield, selectivity and initial specific activity of both enzymes (Shi et al., 2012). In another example, an enzymatically coupled sequential reduction of carbon dioxide to methanol was catalyzed by three different dehydrogenases, formate dehydrogenase (FateDH), formaldehyde dehydrogenase (FaldDH) and alcohol dehydrogenase (ADH), assembled together by encapsulation within sol-gel silica spheres. It was found that the silica shell provided protection for the deposited enzymes. Moreover, the confinement of the multienzymatic system in the nanopores of the silica sol-gel affected the reaction thermodynamics and shifted the process equilibrium towards the products, resulting in up to 90% enhanced methanol productivity. The high nanoporosity of the support limited the pore volume, leading to locally increased concentrations of enzymes and reactants. As a consequence, the substrate supply to the enzyme was well ensured, the overall single enzyme reaction efficiency was improved, so that the final equilibrium was shifted towards final product (Obert and Dave, 1999). Another approach consists of co-encapsulation of superoxide dismutase and catalase into hollow silica nanospheres for the creation of cascade for cell-implanted nanoreactors enzyme therapeutics. Silica sol–gel templating of water-in-oil microemulsions supported by polyethyleneimine modification was used to produce spheres with co-immobilized enzymes. It was found that for high reaction efficiency, detailed quantitative control of enzyme loading was required in order to avoid enzyme inhibition by substrate and products of the reaction. Excellent activities of superoxide dismutase was proved, that facilitated the cascade transformation of superoxide through hydrogen peroxide to water. Nevertheless, the main idea of this study was to prepare stable and reusable multienzymatic systems for acting inside the living cells (Chang et al., 2014). Besides natural enzymes, also artificial enzymes including nanoenzyme, might be used to create cascade systems in natural-artificial hybrid structures based on hollow silica microspheres in order to mimic mitochondria oxidative phosphorylation. In a recent study, gold nanoparticles containing microspheres were produced to show dual enzyme activity and act as GOX-like nanoenzyme and peroxidase-like nanoenzyme to transform glucose into gluconic acid in the presence of oxygen. These spheres were then then coated by proteoliposome membrane containing ATP synthase to promote the synthesis of ATP. Due to the action of artificial enzymes placed inside inorganic capsules, the proton gradient increases and H^+ ions drives the membrane-placed ATP synthase to convert ADP and inorganic phosphate (both presented in the environment outside the sphere) into ATP. It was proved that due to a proper enzyme assembly, the proposed system possessed high activity for oxidative phosphorylation, which was comparable to that of natural mitochondria (Xu et al., 2019). In another study, biomimetic cascade enzyme-initiated toxic-radical-generating device was produced by incorporating of glucose oxidase, sesquiterpene lactone endoperoxide derived dihydroartemisinin and artificial nanozyme hemin, which might act as a peroxidase-like enzyme, and glutathione peroxidase-like enzyme into zeolitic imidazolate framework for enhanced biocatalytic immunotherapy. The zeolitic network provided-a proper matrix for enzyme assembly, which resulted in effective cascade reactions in artificial nanoscale proximity. The presented solution, after further optimization, might be applied in hindering the growth of distant tumors and lung metastasis (Zhao et al., 2021).

7. Conclusions, future remarks and research trends

Many industrial processes involve sequential enzymatic reactions. The development of new multi-enzyme complex structures that can improve enzyme activity, stability and efficiency would be a breakthrough for industrial productivity. A next step towards enhancing the efficiency of microreactors would be designing a structure that is adaptive, is dynamic in nature and is able to interact with the environment. A fundamental understanding of multi-enzymatic reactions in living cells thus helps us reveal the operating rules of this complicated process and offers valuable guidance for the design of microreactors providing efficient sequential enzymatic reactions. In the multi-enzymatic cascades, to achieve high process efficiency, it is essential to reduce limitations in substrate transport and enzyme inhibition. In this term, in the future work, of particular interest should be precise assembly techniques including self-assembly, scaffold-assembly and scaffold free-assembly, based on proper enzyme orientation and/or localization. For complex multienzymatic structures catalyzing cascade reactions, enzyme assembly and orientation are crucial to achieve a desired biomolecule conformation, exposure of its active site, intermediates transport and structure stabilization (Sweetlove and Fernie, 2018. Recently, scaffolds-based techniques, based mainly on proteins, lipids, nucleic acids and polysaccharides are explored (Myhrvold et al., 2016). Nowadays, however, more and more attention, due to their biocompatibility and regenerability, arouse bioscaffolds, such us bacteria, fungi, viruses and biofilm microorganisms (Nguyen et al., 2014). By contrast, scaffold-free techniques base on bio/chemical mediated affinity by use of affinity tags (such as streptavidin-biotin), protein-peptide pairs, nucleic acids pairs, etc. offer flexibility to enzyme structure (Dong et al., 2021). Application of enzyme assembly strategies gain the molecules proper spatial orientation, with reduced diffusional limitations and active site capable for substrate binding. Further, biomolecules are localized in the suitable microenvironment. Consequently, by proper enzyme orientation, substrate channeling is also reinforced, resulting in lower substrate transport limitations. Therefore, proper enzyme placement in the nature-mimicking structure is crucial for enhancement of process efficiency. Despite the difficulties in mimicking living cells, current developments in creating stimuli-responsive compartments lead to the architecture needed for a self-regulated polymeric compartment and enhance the efficiency of multi-enzymatic reactions. Based on the presented and discussed literature examples it is clear that high enzyme activity and in consequence also high efficiencies of single and multienzymatic reactions might be achieved. However, it should be clearly stated that so far the described approaches for fabrication of these systems is actually limited to the laboratory and small scale solutions. Pilot scale and further industrial application will only be possible when the preparation of these complex structures will be scalable and economically reasonable. Therefore, there are numerous research gaps that need to be filled to facilitate further development of a one-pot single and multi-enzymatic reactors for conducting sequential enzyme reactions. For that reason, several parameters need to be considered in order to create an appropriate micro-environment for enhancing enzyme stability and its reusability against negative external factors. Solution condition and nature of

Table 3
The summary of the advantages and disadvantages of the artificial cells architectures and polyelectrolyte LbL microcapsules for enzyme compartmentalization.

	Advantages	Disadvantages
Artificial cells architectures (liposomes, polymersomes and capsosomes	• facilitation of the control of enzymatic cascade reactions within a cell • ease of preparation via self-assembly, and to assemble the protective layer for the fragile or small cargo inside • prone to outer surface and the inner side functionalization • easy control of the permeability and wall thickness of multi-shell capsules at the stage of their production • enhancement of substrate channeling by oriented immobilization • possibility to entrap various biological molecules due to the presence of multi-hydrophobic and hydro-philic domains	• insufficient study on toxicity and safety • necessity for more study on of development and optimization of easy, fast, safe and inexpensive methods of production with the ability to direct scale-up • need for evaluation and mimic the biological parameters such as shape, softness, and surface functionality • requirements for detail determination o effect of process parameters on stability of artificial cells and activity of immobilize biomolecules
Polyelectrolyte LbL microcapsules	• prone to functionalization with various molecules to create multifunctional materials with hybrid properties. • very versatile platforms for encapsulation, storage and delivery systems • selective permeability facilitates loading of different types of molecules • relatively low cost, and ease of preparation • stimulated release of molecules by physiological stimuli or physical stimuli • entrapment and localization of the enzymes for cascade reactions in confined volumes • improvement and controlled enzyme stability and reusability as well as facilitation of their accessibility to the substrates • flexibility in terms of materials' selection, and possibility of creation of architectures at various shape	• limited large-scale application • low dispersion of the microcapsules and tendency to aggregation • insufficient study on toxicity of the polyelectrolytes microcapsules • requirements for optimization study of capsules fabrication • risk of damage of microcapsule integrity during internal core removal • diffusional limitations in transport of substrate and products inside microcapsules and vice versa • sensitivity of the membrane to variations of pH, temperature, ionic strength, etc.

materials must be taken into account to achieve a sustainable growth of the multilayer with specific properties. Apart from the already highly explored materials for microcapsule construction, polyelectrolytes and specifically weak polyelectrolytes, which are more flexible and controllable, are especially interesting. There have been many studies and achievements on this topic, but issues of the long term stability and activity of enzymes remain to be solved. Studying the influence of parameters on the functional properties of enzymes in contact with polyelectrolytes can therefore help discovering ways to control reaction processes in compartments and polyelectrolyte multilayer membranes. Moreover, further study is needed of new adoptive polymeric materials that dynamically change their size, shape and surface charge for more efficient transfer of substrates and/or products through the microcapsules shells and improved reaction rate and efficiency. It is worth noting that most of the multi-compartments are in the form of microcapsules. However, through using a platter template rather than a spherical core and employing LbL assembly, it should be able to create a multilayer membrane for biocatalytic sequential reactions that can be employed in several industrial applications. Finally, many of the presented systems involve lengthy layer-by-layer assembly, which can be a significant cost factor, thus further studies are also required in order to reduce time and costs of these processes, as these factors are crucial for a larger scale applications. Nowadays, layer by layer assembly and enzyme compartmentalization seems to be suitable for a low-volume applications, such as biosensing, however, we strongly believe that future studies will lead to the application of the discussed solutions for preparative single and multienzymatic biotransformations. A brief summary of the most important advantages and disadvantages of the artificial cells architectures and polyelectrolyte LbL microcapsules for enzyme compartmentalization is provided on Table 3.

Conflict of interest statement

The authors declare no conflict of interest.

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References

Abernathy, M.H., He, L., Tang, Y.J., 2017. Channeling in native microbial pathways: implications and challenges for metabolic engineering. Biotechnol. Adv. 35, 805–814.
Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. Molecular Biology of the Cell, fourth ed. Garland Science, New York.
Andreeva, M.A., Gil, V.V., Pismenskaya, N.D., Dammaka, L., Kononenko, M.A., Larchet, C., Grande, D., Nikonenko, V.V., 2018. Mitigation of membrane scaling in electrodialysis by electroconvection enhancement, pH adjustment and pulsed electric field application. J. Membr. Sci. 549, 129–140.
Ariga, K., Hill, J.P., Ji, Q., 2007. Layer-by-layer assembly as a versatile bottom-up nanofabrication technique for exploratory research and realistic application. Phys. Chem. Chem. Phys. 9, 2319–2340.
Axthelm, F., Casse, O., Koppenol, W.H., Nauser, T., Meier, W., Palivan, C.G., 2008. Antioxidant nanoreactor based on superoxide dismutase encapsulated in superoxide-permeable vesicles. J. Phys. Chem. B 112, 8211–8217.
Barbosa, O., Torres, R., Ortiz, C., Berenguer-Murcia, A., Rodrigues, R.C., Fernandez-Lafuente, R., 2013. Heterofunctional supports in enzyme immobilization: from traditional immobilization protocols to opportunities in tuning enzyme properties. Biomacromolecules 14, 2433–2462.
Barbosa, O., Ortiz, C., Berenguer-Murcia, Á., Torres, R., Rodrigues, R.C., Fernandez-Lafuente, R., 2015. Strategies for the one-step immobilization-purification of enzymes as industrial biocatalysts. Biotechnol. Adv. 33, 435–456.
Bédard, M.F., Braun, D., Sukhorukov, G.B., Skirtach, A.G., 2008. Toward self-assembly of nanoparticles on polymeric microshells: near-IR release and permeability. ACS Nano 2, 1807–1816.
Blankschien, M.D., Pretzer, L.A., Huschka, R., Halas, N.J., Gonzalez, R., Wong, M.S., 2013. Light-triggered biocatalysis using thermophilic enzyme–gold nanoparticle complexes. ACS Nano 7, 654–663.
Brezovsky, J., Chovancova, E., Gora, A., Pavelka, A., Biedermannova, L., Damborsky, J., 2013. Software tools for identification, visualization and analysis of protein tunnels and channels. Biotechnol. Adv. 31, 38–49.
Brezovsky, J., Kozlikova, B., Damborsky, J., 2018. Computational analysis of protein tunnels and channels. In: Bornscheuer, U.T., Hohne, M. (Eds.), Protein Engineering Methods and Protocols. Springer Nature, New York, pp. 25–42.
Burke, S.E., Barrett, C.J., 2003. pH-responsive properties of multilayered poly(L-lysine)/hyaluronic acid surfaces. Biomacromolecules 4, 1773–1783.
Burnett, B.J., Barron, P.M., Choe, W., 2012. Recent advances in porphyrinic metal–organic frameworks: materials design, synthetic strategies, and emerging applications. Cryst. Eng. Commun. 14, 3839–3846.
Caruso, F., Möhwald, H., 1999. Protein multilayer formation on colloids through a stepwise self-assembly technique. J. Am. Chem. Soc. 121, 6039–6046.

Caruso, F., Schüler, C., 2000. Enzyme multilayers on colloid particles: assembly, stability, and enzymatic activity. Langmuir 16, 9595–9603.
Chang, H., Yeh, M.-K., 2012. Clinical development of liposome-based drugs: formulation, characterization, and therapeutic. Int. J. Nanomedicine 7, 49–60.
Chang, F.P., Chen, Y.P., Mou, C.Y., 2014. Intracellular implantation of enzymes in hollow silica nanospheres for protein therapy: cascade system of superoxide dismutase and catalase. Small 10, 4785–4795.
Chen, A.H., Silver, P.A., 2012. Designing biological compartmentalization. Trends Cell Biol. 22, 662–670.
Chen, S., Wen, L., Svec, F., Tana, T., Lv, Y., 2017. Magnetic metal–organic frameworks as scaffolds for spatial co-location and positional assembly of multi-enzyme systems enabling enhanced cascade biocatalysis. RSC Adv. 7, 21205–21213.
Chen, G., Huang, S., Kou, X., Wei, S., Huang, S., Jiang, S., Shen, J., Zhum, F., Ouyang, G., 2019. A convenient and versatile amino-acid-boosted biomimetic strategy for the nondestructive encapsulation of biomacromolecules within metal–organic frameworks. Angew. Chem. Int. Ed. 58, 1463–1467.
Cheng, X., Zheng, Z., Zhou, X., Kuang, Q., 2020. Metal–organic framework as a compartmentalized integrated nanozyme reactor to enable high-performance cascade reactions for glucose detection. ACS Sustain. Chem. Eng. 8, 17783–17790.
Chung, S.T., Hamano, M., Aida, K., Uemura, T., 1968. Studies on ATP deaminase. Agric. Biol. Chem. 32, 1287–1291.
Conrado, R.J., Varner, J.D., DeLisa, M.P., 2008. Engineering the spatial organization of metabolic enzymes: mimicking nature's synergy. Curr. Opin. Biotechnol. 19, 492–499.
Cuomo, F., Lopez, F., Piludu, M., Miguel, M.G., Lindman, B., Ceglie, A., 2015. Release of small hydrophilic molecules from polyelectrolyte capsules: effect of the wall thickness. J. Colloid Interface Sci. 447, 211–216.
Curtis, K.A., Miller, D., Millard, P., Basu, S., Horkay, F., Chandran, P., 2016. Unusual salt and pH induced changes in polyethylenimine solutions. PLoS One 11, 0158147.
de Lorenzo, V., Sekowska, A., Danchin, A., 2015. Chemical reactivity drives spatiotemporal organisation of bacterial metabolism. FEMS Microbiol. Rev. 39, 96–119.
De Temmerman, M.L., Demeester, J., De Vos, F., De Smedt, S.C., 2011. Encapsulation performance of layer-by-layer microcapsules for proteins. Biomacromolecules 12, 1283–1289.
Delcea, M., Möhwald, H., Skirtach, A.G., 2011. Stimuli-responsive LbL capsules and nanoshells for drug delivery. Adv. Drug Deliv. Rev. 63, 730–747.
Deng, N.-N., Yelleswarapu, M., Zheng, L., Huck, W.T.S., 2017. Microfluidic assembly of monodisperse vesosomes as artificial cell models. J. Am. Chem. Soc. 139, 587–590.
Dey, C., Kundu, T., Biswal, B.P., Mallick, A., Banerjee, R., 2014. Crystalline metal-organic frameworks (MOFs): synthesis, structure and function. Acta Cryst., B 70, 3–10.
Dogsa, I., Tomsic, M., Orehek, J., Benigar, E., Jamnik, A., Stopar, D., 2014. Amorphous supramolecular structure of carboxymethyl cellulose in aqueous solution at different pH values as determined by rheology, small angle X-ray and light scattering. Carbohydr. Polym. 111, 492–504.
Dong, H., Zhang, W., Zhou, S., Huang, J., Wang, P., 2021. Engineering bioscaffolds for enzyme assembly. Biotechnol. Adv. https://doi.org/10.1016/j.biotechadv.2021.107721. Article in press.
Dressick, W.J., Wahl, K.J., Bassim, N.D., Stroud, R.M., Petrovykh, D.Y., 2012. Divalent–anion salt effects in polyelectrolyte multilayer depositions. Langmuir 28, 15831–15843.
Dunn, M.F., Aguilar, V., Brzovic, P., Drewe, W.F., Houben, K.F., Leja, C.A., Roy, M., 1990. The tryptophan synthase bienzyme complex transfers indole between the . alpha.- and beta.-sites via a 25–30 .ANG. long tunnel. Biochemistry 29, 8598–8607.
Elżbieciak-Wodka, M., Kolasińska-Sojka, M., Warszyński, P., 2017. Effect of mono- and divalent ions on the formation and permeability of polyelectrolyte multilayer films. J. Electroanal. Chem. 789, 123–132.
Eun, C., Kekenes-Huskey, P.M., Metzger, V.T., McCammon, J.A., 2014. A model study of sequential enzyme reactions and electrostatic channeling. J. Chem. Phys. 140, 105101.
Fu, J., Schlenoff, J.B., 2016. Driving forces for oppositely charged polyion association in aqueous solutions: enthalpic, entropic, but not electrostatic. J. Am. Chem. Soc. 138, 980–990.
Furukawa, H., Cordova, K.E., O'Keeffe, M., Yaghi, O.M., 2013. The chemistry and applications of metal-organic frameworks. Science 341, 1230444.
Gao, C., Leporatti, S., Moya, S., Donath, E., Möhwald, H., 2003. Swelling and shrinking of polyelectrolyte microcapsules in response to changes in temperature and ionic strength. Chem. - A Eur. J. 9, 915–920.
Gao, Y., Roberts, C.C., Zhu, J., Lin, J.L., Chang, C.A., Wheeldon, I., 2015. Tuning enzyme kinetics through designed intermolecular interactions far from the active site. ACS Catal. 5, 2149–2153.
Garcia-Galan, C., Berenguer-Murcia, A., Fernandez-Lafuente, R., Rodrigues, R.C., 2011. Potential of different enzyme immobilization strategies to improve enzyme performance. Adv. Synth. Catal. 353, 2885–2904.
Ge, L., Möhwald, H., Li, J., 2003a. Phospholipid liposomes stabilized by the coverage of polyelectrolyte. Colloids Surf. A Physicochem. Eng. Asp. 221, 49–53.
Ge, L., Möhwald, H., Li, J., 2003b. Polymer-stabilized phospholipid vesicles formed on polyelectrolyte multilayer capsules. Biochem. Biophys. Res. Commun. 303, 653–659.
Giannakopoulou, A., Gkantzou, E., Polydera, A., Stamatis, H., 2020. Multienzymatic nanoassemblies: recent progress and applications. Trends Biotechnol. 38 (38), 202–216.
Goicoechea, J., Arregui, F.J., Corres, J.M., Matias, I.R., 2008. Study and optimization of self-assembled polymeric multilayer structures with neutral red for ph sensing applications. J. Sensors 2008, 1–7.
Gözen, I., Jesorka, A., 2015. Lipid nanotube networks: biomimetic cell-to-cell communication and soft-matter technology. Nanofabrication 2, 27–31.
Gun, W.J., Routh, A.F., 2013. Formation and characterization of pH-responsive liquid core microcapsules. Langmuir 29, 12541–12548.
Hattori, T., Hallberg, R., Dubin, P.L., 2000. Roles of electrostatic interaction and polymer structure in the binding of β-lactoglobulin to anionic polyelectrolytes: measurement of binding constants by frontal analysis continuous capillary electrophoresis. Langmuir 16, 9738–9743.
Haug, A., Larsen, B., Smidsrod, O., 1974. Uronic acid sequence in alginate from different sources. Carbohydr. Res. 32, 217–225.
Hinzpeter, F., Gerland, U., Tostevin, F., 2017. Optimal compartmentalization strategies for metabolic microcompartments. Biophys. J. 112, 767–779.
Hosta-Rigau, L., Zhang, Y., Teo, B.M., Postma, A., Städler, B., 2013. Cholesterol – a biological compound as a building block in bionanotechnology. Nanoscale 5, 89–109.
Huang, X., Holden, H.M., Raushel, F.M., 2001. Channeling of substrates and intermediates in enzyme-catalyzed reactions. Annu. Rev. Biochem. 70, 149–180.
Huang, Y., Lin, Y., Ran, X., Ren, J., Qu, X., 2016. Self-assembly and compartmentalization of nanozymes in mesoporous silica-based nanoreactors. Chem. Eur. J. 22, 5705–5711.
Hui Chong, L.S., Zhang, J., Bhat, K.S., Yong, D., Song, J., 2021. Bioinspired cell-in-shell systems in biomedical engineering and beyond: comparative overview and prospect. Biomaterials 266, 120473.
Hwang, E.T., Lee, S., 2019. Multienzymatic cascade reactions via enzyme complex by immobilization. ACS Catal. 9, 4402–4425.
Ibarra-Montaño, E.L., Rodríguez-Laguna, N., Sánchez-Hernández, A., Rojas-Hernández, A., 2015. Determination of pKa values for acrylic, methacrylic and itaconic acids by 1H and 13C NMR in deuterated water. J. Appl. Solution Chem. Model. 4, 7–18.
Itoh, Y., Matsusaki, M., Kida, T., Akashi, M., 2004. Preparation of biodegradable hollow nanocapsules by silica template method. Chem. Lett. 33, 1552–1553.
Jasim, M.A., Hall, G.M., Mann, J., Taylor, K.D.A., 1987. A comparison of immobilised protease. J. Chem. Technol. Biotechnol. 40, 251–258.
Jesionowski, T., Zdarta, J., Krajewska, B., 2014. Enzyme immobilization by adsorption: a review. Adsorption 20, 801–821.
Ji, M., Li, X., Omidvar, M., Sigurdardóttir, S.B., Woodley, J.M., Daugaard, A.E., Luo, J., Pinelo, M., 2020. Charge exclusion as a strategy to control retention of small proteins in polyelectrolyte-modified ultrafiltration membranes. Sep. Purif. Technol. 247, 116936.
Jia, X., Sua, Z., Maa, M., Zhang, S., 2018. Sandwiching multiple dehydrogenases and shared cofactor betweendouble polyelectrolytes for enhanced communication of cofactor ande nzymes. Biochem. Eng. J. 137, 40–49.
Jiang, Y.J., Sun, Q.Y., Zhang, L., Jiang, Z.Y., 2009. Capsules-in-bead scaffold: a rational architecture for spatially separated multienzyme cascade system. J. Mater. Chem. 19, 9068–9074.
Karlsson, M., Davidson, M., Karlsson, R., Karlsson, A., Bergenholtz, J., Konkoli, Z., Jesorka, A., Lobovkina, T., Hurtig, J., Voinova, M., Orwar, O., 2004. Biomimetic nanoscale reactors and networks. Annu. Rev. Phys. Chem. 55, 613–649.
Karsenti, 2008. Self-organization in cell biology: a brief history. Nat. Rev. Mol. Cell Biol. 9, 255–262.
Kayitmazer, A.B., Seeman, D., Minsky, B.B., Dubin, P.L., Xu, Y., 2013. Protein–polyelectrolyte interactions. Soft Matter 9, 2553.
Kienle, D.F., Chaparro Sosa, A.F., Kaar, J.L., Schwartz, D.K., 2020. Polyelectrolyte multilayers enhance the dry storage and ph stability of physically entrapped enzymes. ACS Appl. Mater. Interfaces 12, 22640–22649.
Klein, W.P., Thomsen, R.P., Turner, K.B., Walper, S.A., Vranish, J., Kjems, J., Ancona, M. G., Medintz, I.L., 2019. Enhanced catalysis from multienzyme cascades assembled on a DNA origami triangle. ACS Nano 13, 13677–13689.
Kozintsev, A., Sugihara, K., 2017. Artificial tubular connections between cells based on synthetic lipid nanotubes. RSC Adv. 7, 20700–20708.
Kurinomaru, T., Tomita, S., Hagihara, Y., Shiraki, K., 2014. Enzyme hyperactivation system based on a complementary charged pair of polyelectrolytes and substrates. Langmuir 30, 3826–3831.
Langowska, K., Palivan, C.G., Meier, W., 2013. Polymer nanoreactors shown to produce and release antibiotics locally. Chem. Commun. 49, 128–130.
Larrañaga, A., Lomora, M., Sarasua, J.R., Palivan, C.G., Pandit, A., 2017. Polymer capsules as micro−/nanoreactors for therapeutic applications: current strategies to control membrane permeability. Prog. Mater. Sci. 90, 325–357.
Lawrence, R.E., Zoncu, R., 2019. The lysosome as a cellular Centre for signalling, metabolism and quality control. Nat. Cell Biol. 21, 133–142.
Lewis, S.R., Datta, S., Gui, M., Coker, E.L., Huggins, F.E., Daunert, S., Bachas, L., Bhattacharyya, D., 2011. Reactive nanostructured membranes for water purification. Proc. Natl. Acad. Sci. U. S. A. 108, 8577–8582.
Li, P., Chen, Q., Wang, T.C., Vermeulen, N.A., Mehdi, L., Dohnalkova, A., Browning, N. D., Shen, D., Anderson, R., Gomez-Gualdron, D.A., Cetin, F.M., Jagiello, J., Asiri, A. M., Stoddart, F., Farhal, O.K., 2018. Hierarchically engineered mesoporous metal-organic frameworks toward cell-free immobilized enzyme systems. Chem 4, 1022–1034.
Liu, X., Formanek, P., Voit, B., Appelhans, D., 2017. Functional cellular mimics for the spatiotemporal control of multiple enzymatic cascade reactions. Angew. Chem. Int. Ed. 56, 16233–16238.
Mateo, C., Palomo, J.M., Fernandez-Lorente, G., Guisan, J.M., Fernandez-Lafuente, R., 2007. Improvement of enzyme activity, stability and selectivity via immobilization techniques. Enzym. Microb. Technol. 40, 1451–1463.
Mauser, T., Déjugnat, C., Sukhorukov, G.B., 2006. Balance of hydrophobic and electrostatic forces in the pH response of weak polyelectrolyte capsules. J. Phys. Chem. B 110, 20246–20253.

McNaught, A.D., Wilkinson, A., 1996. Glossary of basic terms in polymer science (IUPAC recommendations 1996). Pure Appl. Chem. 68, 2287–2311.
Mehta, J., Bhardwaj, N., Bhardwaj, S.K., Kim, K.H., Deep, A., 2016. Recent advances in enzyme immobilization techniques: metal-organic frameworks as novel substrates. Coord. Chem. Rev. 322, 30–40.
Milani, M., Pesce, A., Bolognesi, M., Bocedi, A., Ascenzi, P., 2003. Substrate channeling: molecular bases. Biochem. Mol. Biol. Educ. 31, 228–233.
Miles, E.W., Rhee, S., Davies, D.R., 1999. The molecular basis of substrate channeling. J. Biol. Chem. 274, 12193–12196.
Moss, J.M., Van Damme, M.-P.I., Murphy, W.H., Preston, B.N., 1997. Dependence of salt concentration on glycosaminoglycan–lysozyme interactions in cartilage. Arch. Biochem. Biophys. 348, 49–55.
Moya, S., Donath, E., Sukhorukov, G.B., Auch, M., Bäumler, H., Lichtenfeld, H., Möhwald, H., 2000. Lipid coating on polyelectrolyte surface modified colloidal particles and polyelectrolyte capsules. Macromolecules 33, 4538–4544.
Murata, H., Cummings, C.S., Koepsel, R.R., Russel, A.J., 2014. Rational tailoring of substrate and inhibitor affinity via ATRP polymer-based protein engineering. Biomacromol 15, 2817–2823.
Muthukumar, M., 2017. 50th anniversary perspective: a perspective on polyelectrolyte solutions. Macromolecules 50, 9528–9560.
Myhrvold, C., Polka, J.K., Silver, P.A., 2016. Synthetic lipid-containing scaffolds enhance production by colocalizing enzymes. ACS Synth. Biol. 5, 1396–1403.
Nguyen, P.Q., Botyanszki, Z., Tay, P.K.R., Joshi, N.S., 2014. Programmable biofilm-based materials from engineered curli nanofibres. Nat. Commun. 5, 4945.
Obata, T., 2020. Toward an evaluation of metabolite channeling in vivo. Curr. Opin. Biotechnol. 64, 55–61.
Obert, R., Dave, B.C., 1999. Enzymatic conversion of carbon dioxide to methanol: enhanced methanol production in silica sol-gel matrices. J. Am. Chem. Soc. 121, 12192–12193.
Pan, P., Woehl, E., Dunn, M.F., 1997. Protein architecture, dynamics and allostery in tryptophan synthase channeling. Trends Biochem. Sci. 22, 22–27.
Parakhonskiy, B.V., Yashchenok, A.M., Konrad, M., Skirtach, A.G., 2014. Colloidal micro- and nano-particles as templates for polyelectrolyte multilayer capsules. Adv. Colloid Interf. Sci. 207, 253–264.
Peters, R.J.R.W., Marguet, M., Marais, S., Fraaije, M.W., van Hest, J.C.M., Lecommandoux, S., 2014. Cascade reactions in multicompartmentalized polymersomes. Angew. Chem. Int. Ed. 53, 146–150.
Peyratout, C.S., Dähne, L., 2004. Tailor-made polyelectrolyte microcapsules: from multilayers to smart containers. Angew. Chem. Int. Ed. 43, 3762–3783.
Pravda, L., Berka, J.K., Svobodova, Vařeková, R.S., Sehnal, D., Banáš, P., Laskowski, R. A., Koča, J., Otyepka, M., 2014. Anatomy of enzyme channels. BMC Bioinformatics 15, 379.
Rae, B.D., Long, B.M., Badger, M.R., Price, P.G.D., 2013. Functions, compositions, and evolution of the two types of carboxysomes: polyhedral microcompartments that facilitate CO_2 fixation in cyanobacteria and some proteobacteria. Microbiol. Mol. Biol. Rev. 77, 357–379.
Rashid, J.I.A., Yusof, N.A., 2017. The strategies of DNA immobilization and hybridization detection mechanism in the construction of electrochemical DNA sensor: a review. Sens. Bio-Sens. Res. 16, 19–31.
Raushel, F.M., Thoden, J.B., Holden, H.M., 2003. Enzymes with molecular tunnels. Acc. Chem. Res. 36, 539–548.
Ricard, J., 1999. Biological Complexity and the Dynamics of Life Processes. Elsevier, New York.
Rodrigues, R.C., Ortiz, C., Berenguer-Murcia, Á., Torres, R., Fernández-Lafuente, R., 2013. Modifying enzyme activity and selectivity by immobilization. Chem. Soc. Rev. 42, 6290–6307.
Rustom, A., Saffrich, R., Markovic, I., Walther, P., Gerdes, H.-H., 2004. Nanotubular highways for intercellular organelle transport. Science 303, 1007–1010.
Scheepers, D., Chatillon, B., Borneman, Z., Nijmeijer, K., 2021. Influence of charge density and ionic strength on diallyldimethylammonium chloride (DADMAC)-based polyelectrolyte multilayer membrane formation. J. Membr. Sci. 617, 118619.
Schmitt, D.L., An, S., 2017. Spatial organization of metabolic enzyme complexes in cells. Biochemistry 56, 3184–3196.
Seyrek, E., Dubin, P.L., Tribet, C., Gamble, E.A., 2003. Ionic strength dependence of protein-polyelectrolyte interactions. Biomacromolecules 4, 273–282.
Shalova, I.N., Asryants, R.A., Sholukh, M.V., Saso, L., Kurganov, B.I., Muronetz, V.I., Izumrudov, V.A., 2005. Interaction of polyanions with basic proteins. Macromol. Biosci. 5, 1184–1192.
Shalova, I.N., Naletova, I.N., Saso, L., Muronetz, V.I., Izumrudov, V.A., 2007. Interaction of polyelectrolytes with proteins: influence of complexing polycations on the thermoaggregation of oligomeric enzymes. Macromol. Biosci. 7, 929–939.
Sharma, V., Sundaramurthy, A., 2020. Multilayer capsules made of weak polyelectrolytes: a review on the preparation, functionalization and applications in drug delivery. Beilstein J. Nanotechnol. 11, 508–532.
Shatalin, K., Lebreton, S., Rault-leonardon, M., Vélot, C., Srere, P.A., 1999. Electrostatic channeling of oxaloacetate in a fusion protein of porcine citrate synthase and porcine mitochondrial malate dehydrogenase. Biochemistry 38, 881–889.
Shenoy, D.B., Antipov, A.A., Sukhorukov, G.B., Möhwald, H., 2003. Layer-by-layer engineering of biocompatible, decomposable core−shell structures. Biomacromolecules 4, 265–272.
Shi, J., Zhang, L., Jiang, Z., 2011. Facile construction of multicompartment multienzyme system through layer-by-layer self-assembly and biomimetic mineralization. ACS Appl. Mater. Interfaces 3, 881–889.
Shi, J., Wang, X., Jiang, W., Liang, Y., Zhu, Y., Zhang, C., 2012. Constructing spatially separated multienzyme system through bioadhesion-assisted bio-inspired mineralization for efficient carbon dioxide conversion. Bioresour. Technol. 116, 359–366.
Shi, J., Wu, Y., Zhang, S., Tian, Y., Yang, D., Jiang, Z., 2018. Bioinspired construction of multi-enzyme catalytic systems. Chem. Soc. Rev. 47, 4295–4313.
Sigurdardóttir, S.B., Lehmann, J., Ovtar, S., Grivel, J.C., Negra, M.D., Kaiser, A., Pinelo, M., 2018. Enzyme immobilization on inorganic surfaces for membrane reactor applications: mass transfer challenges, enzyme leakage and reuse of materials. Adv. Synth. Catal. 360 (14), 2578–2607.
Sigurdardóttir, S.B., Lehmann, J., Grivel, J.C., Zhang, W., Kaiser, A., Pinelo, M., 2019. Alcohol dehydrogenase on inorganic powders: zeta potential and particle agglomeration as main factors determining activity during immobilization. Colloids Surf. B Biointerface 175, 136–142.
Sigurdardóttir, S.B., Du Chanois, R.M., Epsztein, R., Pinelo, M., Elimelech, M., 2020. Energy barriers to anion transport in polyelectrolyte multilayer nanofiltration membranes: role of intra-pore diffusion. J. Membr. Sci. 603, 117921.
Sirisha, V.L., Jain, A., Kim, S.K., Toldra, F., 2016. Advances in Food and Nutrition, Marine Enzymes Biotechnology: ResearchProduction and Industrial Applications, Pt II - Marine Organisms Producing Enzymes. Elsevier, New York.
Song, J., Shen, H., Yang, Y., Zhou, Z., Su, P., Yang, Y., 2018. Multifunctional magnetic particles for effective suppression of non-specific adsorption and coimmobilization of multiple enzymes by DNA directed immobilization. Mater. Chem. B 6, 5718–5728.
Song, J., He, W., Shen, H., Zhou, Z., Li, M., Su, P., Yang, Y., 2019. Construction of multiple enzyme metal–organic frameworks biocatalyst via DNA scaffold: a promising strategy for enzyme encapsulation. Chem. Eng. J. 363, 174–182.
Souffriau, B., den Abt, T., Thevelein, J.M., 2012. Evidence for rapid uptake of D-galacturonic acid in the yeast *Saccharomyces cerevisiae* by a channel-type transport system. FEBS Lett. 586, 2494–2499.
Spivey, H.O., Ovadi, J., 1999. Substrate channeling. Methods 19, 306–321.
Sukhishvili, S.A., Granick, S., 2000. Layered, erasable, ultrathin polymer films. J. Am. Chem. Soc. 122, 9550–9551.
Sweetlove, L.J., Fernie, A.R., 2018. The role of dynamic enzyme assemblies and substrate channelling in metabolic regulation. Nature Commu. 9, 2136.
Tikhonenko, S.A., Saburova, E.A., Durdenko, E.N., Sukhorukov, B.I., 2009. Enzyme-polyelectrolyte complex: salt effects on the reaction of urease with polyallylamine. Russ. J. Phys. Chem. 83, 1781–1788.
Torchilin, V., 2014. Handbook of Nanobiomedical Research. World Scientific Publishing, Singapore.
van der Straeten, A., Bratek-Skicki, A., Jonas, A.M., Fustin, C.-A., Dupont-Gillain, C., 2018. Integrating proteins in layer-by-layer assemblies independently of their electrical charge. ACS Nano 12, 8372–8381.
Vinogradova, O.I., Lebedeva, O.V., Vasilev, K., Gong, H., Garcia-Turiel, J., Kim, B.-S., 2005. Multilayer DNA/Poly(allylamine hydrochloride) microcapsules: assembly and mechanical properties. Biomacromolecules 6, 1495–1502.
Vriezema, D.M., Hoogboom, J., Velonia, K., Takazawa, K., Christianen, P.C.M., Maan, J. C., Rowan, A.E., Nolte, R.J.M., 2003. Vesicles and polymerized vesicles from thiophene-containing rod-coil block copolymers. Angew. Chem. Int. Ed. 46, 772–776.
Vriezema, D.M., Kros, A., de Gelder, R., Cornelissen, J.J.L.M., Rowan, A.E., Nolte, R.J.M., 2004. Electroformed giant vesicles from thiophene-containing rod−coil diblock copolymers. Macromolecules 37, 4736–4739.
Vriezema, D.M., Garcia, P.M.L., Oltra, N.S., Hatzakis, N.S., Kuiper, S.M., Nolte, R.J.M., Rowan, A.E., van Hest, J.C.M., 2007. Positional assembly of enzymes in polymersome nanoreactors for cascade reactions. Angew. Chem. Int. Ed. 46, 7378–7382.
Wang, Y., Yu, A., Caruso, F., 2005. Nanoporous polyelectrolyte spheres prepared by sequentially coating sacrificial mesoporous silica spheres. Angew. Chem. Int. Ed. 44, 2888–2892.
Wang, S.Z., Zhang, Y.H., Ren, H., Wang, Y.L., Jiang, W., Fang, B.S., 2017a. Strategies and perspectives of assembling multienzyme systems. Crit. Rev. Biotechnol. 37, 1024–1037.
Wang, C., Zhang, Q., Wang, X., Chang, H., Zhang, S., Tang, Y., Xu, J., Qi, R., Chen, Y., 2017b. Dynamic modulation of enzyme activity by near-infrared light. Angew. Chem. Int. Ed. 56, 6767–6772.
Waqas, M., Lee, H.-M., Kim, J., Telling, G., Kim, J.-K., Kim, D.-H., Ryou, C., 2017. Effect of poly-L-arginine in inhibiting scrapie prion protein of cultured cells. Mol. Cell. Biochem. 428, 57–66.
Wheeldon, I., Minteer, S.D., Banta, S., Barton, S.C., Atanassov, P., Sigman, M., 2016. Substrate channelling as an approach to cascade reactions. Nat. Chem. 8, 299–309.
Wilson, D.S., Szostak, J.W., 1999. In vitro selection of functional nucleic acids. Annu. Rev. Biochem. 68, 611–647.
Winkel, B.S.J., 2004. Metabolic channeling in plants. Annu. Rev. Plant Biol. 55, 85–107.
Wisniewska, M., Urban, T., Grzadka, E., Zarko, V.I., Gunko, V.M., 2014. Comparison of adsorption affinity of polyacrylic acid for surfaces of mixed silica–alumina. Colloid Polym. Sci. 292, 699–705.
Wu, F., Minteer, S., 2015. Krebs cycle metabolon: structural evidence of substrate channeling revealed by cross-linking and mass spectrometry. Angew. Chem. Int. Ed. 54, 1851–1854.
Xu, Y., Fei, J., Li, G., Yuan, T., Xu, X., Li, J., 2019. Nanozyme-catalyzed cascade reactions for mitochondria-mimicking oxidative phosphorylation. Angew. Chem. Int. Ed. 58, 5572–5576.
Yang, Y., Zhang, R., Zhou, B., Song, J., Su, P., Yang, Y., 2017. High activity and convenient ratio control: DNA-directed coimmobilization of multiple enzymes on multifunctionalized magnetic nanoparticles. ACS Appl. Mater. Interfaces 9, 37254–37263.
York-Duran, M.J., Godoy-Gallardo, M., Labay, C., Urquhart, A.J., Andresen, T.L., Hosta-Rigau, L., 2017. Recent advances in compartmentalized synthetic architectures as

drug carriers, cell mimics and artificial organelles. Colloids Surf. B Biointerface 152, 199–213.

You, C.C., Agasti, S.S., De, M., Knapp, M.J., Rotello, V.R., 2006. Modulation of the catalytic behavior of α-chymotrypsin at monolayer-protected nanoparticle surfaces. J. Am. Chem. Soc. 128, 14612–14618.

Yu, A., Wang, Y., Barlow, E., Caruso, F., 2005. Mesoporous silica particles as templates for preparing enzyme-loaded biocompatible microcapsules. Adv. Mater. 17, 1737–1741.

Yun, J., Kim, H.-I., 2010. Control of release characteristics in pH-sensitive poly(vinyl alcohol)/poly(acrylic acid) microcapsules containing chemically treated alumina core. J. Appl. Polym. Sci. 115, 1853–1858.

Yurekli, Y., 2020. Layer-by-layer self-assembly of multifunctional enzymatic UF membranes. J. Appl. Polym. Sci. 137, 48750.

Zappi, D., Coria-Oriundo, L.L., Piccinini, E., Gramajo, M., von Bilderling, C., Pietrasanta, L.I., Azzaroni, O., Battaglini, F., 2019. The effect of ionic strength and phosphate ions on the construction of redox polyelectrolyte–enzyme self-assemblies. Phys. Chem. Chem. Phys. 21, 22947–22954.

Zdarta, J., Meyer, A.S., Jesionowski, T., Pinelo, M., 2019. Multi-faceted strategy based on enzyme immobilization with reactant adsorption and membrane technology for biocatalytic removal of pollutants: a critical review. Biotechnol. Adv. 37, 107401.

Zdarta, J., Nguyen, L.N., Jankowska, K., Jesionowski, T., Nghiem, L.D., 2021. A contemporary review of enzymatic applications in the remediation of emerging estrogenic compounds. Crit. Rev. Environ. Sci. Technol. https://doi.org/10.1080/10643389.2021.1889283.

Zhang, Y.H.P., 2011. Substrate channeling and enzyme complexes for biotechnological applications. Biotechnol. Adv. 29, 715–725.

Zhang, Y., Hess, H., 2020. Microenvironmental engineering: an effective strategy for tailoring enzymatic activities. Chin. J. Chem. Eng. 28, 2028–2036.

Zhang, D.Y., Seelig, G., 2011. Dynamic DNA nanotechnology using strand-displacement reactions. Nat. Chem. 3, 103–113.

Zhang, Y., Wang, Q., Hess, H., 2017. Increasing enzyme cascade throughput by pH-engineering the microenvironment of individual enzymes. ACS Catal. 7, 2047–2051.

Zhao, Q., Li, B., 2008. pH-controlled drug loading and release from biodegradable microcapsules. Nanomedicine 4, 302–310.

Zhao, Y., Xiao, X., Zou, M., Ding, M., Xiao, H., Wang, M., Jiang, F., Cheng, Z., Ma, P., Lin, J., 2021. Nanozyme-initiated in situ cascade reactions for self-amplified biocatalytic immunotherapy. Adv. Mater. 33, 2006363.