# Adaptive polymersome nanoreactors

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Minireview

# Adaptive Polymersome Nanoreactors

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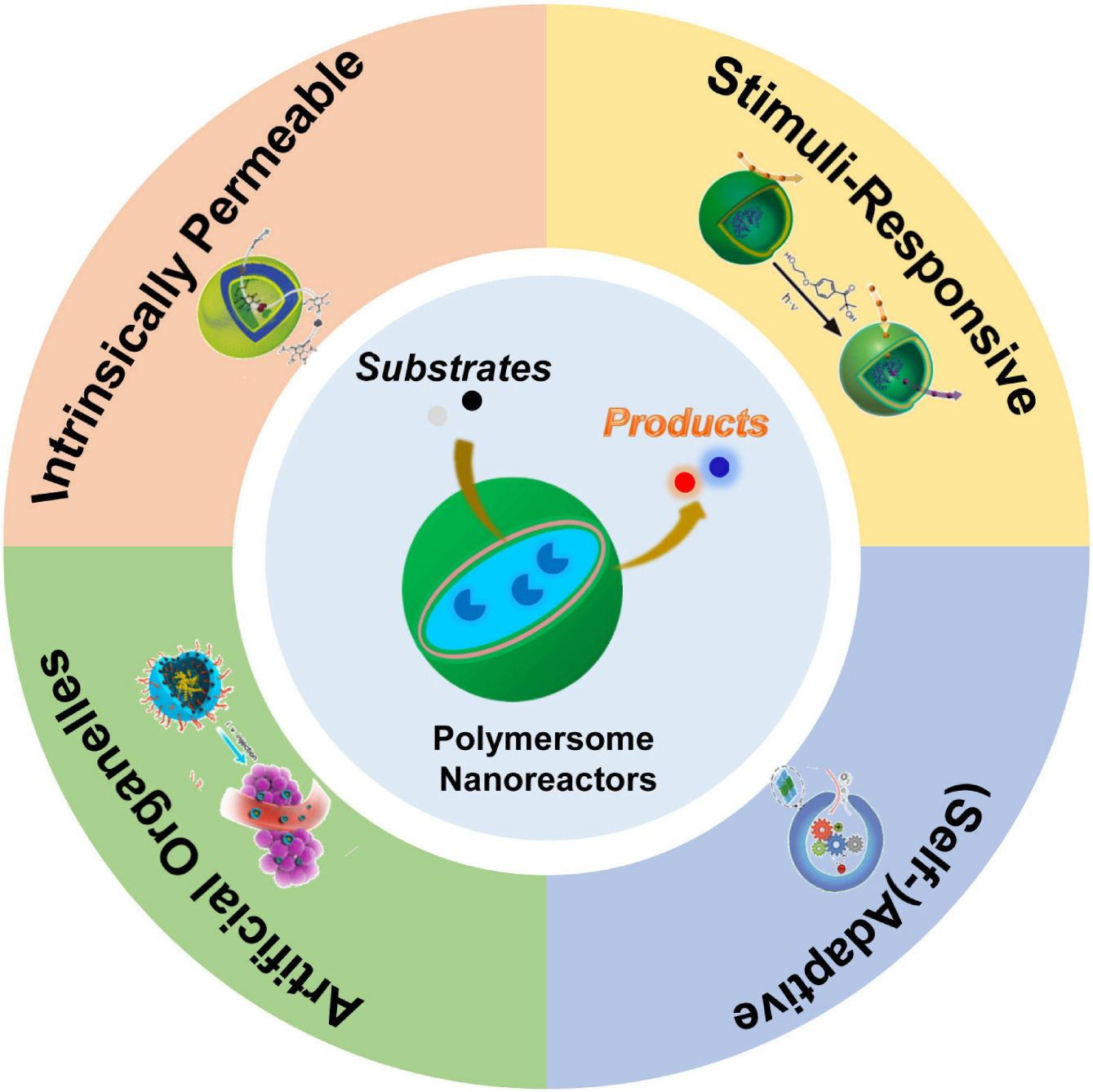

**Abstract:** Adaptive polymersome systems have gained much interest in a wide variety of research fields, ranging from cell mimics to nanomedicine, because of their high stability, tunable shape and size. Furthermore, polymersomes can be effectively transformed into nanoreactors *via* the incorporation of catalytic species. By employing polymersomes which are adaptive in structure and function the features of polymersome nanoreactors can be even further extended. In this review, we focus on recent impressive developments of smart polymersomes as functional nanoreactors with an emphasis on the type of adaptivity that is installed, which includes intrinsic permeability, stimuli-responsiveness and self-adaptivity. Moreover, particular attention is given to the utility of polymersome nanoreactors *in vitro* and *in vivo,* which paves the next step forward towards the engineering of artificial organelles as therapeutic materials.

## 1. Introduction

Recent advances in synthetic chemistry and self-assembly have provided researchers with the necessary tools to create artificial architectures that mimic living systems. Over the years, a plethora of low molecular weight amphiphiles derived from fatty acids and phospholipids capable of self-assembling into bilayer vesicular structures have been developed, aimed to mimicking the complex structure and function of the cell.[1] Analogous to these liposomes, polymersomes, formed from amphiphilic block copolymers, have received widespread scientific attention in view of their specific characteristics such as improved stability, architectural control, and intriguing shape-transformation capability.[2] Generally, polymersomes can be created by a wide variety of techniques, such as film rehydration, bulk hydration, electroformation, and nano-precipitation.[3] The chemical and physical properties of the polymersomes are highly dependent on the type of amphiphilic copolymers applied. The properties of each block as well as the overall polymer chains, including molecular weight, dispersity (Đ) and hydrophilic-hydrophobic volume ratio have a significant influence on the properties of the polymersomes. To further increase the versatility and applicability of the polymersomes, in some cases, regular block copolymers have been combined with low molecular weight components and biomolecules (e.g. phospholipids, nucleic acid, proteins, peptides).[3a]

During the last decade, significant advances have been made in the development of polymersomes since they were first reported.[2f] One of the most inherent and interesting properties of polymersomes is their capability to encapsulate both hydrophobic molecules in their membranes and hydrophilic compounds within the aqueous lumen which allows for numerous applications in nanomedicine.[4] For example, polymersomes have demonstrated their use as drug delivery system (DDS) not only owing to their well-tailored surface modification, mechanical stability, and tunable morphology and size, but also because of their high loading drug efficiency as a result of the large hydrophobic domain of the membranes.[5] The usefulness of polymersomes in the area of DDS has been extensively covered in many reviews[6] and will not be discussed in much detail in this article. Here, we will focus on the applicability of polymersomes as nanoreactors. Polymersomes are transformed into nanoreactors by incorporating enzymes or other catalytic species into the lumen, hydrophobic membrane or on the particle surface.[7] Such synthetically developed compartments hold great potential for confined catalysis, to separate catalytic species from each other and to protect catalysts from undesired influences from the environment. Besides applications in regular organic synthesis they can also be applied as therapeutic modalities by efficiently operating biological tasks in living cells, thereby replacing dysfunctional enzymes. Furthermore, the integration of biologically active segments associated with the polymersome architecture could provide a useful strategy for the construction of artificial cells, which contributes to origin of life research.

Most benefits of polymersomes as nanoreactors are very similar to the use of polymersomes for DDS, with one exception: poor bilayer permeability; the high molecular weight constituents of the polymersome bilayer membrane not only endow the structure with robustness and stability, but also with an increased barrier against diffusion in comparison to their counterparts, liposomes. For nanoreactor applications this is a crucial feature, as the membrane permeability plays a key role in transporting substrates and products to and from the catalytic centre. With this challenge in mind, facile approaches need to be employed to produce membranes with tunable permeability, imparting sufficient efficiency to the polymersome nanoreactors. To date, only a few polymersomes are found to be intrinsically permeable enough to serve as a versatile platform for the construction of nanoreactors. For instance, polymersomes composed of polystyrene-b-poly(isocyanoalanine(2-thiophen-3-yl-ethyl)amide) (PS-*b*-PIAT) are proven to be semipermeable in nature, by accommodating enzymes in their structure while small molecules can diffuse in and out of the nanoparticles.[8] A more bio-inspired strategy is to embed channel proteins in the polymer membranes, and in such a way transportation of small molecules across the polymersome membranes is achieved.[7b] Also, the exploitation of layer-by-layer self-assembly provides a new avenue for the development of permeable polymersomes or polymer capsules, which have been extensively used to develop a wide range of nanoreactors.[9] Moreover, a recent development is the construction of polymersomes that can adjust their chemical or

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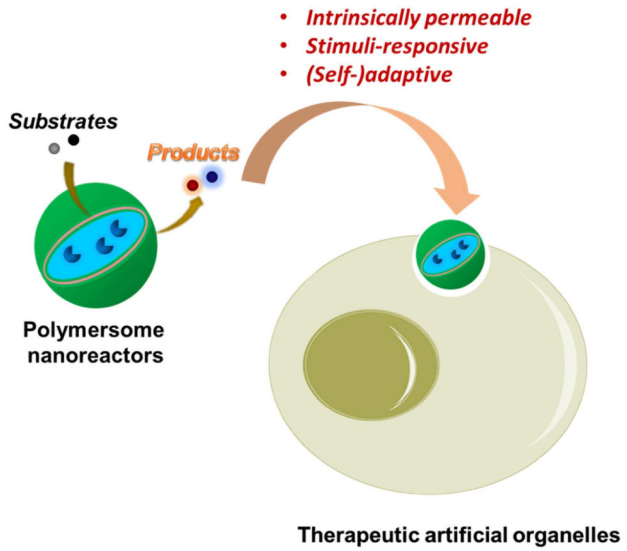
physical membrane properties in response to external stimuli, which permits the tailor-made regulation of permeability.[10]

In this review, we will describe the state of the art concerning functional polymersomes as adaptive nanoreactors, with a particular focus on examples that utilize changes of external signals to mediate the permeability of the membranes. Subsequently, we highlight recent progress in the systematic engineering of polymersome nanoreactors to be artificial organelles *in vitro* and *in vivo* which is regarded as a significant step forward towards their biomedical applications (Scheme 1). Finally, the opportunities and future challenges of adaptive polymersome nanoreactors will be discussed.




**Scheme 1.** Schematic representation for the adaptive polymersome nanoreactors and their applications as artificial organelles.

## 2. Intrinsically Permeable Polymersome Nanoreactors

As already mentioned, in some limited cases, polymersomes are inherently permeable. Van Hest and co-workers have shown that the amphiphilic copolymer PS-*b*-PIAT is able to self-assemble into polymersomes which are inherently porous, owing to the frustrated packing of the polymers in the membrane.[8a,d,11] When these polymersomes were functionalized by encapsulating enzymes, small molecules like substrates and products could diffuse across the polymer bilayers. For example, to regulate the levels of reactive oxygen species (ROS), superoxide dismutase (SOD1, to neutralize superoxide radicals ($O_2^{\bullet -}$)) and catalase (CAT, to neutralize $H_2O_2$) were encapsulated in PS-*b*-PIAT polymersomes (Figure 1a).[8d] When a single enzyme was loaded, it was noted that the activity of SOD1 in PS-*b*-PIAT polymersomes was higher than that in PS-*b*-PEG polymersomes, suggesting that the PS-PIAT polymersome are more permeable for $H_2O_2$. However, when two enzymes were encapsulated, and $H_2O_2$ was produced inside the polymersomes, both nanoreactors showed similar enzymatic activity. This can be explained by the fact that the diffusion of $O_2$ across the PS-*b*-PIAT and polystyrene-b-poly(ethylene glycol) (PS-b-PEG) polymersomes is similar. In addition, a multi-enzyme containing PS-*b*-PIAT nanoreactor was successfully constructed by not only encapsulating enzymes inside the lumen but also positioning different types of enzymes in the polymersome membrane (Figure 1b).[8a] The three-enzyme-polymersome system contained glucose oxidase (GOX) in the inner aqueous pool of the polymersomes, horseradish peroxidase (HRP) in the hydrophobic domain of the polymersome membrane, and *Candida Antarctica lipase* B (CALB), 1,2,3,4-tetra-O-acetyl-b-glucopyranose (GAc4) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in the bulk solution. The cascade reaction assessment showed that the reaction performed smoothly demonstrating that the generated glucose can readily permeate the membrane and be converted by GOX to its lactone, thus leading to the release of $H_2O_2$. Then, the produced $H_2O_2$ inside the nanoreactors was subsequently used to convert ABTS to $ABTS^{\bullet +}$ by HRP.

Despite the fact that various single compartmentalized structures have been reported, cell mimetic systems in terms of multi-compartmentalization are limited, especially those that combine structural intricacy with cascade catalysis. One of the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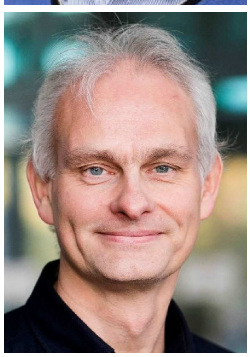

***Jan C.M. van Hest*** *obtained his PhD from Eindhoven University of Technology in 1996 in macro-organic chemistry with prof E.W. Meijer. He worked as a postdoc with prof D.A. Tirrell on protein engineering. In 1997 he joined the chemical company DSM in the Netherlands. In 2000 he was appointed full professor in Bio-organic chemistry at Radboud University Nijmegen. As of September 2016 he holds the chair of Bio-organic Chemistry at Eindhoven University of Technology. The group's focus is to develop well-defined compartments for nanomedicine and artificial cell research. Using a combination of techniques from polymer science to protein engineering, well-defined carriers and scaffolds are developed for application in e.g. cancer treatment, immunology and ophthalmology.*

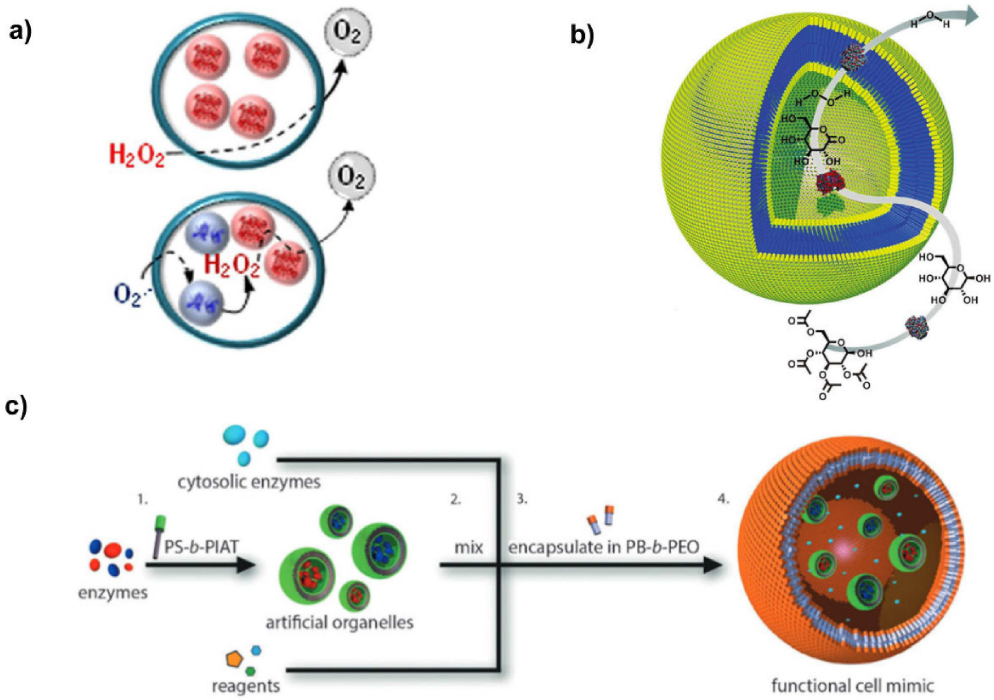


**Figure 1. a)** Permeability effects on the efficiency of antioxidant nanoreactors. The enzymatic activities of the nanoreactors were evaluated either by the addition of $H_2O_2$ or by the production of $O_2^{\bullet-}$ in situ. Reproduced with permission from Ref 8d. Copyright 2013, American Chemical Society. **b)** Schematic representation of the multistep reaction taking place in the three-enzyme-polymersome system. Reproduced with permission from Ref 8a. Copyright 2007, Wiley-VCH. **c)** Illustration of the functional cell mimic, which demonstrates the initial encapsulation of different enzymes in PS-*b*-PIAT polymersomes (1), followed by mixing of the nanoreactors, cytosolic enzymes, and substrates (2), and encapsulation of the reaction mixture in PB-*b*-PEO vesicles (3) to create artificial cells (4), inside which enzymatic multicompartment catalysis takes place. Reproduced with permission from Ref 11c. Copyright 2015, Wiley-VCH.

first steps in this direction was the development of a eukaryotic cell mimetic polymersome-in-polymersome system in which the encapsulated polymersome nanoreactors operated as artificial organelles in multistep biocatalytic reactions. For the construction of the organelles, the abovementioned $PS_{40}$-*b*-$PIAT_{50}$ polymersomes were utilized. The formed PS-*b*-PIAT nanoreactors, together with free (cytosolic) enzymes, reagents and cofactors were quantitatively[12] encapsulated in micron-sized polybutadiene-*b*-poly(ethylene oxide) (PB-*b*-PEO) polymersomes by an emulsion-centrifugation strategy[13] to generate multi-compartmentalized polymersomes-in-polymersome structure (Figure 1c).[11c] Activity analysis showed that enzymatic reactions inside the synthetic cell proceeded smoothly as the substrate could diffuse from one artificial organelle to the next. To better understand the location of the nanoscaled polymersome nanoreactors, one of the artificial cell structures was reconstructed in 3D, which clearly revealed that the organelle sub-compartments were well confined and the enzymatic reactions proceeded exclusively in the small polymersome nanoreactors. This polymersomes-in-polymersome multi-compartmentalized system can serve as structural and functional cell mimic, and the design principle in this system can be further exploited for the study of cellular processes with multiple successive reactions.

Another widely reported polymersome nanoreactor system developed by Meier et al. is comprised of poly(2-methyl-2-oxazoline)-block-polydimethylsiloxane-block-poly(2-methyl-2-oxazoline) (PMOXA-*b*-PDMS-*b*-PMOXA). These polymersomes were intrinsically permeable for $O_2$ and $O_2^{\bullet-}$, allowing them to be used as antioxidant nanoreactors *via* the encapsulation of Cu,Zn superoxide dismutase.[7b] When the polymer membrane was accommodated with channel proteins, such as aquaporin Z, the polymersomes became permeable for a wider variety of small molecule substrates.[14] Recently, the same group developed functional PMOXA-*b*-PDMS-*b*-PMOXA polymersome nanoreactors which allowed for the local production and release of antibiotics (Figure 2a).[14b] The bacterial porin Outer membrane protein F (OmpF) was inserted into the polymeric membranes, resulting in efficient traveling of small molecules up to 600 Da. Penicillin acylase from *E. coli* was encapsulated inside the polymersomes to create nanoreactors which could convert 7-aminodesacetoxycephalosporanic acid (7-ADCA) and phenylglycine methyl ester (PGME) into cephalexin (semisynthetic cephalosporin antibiotic), capable of inhibiting bacterial growth. Additionally, they demonstrated the immobilization of polymersome nanoreactors on a solid support to generate "active surfaces", which were utilized as efficient biosensors for sugar alcohols (Figure 2b).[15] *E. coli* glycerol facilitator (GlpF) was selected to transport sugar alcohols through the polymer membranes. Ribitol dehydrogenase (RDH), which is able to catalyze a variety of sugar alcohol reactions, was encapsulated in PDMS-b-PMOXA polymersomes. The active surface was accomplished by immobilizing the nanoreactors, and the subsequent "smart" surface demonstrated precise spatial and temporal responses to ribitol.

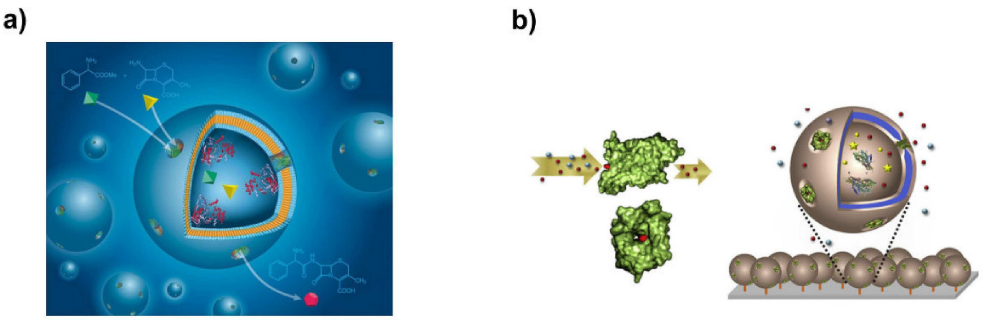


**Figure 2. a)** Schematic representation of a penicillin acylase-loaded nanoreactor. PMOXA-*b*-PDMS-*b*-PMOXA polymersomes were permeabilised for substrates and products by inserting the bacterial porin Outer membrane protein F (OmpF) into their polymeric membranes. Reproduced with permission from Ref 14b. Copyright 2013, Royal Society of Chemistry. **b)** Left: Molecular representation of the selective membrane protein GlpF (green) and ribitol as a model sugar alcohol (red): side view (upper part of the image), front view (lowerpart). Right: Schematic representation of an "active surface" serving as a sugar alcohol biosensor based on immobilized protein-polymersome nanoreactors with reconstituted membrane protein (GlpF) for selective transport of sugar alcohols, and encapsulated enzymes (RDH) for sensitive detection of sugar alcohols. Reproduced with permission from Ref 15. Copyright 2016, Elsevier.

Polymersomes with a semipermeable membrane can easily be prepared by mixing pairs of oppositely charged polyelectrolytes in an aqueous medium; these polymeric vesicles are also known as polyion complex vesicles (PICsomes). The oppositely charged block copolymers can form stable micro and nano-scaled vesicles by simply adjusting the polymer length and concentrations. Because of their easy and biologically friendly (no organic solvent involved) preparation, PICsomes have shown much promise as biomedical nanoreactors, as will be discussed later.[16]

Polymerization-induced self-assembly (PISA) has become a powerful technique for the preparation of polymeric nanoparticles in the form of micelles, worm-like micelles, and vesicles.[17] Hence, PISA may provide a versatile approach to the construction of polymersome nanoreactors by simply blending enzymes with the monomers during the process of polymerization. For example, O'Reilly et al. described the preparation of intrinsically permeable polymersomes loaded with functional enzymes by the method of visible light mediated reversible addition-fragmentation chain-transfer polymerization-induced self-assembly (photo-RAFT-PISA) in aqueous media.[18] The aqueous photo-PISA was performed in water in the presence of $PEG_{115}$ macro-chain-transfer agent (macro-CTA), 2-hydroxypropyl methacrylate (HPMA) and HRP or GOX (Figure 3 a). The produced enzyme encapsulated polymersomes were permeable due to the highly hydrated nature of the PHPMA membrane,[19] which allows for the transportation of small molecules while preventing the leakage of macromolecules. They found that both the single enzyme and two enzyme-loaded polymersomes showed high enzymatic activity.

More recently, the same group expanded the scope of enzyme-loaded PISA vesicles to the field of immunotherapy by successfully preparing L-Asparaginase (ASNS) loaded polymersome nanoreactors.[20] As certain tumor cells have lost the ability to produce asparagine, this enzyme will deplete these cells from this crucial metabolite, leading to cell death. Although this is a widely used injectable treatment for acute lymphoblastic leukemia, ASNS is also associated with a set of side-effects which are induced by the production of anti-ASNS antibodies.[21] To increase the proteolytic stability and reduce the antibody recognition compared to the free ASNS, the authors demonstrated the one-pot synthesis of ASNS-loaded PEG-*b*-PHPMA vesicles using PISA (Figure 3 b). This approach yielded well-defined enzyme loaded polymersomes at high solids content (11 wt%) in short reaction times. Compared to both the free enzyme and the PEGylated conjugate, the binding of ASNS antibodies was significantly reduced and the stability of the enzymes to proteolytic degradation was greatly enhanced when ASNS was encapsulated in these vesicles. The cell viability of these nanoreactors with vesicle concentrations up to 2 mg mL$^{-1}$ was found to be $\geq$90% after incubating cells for 7 days, suggesting low cytotoxicity. This work provides a unique tool for the preparation of functional polymersome nanoreactors with improved stability and reduced immunogenicity.

## 3. Stimuli-Responsive Polymersome Nanoreactors

In order to endow polymersomes with adaptivity, they have been designed to respond to a diverse set of biological signals (e.g. glucose) or external stimuli (e.g. pH, temperature, light). Stimulus-responsiveness can easily be introduced by the tailor-made design of block copolymers with specific functional groups that can intrinsically sense minimal changes in the local environment. Under specific stimulation, these polymersomes can undergo morphology or phase changes, which induce membrane disruption to release cargo or regulate membrane permeability to allow catalytic reactions to occur.[22] This latter feature provides a unique tool for the engineering of polymersomes to be adaptive nanoreactors. Although responsiveness to a wide range of stimuli has been incorporated in polymersomes,[6b,10b,23] there have only been very limited reports on responsive polymersome nanoreactors, and existing examples to date have only involved pH, temperature, carbon dioxide ($CO_2$) or light as the stimulus.

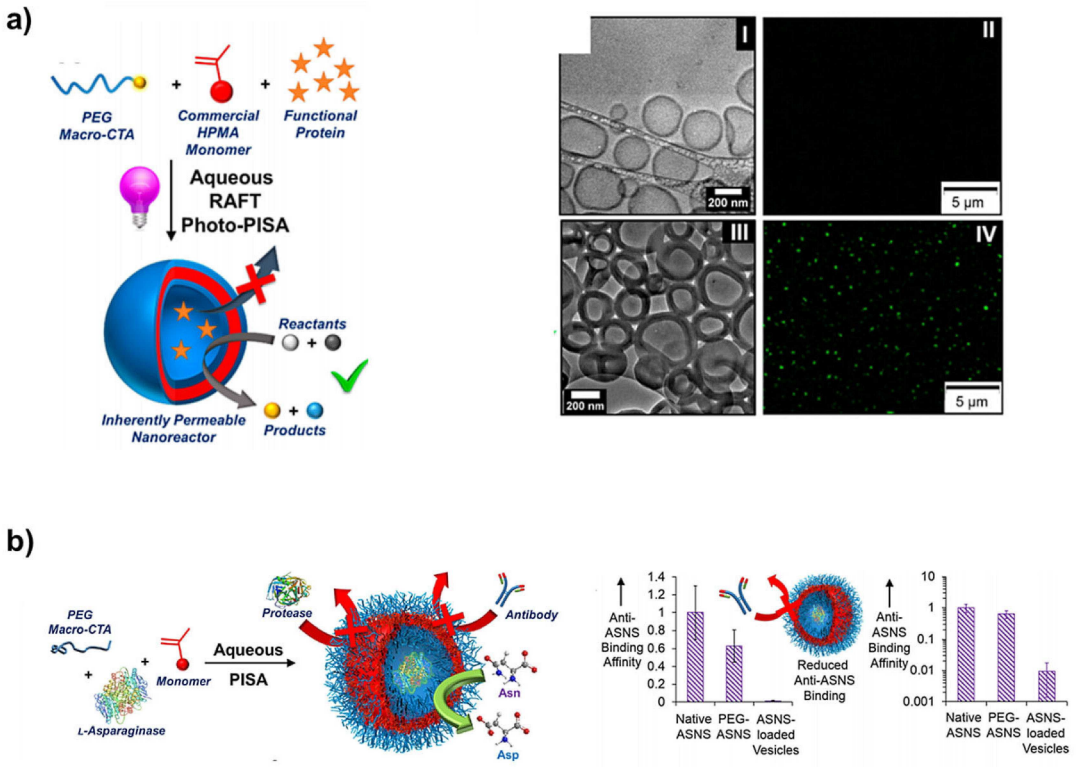


**Figure 3. a)** Left: Schematic representation of the preparation of inherently permeable protein-loaded nanoreactors by aqueous PISA. Right: Cryo-TEM image and fluorescence micrograph of empty vesicles (I, II) and GFP-loaded vesicles (III, IV). Reproduced with permission from Ref 18. Copyright 2017, American Chemical Society. **b)** Left: Schematic of the ASNS-loaded vesicle preparation by PISA. Right: Anti-ASNS binding affinity toward native ASNS, PEG-ASNS, and ASNS-loaded vesicles shown on linear and logarithmic scales. Reproduced with permission from Ref 20. Copyright 2018, American Chemical Society.

### 3.1. pH-Responsive Polymersome Nanoreactors

pH can be considered as one of the most important parameters in biological systems, (e.g. the lower pH value in endosomes and lysosomes of cells to give rise to the degradation of biomacromolecules). pH-responsive polymersomes can be easily obtained by including a pH-sensitive block in the copolymer chains. Upon a change in pH, the sensitive part of such polymersomes will undergo a hydrophobic-hydrophilic transition, leading to fine-tuning of the permeability.

Voit et al. reported a series of pH-responsive polymersome systems based on poly(2-(diethylamino)ethyl) methacrylate (PDEAEMA), which have a p$K_a$ suitable for application in the cellular environment.[24] For example, a polymersome nanoreactor with pH controllable permeability was developed on the basis of pH-sensitive copolymers with PEG as the hydrophilic part and PDEAEMA with photo-cross-linking unit 3,4-dimethyl maleic imidoethyl methacrylate (DMIEM,) or 3,4-dimethyl maleic imidobutyl methacrylate (DMIBM) as hydrophobic part (Figure 4).[24b] These polymersomes had high mechanical stability after crosslinking, and their membrane pores or permeability could be finely controlled by the shear rate, cross-linking degree, and the pH value, which allowed them to be used as nanoreactors.

The reconstitution of channel proteins into polymer membranes is an interesting option to construct functional nano-

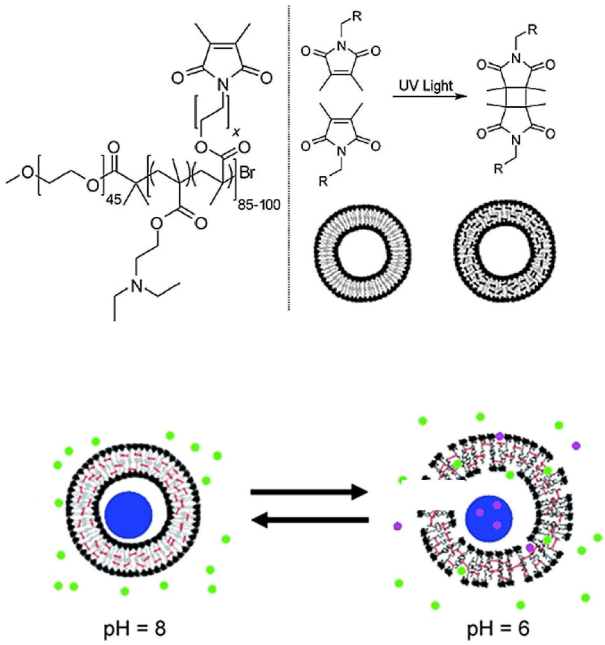


**Figure 4.** Crosslinked pH-responsive polymersomes as bionanoreactors. Reproduced with permission from Ref 24b. Copyright 2012, Wiley-VCH.

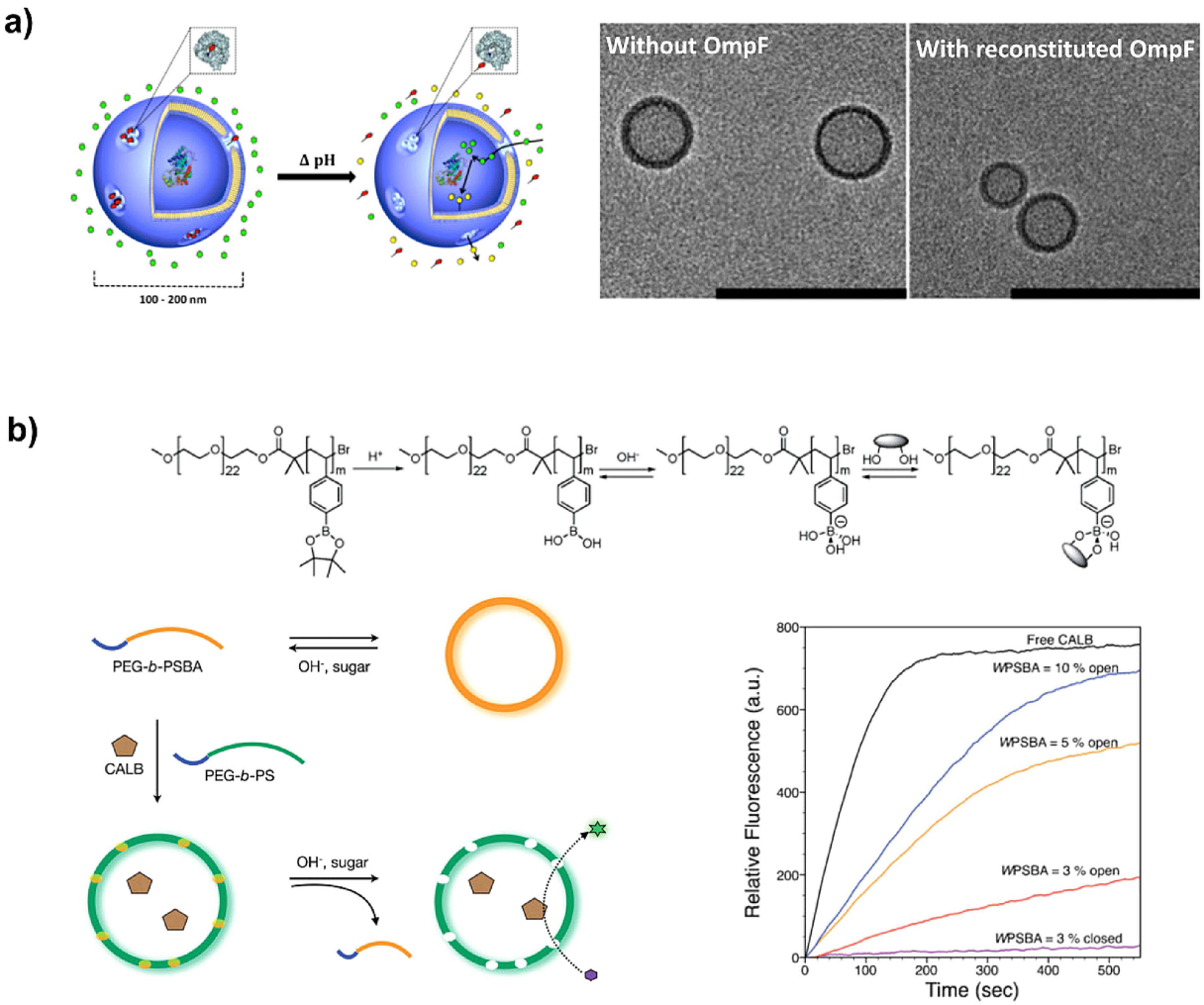


**Figure 5. a)** Left: Schematic representation of a nanoreactor with triggered activity by a chemically engineered protein "gate" inserted in a polymersome membrane. A change in pH induces the release of the sensitive molecular cap (green dots) from the protein "gate" allowing the entrance of substrates (red dots), and the release of the products of the enzymatic reaction (yellow dots). Right: Cryo-TEM micrographs of: nanoreactors without OmpF, and with reconstituted OmpF-WT, Scale bar = 200 nm. Reproduced with permission from Ref 25. Copyright 2015, American Chemical Society. **b)** Left: Schematic representation of the formation of polymersome nanoreactors with a permeable membrane utilizing the pH responsiveness of the block copolymer PEG-b-PSBA. Right: Activity assay of the bioreactors prepared from the permeable polymersomes with $W_{PSBA}$ 3, 5, and 10%, compared to unencapsulated CALB and the closed polymersomes with $W_{PSBA}$ 3% encapsulating CALB in the inner compartment. Reproduced with permission from Ref 26. Copyright 2009, Wiley-VCH.

reactors. Palivan et al. demonstrated a pH-responsive $PMOXA_6$-*b*-$PDMS_{44}$-*b*-$PMOXA_6$ polymersome nanoreactor by inserting channel protein OmpF in the membranes.[25] OmpF was chemically modified with a pH-responsive molecular cap to serve as sensitive "gate". Therefore, the "on-off" switch of the OmpF gates was achieved by tuning the pH value of the nanoreactor solution, which regulated the diffusion of molecules through the channel in the polymersome membrane. When the catalytic activity of HRP-filled nanoreactors was investigated, the enzyme activity was shown to be much higher at pH = 5.5 than at pH = 7.4, suggesting the pH-mediated control of the accessibility of the enzyme for its substrate (Figure 5a). This system opens new opportunities for the design of adaptive polymersome nanoreactors capable of responding "on demand".

Another versatile approach towards permeable nanoreactors is to introduce a sacrificial copolymer, which is blended with normal amphiphilic copolymers during the formation of polymersomes. The sacrificial hydrophobic block bearing stimulus-responsive groups becomes hydrophilic in response to the trigger, and therefore is removed from the polymersome membranes, leading to a porous structure. van Hest et al. have shown this principle by developing a PEG-*b*-PS polymersome mixed with PEG-poly(styrene boronic acid) (PSBA) which is pH-responsive.[26] In the presence of alkaline media and upon addition of glucose or fructose, the boronic acid moieties were ionized to boronate and complexed with the saccharides, giving rise to an increased solubility of the PSBA block in water, and subsequent removal from the polymer bilayer, leaving behind permeable polymersomes. The degree of porosity of the polymersomes was adjusted by varying the ratio between PEG-*b*-PS and PEG-*b*-PSBA, allowing for the transportation of

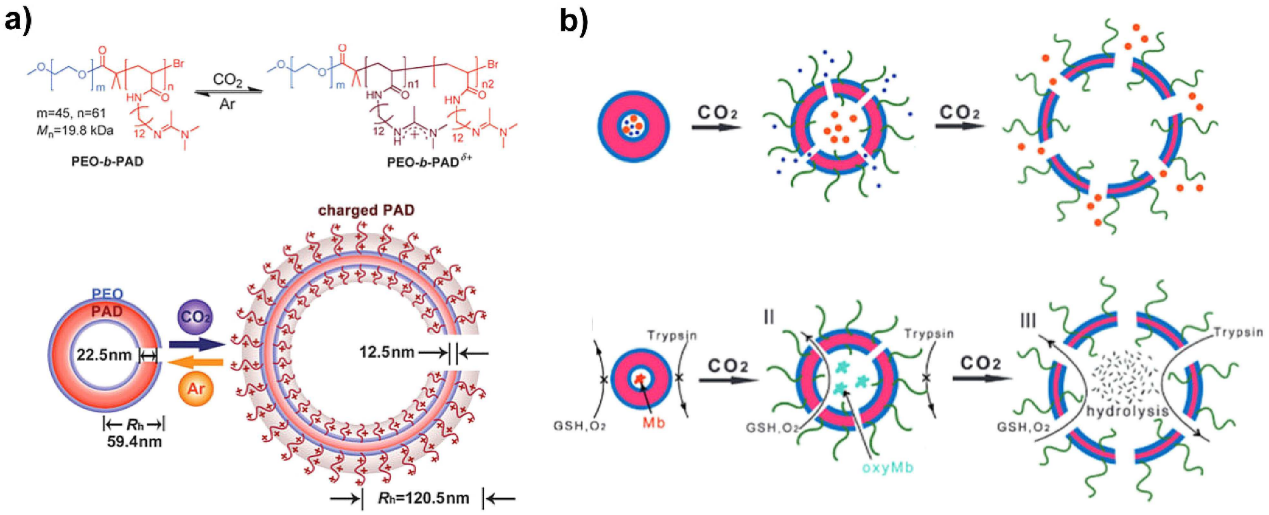


**Figure 6. a)** Schematic representation of the self-assembly of diblock copolymer PEO-b-PAD into vesicles and their reversible gas-responsive "breathing" in aqueous media as $CO_2$-responsive polymersomes. Reproduced with permission from Ref 28a. Copyright 2011, Wiley-VCH. **b)** Illustration of the compartmentalization of two enzymatic reactions in PEG-b-PAD polymersomes modulated by $CO_2$ levels. Reproduced with permission from Ref 30. Copyright 2013, Wiley-VCH.

substrates across the membranes and the execution of enzymatic reactions inside the polymersomes (Figure 5b).

### 3.2. $CO_2$-Responsive Polymersome Nanoreactors

As one of the well-known intracellular biosignals, $CO_2$ has been widely used to mediate responsive systems because of its fascinating features such as easy manipulation, good biocompatibility, and excellent biomembrane permeability.[27] It can efficiently interact with some specific functional groups such as tertiary amines, amidine, and guanidine groups to render them hydrophilic. Since these reactions are reversible, $CO_2$ can be readily removed by introducing an inert gas (e.g. argon or nitrogen) into the system or by heating, which allows the $CO_2$-sensitive species to be restored to their initial state. Compared to traditional responsive systems, the $CO_2$-switched on/off cycles can be performed many times by repeated addition and removal of $CO_2$ without any contamination or dilution derived from the inevitable accumulation of chemicals. Moreover, the strength or magnitude of $CO_2$ responsiveness can be easily achieved by regulating the amount of $CO_2$ by controlling the flow of the gas. Hence, $CO_2$ is particularly regarded as a truly "green" and smart stimulus to the system, and great efforts have been devoted to this concept.[28]

In a similar fashion to the design of pH-sensitive polymersomes, $CO_2$-responsive polymersomes were obtained by integrating $CO_2$-sensitive units in amphiphilic block copolymers.[29] For example, Yuan et al. reported the first example of $CO_2$-responsive polymersomes consisting of amidine-containing amphiphilic copolymer PEG-b- poly(N-amidino) dodecyl acrylamide (PAD).[28a] The polymer self-assembled into a vesicular structure with a radius of 60 nm. When these polymersomes were treated with $CO_2$ for 20 min, they swelled to a size of 120 nm, because of the $CO_2$ induced protonation of PAD, and their volume underwent a remarkable increase up to 800%. Interestingly, these polymersomes were able to contract back to the original size when Ar was passed through the solution to remove $CO_2$. In such a way, the size and volume of these polymersomes were well modulated by alternating treatment with $CO_2$ and Ar (Figure 6a). These polymersomes with intriguing expansion and contraction can be regarded as functional "breathing" nanocontainers for controlled drug release.

Inspired by the exclusive "breathing" feature of these polymersomes, this principle was extended to an intelligent gas-tuned polymersome nanoreactor to mimic the selectivity of cell membranes.[30] The volume expansion and contraction of the polymersomes was expected to give rise to a significant change in polymer membrane thickness, which allowed for the selective transportation of small molecules across the membrane. Indeed $CO_2$-triggered polymersome swelling regulated the membrane permeability and enabled the selective release of nanoparticles modulated by $CO_2$ levels. Furthermore, myoglobin (Mb) was loaded in the lumen of the polymersomes to serve as a nanoreactor. When the nanoreactor solution was treated with $CO_2$ for 5 min, glutathione (GSH) was able to travel through the bilayer to undergo an enzymatic reaction generating $O_2$-carrying myoglobin (oxyMb). When the $CO_2$ stimulus was prolonged to 15 min, further swelling of the membrane led to enhanced permeability, which allowed trypsin, a relative higher molecular weight protease, to pass the membrane to degrade Mb (Figure 6b). Hence, through controlling $CO_2$ stimulation strength, these smart polymersomes with controlled permeability can be utilized as a good platform to specifically perform two different enzymatic reactions, which show promise for the development of biomimetic cytomembranes.

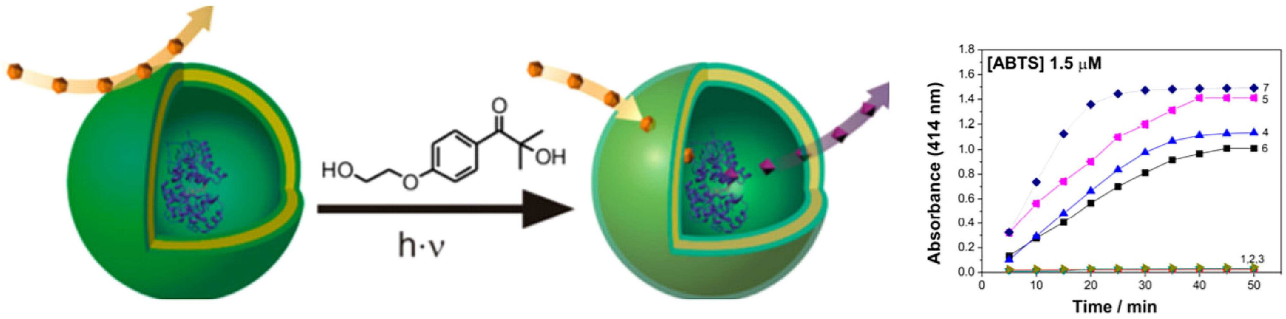


**Figure 7.** Left: Illustration of the photoreaction of a hydroxyalkyphenone with the membrane of polymersomes enabling the generation of semipermeable photo-responsive polymersome nanoreactors. Right: HRP activity assays with ABTS as a substrate to prove the concept of light-induced activation of nanoreactors. (1–3) HRP-filled polymersomes before photoreaction with PP–OH. (4–6) HRP-filled polymersomes after photoreaction with PP–OH. (7) Free HRP. Reproduced with permission from Ref 32. Copyright 2013, American Chemical Society.

### 3.3. Light-Responsive Polymersome Nanoreactors

Photo-responsive self-assembled systems have recently gathered considerable attention because the sensitive behavior of the assemblies can be rapidly and conveniently triggered at a specific time and location upon exposure to visible, ultraviolet, and near-infrared light.[31] In general, photosensitive species are incorporated in copolymers that function as light-cleavable linkers undergoing light-mediated degradation, or that can adopt light-responsive conformational changes, such as azobenzene. Integrating these light-responsive groups in polymersomes can be employed to regulate polymer membrane properties, such as permeability. This principle has already been utilized to some extent to design photo-responsive polymersome nanoreactors.

For example, Bruns et al. described a convenient and versatile approach to create UV-responsive nanoreactors by encapsulating enzymes inside the polymersomes which contained a hydroxyalkylphenone within the membrane (Figure 7 a).[32] The polymersomes were constructed from α,ω-hydroxy-end-capped PMOXA-*b*-PDMS-*b*-PMOXA, α,ω-acrylate-end-capped PMOXA-*b*-PDMS-*b*-PMOXA, or PEO-b-PB, respectively. The two latter polymers, bearing double bonds at the chain ends or in the main chain PB block tend to be attacked by radicals. To generate radicals, one water-soluble α-hydroxyalkylphenone, 2-hydroxy-4-2-(hydroxyethoxy)-2-methylpropiophenone (PP–OH), was embedded in the membranes. Applying UV irradiation to the polymersome solution, PP–OH produced two primary radicals which reacted with the polymer membranes. By choosing the appropriate reaction conditions, the formed radicals did not lead to crosslinking but instead were added to the hydrophobic polymer domains, thereby increasing their hydrophilicity, which enhanced the permeability of the polymersome membranes. HRP was encapsulated inside the polymersomes to generate nanoreactors with a photo-triggered "ON" state; the protective effect of the nanoreactors was shown as the encapsulated enzymes were protected against degradation by proteases.

Another interesting work regarding light-responsive polymersome nanoreactors based on Donor-Acceptor Stenhouse Adducts (DASAs) was recently reported by Bruns and coworkers.[33] The polymers consisted of hydrophilic PEG and hydrophobic poly(pentafluorophenyl methacrylate) (PFPMA) and poly(hexyl methacrylate) (HMA). The aromatic amine precursor N-(4-methoxyphenyl)-1,3-diaminepropane (MPDP) was conjugated to the randomly distributed activated ester segments over the hydrophobic block, yielding DASA-bearing block copolymers. DASAs were able to isomerize upon irradiation with visible light, which gives rise to a permeability change of the polymersome membranes by a transition from a nonpolar triene-enol state to a cyclopentenone form with increased polarity. The release of hydrophilic payload could be triggered by light and stopped as soon as the light was turned off. Encapsulating enzymes inside these polymersomes led to the formation of photo-responsive nanoreactors in which case enzymatic reactions could be triggered "on" by light and stopped immediately when the light irradiation was removed. Interestingly, mixing two classes of polymersome nanoreactors, which were switched with green light and red light respectively, allowed for light-controlled wavelength-selective catalysis that enabled specific regulation of individual biocatalytic steps of a cascade reaction in a one pot process (Figure 8).

## 4. Self-Adaptive Polymersome Nanoreactors

Out-of-equilibrium processes are ubiquitous in nature and often characterized by adaptive, transient behavior. Translating this behavior onto functional self-assemblies is an exciting way to create complex, biomimetic systems. In this regard, fuel-driven structural and functional operations under temporal control are thought to play a key role in bridging man-made materials and biology in terms of natural organization and function. Over the years, synthetic molecular or nanoscaled out-of-equilibrium systems have been demonstrated for active or self-adaptive materials with exclusive properties such as dissipative fibers,[34] transient peptide hydrogels,[35] temporally programmed supramolecular polymers[36] and non-equilibrium molecular recognition and colloidal systems.[36–37] Regardless of the excellent performance of these systems, it still remains a major

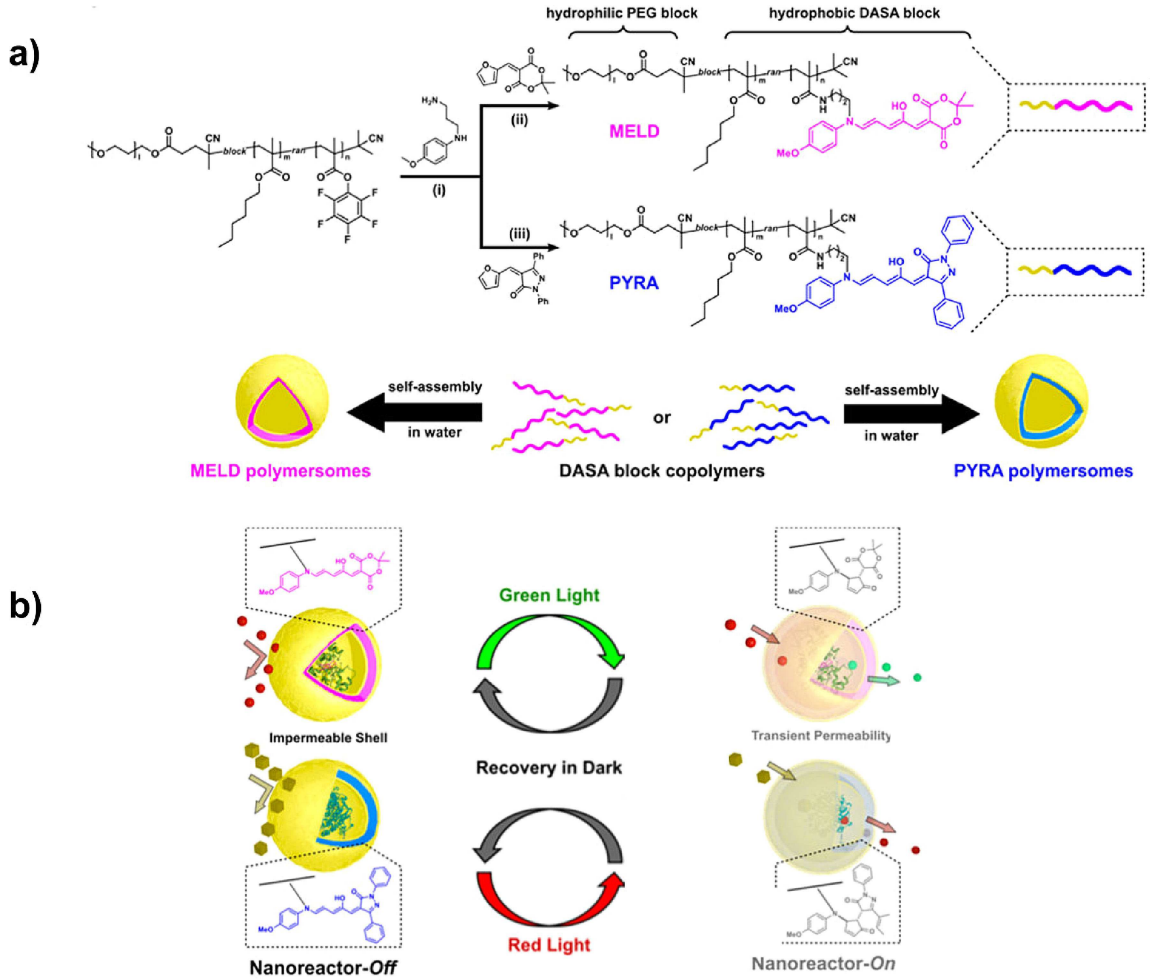


**Figure 8. a)** Preparation of DASA-functionalized visible light-responsive polymersomes. DASA-functionalized block copolymers MELD and PYRA were synthesized in two steps from an active ester block copolymer. **b)** Schematic representation of DASA-bearing visible light-responsive polymersome nanoreactors. Two different DASAs were conjugated with the polymersomes to serve as enzyme-loaded nanoreactors which responded to irradiation with green light and red light respectively. The DASA moieties in the hydrophobic part of the polymer membrane underwent a conformational change upon applying the light stimulus, leading to an increased permeability of the polymersome membrane and activation of the enzyme nanoreactor. The nanoreactors were restored back to their initial impermeable state when the light was turned off. Reproduced with permission from Ref 33. Copyright 2018, American Chemical Society.

challenge to include this behavior in polymersome systems, creating self-adaptive polymersome nanoreactors which are mediated by energy input and consumption.

Limited examples come from van Hest and coworkers. For example, they described a compartmentalized out-of-equilibrium enzymatic reaction network for sustained autonomous movement.[38] The system was based on bowl-shaped PEG-*b*-PS polymersomes, named stomatocytes, which are formed *via* the osmotic pressure induced shape transformation of PEG-*b*-PS spherical polymersomes. These stomatocytes were turned into nanoreactors by the encapsulation of a multienzyme metabolic network (Figure 9a). The bowl-shaped nanoreactor structures were confirmed with cryo-transmission electron microscopy (cryo-TEM) (Figure 9b). As basic sources of fuel glucose and phosphoenol pyruvate (PEP) were used. Glucose was converted by hexokinase (HK) in an ATP dependent manner. ATP was recreated using Pyruvate Kinase (PK). As the conversion of glucose was dependent on the ATP concentration, the flux of glucose through the system was decoupled from its initial concentration. PK also contributed to the formation of pyruvate, which entered the pyruvate-L-lactate cycle, where L-lactate dehydrogenase (LDH) consumed pyruvate; this reaction was at the same time opposed by L-lactate oxidase (LO). Continuous addition of pyruvate allowed for the acceleration of this cycle until a steady state was reached which was caused by the feedforward inhibition at high concentrations of pyruvate on LDH (pyruvate-L-lactate cycle). More importantly, the function of the pyruvate-L-lactate cycle was coupled to a motor cycle containing catalase (CAT) which catalyzed the production of $O_2$ from $H_2O_2$ to activate the motion of the system (motor cycle) (Figure 9c). Compared to a simple one-step enzymatic reaction, the out-of-equilibrium enzymatic network in this work was able to control the energy consumption while keeping the constant

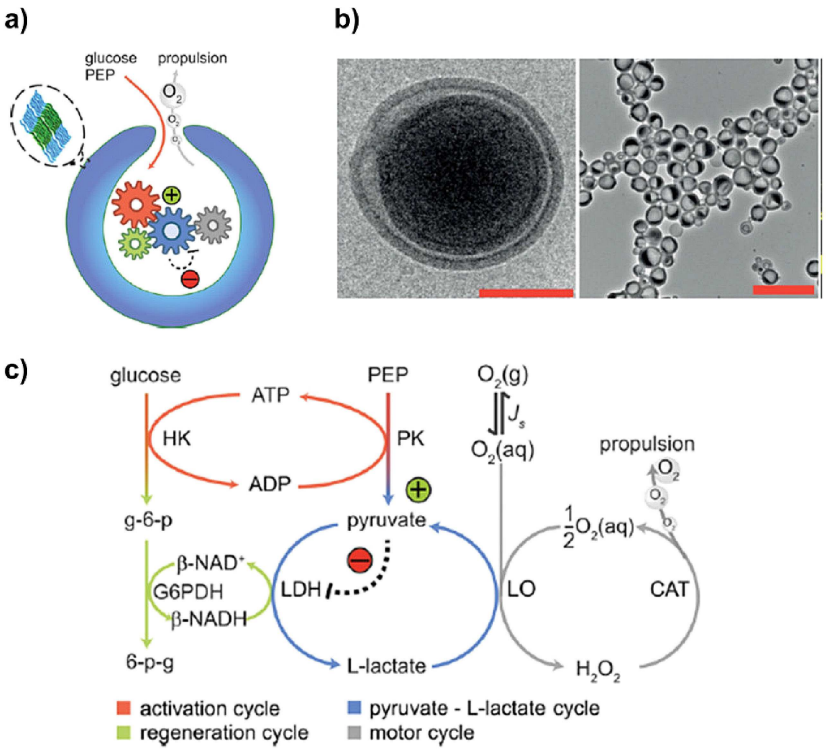


**Figure 9. a)** Schematic representation of the stomatocyte nanoreactors containing four enzymatic cycles which are utilized to convert glucose and phosphoenolpyruvate (PEP) into movement of the nanoparticles. **b)** Cryo-transmission electron microscopy (cryo-TEM, left) and TEM (right) images of the nanoreactors loaded with the enzymatic network. Scale bars 100 nm (left) and 1 μm (right). **c)** Rational design of a metabolic pathway for double cycling of natural substrates leading to autonomous movement. Reproduced with permission from Ref 38. Copyright 2016, American Chemical Society.

velocity of the stomatocytes, even with widely varying concentrations of fuel (glucose).

More recently, van Hest et al. demonstrated the construction of a self-regulated and time programmed polymersome nanoreactor.[39] This system was based on an earlier developed "breathing" microgel system. Herein, pH sensitive diethylamine moieties incorporated in a crosslinked polymer network were protonated upon addition of acid, which resulted in an increase in polarity and subsequent swelling of the gel. The encapsulated enzyme urease at the same time converted urea into ammonia, thereby restoring the initial high pH of the solution, which led to deprotonation and deswelling.[40] Upon the repeated addition of chemical fuel, the size switch of the microgel could be driven several cycles, endowing the microgel with cell-like "breathing" features.

To expand the microgel to the feedback-induced temporal control of "breathing" polymersomes in order to create self-adaptive nanoreactors, they prepared pH-responsive polymersomes consisting of hydrophilic PEG, hydrophobic PDEAEMA and poly[2-hydroxy-4-(methacryloyloxy) benzophenone] (PBMA) and fluorescein as a fluorescent read out system. To create nanoreactors, urease, which controlled the pH change, and HRP, which acted as a model enzyme, were encapsulated in the polymersomes. In the presence of high pH media, the polymersomes shrunk as a consequence of deprotonation of the pH-sensitive PDEAEMA blocks. In this situation, the membranes of the nanoreactors were impermeable and substrates were unable to pass through the membranes, giving rise to a nanoreactor "OFF" state. Introducing chemical fuel (HCl and urea) resulted in a rapid pH decrease, thereby leading to an increasing size of the polymersomes. In this case, the permeability of the membranes was greatly increased, allowing the substrate to penetrate into the polymersomes and turning the nanoreactor in the "ON" state. However, this nanoreactor "ON" state was transient. Over time, a gradual increase in pH was observed by virtue of the conversion of urea into ammonia. Hence, polymersomes recovered to their initial impermeable state and the enzyme catalysis automatically changed to the "OFF" state again (Figure 10a). In addition, it was found that the higher the concentration of urea was used, the faster the catalysis reached its end point (Figure 10b). Similar to the transient behavior of the polymersome swelling and shrinking, consecutive nanoreactor "OFF-ON-OFF" cycles could be accomplished upon multiple additions of chemical fuel (Figure 10c). In comparison with other reported polymersome nanoreactors, the main difference is the fuel-mediated self-adaptive behavior; the basic design rules in this work will facilitate engineering of artificial organelles with adaptive features.

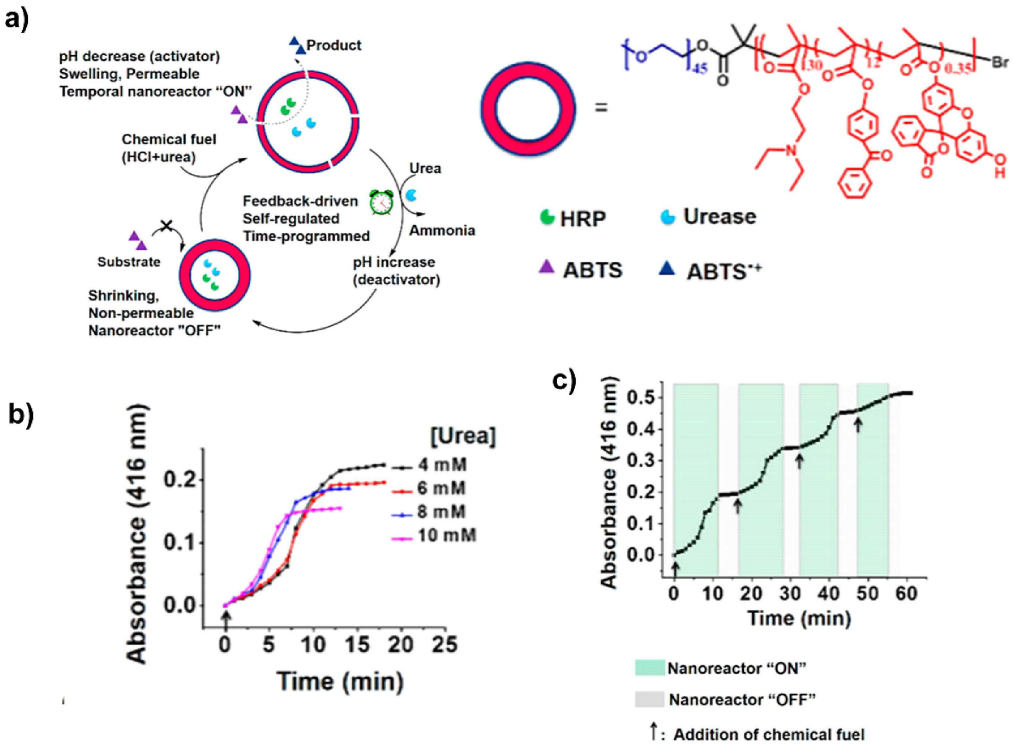


**Figure 10. a)** Schematic overview of feedback-induced temporal control of polymersome nanoreactors. **b)** UV absorbance at 416 nm of the oxidation of ABTS by nanoreactors upon the addition of different concentrations of urea. **c)** Reversible nanoreactor "ON-OFF" regulation in time following repeated additions of urea. Reproduced with permission from Ref 39. Copyright 2018, American Chemical Society.

## 5. Polymersome Nanoreactors as Artificial Organelles in vitro and in vivo

By making use of various approaches to trigger porosity or permeability in an adaptive manner, polymersome nanoreactors have become a more complex model of naturally occurring systems. From an application perspective, a next step forward, following these advances, is the utility of polymersome nanoreactors as actual artificial organelles *in vitro* and finally *in vivo*.[41] For example, polymersome nanoreactors can treat certain diseases by *in cellulo* converting toxic substances into nontoxic ones. In some cases, the *in situ* conversion of nontoxic prodrugs into therapeutic agents by polymersome nanoreactors shows great promise to address challenging diseases such as cancer. In recent years, the capacity of polymersome nanoreactors to be active in specific biological environments is now being harnessed for their application as artificial organelles. Several groups have already made fascinating progress in this emerging field of research.

An early example of a polymersome nanoreactor system exploited in cells was reported by Hunziker et al. who introduced trypsin-loaded PMOXA-b-PDMA-b-PMOXA polymersomes into cells, to serve as specific functional artificial organelles.[7a] The same polymersomes were utilized to encapsulate superoxide dismutase (SOD) and actoperoxi-dase (LPO) to lower artificially increased ROS levels.[8e] This system was extended to show the capability of these nanoreactors to escape the endolysosomal pathway to function inside the cytoplasm of HeLa cells despite of their large size (200 nm).[41c]

To effectively improve the uptake of polymersome nanoreactors in mammalian cells, van Hest et al. developed functional cell-penetrating peptide (CPPs) modified polymersome nanoreactors to mediate cellular uptake of the artificial organelles.[11b] In particular the HIV-derived tat peptide was used as CPP. The polymersomes were prepared by coassembly of PS-PIAT with 10 wt% PS-PEG-tat, enabling access to a tat-polymersome. Compared to tat-free polymersomes, the uptake of tat-polymersomes in HeLa cells was greatly promoted. To prove that the polymersome nanoreactors were able to function *in vitro,* HeLa cells were incubated with HRP-loaded tat-polymersomes. It was found that after incubation for 16 h, the nanoreactors still maintain 42% of the initial activity which is higher than free HRP trafficked to lysosomes, suggesting significant progress towards a functional artificial organelle (Figure 11).

In order to make the application of polymersome nanoreactors as therapeutic modalities viable, the structures need to be composed of biodegradable components. Recently, van Hest and coworker described enzyme-filled, biodegradable semipermeable poly(ethylene glycol)-block-poly(caprolactone-gradient-trimethylene carbonate) (PEG-P(CL-**g**-TMC)) polymersomal nanoreactors, readily assembled with the help of a direct hydration methodology (Figure 12).[42] Using the hydration method for the fabrication of biodegradable polymersomes avoided the involvement of organic solvent and facilitated

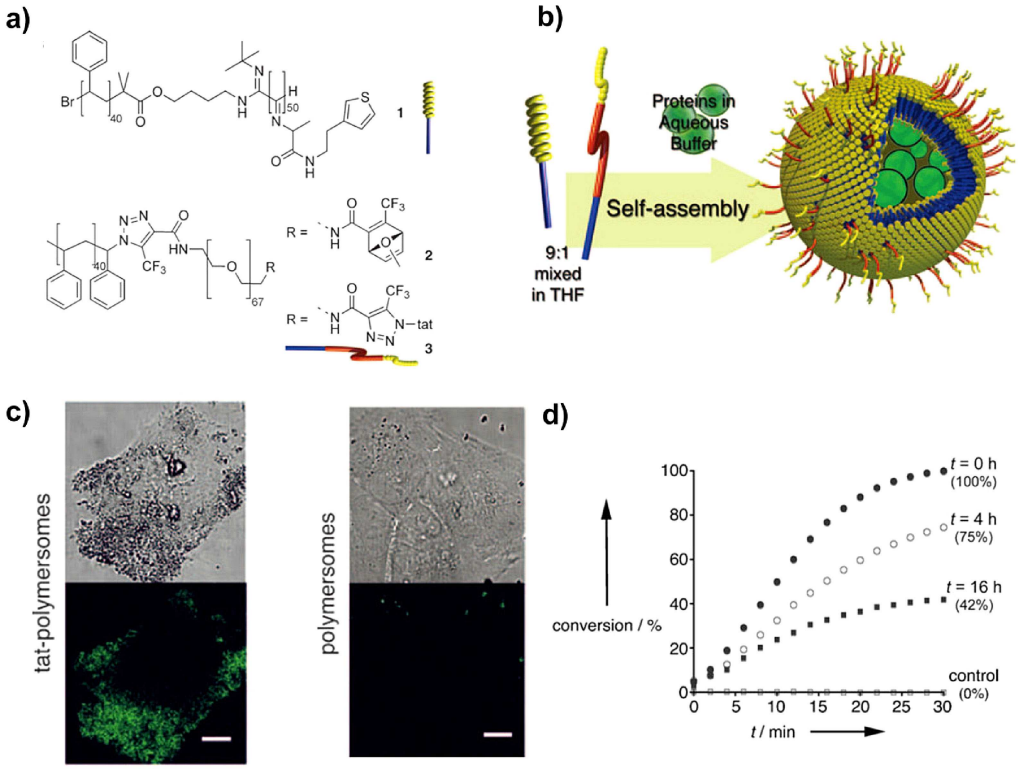


**Figure 11. a)** Structures and representations of the polymers used for the construction of a tat functional polymersome nanoreactor. **b)** Formation of enzyme loaded tat-polymersomes. **c)** Confocal micrographs of HeLa cells incubated with GFP-loaded polymersomes with or without tat. Scale bars: 10 mm. **d)** Activity essay of intracellular HRP-loaded tat-polymersomes over time. Reproduced with permission from Ref 11b. Copyright 2010, Wiley-VCH.

biomolecular encapsulation; as such, this method can be regarded as a "green" approach to the preparation of polymersomes.[43] To induce the uptake of these polymersomes, they were also functionalized with the cell-penetrating peptide tat. Cell uptake evaluation of the particles in HEK293T cells indicated the strong uptake-facilitating properties of tat-polymersomes relative to unmodified polymersomes. To generate biodegradable antioxidant nanoreactors (AONs), two isoforms of catalase, from bovine (CBOV) and bacterial (CBAC) sources were encapsulated in tat-PEG-P(CL-*g*-TMC) polymersomes. To impart the functionality of the AONs as synthetic organelles to these nanoreactors, their capability to shield HEK293T cells against the detrimental effects of exogenous $H_2O_2$ was studied. Crystal violet analysis displayed that both AONs can fully protect against $H_2O_2$ cytotoxicity. More importantly, using these AONs, for the first time, patient derived human-complex-I-deficient primary fibroblasts were effectively protected against the toxicity of exogenous $H_2O_2$, which indicated their therapeutically relevant application potential.

Considering the fact that nanoreactors are capable of actively transforming prodrugs into original drugs for cancer killing, which leads to higher therapeutic efficacy and lower side effects, the investigation of polymersome nanoreactors for this *in vivo* therapeutic application as artificial organelles is a logic step forward. For example, Kataoka et al. developed an injectable enzyme-loaded PICsome system as a model *in vivo* nanoreactor that functions in tumors.[44] The PICsomes with a well-controlled size were obtained by simply mixing PEG-based block aniomers (PEG-*b*-PAsp), catiomers (Homo-P(Asp-AP)), and β-galactose (β-gal) or lysozyme. Cross-linked β-gal@PICsomes nanoreactors were obtained by cross-linking them with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Figure 13a). The formed nanoreactors with a diameter of 100 nm displayed selective accumulation in C26 tumor tissues, which is in agreement with the enhanced permeability and retention (EPR) effect.[45] To prove the feasibility of their utility as *in vivo* nanoreactors, β-gal@Cy5-PICsomes and β-gal were injected intravenously into the tail vein of mice bearing C26 tumors. As a model prodrug, HMDER-β-Gal, was introduced after the injection of nanoreactors, and the *in vivo* catalytic behavior was studied by employing an *in vivo* imaging system (IVIS). As expected, only the tumor site of the mouse treated with β-gal@Cy5-PICsomes showed substantial HMDER fluorescence, while the tumor site treated with free β-gal demonstrated negligible fluorescence (Figure 13b). Interestingly, *ex vivo* fluorescence imaging indicated that negligible fluorescence was found in major organs (liver, lung, spleen, kidney; Figure 13c) indicating detected fluorescence of HMDER was only from the tumor tissue.

A challenging step in development of artificial organelles *in vivo* is the activation of the polymersome nanoreactors by a specific endogenous trigger inside cells. The design and construction of artificial organelles with triggered activity *in vivo* demonstrate necessary steps towards the creation of cell

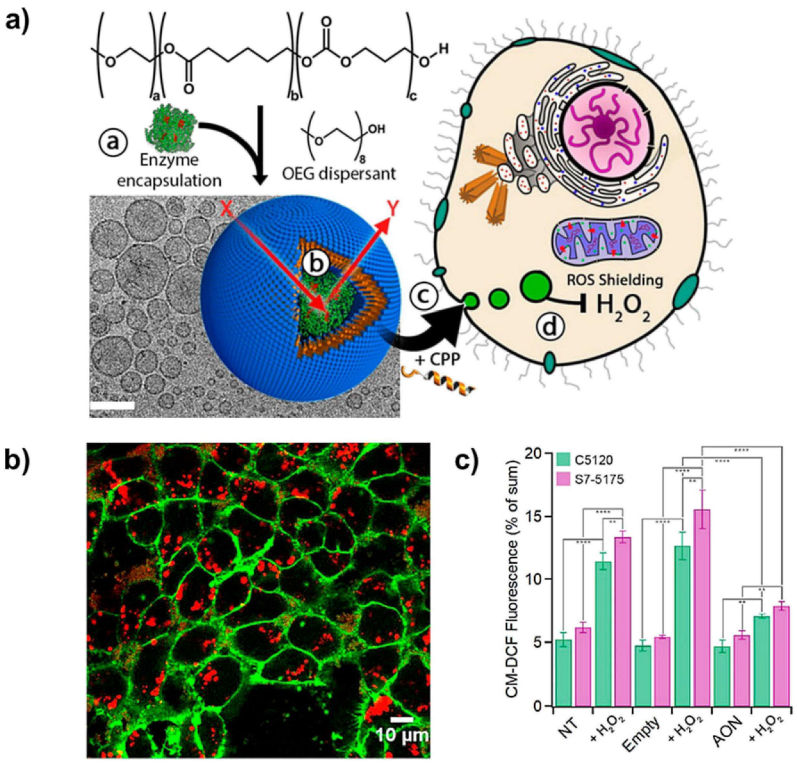


**Figure 12. a)** Preparation of biodegradable tat-PEG-PCLgTMC polymersomes by the direct hydration method, and their use as antioxidant polymersomes (containing catalase) demonstrating ROS shielding in both HEK 293 and (primary) human fibroblasts. Scale bar: 100 nm. **b)** Confocal microscopy images showing the subcellular distribution of 2.5% tat-polymersomes encapsulating AF647 labeled BSA (red) after 24 h of incubation at a concentration of 0.4 mg/mL (plasma membrane stained with CellMask green). **c)** Intracellular ROS assessed by CM-DCF fluorescence intensity measurements after treatment with 0.4 mg/mL polymersomes for 24 h prior to challenge with or without 0.5 mM $H_2O_2$ for 5 min. Reproduced with permission from Ref 42. Copyright 2018, American Chemical Society.

implants, and holds great potential for personalized medicine by a straightforward change of the biomolecules inside the artificial organelles. More recently, Palivan's group presented the design of smart polymersome nanoreactors with an *in situ* enzymatic reaction that is induced by the presence of an intracellular trigger, and demonstrated *in vitro* and *in vivo* utility.[46] The $PMOXA_6$-*b*-$PDMS_{44}$-*b*-$PMOXA_6$ polymersome membranes were equipped with a cysteine double mutant of OmpF (OmpF–M), which responds to changes in GSH concentrations in intracellular environments, thereby blocking/ unblocking the protein pores (Figure 14a and 14b). The *in situ* activity regulation of the encapsulated HRP in the vertebrate zebrafish embryo (ZFE) model showed the *in vivo* functionality of these artificial organelles (Figure 14c). This work presents the feasibility of using polymersome nanoractors as cellular implants in living organisms, and is expected to open new perspectives for patient-oriented protein therapy.

In some cases, cooperative cancer therapy is needed when engineering a nanoreactor system with tumor activable cascade reactions. Recently, Ge et al. rationally designed ultrasmall iron oxide nanoparticles (USIONs) and GOX encapsulated hybrid polymersome nanoreactors (Fe/G@R-NRs) for cooperative cancer therapy on the basis of hydroxyl radicals (•OH) and pH-responsive block copolymer prodrugs (Figure 15).[47] The amphiphilic block copolymers bearing prodrugs were synthesized *via* the reversible addition-fragmentation chain transfer (RAFT) copolymerization of the camptothecin (CPT) monomer (CPTKMA or CPTMA) and 2-(pentamethyleneimino) ethyl methacrylate (PEMA) by using PEG-CTA. USIONs and GOX were coloaded into the polymer membrane and inner cavity of PEG-*b*-P(CPTKMA-co-PEMA) polymersomes, respectively, to generate ROS-responsive therapeutic nanoreactors (Fe/G@R-NRs). Considering the ultrasensitive pH-responsive property of PEMA segments with a $pK_a$ approximate to 7.0, the low pH media at the tumor site offered the nanoreactors acid-induced membrane permeability for selective transportation of small molecules. *In vitro* cell experiments showed efficient ROS production and CPT release from Fe/G@R-NRs at pH 6.5. To evaluate the *in vivo* antitumor efficacy, A549 tumor models were treated with Fe/G@R-NRs. The most efficient tumor growth inhibition was found when the tumor was treated with Fe/G@NRs, while ROS-nonresponsive nanoreactors (N-NRs) and other control groups showed lower anti-tumor efficacy. These fascinating findings in this study demonstrate a robust and versatile nanomedicine engineering approach to orchestrate cooperative cancer therapy.

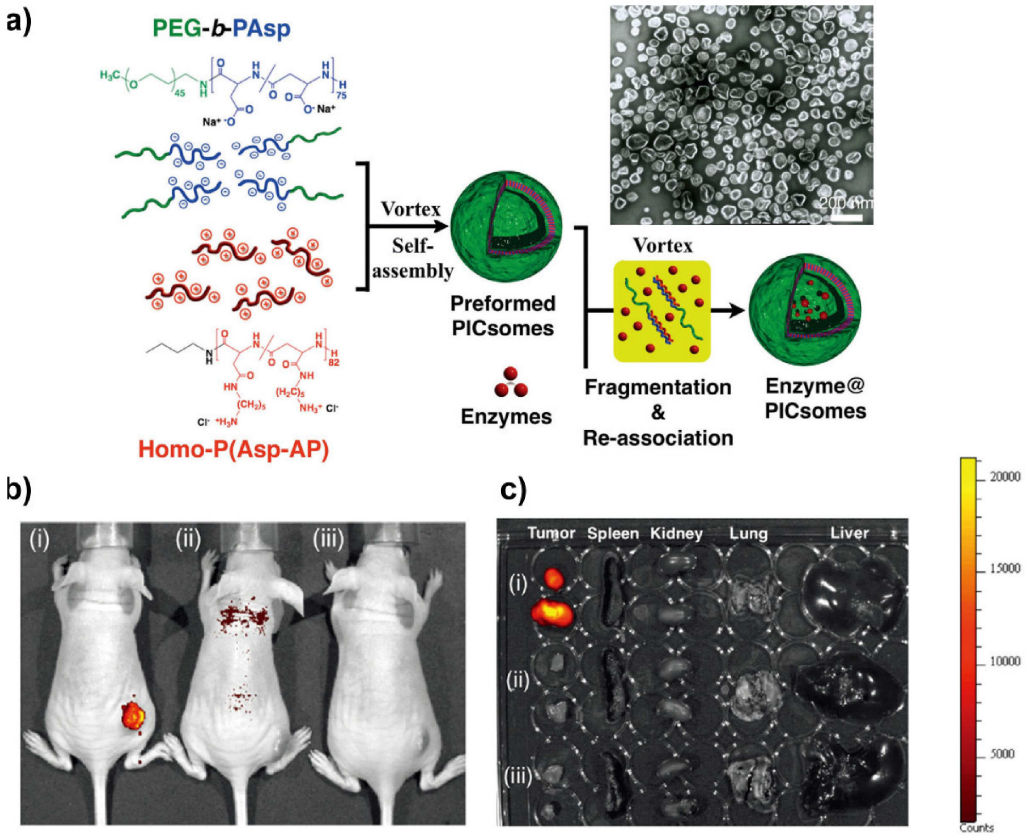


**Figure 13. a)** Chemical structures of the constituent polymers of PICsomes, and preparation of enzyme@PICsomes *via* preformed PICsomes. Top right: TEM image of β-gal@PICsomes (scale bar: 200 nm). **b)** *In vivo* imaging of C26 tumor mice. The tumor site of the mouse was treated with βGal@Cy5-PICsomes (i), free βGal (ii), and HMDER-βGal (iii), respectively. Filter set: $E_x/E_m = 500/560$ nm for HMDER. **c)** *Ex vivo* imaging of the excised tumor and organs. Filter set: $\lambda_{ex}/\lambda_{em} = 500/560$ nm. Reproduced with permission from Ref 44. Copyright 2016, Wiley-VCH.

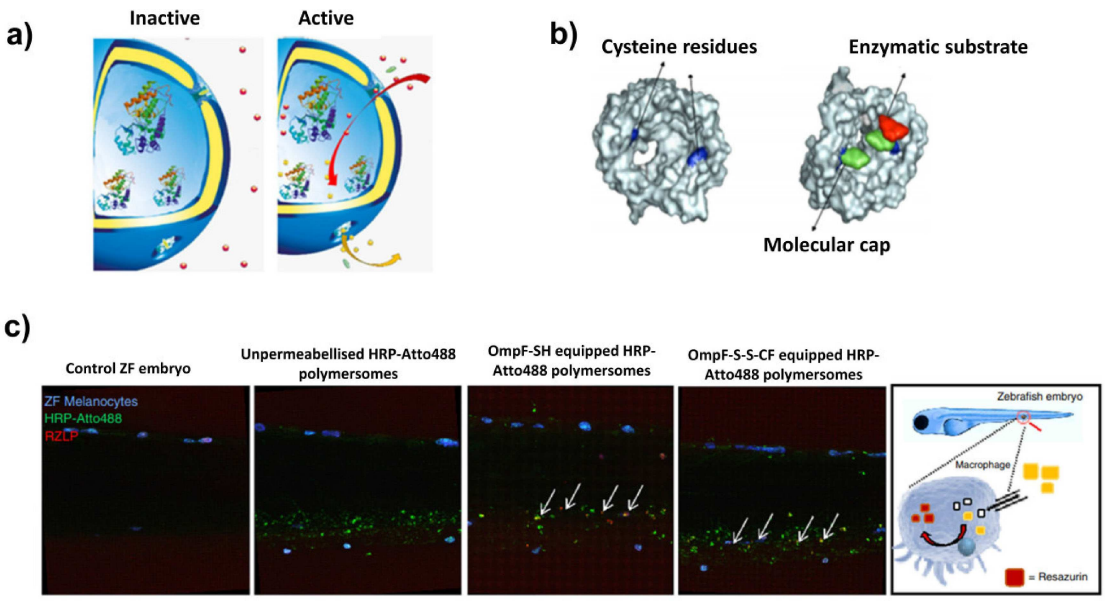


**Figure 14.** a) Schematic representation of modified OmpF acting as a gate in catalytic nanocompartments. b) Molecular representation of the OmpF–M cysteine mutant. c) *In vivo* ZFE biodistribution and activity of artificial organelles. Blue signal: ZFE melanocytes. Green signal: HRP-Atto488. Red signal: Resazurin-like product (RZLP). Arrows show regions of enzymatic activity of artificial organelles. Reproduced with permission from Ref 46. Copyright 2018, Nature Publishing Group.

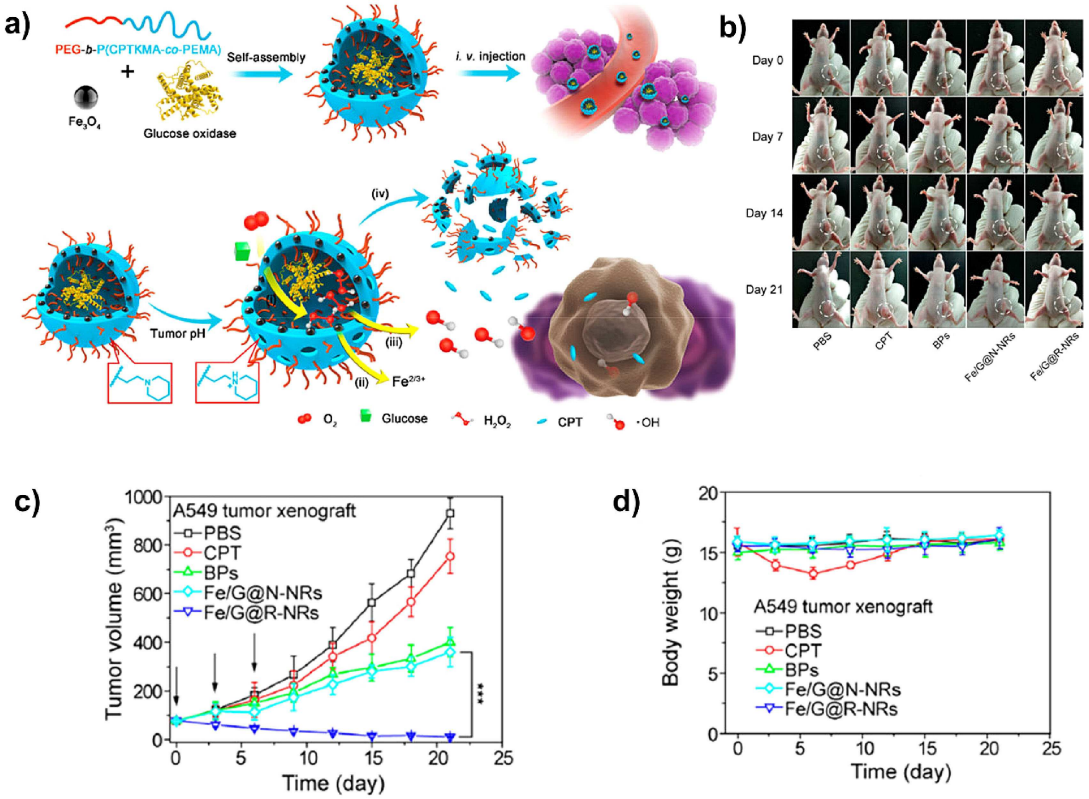


**Figure 15. a)** Preparation of hybrid polymersome nanoreactors Fe/G@R-NRs for cooperative cancer therapy and cascade reactions at tumor sites due to the tumor acid-triggered membrane permeability enhancement. **b)** Typical images of A549 tumor-bearing mice at predetermined time intervals after treatment with various formulations. **c)** Time-dependent A549 tumor growth profiles. The arrows indicate the injection time. Mean ± s.d., n = 5. ***$p < 0.005$ (t-test). **d)** Mouse body weight change over the time during the treatment. Mean ± s.d., n = 5. Reproduced with permission from Ref 47. Copyright 2019, American Chemical Society.

## 6. Conclusion and Perspectives

Adaptive self-assembly plays an important role in nature and shows much promise for a next generation of synthetic materials. Specifically in the fields of nanomedicine and artificial cell research, introducing adaptivity into nanoparticle systems provides possibilities of mimicking more closely cellular structures and engineering functional nanoreactors as artificial organelles. The past decade has witnessed growing interest in the possibilities polymersomes have to serve as nanoreactors. Specifically the design and construction of multicompartment enzymatic nanosystems has developed to such an extent that we can attain control over permeability, catalyst accessibility and positional assembly. In this review, we have summarized recent developments that provide the necessary next step in nanoreactor performance, namely to include adaptive behavior in these polymersome nanoreactors, which extends their application potential as artificial organelles for both *in vitro* and *in vivo* investigations.

To yield permeable polymersomes that can serve as nanoreactors, several approaches utilized by different groups have been established. For example, polymersomes comprised of PS-*b*-PIAT, biodegradable PEG-P(CLgTMC) or polyelectrolytes have been successfully employed as nanoreactors because of their intrinsically permeable polymer bilayer. Also, the introduction of channel proteins like the outer-membrane-protein F (OmpF), into PDMS-*b*-PMOXA-*b*-PDMS membranes allows polymersomes to serve as nanoreactors. Another more bio-inspired and versatile method is to develop environmentally adaptive polymersomes, which can be accomplished by employing stimuli-responsive macromolecules, a class of "smart" polymers that are capable of converting specific environmental changes to functional outputs. The switchable porosity and permeability of such a sensitive polymersome system can be well controlled, allowing for the activity regulation of the enzymatic reaction within the nanoreactors. In addition, we have also put a spotlight on very intriguing but less reported self-adaptive polymersome nanoreactor systems which show real life-like behavior by keeping the nanoreactors in an out-of-equilibrium fashion. The concept of self-adaptive polymersome nanoreactors would enable access to intelligent polymersomes with biomimetic cellular biological functions. Furthermore, recent impressive research has shown that polymersome nanoreactors can be employed *in vivo* as therapeutically artificial organelles.

Despite the above highlighted exciting progress in engineering adaptive polymersome nanoreactors, many pitfalls still remain to be resolved or be taken into consideration on performing follow-up research. Most of the existing achieve-

ments on adaptive polymersome nanoreactors are only at the earlier conceptual stage, and it is highly desired to move forward towards the realization of their implantation in cells and organs. For example, to further enhance the utility of polymersome nanoreactors, an important consideration is using biodegradable and biocompatible polymer building blocks, which allows for the cellular integration without any toxic side effects that would undermine biomedical application.[42] Another pitfall of designing polymersome nanoreactors is that although there has been substantial progress toward stimuli-responsive polymersomes, more attention should be paid to employ these responsive systems to create adaptive nanoreactors, especially the ones functioning in a self-adaptive manner. Last but not least, the nanoreactors are often trapped in endosomes when they are localized inside cells, which undoubtedly limits their applicability *in vitro* and *in vivo*. Hence, new approaches should be developed to precisely redirect the nanoreactors to the cytoplasm, rendering them to serve their purpose as therapeutic artificial organelles.

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## Conflict of Interest

The authors declare no conflict of interest.

**Keywords:** adaptive · self-assembly · polymersomes · nanoreactors · artificial organelles

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