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Controlled Membrane Transport in Polymeric Biomimetic Nanoreactors

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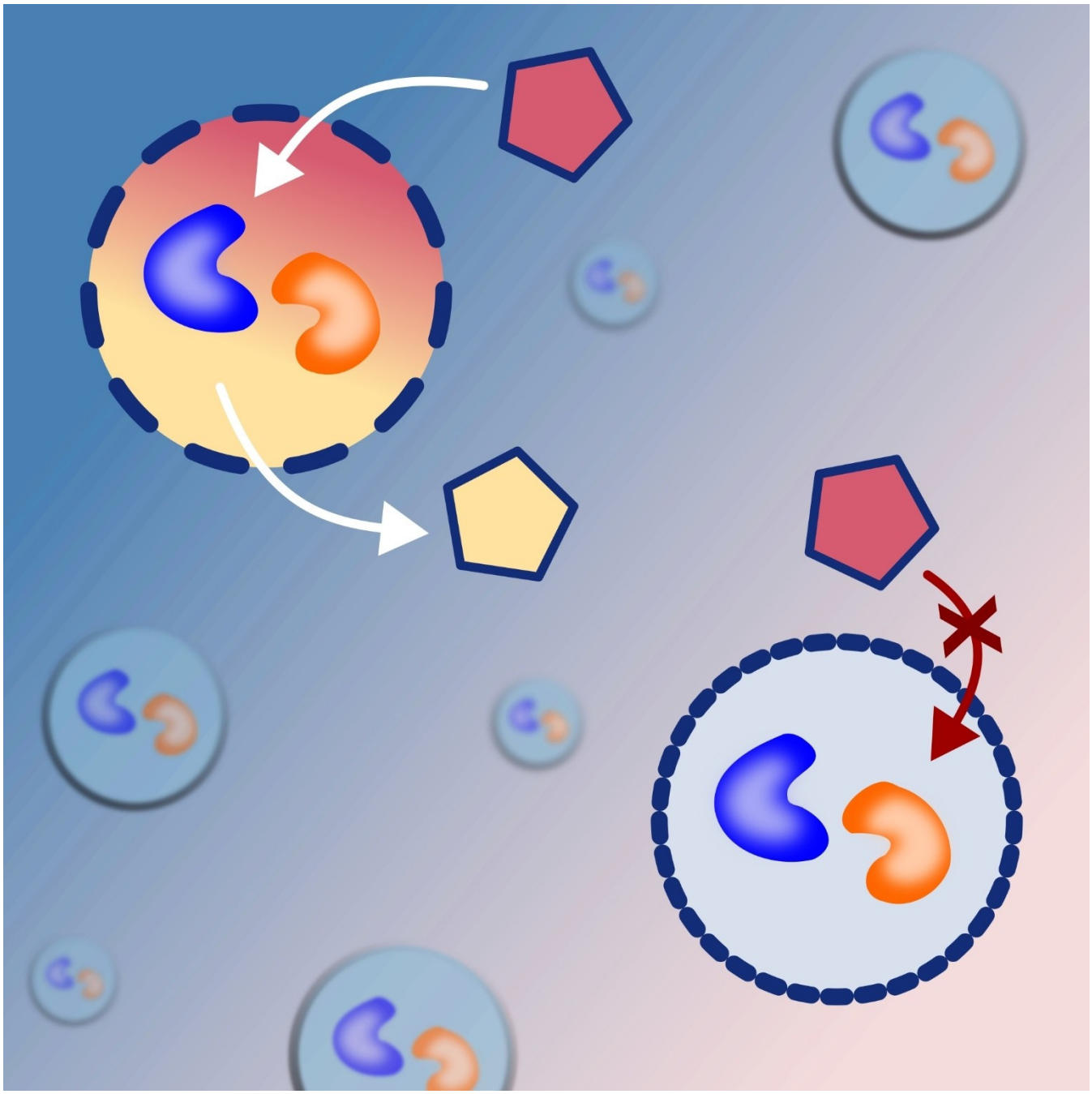

Polymersome-based biomimetic nanoreactors (PBNs) have generated great interest in nanomedicine and cell mimicry due to their robustness, tuneable chemistry, and broad applicability in biologically relevant fields. In this concept review, we mainly discuss the state of the art in functional polymersomes as biomimetic nanoreactors with membrane-controlled transport. PBNs that use environmental changes or external stimuli to adjust membrane permeability while maintaining structural integrity are highlighted. By encapsulating catalytic species, PBNs are able to convert inactive substrates into functional products in a controlled manner. In addition, special attention is paid to the use of PBNs as tailored artificial organelles with biomedical applications in vitro and in vivo, facilitating the fabrication of next-generation artificial organelles as therapeutic nanocompartments.

1. Introduction

The presence of membrane-bound compartments in living cells enables the fine control of important biological processes through various chemical/enzymatic networks. Biological compartments allow cells to modulate behaviour and functions through selective molecular transport and separation of incompatible processes. For example, membrane transport in lipid vesicles can be modelled by mechanisms such as solubility-diffusion, transient pore formation, head-group gated transport, and lipid flipping.[1] The versatility of biological compartments inspired researchers to develop biomimetic nanoreactors that use biological compartmentalization strategies to synthesize complex molecules (Figure 1a).[2]

Compartments are essential for the development of biomimetic nanoreactors. They can be formed from various materials such as lipids (e.g., liposomes), droplets (e.g., coacervates) and capsules (e.g., silica, multilayer polymers).[3] Among these, polymeric vesicles have become an excellent choice for the development of biomimetic nanoreactors.[4] The structural, chemical, and mechanical properties of polymer vesicles can be largely tailored because the diversity of their building blocks (i.e., monomers) is virtually unlimited.[5]

The integration of catalytic species into polymeric vesicles can create a wide range of functional polymeric biomimetic nanoreactors (PBNs). Compared with lipid vesicles and biological vesicles (i.e., extracellular vesicles), the versatility of polymer vesicles allows for customization of properties such as morphology, flexibility, and surface and membrane chemistry.[6] The resulting polymer architectures have been shown to exhibit improved mechanical stability compared to lipid vesicles, allowing the functionality of membrane and surface chemistry to be explored in the development of PBNs.[7]

Structurally, PBNs consist of polymer vesicles containing a catalytic or reactive core with a membrane that separates the internal environment from the external medium and modulates the internal chemical process through molecular transport (Figure 1a).[8]

Due to their versatility, PBNs have found a variety of technological applications in different fields (Figure 1b).[9] For example, protein-loaded PBNs, when immobilized on a solid support, can be used as active biosensors to detect sugar alcohols.[10] As molecular nanofactories, PBNs can be designed to produce and release antibiotics locally.[11] Moreover, by encapsulating catalytic enzymes, PBNs can convert a nontoxic prodrug into a therapeutic drug in dysfunctional target cells while reducing damage to healthy cells.[12] Compared to native enzymes, biocatalysts integrated into PBNs can be protected from potentially harmful environmental effects by integrating biocatalytic species. This enables the development of PBNs as active synthetic organelles and cellular implants in living organisms and opens up the possibility of programming and controlling cellular processes with synthetic analogues.[13]

A major challenge in the field is to develop mechanisms to regulate the permeability of PBNs while maintaining structural integrity. Polymer vesicles prepared with high molecular weight polymers usually exhibit limited permeability to molecules and ions compared to lipid vesicles.[4] To overcome this challenge, simple approaches are needed to fabricate polymer vesicles

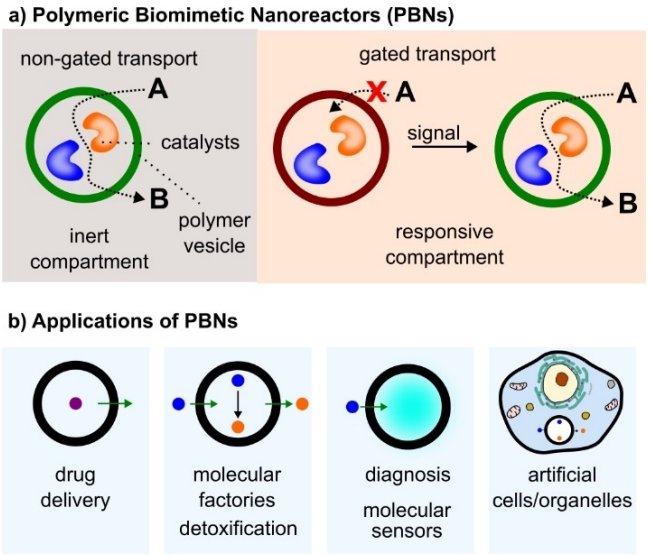

Figure 1. Definition and applications of polymeric biomimetic reactors. The examples mainly refer to polymeric vesicles with encapsulated catalytic species. The scheme assumes that molecules in the surrounding medium can permeate the membrane in a controlled manner.

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with adjustable membrane permeability, which is essential for the functionality and applicability of PBNs. In this concept review, we discuss various strategies to regulate the membrane permeability of PBNs for gated transport, as well as future opportunities and challenges in this field.

2. PBNs with Stimuli-Responsive Permeability

An increasing focus in the development of polymer-based nanoreactors is the design of polymersomes that can adjust their membrane permeability in response to stimuli while maintaining structural integrity.[14] Controlled transport can be explored by developing polymers whose amphiphilic properties can change upon stimulation, which can be realized by introducing responsive components as building blocks.[15] A range of stimuli such as pH, redox, oxidation, and light are used to control membrane permeability in PBNs.[16]

A common mechanism of stimulus-induced permeability in polymersomes is modulation of the hydrophilic-hydrophobic ratio. For example, Wang et al. developed stimuli-responsive polymersomes by incorporating a pH-responsive 2-(*N*,*N*′-diisopropylamino) ethyl methacrylate (DPAEMA) block (Figure 2) in the polymer design.[17] Membrane swelling due to the protonation of the DPAEMA block at acidic pH resulted in increased size of the polymersomes, which induced the increase of membrane permeability. Crosslinking of the membrane protected the structural integrity of the vesicles. The polymersomes were able to dock (bio)macromolecules in tailored sites in a pH- and size-dependent manner, mimicking artificial organelles that could also be useful for the development of synthetic bioreactors.

Light is a powerful tool for spatial control of membrane permeability.[18] In a recent work by our group, we have developed light-activated giant polymersomes with controlled membrane transport (Figure 3a).[19] Polymersomes were prepared from poly(butadiene)-block poly(ethylene oxide), which initially exhibited limited permeability. By incorporating a photosensitive permeability modulator (spiropyran derivative) into the membrane, the membrane permeability could be modulated by photoisomerization of the spiropyran, resulting in increased permeability for small molecules (MW $<$ 4 kDa). The potential of this approach was demonstrated by designing an enzymatic microreactor (Figure 3b). An enzyme, β-galactosidase, was encapsulated inside the polymersomes. Light activation allowed diffusion of substrates that were then hydrolysed by the enzymes inside the vesicles. In a more complex design, a hybrid coacervate-in-polymersome system was created that mimics the adaptive formation of biological condensates in cells (Figure 3c). This was achieved by encapsulating poly-L-lysine (PLL) in polymersomes. Light irradiation allowed adenosine triphosphate (ATP) to cross the membrane, resulting in complex coacervation between ATP and PLL. This process created membrane-less sub-coacervates within polymersomes.

Polymeric vesicles with stimuli-responsive permeability can be used to engineer PBNs that exhibit transient properties that depend on the availability of chemical fuels. For example, van Hest and co-workers prepared pH-dependent polymersomes from hydrophilic PEG and hydrophobic poly[2-(diethylamino)-ethyl methacrylate] (PDEAEMA). The vesicles were loaded with urease for pH control and HRP as a functional model enzyme (Figure 4).[20] The polymersomes were crosslinked to avoid loss of structural integrity upon pH changes. In the presence of chemical fuels (HCl and urea) and substrates (H_2O_2 and ABTS),

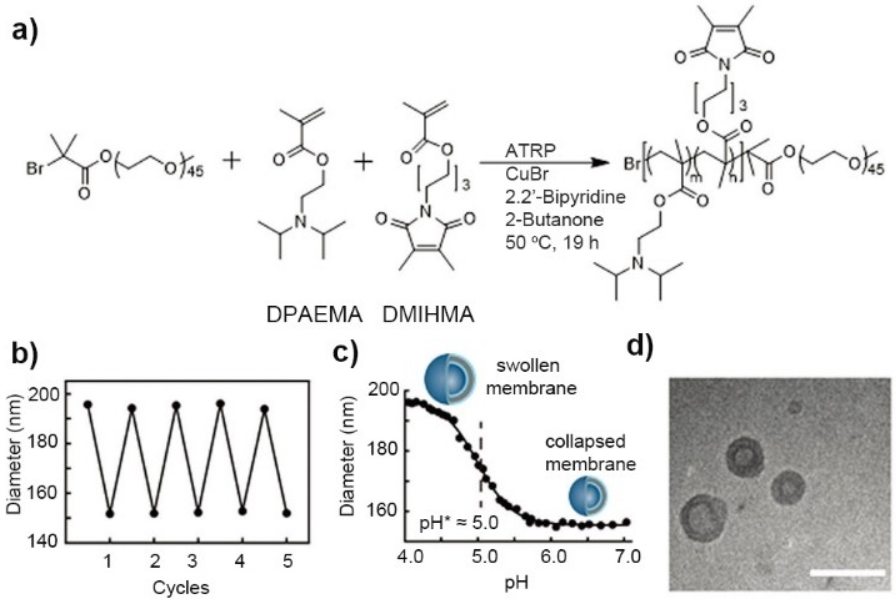

Figure 2. a) Schematic representation of pH-sensitive polymersomes composed of crosslinked amphiphilic block copolymers. b) The pH sensitivity allows reversible swelling/deswelling of the polymersomes at pH 4 and 7 in water. c) DLS data of polymersomes solution at different pH. d) Cryo-TEM imaging of typical polymersomes; scale bar: 200 nm. Adapted with permission from ref. [17]. Copyright: 2021, Wiley-VCH.

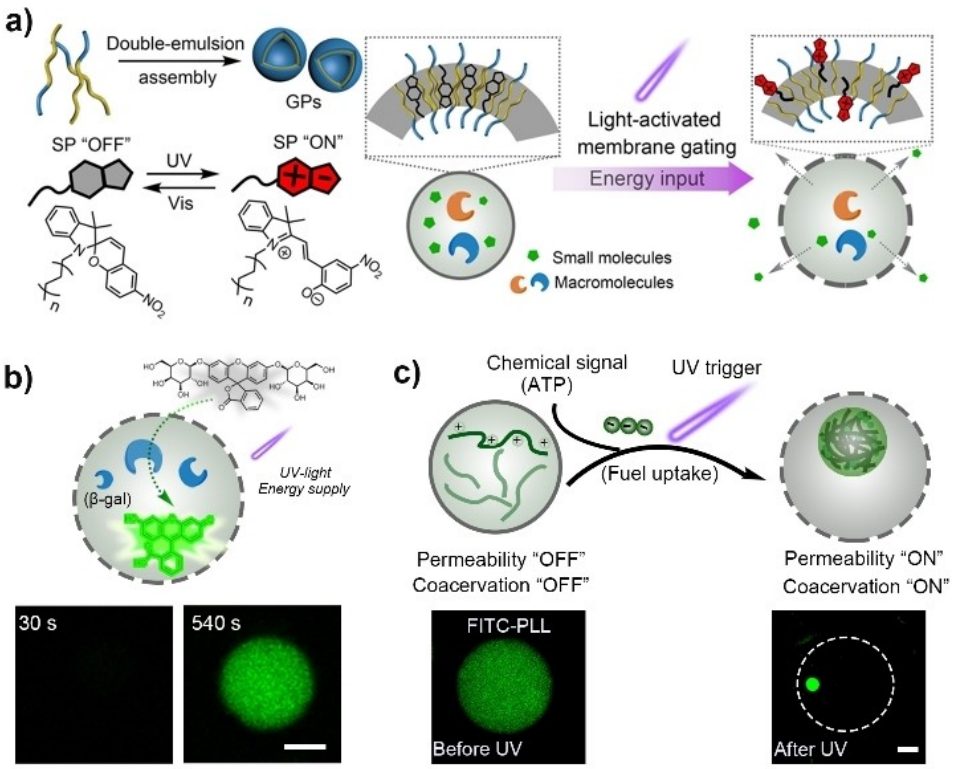

Figure 3. a) Schematic representation of the design and construction of the photoactivated giant polymersomes. b) The giant polymersomes were used to construct light-activated microreactors. c) Light-activated formation of sub-compartments inside the giant polymersomes. Reproduced with permission from ref. [19]. Copyright: 2022, Wiley-VCH.

the PDEAEMA block was protonated, causing the polymersome to swell and increase its permeability. This allowed the substrate to enter the polymersomes and initiate the nanoreactor process (oxidation of ABTS). Over time, the pH gradually increased due to the conversion of urea to ammonia. This resulted in the eventual shrinkage of polymersomes and a decrease in permeability. As a result, the enzymatic catalysis automatically entered the "OFF" state. The catalytic "on-off" cycle of the polymersome-based nanoreactor could be repeated by adding chemical fuels. The designed system exhibited fuel-mediated self-adaptive behaviour, facilitating the development of artificial organelles with out-of-equilibrium properties.

3. PBNs as Artificial Organelles for Biomedical Applications

Due to their versatile encapsulation capabilities and tuneable permeability, PBNs have been recognized as models for artificial organelles with potential biomedical applications. An important feature is that enzyme-containing PBNs are able to convert prodrugs into active drugs in situ and restore the original therapeutic form only at the target sites. Therefore, PBN-based therapeutic systems can be efficient and have fewer side effects, which shows promise for treating difficult diseases such as cancer.

For example, Akiyoshi and co-workers developed a catalytic therapeutic approach based on polymersome nanoreactors.[12] The polymersomes were composed of carbohydrate-*b*-poly-(propylene glycol) and exhibited inherent permeability to molecules below 5 kDa. The selective permeability combined with the ability to retain enzymes inside the polymersomes allowed their use in vitro as biocatalyst nanoreactors that could maintain long-term activity. Polymersomes encapsulating β-galactosidase- were taken up by cells, allowing these nano-factories to effectively convert a DOX prodrug into active DOX in several cancer cell lines and inhibit tumour cell growth. The ease of enzyme encapsulation with high efficiency, controlled permeability, and accumulation of at target sites provide the opportunity to use polymersome-based nanoreactors as more effective tools in biomedicine compared to conventional drug delivery systems.

The ability of PBNs to become active in specific biological environments offers new therapeutic strategies. For example, Kataoka and co-workers have produced glucose oxidase-loaded PBNs equipped with a phenylboronic ester block and a pH-sensitive piperidine block (Figure 5).[21] In the acidic tumour microenvironment, PBNs exhibited increased membrane permeability due to protonation of the piperidine block. This allowed the diffusion of small molecules such as glucose into the PBNs, which initiated an oxidation reaction that generated and released abundant H_2O_2. The increased H_2O_2 concentration led to oxidation of the phenylboronic ester block, producing

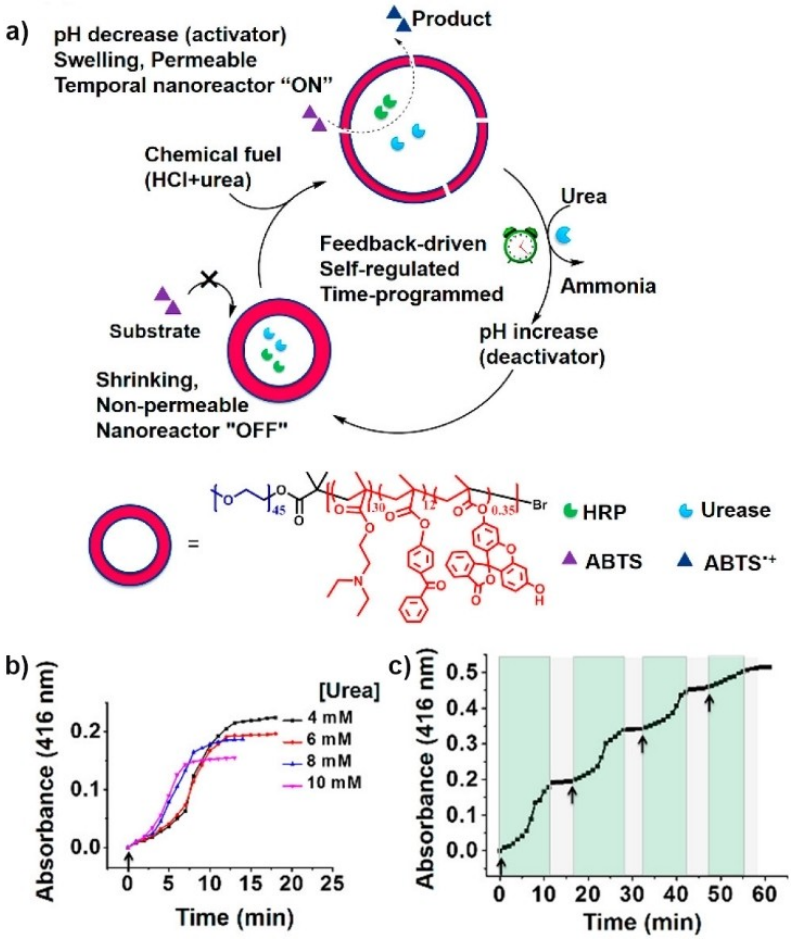

Figure 4. a) Schematic representation of polymersome nanoreactors with temporal control. b) UV absorbance at 416 showing the oxidation of ABTS by polymersome nanoreactors at different urea concentrations. c) Sequential ON-OFF regulation with repeated addition of urea. Reproduced with permission from ref. [20]. Copyright: 2018, the authors under a CC-BY-NC-ND license.

quinone methide (QM) as a by-product. The released QM decreased the intracellular GSH level, which weakened the antioxidant ability of cancer cells. The production of H_2O_2 and depletion of GSH synergistically increased oxidative stress on tumour cells and inhibited their growth in vivo.

4. Summary and Outlook

In recent decades, there has been great interest in the use of polymersomes as versatile platforms for the development of biomimetic nanoreactors. The development of polymer biomimetic nanoreactors has combined expertise from various interdisciplinary fields such as polymer synthesis, self-assembly, nanoscience, catalysis, chemical biology and synthetic biology. Significant progress has been made in the development of polymer vesicles with controlled properties such as chemical composition, surface chemistry, membrane permeability and catalyst accessibility. In this article, we have summarized recent developments in controlled membrane permeability and gated transport in polymeric biomimetic nanoreactors. Controlled membrane permeability allows information and biologically relevant compounds (substrates/products) of small size to pass through the membrane barrier of the polymersome on demand, while the active biomolecules (e.g., enzymes) are protected in their inner cavity. In this way, effective enzymatic conversion, activity enhancement and function transfer can occur in situ and synergistically while maintaining the structural integrity of the carrier.

Several challenges remain in the development and application of PBNs. These include the development of biodegradable building blocks, scalable formulations, increasing the encapsulation efficiency of active species and optimizing their application and stability in dysfunctional cells and in vivo organs with complex microenvironments. The future development and success of PBNs as functional artificial organelles depends on the collaboration and joint efforts of materials scientists, biochemists and medical scientists. This is particularly important to refine our understanding of the essential criteria for making PBNs and to establish principles for further designing more complex biomimetic compartments with multiple functions. For example, by coating them with a cell membrane, the integrated

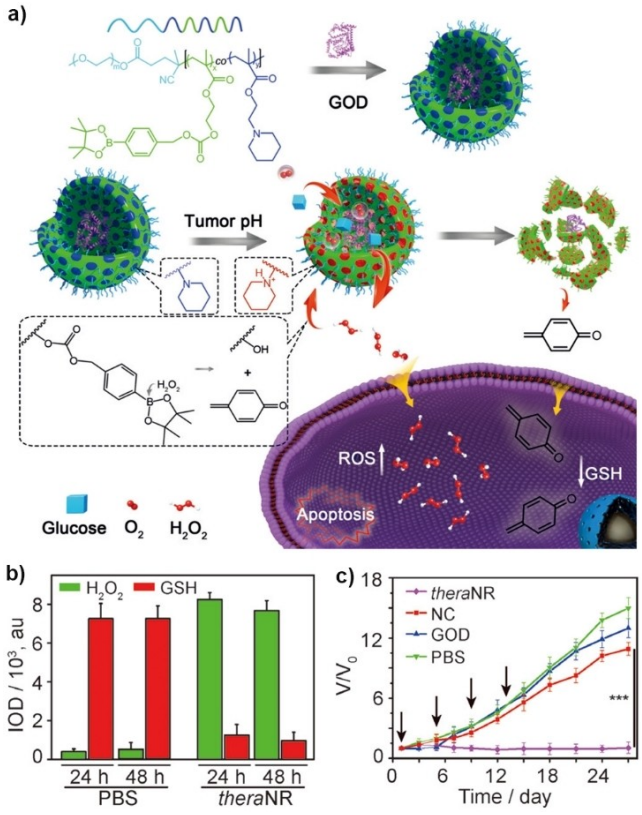

Figure 5. a) Schematic of polymeric nanoreactors responding to a tumour microenvironment. b) Quantification of H_2O_2 and GSH levels in tumour tissues after treatment with PBS or therapeutic nanoreactors. c) Tumour growth curves of mice treated with PBS, glucosidase, blank nanocarriers, and therapeutic nanoreactors, respectively. Reproduced with permission from ref. [21]. Copyright: 2017, Wiley-VCH.

PBNs could potentially be used more effectively in the complex in vivo environment due to reduced systemic clearance and improved circulation in the blood. In addition, biomimetic nanoreactors also hold great potential for application in tumour immunotherapy, which would be a more effective approach to achieve better performance and fewer systemic side effects. PBN-derived artificial organelles appear to be an effective strategy for treating various pathologies, with further exciting advances expected through the use of stable and active nanoreactors.

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Conflict of Interest

The authors declare no conflict of interest.

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