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# Polymersomes: Breaking the Glass Ceiling?

*Tamuka Chidanguro, Elina Ghimire, Cheyenne H. Liu, and Yoan C. Simon**

**Polymer vesicles, also known as polymersomes, have garnered a lot of interest even before the first report of their fabrication in the mid-1990s. These capsules have found applications in areas such as drug delivery, diagnostics and cellular models, and are made via the self-assembly of amphiphilic block copolymers, predominantly with soft, rubbery hydrophobic segments. Comparatively, and despite their remarkable impermeability, glassy polymersomes (GPs) have been less pervasive due to their rigidity, lack of biodegradability and more restricted fabrication strategies. GPs are now becoming more prominent, thanks to their ability to undergo stable shape-change (e.g., into non-spherical morphologies) as a response to a predetermined trigger (e.g., light, solvent). The basics of block copolymer self-assembly with an emphasis on polymersomes and GPs in particular are reviewed here. The principles and advantages of shape transformation of GPs as well as their general usefulness are also discussed, together with some of the challenges and opportunities currently facing this area.**

## 1. Introduction

Water is at the origin of life. This three-atom molecule is responsible for the development of living organisms, which by definition are constituted of organic matter, i.e., oftentimes water-insoluble, carbon-based molecules. The key to uniting these seemingly incompatible media are amphiphiles. Amphiphilic structures (from the Greek *amphis*: both and *philia*: love, friendship) have the unique ability to connect otherwise immiscible media (e.g., in soaps and detergents), but also, in the case of organisms, to create divisions between the inside and outside of cells, or organelles. This division is embodied by the term vesicle (from the Latin *vesicula*, small bladder), which designates a compartment, formed in an aqueous medium and separated from the outside by an amphiphilic membrane. Multiple technological applications from cleaning products to drug delivery to catalysis capitalize on the unique properties of amphiphiles and the self-assembled structures that they form.[1–3] While the first reported use of soaps dates back to 2800 BC in the Middle East,[4] the real initial foray in controlling assemblies of amphiphiles came from hematology and the manipulation of phospholipids by Bangham and Horne.[5,6] When attempting to image dry phospholipids by electron microscopy, the two observed phospholipid assemblies or liposomes arranged in a bilayer structure similar to that of the cellular membrane. The ability of liposomes to encapsulate active principles became quickly apparent and helped realize their potential in drug delivery,[7] cosmetics,[8] and cell mimicry.[9]

In parallel, the self-assembly of amphiphilic block copolymers had already garnered a lot of interest. Early on, scientists recognized their fantastic potential owing to their unique phase separation behavior,[10,11] their ability to form nanostructures,[12] and their usefulness as surfactants in consumer products in hair and skin care.[13] While accounts suggest prior discussions,[14] the first reports of polymer vesicles, or polymersomes date back to 1995.[15,16] Since then, a growing number of groups has worked on the fabrication and characterization of polymersomes worldwide. Stimulated by the improvements in controlled polymerization methods, these groups have now access to an endless library of polymeric di-, tri- and other multiblock copolymers, which permits ever more intricate studies.

Early work by the Eisenberg and Discher groups helped unravel some of the underpinnings of amphiphilic block copolymer self-assembly with glassy and rubbery hydrophobic segments, respectively.[15,17–19] Early on, it was suggested that polymersomes with glassy hydrophobic blocks, referred to here as glassy polymersomes (GPs), are less desirable for biomedical applications, due to their inherent lack of fluidity.[20] This rigidity (unlike their liposome counterparts) would limit their usefulness in drug delivery by restricting circulation through narrow vessels for instance. However, over the past decade, multiple researchers have highlighted the benefits of GPs and their unique properties, such as their remarkable impermeability and their ability to change shapes into long-lived metastable topologies. The early dismissal of GPs seems to have skewed the research in favor of polymersomes with fluid membranes, or 'soft' polymersomes (SPs). Consequently, most reviews on the self-assembly of block copolymers and their applications have focused systems with soft membranes, e.g. poly(dimethyl siloxane) (PDMS), poly(ethylethylene) (PEE) and poly(butadiene) (PBD). Given the increasing body of work on GPs, their specificities, and the advent of novel methodologies to access them (e.g., polymerization-induced self-assembly, PISA), the time is now ripe to review some of the work concerned with the fabrication, characterization and potential usefulness of these tantalizing structures.

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In this overview, we succinctly touch upon the basics behind the self-assembly of block copolymers. We specifically emphasize the fundamentals that allow one to attain polymersomes via self-assembly, with an obvious emphasis on glassy polymers. To do so, we take advantage of some of the pioneering work that is now nearly two decades old.[15,17,21,22] Due to the inherent kinetic issues associated with the formation of GPs, we look at the various strategies to make them. In particular, we highlight some of the limitations associated with rigid walls in terms of fabrication methodology and contrast them with SPs.

The seminal report on the use of osmotic pressure changes to transform polymersomes into trapped, yet kinetically stable, stomatocytes by Kim et al. is partially responsible for the mini-renaissance of GPs.[23] Shape-change and its usefulness are therefore central to the subsequent discussion. We also address the recent developments aiming to circumvent the main drawbacks of prevalent vinylic systems, i.e., lack of biodegradability and biocompatibility. Strategies implementing shape-changes in benign and hydrolyzable systems, such poly((D,L) lactide) (PDLLA) copolymers, are reviewed as promising alternatives. Lastly, we discuss some of the challenges (notably in terms of characterization and fidelity) and potential opportunities for GPs.

## 2. Self-Assembly of Block Copolymers

Amphiphilic block copolymers can self-assemble into a variety of morphologies.[24] The most commonly discussed morphologies (**Figure 1**) include spherical micelles (solid spheres with hydrophobic blocks huddled in the center), worm-like rods (cylindrical micellar structures) and polymersomes (hollow spheres with a hydrophilic lumen and corona).[25,26] Block copolymer composition plays an important role in determining the final morphology of the self-assembled structures. In the simplest systems, block copolymers with high hydrophilic content will self-assemble into micelles. With gradually increasing hydrophobic content, rod-like structures start to form and eventually yield way to polymersomes.[27–29]

The tools to analyze and understand the assembly behavior of amphiphilic block copolymers have been largely derived from the study of smaller amphiphiles. In the 1970s, Israelachvili and coworkers defined a critical packing parameter, $p$, which can be used to predict the morphology of the self-assembled aggregates.[31] For a molecule of length $l_c$ (Equation (1)), the packing parameter $p$ is influenced by the volume of the hydrophobic chain, $v$, and the area occupied by the hydrophilic group, $a_0$.[32,33]

$$p = \frac{v}{a_0 l_c} \tag{1}$$

*Grosso modo*, for a value of $p < 1/3$, one expects spherical micelles; for $1/3 < p < 1/2$ cylindrical micelles are likely to form, and finally (and desirably here), for $1/2 < p < 1$, polymersomes are the most probable architecture.[25,34,35] Values of $p$ greater than one led to the formation of other inverted aggregates.[36] Adapting Israelachvili's work on small molecules to polymeric amphiphiles requires a few adjustments. For instance, the parameters $l_c$, $a_0$ and $v$ are difficult to obtain. Instead, molecular weight and degree of polymerization of different blocks are more readily accessible by conventional characterization methods (e.g., size-exclusion chromatography, static light scattering, colligative properties). Therefore, the hydrophilic weight fraction, $f$, is often used in lieu of the packing parameter. This fraction works as a good substitute for $p$ in most block copolymer systems.[37] Generally speaking, $f = 0.35 \pm 0.1$ leads to the formation of vesicles, while $f > 0.45$ usually afford spherical micelles and cylindrical systems such as rods appear for $0.4 < f < 0.5$.[38] Similarly to lipophilic emulsifiers, high hydrophobic content, i.e., values of $f < 0.25$, inverted structures form.[21,39,40] Using empirical data from various studies and block copolymers and utilizing simulations, Discher et al.

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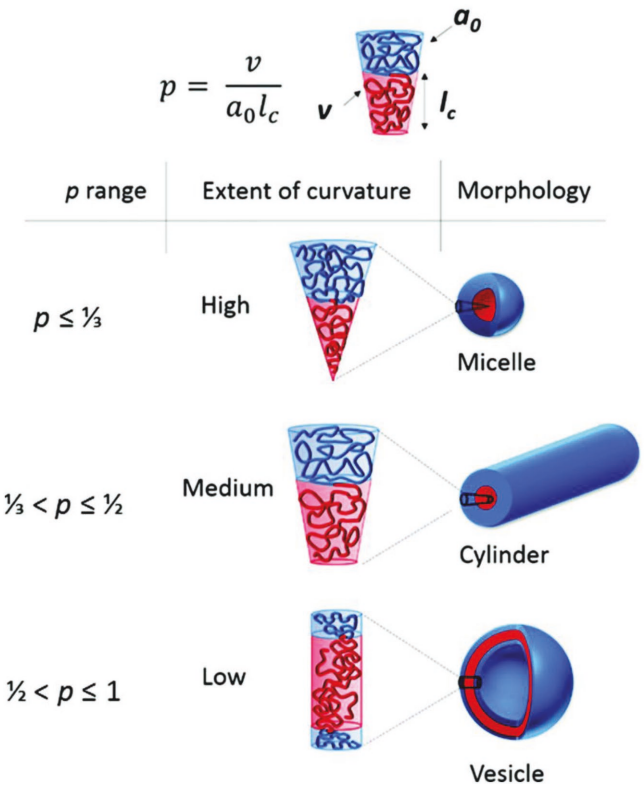


**Figure 1.** The different morphologies obtained by targeting different packing parameters, *p*. Reproduced under the terms and conditions of the Creative Commons Attribution 3.0 Unported Licence.[30] Copyright 2017, the authors, Royal Society of Chemistry.

were able to relate the packing parameter $p$ to $f$, through the following Equation (2):[37]

$$f = e^{-\frac{p}{\beta}} \quad (2)$$

where $\beta$ is a fitting constant with a value of $\beta = 0.66$.

Interestingly, these general considerations about hydrophilic fractions tend to break down for polymers with glassy hydrophobic segments. Therefore, the difference with low $T_g$ polymeric systems becomes clearly noticeable. In their investigation of so-called "crew-cut" aggregates made from polystyrene-*block*-poly(acrylic acid) (PS-*b*-PAA), Yu et al. did not observe the expected vesicle morphology for the ranges of $f$ typically associated with polymersomes ($0.25 < f < 0.45$).[19,41] Surprisingly, cylindrical micelles only started to appear at $f = 0.18$, significantly below the usual hydrophilic volume fraction. As for the polymersomes, they only formed at a remarkable $f = 0.12$. The vesicles at this ratio were small in size and larger vesicles were obtained for hydrophilic compositions as low as 0.06.[19,22,42] In contrast, for SPs, vesicles have formed with hydrophobic ratios as low as 60%.[43]

Another critical factor in determining the morphology is the nature of pairwise interactions. As will be apparent later, the formation of GPs necessitates the use of water and a plasticizing water-miscible organic solvent. The latter is often present at the end of the GP formation and therefore plays an important role in the nature of the aggregates. The presence of a second solvent complexifies even more the formation of GPs, unlike straightforward hydration methods commonly employed for SPs. The Flory-Huggins interaction parameter often depicts the polymer-solvent interactions.[44,45] Each block therefore interacts differently with the solvent(s). For the addition of each new component (solvent or block) to a mixture, pairwise interactions of said component with all of the other constituents need to be taken into account. As a result, the number of possible assemblies increases rapidly with an increasing number of constituents. For example, Mai and Eisenberg pointed out that "the self-assembly of [...] PS-*b*-PAA copolymers in dioxane–water or other solvent–nonsolvent mixtures involves six $\chi$-parameters, namely $\chi_{AB}$, $\chi_{AS}$, $\chi_{AN}$, $\chi_{BS}$, $\chi_{BN}$, $\chi_{SN}$," with the subscripts A and B representing each blocks, S the cosolvent for the blocks (here dioxane), and N denoting the nonsolvent (here water).[25] Among other things, these pairwise interactions affect the dimensions of the resulting vesicles. Each size corresponds to a given bending energy, i.e., the energy required to bend the membrane bilayer. In a general sense, smaller amounts of 'good' solvents are necessary to solubilize the hydrophobic blocks and lower their bending energy, leading to vesicle formation. Conversely, it takes more 'poor' solvent to achieve the same result.[32,46]

Other factors affecting the self-assembled structures include the chemical composition and dispersity ($Đ$) of the blocks,[47] temperature, and concentration.[48] Higher $Đ$ of the block copolymers results in the formation of smaller vesicles at equivalent number-average chain length, while higher concentrations of block copolymers accelerate vesicle formation.[49] As expected, GPs will behave similarly to SPs for temperatures higher than $T_g$.[50]

Considering polymeric factors (dispersity, molecular weight, composition), experimental conditions (concentration, temperature), and pairwise interactions (Flory-Huggins parameters, solvency), it is easy to imagine that micelles, rods and vesicles, though most commonly reported, are just three of many morphologies that can be obtained via block copolymer self-assembly. As often in science, some of the more intricate problems take longer to be solved and can lead to dismissal of initial results.[51–54] It is probable that some of these more esoteric structures (*vide infra*) were observed but were simply not formally reported (c.f. nulltiples)[55] due to complexity or lack of theoretical framework available. By manipulating the PS-*b*-PAA copolymer composition and solvent effects, the Eisenberg group isolated various aggregates including large compound vesicles, large compound micelles, micellar rods and hollow hoops (**Figure 2**). Their study investigated how 'crew-cut' aggregate morphology could be tuned by simply tweaking solvent compositions while keeping the very same polymers (e.g., Figure 2b-c and 2f-g).

## 3. Effect of Glassiness in Polymer Self-Assembly

Polymersomes and other self-assembled block-copolymer aggregates owe their rapid rise and development mostly to the data gathered from the many extensive studies on liposomes behavior, properties and fabrication. These studies served as a starting point for the first polymer vesicles almost three


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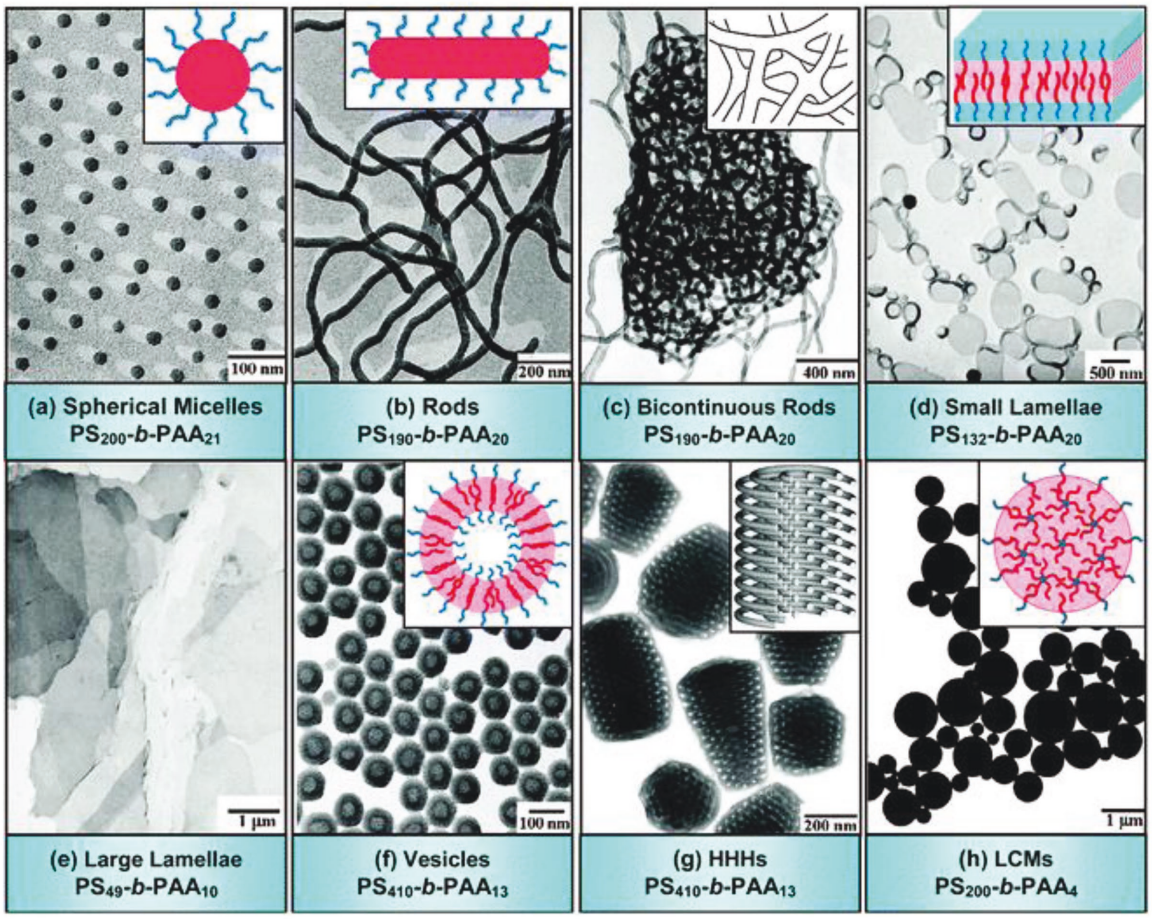


**Figure 2.** Transmission electron microscopy (TEM) images and corresponding schematic diagrams of various morphologies formed from amphiphilic $PS_m$-*b*-$PAA_n$ copolymers. In the schematic diagrams, red represents hydrophobic PS parts, while blue denotes hydrophilic PAA segments. HHHs: hexagonally packed hollow hoops; LCMs: large compound micelles, in which inverse micelles consist of a PAA core surrounded by PS coronal chains. Generally, the hydrophilic segments (e.g., coronas) of the crew-cut aggregates cannot be seen in TEM images if they are not stained. Reproduced with permission.[56] Copyright 1999, NRC Research Press. Reproduced with permission.[25] Copyright 2012, Royal Society of Chemistry.

decades later and continue to be a source of inspiration for polymer scientists. Notably, strategies developed for liposome fabrication (e.g., film rehydration and electroformation) were readily adapted to form polymersomes.[57]

Self-assembly approaches can be divided broadly into two groups: solvent-free and solvent-displacement techniques.[29] The latter usually involve the dissolution of block copolymers in an organic solvent, followed by addition of an aqueous phase (water or buffer solution) to form vesicles.[58] In the solvent-free techniques, the amphiphilic block copolymers are directly hydrated by the aqueous phase.[59] In the film rehydration technique, perhaps the most common solvent-free method, the block copolymers are first dissolved in an organic solvent, which is then (vacuum) dried, leaving a thin layer of amphiphile.[60] Upon hydration, vesicles (and other self-assembled aggregates) form. Similarly, in the direct hydration method, another solvent-free method, block copolymers are directly hydrated as a powder.[61] Unfortunately, the extreme hydrophobicity of glassy polymers essentially excludes them from being fabricated via either of these solvent-free techniques. As a result, most glassy polymer vesicles have been fabricated using solvent-displacement strategies.[29] Discussed below are some of the methods that are currently being used in the fabrication of GPs. It is worth noting that the methods described here are also common for SPs. First, we will discuss the solvent injection method (and its derivatives), then the heating method, and, lastly, the polymerization-induced self-assembly (PISA) process. Finally, we will comment on size control and strategies to resize GPs.

### 3.1. Solvent Injection

In this method, an organic solvent dissolves both the hydrophobic and hydrophilic blocks of a copolymer. Common organic solvents, such as tetrahydrofuran (THF),[62] chloroform,[63] dioxane and *N*,*N*-dimethylformamide (DMF) are often used to fabricate GPs.[64] Once the copolymer is dissolved, the aqueous phase is slowly added (typically with a syringe pump) to the mixture under constant agitation (**Figure 3**a). Interestingly, the stirring speed has an influence on the dimensions of the aggregates.[65] Higher stirring rates lead to smaller sized vesicles, on


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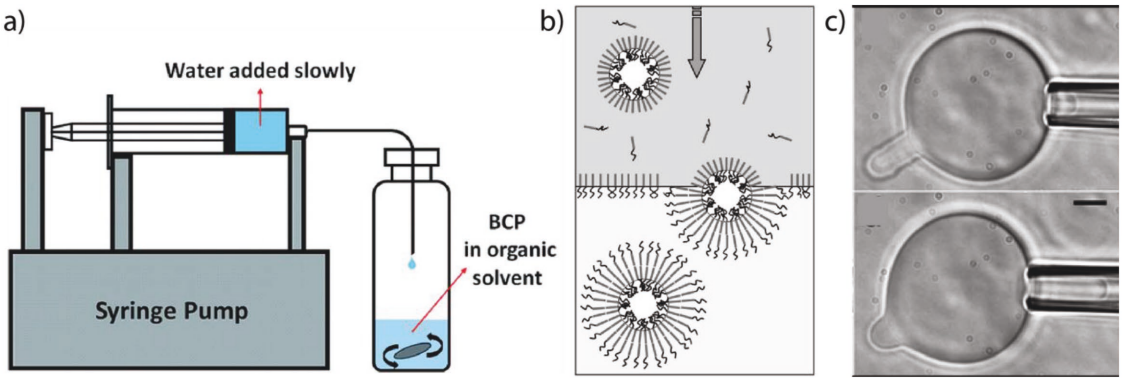


**Figure 3.** a) Illustration of the solvent injection method showing slow addition of water into a block copolymer (BCP) organic solution. Reproduced with permission.[66] Copyright 2016, Royal Society of Chemistry. b) The inverse emulsion method. c) The tongue deformations observed after micropipette aspiration. Reproduced with permission.[67] Copyright 2009, Royal Society of Chemistry.

account of stronger shear during agitation. Too large of a stirring speed, however, results in the polymer precipitating due to bilayer disruption. Likewise, the nature of the solvent utilized influences the size of the resulting polymersomes. Solvent interactions lead to various degrees of swelling of the hydrophobic block, which directly affects the degree of curvature of GPs and ultimately their size. Consequently, this method allows one to tune the dimensions of GPs by adjusting the solvent and addition conditions. In some cases, solvent mixtures (commonly THF:dioxane) are used to control the self-assembly process. Depending on the intended goal, quenching of the GPs occurs either into a large amount of water or by plunging the sample in liquid nitrogen (mainly for DMF-based samples) to trap the structures and rigidify the membrane. Organic solvent removal by dialysis often results in GP shape transformation as discussed further.

While the organic solvent is important in determining the morphology of the self-assembled structures, Luo et al. showed that the aqueous-to-organic solvent ratio directly influences the size of the polymersomes – even for a system of identical composition.[68] Using a PS-*b*-PAA copolymer, they formed polymersomes of increasing sizes by simply increasing the amount of water used during the formation process. At water compositions (in a THF/dioxane/water system) of 24.5%, vesicles with diameters around 90 nm formed, but upon increasing the water content to 67%, they observed polymersomes with diameters around 200 nm.

#### *3.1.1. Inverted Emulsion*

Due to their incompatibility with solvent-free fabrication techniques, achieving larger vesicle sizes, or giant unilamellar vesicles (GUVs) has proven challenging for GPs. GUVs are often useful in the determination of the characteristic properties of polymersomes, including membrane elasticity and permeability. Although atomic force microscopy (AFM) can serve in the investigation of membrane elasticity through force-displacement schemes, the optical microscopy measurements, e.g. through micropipette aspiration, are not only more accurate but also significantly more straightforward.[17] In most SPs, electroformation often produces GUVs of specific sizes (by controlling frequency, current and voltage). Unfortunately, electroformation tends to give poor yield for glassy polymers, if at all. To circumvent these limitations, Mabrouk et al. adapted a fabrication scheme developed for liposomes by Pautot et al. to attain glassy GUVs (Figure 3b).[67,69] They efficiently fabricated GUVs from two amphiphilic block copolymers consisting of PEG and, either poly((40-acryloxybutyl) 2,5-di(49-butyloxybenzoyloxy) benzoate) (PA444), or poly(4-butyloxy-29-(4-methacryloyloxybutoxy)-49-(4-butoxybenzoyloxy)azobenzene) (PMAazo444). This method has since then been extended to other types of systems.[68] We describe the procedure here briefly. First, the polymer is dissolved in an organic solvent less dense than water. Then, by adding a small amount of aqueous phase to the block copolymer solution and by repeatedly drawing and pushing the mixture with a pipette, an emulsion forms. This emulsion is then carefully laid on the top of an aqueous buffer. Upon centrifugation, the diblock copolymers diffuse from the upper emulsion phase towards the interface and form GUVs. Interestingly, despite their glassy nature, the latter exhibited enough fluidity to distort under micropipette aspiration, leaving 'tongue'-deformations when suction was removed (Figure 3c). These deformations retained their shape for tens of minutes after release, similar to gel-phase liposomes. This method could be tailored to carefully shape these micro-containers.

### 3.2. Heating Method

High temperature is an interesting 'knob' to control the self-assembly of block copolymers. While thermodynamic considerations impose the formation of vesicular assemblies, the presence of glassy hydrophobic blocks lead to kinetic jamming and preclude the advent of equilibrium structures. By imparting thermal energy to the system, one can overcome these kinetic traps and attain the most stable morphology. In the heating method, water and an organic solvent are first mixed together with the block copolymer. The mixture is then heated to form the vesicles. In a study comparing solvent injection and the heating method for PS-*b*-PAA, Liu et al. observed that vesicles appeared at higher water content, and over a shorter period of time using the heating method.[66]


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### 3.3. Polymerization-Induced Self-Assembly (PISA)

First brought to prominence by the seminal work of Charleux and coworkers[70,71] and the significant contributions by Armes and coworkers,[26,72] PISA is rapidly becoming one of the most commonly used methods in block copolymer self-assembly.[73–77] It offers the opportunity for tunable self-assembly via direct control of the polymerization process. Commonly, a hydrophilic macroinitiator or macrochain-transfer agent (macro-CTA) is often used in to polymerize the hydrophobic block and append the water-soluble segment. As the block length increases, morphologies progress from high curvature structures, such as micelles (spherical to rod-like), towards the lower curvature morphologies, such as vesicles (**Figure 4**). Recent work by the Armes group and collaborators has shown that these morphologies can be monitored in situ using techniques, such as small angle X-ray scattering.[78–80] One of the most significant advantages of PISA is the use of group-tolerant, controlled polymerization procedures, which ensures low $Đ$ and controlled degree of polymerization. The bulk of work on PISA was done using reversible addition-fragmentation chain-transfer (RAFT) polymerization due to its versatile and facile implementation. For instance, various hydrophobic monomers, including lauryl methacrylate, benzyl methacrylate, and styrene were successfully polymerized in the presence of hydrophilic macro-CTAs to form amphiphilic block copolymers. Since most of these polymerizations are conducted above 60 °C, the high-temperature morphology can be kinetically trapped upon cooling to room temperature. PISA has also been reported with other polymerization techniques, such as atom-transfer radical polymerization,[81] organotellurium-mediated radical polymerization,[82,83] and ring-opening metathesis polymerization.[84] Interestingly, many of the hydrophobic blocks grown via the PISA process have been glassy in nature. Consequently, PISA offers a unique platform to fabricate and study a wide variety of GPs. Several reviews on PISA offer a glimpse into the rapid development of the field and its potential applicability.[72,85–87]

## 4. Size Control and Shape Transformation

### 4.1. Size Control

Once SPs are formed, their fluid nature allows one to readily resize them, most commonly, via extrusion through a membrane. This method was swiftly adapted from the liposome 'playbook'.[91,92] GPs are however usually difficult to extrude through a membrane due to their intrinsic rigidity. As a result, plasticizing agents are often used to 'soften' the membrane and allow them to be reconfigured into uniformly sized vesicles with narrow $Đ$. Once the membrane reconstitution is complete, the solvents can then be readily removed (e.g., evaporation, dialysis). For example, Men et al. used a (THF/dioxane/water) solvent injection method to fabricate polymersomes of diameters around 400 nm from a PS-*b*-PEG block copolymer, at water contents of 20%, 33% and 67% respectively.[93] Upon extrusion with a 0.2 um nylon membrane, they obtained polymersomes of sizes below 100 nm from the polymersomes consisting of 20% and 33% water, while the 67% system was too rigid to pass through the membrane. However, upon addition of more plasticizer (THF/dioxane mixture), they were successfully extruded to yield sub 100 nm vesicles. Alternatively, Men et al. explored sonication as a means to resize GPs.[93] Again, plasticization was necessary to induce size change. At high plasticizer content (i.e., water content = 33%), the larger polymersomes were readily resized to below 100 nm within 30 s. However, at lower plasticizer content (water content = 37%), the size did not vary even after 15 min of continuous sonication.

### 4.2. Shape Transformation

Perhaps one of the most impressive qualities of vesicular structures is their ability to deform to adapt continuously to their surroundings. This behavior is exemplified in lipid-based vesicles.[94] For instance, cells change their shape during processes

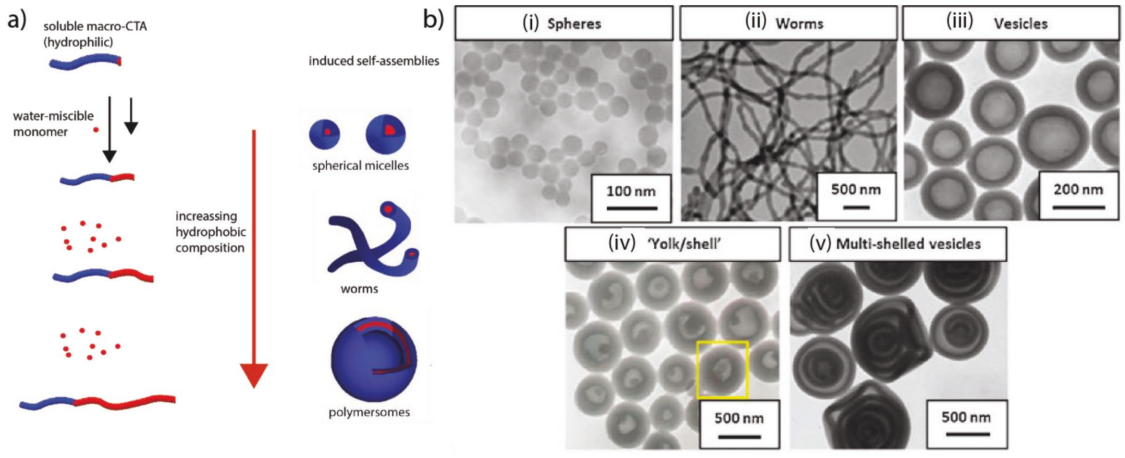


**Figure 4.** a) Schematic representation of PISA via RAFT polymerization. b) TEM images of the morphologies obtained from PISA via RAFT dispersion polymerization of styrene in methanol using various poly(4-vinylpyridine) (P4VP) macro-CTAs: (i) spheres, (ii) worms, (ii) vesicles. Adapted with permission.[88] Copyright 2010, Royal Society of Chemistry. (iv) yolk/shell. Adapted with permission.[89] Copyright 2010, American Chemical Society. (v) multi-shelled vesicles.Adapted with permission.[90] Copyright 2014, American Chemical Society. Collage adapted with permission.[85] Copyright 2016, Elsevier.


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such as signaling,[95] wound repair,[96] growth and death,[97] and many other functions required to maintain homeostasis. Strikingly, in phagocytosis, white blood cells engulf pathogens and retain their integrity despite the massive rearrangement.[98] SPs, with constitutive blocks like PBD, and poly(dimethylsiloxane) (PDMS),[99] are elastic and fluid,[99] and constitute excellent candidates for biomedical applications.[100] To achieve continuous and rapid deformation, glassy polymers would require the constant presence of a plasticizer (typically an organic solvent) or a constant heat influx, neither of which are really practical. Nevertheless, the rigidity of their polymer membranes enables one to program the deformation of the assemblies to fabricate metastable nanoobjects that transcend the conventional spherical vesicles. Like many biological membranes, GPs can resist very strong osmotic pressures, while SPs will either shrivel or burst in response to hyper- and hypoosmotic stresses, respectively. These metastable nanoobjects are obtained by shaping the polymersomes in the presence of a stimulus (e.g., solvent, pH,) and trapping them upon removal of said stimulus.[62] GPs that have undergone shape transformation exhibit behaviors that would be otherwise unattainable with conventional spherical morphologies. A number of studies reported how cells exhibit better adhesion to non-spherical structures,[101,102] an encouraging sign for the rising interest in shape transformation. In parallel to the polymersome efforts, it is interesting to notice the simultaneous efforts to synthesize non-natural phospholipids for the fabrication of non-spherical liposomal structures with unique properties. For instance, Zumbuehl and coworkers designed lenticular structures to fall apart under specific shear conditions and constitute promising candidates for flow-responsive programmed drug delivery in blood vessels.[103–105] While the richness of origami morphologies attained with polymersomes has not yet rivaled their liposome counterparts, we describe here a few promising polymer-based candidates.

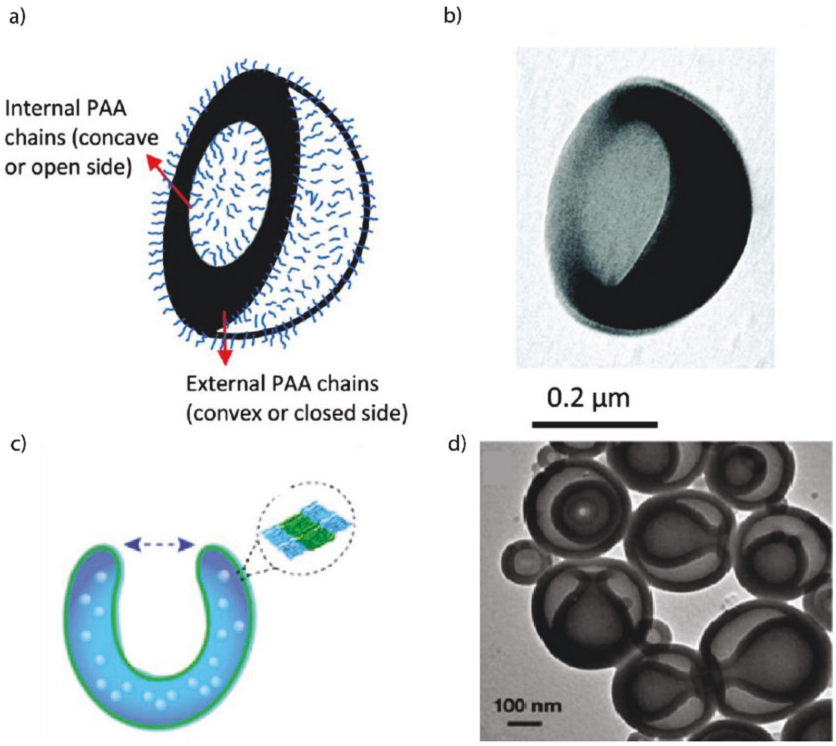


**Figure 5.** a) Schematic representation of a typical kippah vesicle (initially open-side-up) after counterclockwise tilting by −60°. b) A TEM image of a kippah vesicle at −60° tilt (i.e., counterclockwise). Reproduced with permission.[106] Copyright 2010, American Chemical Society. c) Schematic representation of a stomatocyte. Adapted with permission.[107] Copyright 2016, American Chemical Society. d) dry TEM images of stomatocytes at various angles. Reproduced with permission.[23] Copyright 2010, American Chemical Society.

In the past decade, there has been a mini-renaissance in the self-assembly of glassy systems beyond regular polymersomes and micelles. In 2010, Azzam and Eisenberg attained *kippah* (Hebrew, meaning dome, commonly referring to a traditional skullcap) morphologies in PS-*b*-PAA-based polymersomes as they were investigating the effect of drying mechanisms on their transmission electron micrographs (**Figure 5**a,b).[106] The kippah morphology was observed upon rehydration of freeze-dried vesicles, or upon vacuum drying. This observation led the authors to believe that wall flexibility and pressure gradients played a role in their formation. Kim et al. further fueled this recrudescence when they reported a simple way to deform polymersomes into stomatocytes (from Greek, *stoma*, mouth and *kutos*, vessel and named after the bowl-shaped erythrocytes,) (Figure 4c,d).[106,108,109] After fabricating polymersomes via solvent displacement, Kim et al. dialyzed them against pure water. As a result of the osmotic imbalance between the inner compartment and the outside of the vesicles, the membrane deformed, invaginated and ultimately led to the formation of stomatocytes.

Early on, the Thordason group investigated the co-assembly of mismatched block copolymers (PS-*b*-PEG and PS-*b*-PAA) to obtain polymersomes.[110] More recently, they expanded on their GP expertise to achieve multiple non-spherical polymersomes. The latter capitalized on the anisotropic interactions between perylene units constituting their hydrophobic blocks: a poly(*N*-isopropylacrylamide-*co*-perylene diester monoimide) tethered to a PEG hydrophilic block.[111] By tweaking the solvent composition, the solvation of perylene H-aggregates could be adjusted to afford more ellipsoidal and tubular morphologies.[112,113]

The bending energy, E, of the structures plays a pivotal role in these approaches.[114] It can be described (Equation (3)) as a function of three parameters: κ, the bending rigidity, *C* the mean surface curvature, and $C_0$, the spontaneous curvature.[62]

$$E = \kappa \oint (2C - C_0)^2 dA \tag{3}$$

*C* is an intrinsic value that is determined by the shape, while $C_0$ is caused by asymmetry in the bilayer membrane, between the interior and the outer layer. Therefore, the environment (e.g., osmotic pressure differences, *vide supra*) can affect the


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$C_0$ value and ultimately $E$. With minimal or positive $C_0$ contribution, prolate structures are energetically favored, while a negative $C_0$ contribution favors oblate (stomatocytes, kippot) instead.[115]

## 5. Computational Studies

As for the experimental side, a significant portion of computational work in polymersomes also started as an extension of the self-assembly behavior of phospholipids. Seminal work by the groups of Lipowsky and others has been used to understand lipid bilayer membranes and their behavior. Since the turn of the millennium, these groups also contributed to further the knowledge of aqueous self-assembly, by simulating amphiphilic block copolymer behavior in solution.[116–121] Some of the self-consistent field theories commonly applied to block copolymer melts have also been further adapted to explain micellar assemblies[122] Together, these simulations provide an atom-level understanding of the behavior that lead to the polymer vesicle formation.[37] Over the last two decades, various simulation techniques, such as Monte-Carlo,[123,124] Brownian dynamics,[125] molecular dynamics[116,126] (MD) and dissipative particle dynamics (DPD),[127,128] have been used to study both lipid and polymer membranes.[129] Evidently, simulating polymer self-assembly with atom resolution is limited by computing power. Gratifyingly, the use of coarse-grain models has simplified simulations. By representing several atoms or segments as a sphere, a polymer chain can now be reduced to fewer, more readily modeled spheres.[37] Early work by Srinivas et al. used coarse-grain molecular dynamics (CG MD) to model the assembly of diblock copolymers in water and confirmed the experimentally observed importance of the hydrophilic fraction on morphology.[130,131] This method was further extended to model the aqueous assembly of many other block copolymers.[132,133] Beyond morphological information, modeling also helped elucidating the formation process and corroborated hypotheses from experimental results.[134] While some of the simulations produce results that are designed to fit a variety of polymer block copolymers,[49,135,136] some of the work has been aimed at specific block copolymer systems.[137,138] Recently, Sun et al. used a CG MD to simulate the self-assembly behavior of PS-*b*-PAA diblock copolymers,[139] modeling up to 900 chains per vesicle. By varying block copolymer ratios and concentration, the simulated morphologies were consistent with experimental results. Interestingly, these computational results explained results experimentally observed around two decades earlier.[15,19,22,56]

Many simulations of liposomal transformations[140–143] inspired the more recent computational work on polymer vesicles. Pioneering work by Seifert et al. reported on critical concepts such as spontaneous curvature and bilayer coupling in lipids.[144] Li et al. capitalized on these ideas to model the shape transformation of polymersomes from amphiphilic triblock copolymers using DPD.[145] They varied the repulsive parameters between the hydrophilic blocks to control the spontaneous curvature and obtained various vesicular morphologies, such as starfish, toroidal, or stomatocytes. Compellingly, their results suggested that polymersomes would essentially undergo the same transformations as liposomes provided that they have the same mobility. This assumption was later verified experimentally by Kim et al. and Salva et al. by inducing osmotically driven shape transformation of vesicles.[23,99] Recently, Tan et al. simulated the shape transformations of hyperbranched amphiphilic block copolymers by altering the interaction parameters and hydrophilic fractions.[146] In addition to observing the expected transformations of spherical and tubular vesicles, as well as stomatocytes, they predicted toroidal structures with multiple holes. Such results are already ahead of current experimental observations in the field and are an indicator of the potential shape transformations that may be ultimately attainable.

Gratifyingly, modeled structures are often observed in experimentally prepared samples. However, *in silico* calculations and empirical evidence are often discrepant in that the former focuses chiefly on the thermodynamically most favorable morphology, while kinetic pathways and intricate experimental details often lead to morphologically diverse mixtures. With the explosion in computing power today, one can reasonably poise that more variables can be fed into the simulations to better anticipate the experimentally observed morphologies. Ultimately, there is little doubt that computational advances will make it possible to use simulation to inform the conditions necessary to yield homogeneous assemblies.

## 6. Current Status and Use

### 6.1. Based on Shape-Transformed Vesicles

#### *6.1.1. Nanomotors*

Nanomotors are autonomous robots "capable of swimming in liquid environments by harvesting fuel from their surrounding media or by harnessing power from external energy sources."[147] To comprehend the growing interest in nanomotors and their remarkable potential, one must understand the inspiration behind them: *taxis*. The latter is a cellular process, whereby biomolecular motors harness energy from the hydrolysis of adenosine triphosphate (ATP), to perform their motile functions.[148] Consequently, the promise to create self-propelled systems capable of autonomous transport has been truly stimulating. Since the fabrication of a micro-sized motor by Ismagilov et al.,[149,150] rapid developments have led to micro- and nano-sized motors capable of utilizing chemical,[151] optical,[152,153] rheological,[154] or ultrasonic energy sources to achieve *taxis*.[155] Recent reviews on this burgeoning field highlight these developments, and show that the complexity of these nanomotors cannot be understated.[147,148,156–158] Quintessentially however, a successful system has a few specific requirements: efficient propulsion of the motor, and the ability to carry the cargo successfully, especially in a manner that does not negatively interfere with the propulsion mechanism. Gratifyingly, polymer stomatocytes are well poised to contribute to this tantalizing field of research. Using the solvent injection method, Wilson et al. fabricated spherical GPs with PS-*b*-PEG copolymers and transformed them into stomatocytes by dialysis. The latter was performed in an aqueous solution that containing platinum nanoparticles (PtNp), which were


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trapped in the invagination (or secondary cavity, **Figure 6**a). These PtNps catalyzed the decomposition of $H_2O_2$ into oxygen in water, resulting in an $H_2O_2$-powered 'stomamotor.'

Recently, Tu et al. demonstrated that these PS-*b*-PEG-based stomamotors could potentially be used in stimuli-responsive drug-delivery schemes if the two blocks are connected by disulfide bonds.[159] The GPs were loaded with doxorubicin during self-assembly prior to being deformed into stomamotors as mentioned above. The resulting stomamotors were able to show directed $H_2O_2$-powered motility using a PtNp motor. The addition of glutathione (GSH) prompted the reduction of the disulfide connector leading to the complete disintegration of the stomatocyte (Figure 6b). The simultaneous discharge of the encapsulated doxorubicin and of the PtNPs ensured the targeted release of the therapeutic payload by halting motion at the GSH exposure site.

Obviously, PS-based stomamotors are not optimal carriers due to the limited biomedical relevance of styrenic monomers. Thankfully, recent synthetic advances hint at the imminence of novel, biologically relevant stomamotor for drug-delivery. Tu et al. developed a bio-hybrid stomatomotor (Figure 6c) by blending PS-*b*-PEG with poly(caprolactone)-*block*-poly(ethylene glycol) (PCL-*b*-PEG).[160] These nanomotors showed pH responsiveness, disintegrating upon acidic degradation of the PCL block. Remarkably, Toebes et al. fabricated completely biocompatible and biodegradable nanomotors by using tubular polymersomes instead of stomatocytes. First, they fabricated polymersomes from azide-functionalized poly(*D,L*-lactic acid)-*block*-poly(ethylene

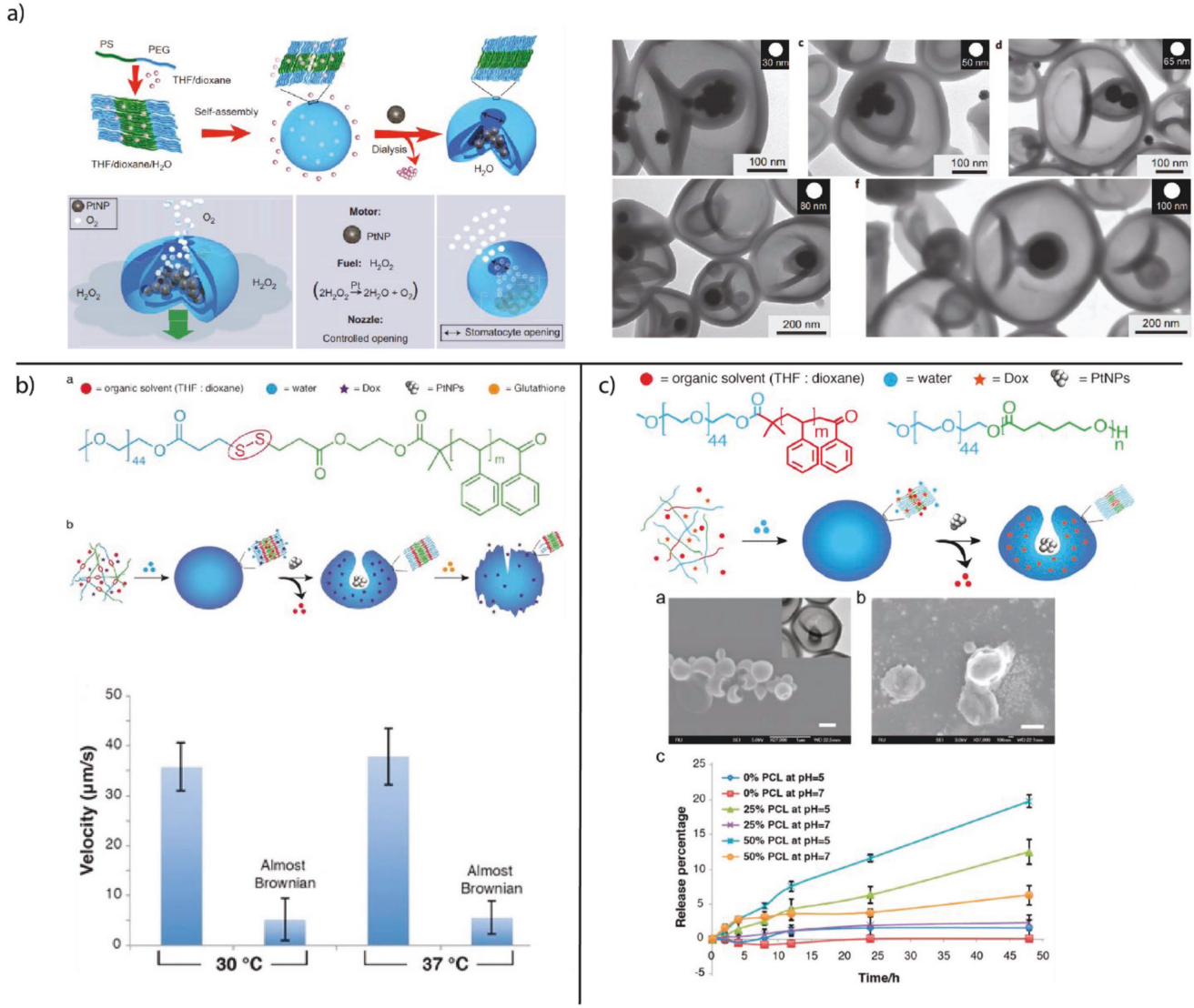


**Figure 6.** a) Design and assembly mechanism of the nanomotor. The TEM images show entrapment of the PtNps in the stomatocyte cavity. The small inserts indicate the size of the nanoparticle entrapped. Reproduced with permission.[150] Copyright 2012, Nature Publishing Group. b) Schematic representation of redox-sensitive stomatocytes made from PS and PEG blocks separated by a disulfide bond. The bottom graph shows that upon reduction, the velocity of the nanomotor follows Brownian motion, suggesting that the PtNp motor has been released upon disintegration. Reproduced with permission.[159] Copyright 2017, Wiley-VCH. c) Biodegradable stomatocytes formation. The graph shows a release profile of encapsulated doxorubin with PCL composition. Reproduced with permission.[160] Copyright 2017, American Chemical Society.


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glycol) (PEG-*b*-PDLLA) diblock copolymers and then transformed them into prolates via dialysis. The azide functionality was used to couple catalase, a ubiquitous enzyme, which, like the aforementioned PtNPs, catalyzes the decomposition of $H_2O_2$ into water and oxygen. Catalase served as the engine driving the nanotubes motions.[161] Instead of utilizing the secondary compartment present in stomatocytes, they directly attached catalase to the vesicle. Advantageously, unlike PtNPs, catalase is biobased and biocompatible. The nanotubes were also successfully loaded with hydrophilic and hydrophobic dyes.

In fact, Pijpers et al. reported completely biodegradable and biocompatible stomatocytes based on PDLLA-*b*-PEG.[162] They blended various diblocks of PDLLA-*b*-PEG into polymersomes via solvent injection (THF/dioxane/water) and then used dialysis in NaCl to shape-transform polymersomes into stomatocytes. They found that changes in PEG solvation upon dialysis were responsible for this shape change. Since similar PDLLA-*b*-PEG had previously formed nanotubes upon dialysis, this report demonstrated how slight changes in chemical composition lead to different assemblies.[23] The next logical step is to use the secondary container to load an appropriate propulsion mechanism.

### 6.1.2. Nanotubes

As shown above, one can transform GPs into kinetically stable ellipsoidal and tubular shapes. The latter exhibit higher adhesion affinity to more peptides and functional proteins compared to spherical aggregates due to their larger surface area at constant volume.[163] Consequently, these non-spherical structures adhere to vascular walls.[164,165] Van Oers et al. reported the shape transformation of GPs into tubules using crosslinking.[166] They copolymerized styrene with an azide-functionalized styrenic monomer using PEG macro-CTA to form the amphiphilic diblock terpolymer. GPs were then obtained by solvent displacement using THF/water. Then, a bicyclo[6.1.0]nonyne derivative was reacted with the azide moiety via strain-promoted 'click' coupling. This reaction introduced a tension into the membrane, which resulted into an elongation into tubules. Building on this work, Abdelmohsen et al. used a PDLLA-*b*-PEG to fabricate GPs by solvent displacement (**Figure 7**). Using dialysis under hypertonic conditions, GPs transformed into nanotubes.[115] The latter could successfully be loaded with both hydrophobic and hydrophilic drugs. Moreover, they also tethered the tubes to functional proteins as shown Figure 7b. This report was one of the first to integrate both biocompatibility and biodegradability.

### 6.1.3. Breathing Polymersomes

Carbon dioxide is a key metabolite with a constant presence in cellular milieu. Cells utilize it to adjust cellular pH, and, when in excess, it can be removed by activating $CO_2$-sensitive pathways.[167] To mimic this property, several groups have successfully imparted physiologically relevant $CO_2$-responsiveness to polymersomes.[167–170] Yan et al. coined the

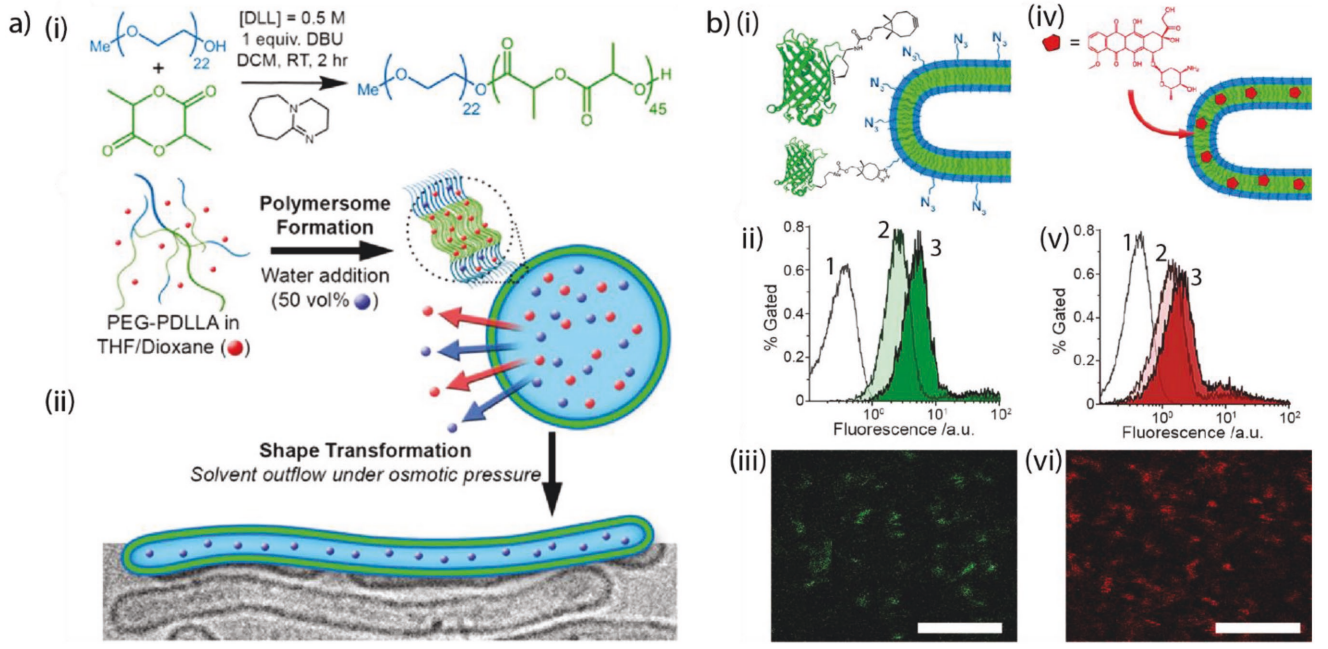


**Figure 7.** a) Schematic outlining the (i) organobase-catalyzed synthesis of PEG-*b*-PDLLA and (ii) the osmotically induced shape transformation of spherical polymersomes into nanotubes. b) (i) Schematic outlining the covalent modification of azide modified nanotubes using $eGFP^{BCN}$, (ii) flow cytometry data for eGFP modification of 5% azido nanotubes using an equimolar (curve 2) or a 3-fold excess (curve 3) of eGFPBCN compared to unmodified tubes (curve 1) and (iii) confocal visualization of green-fluorescent nanotubes. (iv) Schematic outlining the loading of nanotubes with fluorescent doxorubicin (DOX), (v) flow cytometry data of nanotubes loaded with 2 (curve 2) and 5 (curve 3) wt % DOX compared to unloaded tubes (curve 1) and (vi) confocal visualization of DOXloaded nanotubes (5 wt % preparation). All scale bars = 5 µm. Reproduced with permission.[115] Copyright 2016, American Chemical Society.


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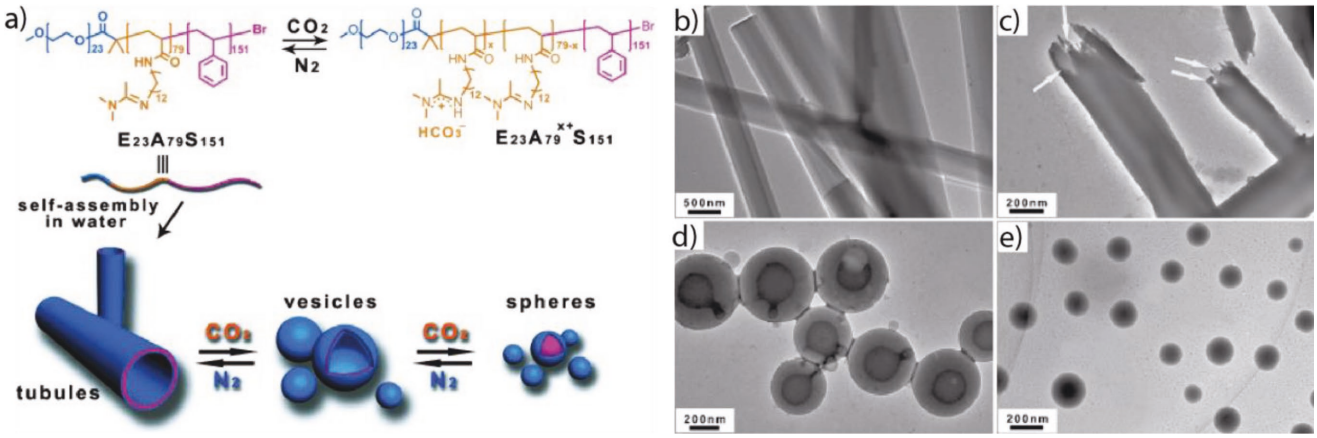


**Figure 8.** a) Schematic representation of the PS-*b*-PDDA-*b*-PEG system and $CO_2$-responsiveness. b) TEM images of the aggregates in different levels of $CO_2$ stimulus: b, c) no stimulus, d) 15 min, e) 25 min. Scale bars: b) 500 nm, c–e) 200 nm. Reproduced with permission.[173] Copyright 2013, Wiley-VCH.

term 'breathing polymersomes' when studying the behavior of poly((*N*-amidine)dodecylacryl amide)-*block*-poly(ethylene glycol) (PDDA-*b*-PEG) copolymer assemblies.[171] They observed that these systems contracted and expanded back (hence the term breathing), when exposed to $CO_2$ and Ar respectively. First used by Liu et al.,[172] amidine is a nitrogen-based molecule readily protonated by $CO_2$ in the presence of water to form a charged amidinium species (**Figure 8**). In doing so, the hydrophilic character of the PDDA block increases, which in turn leads to the reconfiguration of the assembly, as the protonated PDDA blocks move to the corona, while the uncharged PDDA remains in the inner layer. However, the complete protonation of the PDDA leads to its dissolution in water, thereby disintegrating the vesicles and limiting their transformability. In an effort to stabilize the bilayer structure, Yan and Zhao 'sandwiched' PDDA between PEG and PS to form a PS-*b*-PDDA-*b*-PEG triblock terpolymer.[173] While the back-transformation from micelles to vesicles was incomplete with the diblocks, the presence of a permanently hydrophobic PS block in the terpolymer ensured a much stronger phase separation and enabled a complete and reversible transformation from spherical vesicles to nanotubular prolates. The facile reversion to amidine upon bubbling $N_2$ makes this approach a viable gateway towards so-called 'living assemblies' for cell mimicry.

Capitalizing on this seminal work, the breathing vesicle scheme was also implemented in a number of different polymeric systems (including SPs), such as amphiphilic PCL-based star polymer assemblies.[174] In that case, the crystallinity of PCL probably also served as a jamming mechanism on top of the glassy nature. Another system proposed by Liu et al. exhibits dual $CO_2$- and temperature-responsiveness.[175]

Recently, Che and Yuan reported how a poly(2-dimethylaminoethyl methacrylate) (PDEAMA) center block can serve as a $CO_2$-responsive segment. Using a solvent displacement method followed by dialysis, the PS-*b*-PDEAMA-*b*-PEG assembled into stomatocytes and could be transformed into butterfly-shaped wrinkled vesicles upon exposure to $CO_2$.[176] Excitingly, this report marks the first demonstration of a stimuli-responsive, shape-transforming stomatocyte.

Lin et al developed a triple-stimuli responsive system: $CO_2$, light and $O_2$,[177] using a poly[(ethylene glycol)methyl ether]-*block*-poly(*N*,*N*-dimethylamino ethyl methacrylate-*co*-2,2,2-trifluoroethyl methacrylate)-*block*-poly(4-(4-methoxy-phenylazo)phenoxy methacrylate) (PEG-*b*-P(DMAEMA-*co*-TFEMA)-*b*-PMEPPMA) triblock tetrapolymer. The P(DMAEMA-*co*-TFEMA) block was responsive to $CO_2$ and $O_2$ (**Figure 9**). In addition to its role in light responsiveness, the presence of the rigid hydrophobic PMEPPMA block ensured the integrity of the system upon $CO_2$ and $O_2$-induced 'breathing', rather than micelle formation or disintegration. These systems are a good example of how shape transformation can be tailored towards response to physiologically relevant stimuli.

### 6.2. Non-Transforming Glassy Polymersomes

While recent applications of these GPs have been mainly focused on shape transformation, as highlighted above, spherical GPs have still a lot to offer. Molla et al.[178] excitingly demonstrated the actuation of an azobenzene unit connecting the hydrophilic PEG block and the glassy membrane-core-forming PDLLA segment (**Figure 10**). Remarkably, a single azobenzene unit per chain was capable of creating a 'ripple' motion across more than 500 chemical bonds of a GP membrane. The self-assembled polymersomes (ca. 165 nm in diameter) encapsulated the hydrophobic dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate for over 15 days. They also established the on-demand light responsiveness of these assemblies by exploiting "the sensitivity of non-equilibrium glassy systems to interfacial mobility." Azobenzene rearranges from *trans* to *cis* upon exposure to sub-400 nm light.[179–181] Visible light (>400 nm) powers the reverse isomerization. Molla et al. used this UV-responsiveness to elicit the release of both hydrophilic and hydrophobic dyes. Dye release stopped when the UV irradiation ceased. The azobenzene isomerization at the interface of both blocks creates a transient relaxation of the out-of-equilibrium glassy membrane. This relaxation facilitates the propagation of an otherwise small perturbation and leads to fast on-off response times. In polymersomes, most stimuli-responsive schemes rely on the presence of a concentration gradient.


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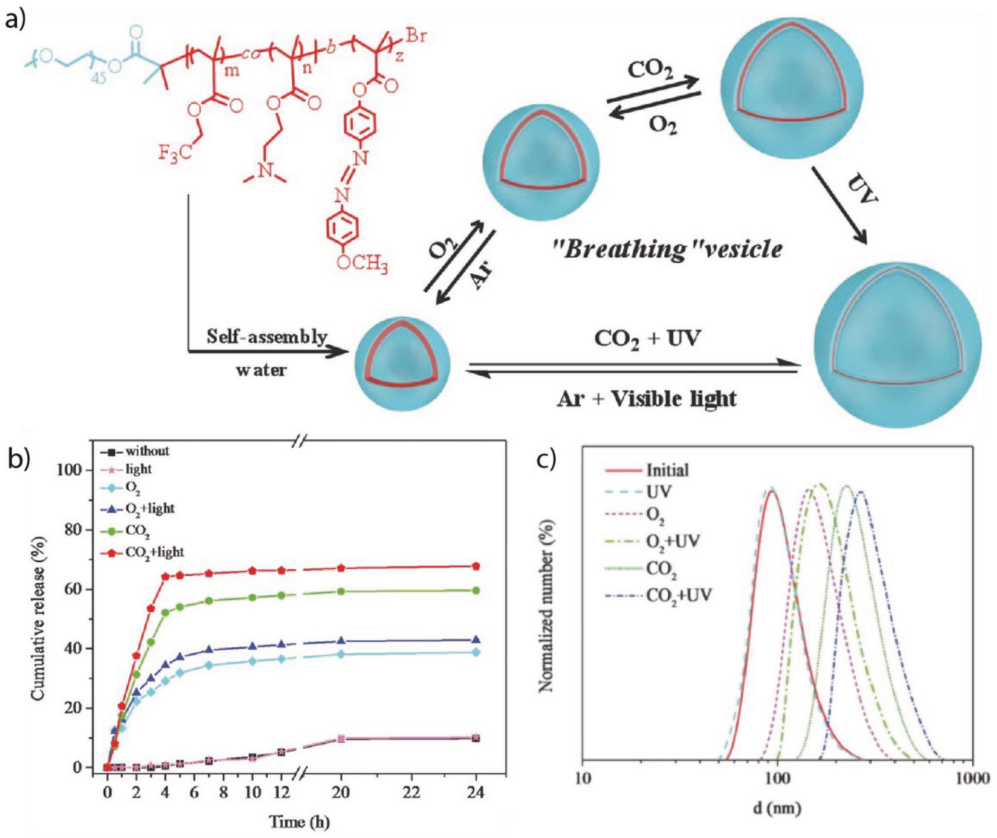


**Figure 9.** a) Chemical structure of a prepared triblock tetrapolymer and illustration of its self-assembly in water into vesicles with a "breathing" responsive behavior upon external stimulation. b) Rate-tunable Calcein release from P2 vesicles upon different single stimuli or various stimuli combinations. (Concentration of P2 vesicular solution: 1 mg $mL^{-1}$). c) DLS results showing the change in size of vesicles with stimuli. Reproduced with permission.[177] Copyright 2017, Wiley-VCH.

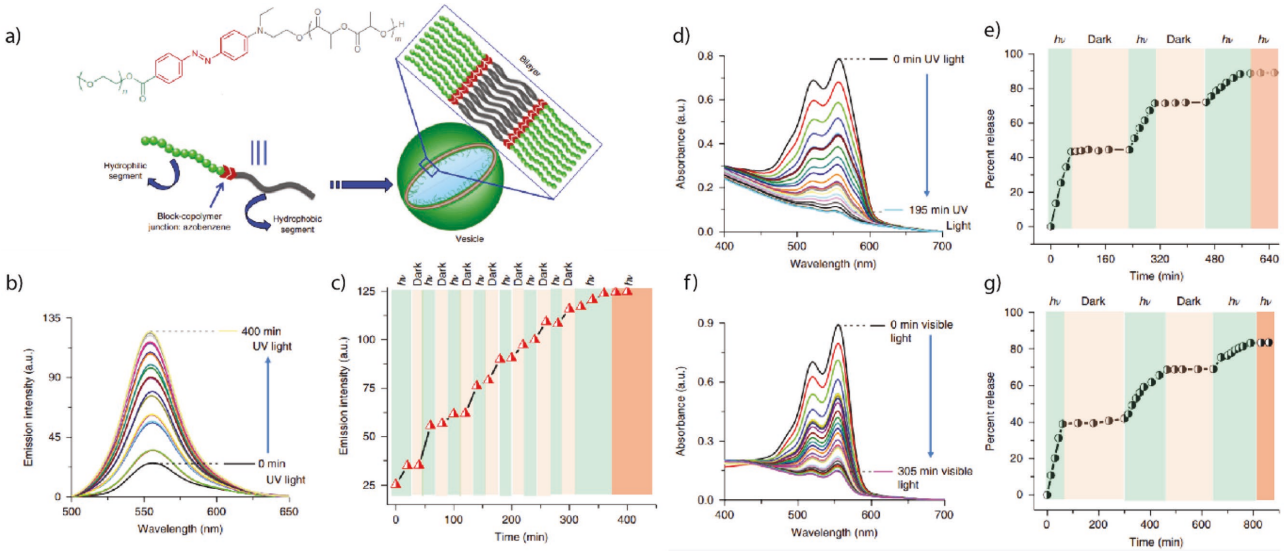


**Figure 10.** a) Chemical structure of the block copolymer azobenzene-modified PDLLA-*b*-PEG diblock used and illustration of the vesicle and the bilayer assembly. b) Vesicles demonstrating non-equilibrium behavior. Increasing emission intensity of the hydrophilic dye R6G in response to its release from P2 vesicles, controlled by alternating cycles of UV light and darkness. c) Emission intensity profile of R6G during light and dark cycles. Absorption spectra indicating DiI release from vesicles during alternating cycles of d) UV light and dark, and f) visible light and dark. Corresponding percent release profile of DiI from P2 vesicles in the alternating presence of e) UV light and dark and g) visible light and dark, respectively deduced from d) and f). Reproduced with permission.[178] Copyright 2018, Springer Nature.


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Molla et al. circumvented this need and demonstrated the versatility of a simple, biodegradable and biocompatible system, by controlling the release of encapsulated hexamethylene diamine into an excess of sebacoyl chloride to synthesize nylon.

Cell biomimicry has driven the exploration of polymersomes. Organelles, such as mitochondria and the Golgi apparatus, play vital cellular functions, respectively in metabolism and protein modification.[182] Oftentimes, a membrane separates these organelles from the cytoplasm. This separation enables each organelle to perform its function successfully without interfering with other organelles. Consequently, compartmentalization (i.e., the fabrication of polymersomes inside polymersomes) is probably the next step in replicating cellular machinery.[183] Despite the advantages of SPs in terms of fluidity and elasticity, the impermeablity of GPs make them pertinent cell models.

Peters et al. developed a multi-compartmentalized system capable of carrying out a cascade reaction, similar to a eukaryotic cell (**Figure 11**).[184] First, they encapsulated enzymes (CalB and ADH) into porous polystyrene-*b*-poly(3-(isocyano-*L*-alanyl-aminoethyl)thiophene) (PS-*b*-PIAT) GPs. These polymersomes were then loaded together with cytosolic enzymes and substrates into an outer PBD-*b*-PEG vesicle. The PS-*b*-PIAT polymersome is porous and allows the diffusion of small molecules (e.g., substrates and products of the cascade reaction), while trapping the proteolytic enzymes.[185]

Industrials have recognized the possibility to encapsulate both hydrophilic and hydrophobic molecules into polymersomes.[186] However, they suffer from poor stability upon exposure to industrial surfactants. Song et al.[187] fabricated rigid PS-*b*-PEG-based crew-cut polymersomes with ultra-thick membranes (up to 47 nm) via PISA and encapsulated the dye Rhodamine B therein. Gratifyingly, the resulting polymersomes were stable despite the presence of strong surfactants. In non-medical related industries, keeping active agents encapsulated appealingly means extended shelf-life for the product (fragrances, coatings etc.). Thanks to their resistance to permeability, GPs can therefore play a more important role.

## 7. Conclusion and Outlook

This compendium aimed to summarize some of the most noticeable GPs literature and to show how polymersomes rapidly rose to prominence thanks to their similitude to their lipid-based analogs.[6] Interestingly, new and theoretically predicted structures are still emerging and their isolation and characterization is ongoing,[111] and shape-transformation continues to be a driver of innovation in the field. In the following paragraphs, we outline our views on un(der)explored uses of GPs as well as the challenges posed by their fabrication, implementation and characterization. For the sake of clarity, the discussion starts with lower hanging fruit and increases gradually in complexity.

The enhanced impermeability of GPs and their resistance to surfactants promote longer shelf lives for encapsulated active agents. These assets are particularly desirable in areas such as self-healing coatings and cosmetics industries. The Moore, Sottos and White groups have developed a healable materials, whereby monomers and catalyst are contained separately in poly(urea-formaldehyde) capsules (made via an in situ emulsion polymerization) embedded in an epoxy matrix.[188] Upon mechanical damage, the capsules break and trigger the polymerization which fills the gap. Nevertheless, the size of the capsules (typically on the order of hundreds of microns) can be problematic in the realization optically clear coatings. Consequently, the ability to reduce the dimensions of the capsules would be very advantageous to reduce the haziness of the

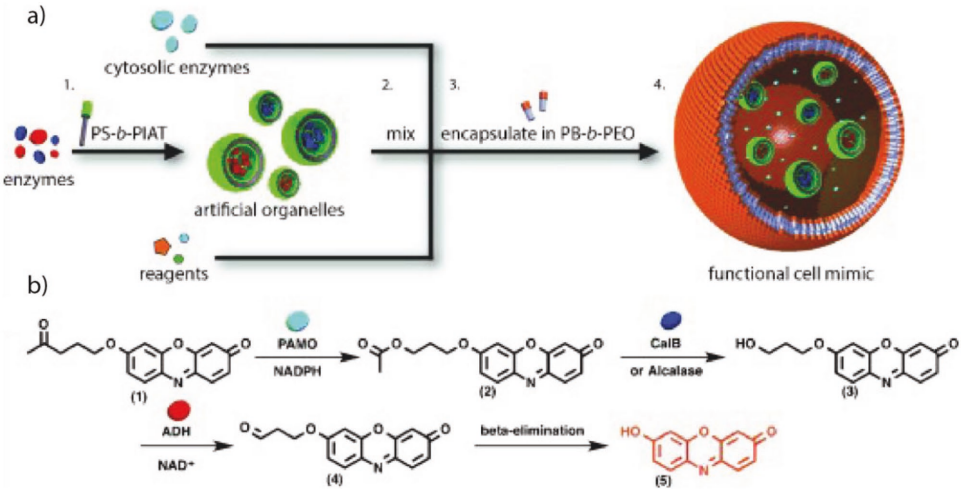


**Figure 11.** a) The concept of the cell mimic, which shows the initial encapsulation of different enzymes in polystyrene-*b*-poly(3-(isocyano-L-alanyl-amino-ethyl)-thiophene) (PS-*b*-PIAT) nanoreactors (1), followed by mixing of the organelle mimics, cytosolic enzymes, and reagents (2), before encapsulation of the reaction mixture in polybutadiene-*b*-poly(ethylene oxide) (PB-*b*-PEO) vesicles (3) to create the functional cell mimic (4), inside which enzymatic multicompartment catalysis takes place. b) Detailed cascade reaction scheme. Profluorescent substrate 1 undergoes a Baeyer–Villiger reaction catalyzed by phenylacetone monooxygenase (PAMO), with one unit of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) being consumed, to yield ester 2, which is subsequently hydrolyzed by Candida antarctica lipase B (CalB) or alcalase to provide primary alcohol 3. Alcohol dehydrogenase (ADH) oxidizes the alcohol, by using the cofactor nicotinamide adenine dinucleotide (NAD+), to give aldehyde 4, which then undergoes spontaneous beta-elimination to yield resorufin (5) as the final fluorescent product. Figure and caption reproduced with permission.[184] Copyright 2013, Wiley-VCH.


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healable layer. Also, typically, these capsules have served to hold organic contents. However, with an increasing need for green chemistry, the possibility to perform healing using water as a solvent could be attractive. Additionally, research in the field of reverse vesicles has been very restricted compared to their normal phase analogs. The ability to encapsulate organic liquids in reverse GPs could prove very advantageous indeed.[189]

Normal-phase rigid polymersomes are also enticing in radionuclide therapy due to their potential to lower the impact of daughter nucleotide recoil. While PBD-*b*-PEG systems required polymersome diameters of up to 800 nm,[190,191] smaller, thick-membraned crew-cut polymersomes would enable longer circulation times and better nuclide retention. Consequently, therein lies an opportunity to develop a more targeted approach to cancer therapy. Likewise, in bioimaging or theranostics, the ability to retain the marker until the carrier reached the desired destination is of paramount importance.[192]

Interestingly, most of the reported work on GPs, especially those in shape-transformations has mainly focused on diblock systems. It is worth noting that amphiphilic triblock copolymers offer more unexplored room for research. ABA triblock copolymers have shown interesting self-assembly behavior; while some triblocks can self-assemble into polymersomes by forming a monolayer membrane, others orient themselves into a U-shape to form bilayer membranes.[193] ABC blocks have also been observed to show preferential orientation of their hydrophilic blocks, with some blocks forming the interior while the other hydrophilic block is oriented towards the exterior.[194,195] The possibility of all these different morphologies is enticing when applied to shape transformation as it may lead to more ways to control shape transformation.

To fully realize the potential of newly isolated morphologies, a bigger effort needs to be placed on their characterization. Early reports on block copolymer solution self-assembly continue to be used as references to verify the nature of the aggregates.[15,196] Images from seminal manuscripts (such as in Figure 2) often serve to identify the self-assembled aggregates.[15,196] There is consequently a need to develop similar knowledge for the various shape-transformed morphologies. Robust methods like dry and cryo-TEM are able to characterize these morphologies since they offer a visual image of the structures. However, even if they tend to become more accessible, they can prove cost-prohibitive (*viz*. onerous equipment and maintenance) and time-consuming particularly as one attempts to refine a fabrication protocol. Consequently, the improvement on the current theories and models for alternative methods, such as light scattering, is highly coveted. Most reports use dynamic light scattering to determine the hydrodynamic size of their assemblies. When used in conjunction with static light scattering, the ratio of the radius of gyration ($R_g$) and the hydrodynamic radius ($R_h$) predicts the morphology of the self-assembled structure. For $R_g/R_h$ close to 1, hollow spheres are expected, while $R_g/R_h$ = 0.77 indicates solid spheres (here micelles). Accordingly, Abdelmohsen et al. used light scattering to differentiate between prolate and oblate self-assembled structures.[197] Using the Perrin approximation, they were able to model the prolates quite accurately but observed some discrepancies with oblate assemblies. Consequently, there is plenty of room to refine theoretical considerations and come up with more representative models of the various morphologies attainable with GPs. This endeavor will in turn prove beneficial for the rapid screening of morphologies by scattering techniques.

As alluded to earlier, compartmentalization is one of the next frontiers in the field of polymersomes.[183] In the cell, multiple organelles have different shapes that enhance their function.[198] The ability to transform polymersomes into function-enhancing shapes is a tantalizing prospect to enhance cell biomimicry. This feat would require the fabrication of intricate co-assemblies of GPs (in GPs). The recent fabrication of the stomatocyte-in-stomatocyte bodes well for this type of complex architecture.[199] Generally speaking, the prospect of controlling the individual shape of each polymersome to make function-enhancing assemblies (e.g., stomatocyte-in-sphere, sphere-in-sphere or tube-in-stomatocyte) is quite alluring. Furthermore, the incorporation of orthogonal stimuli-responsiveness to address each individual organelle mimic and therefore emulate signaling cascade will lead to hitherto unattained intricacy and function. Features, such as breathing and responses to other physiologically relevant stimuli (e.g., light, force) promise to help replicate complex biological functions.

Finally, in SPs, the insertion of membrane proteins into the membranes has enabled transport control.[200–203] Bestowing such properties upon GP systems would also be another important milestone. One must however take heed that most of the current transmembrane polymersome assemblies remain semi-permeable and rely mostly on sieving effects, namely they cannot sustain a gradient. Additionally, the kinetics of the incorporation of bulky pore-forming structures (e.g., DNA nanopores, transmembrane proteins) into a glassy system would be probably vastly different from the SP counterparts and must be carefully considered. Nonetheless, this dearth of knowledge presents itself as a great opportunity to understand and control the co-assembly of amphiphiles with pore-forming elements. The on-command flux control of substrate in and out of the vesicles constitutes another extraordinary opportunity to create hierarchical structures with complex signaling pathways. Overall, the field of GPs is now mature for further studies and we hope that this review will encourage many to investigate their potential.

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## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

block copolymers, glassy polymersomes, shape-transformation

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