Advances and challenges in smart and functional polymer vesicles

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Polymer vesicles prepared by self-assembly techniques have attracted increasing scientific interest in recent years. This is as a result of their numerous potential applications such as tunable delivery vehicles, for the templating of biomineralization, as nanoreactors and as scaffolds for biological conjugation. Presented in this review are the recent advances in the preparation and application of 'smart' and functional block copolymer vesicles such as those which respond to external stimuli to afford a change in structure, morphology or controlled release event. In this Highlight, we first give an overview of the structure of polymer vesicles, followed by a summary of the methods used for their preparation. We then focus on recently developed intelligent polymer vesicles which can respond to the application of external stimuli such as a change in temperature, pH or redox to afford novel nanomaterials. The potential applications of these materials are explored with specific focus on the functionalization of various domains of the polymer vesicles. Finally, the current limitations in the preparation and application of polymer vesicles are explored as are the challenges facing the development of these nanostructures towards real-world applications.

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1. Introduction

Polymer vesicles, or polymersomes, are usually hollow nanometer sized spheres with a hydrophobic bilayer membrane and hydrophilic internal and external coronas.[1,2] The typical structure of a polymer vesicle is shown in Fig. 1. The hydrated hydrophilic coronas (blue in Fig. 1) are expressed on both the inside and outside the hydrophobic membrane (red in Fig. 1) in aqueous solution. This hollow structure is different from

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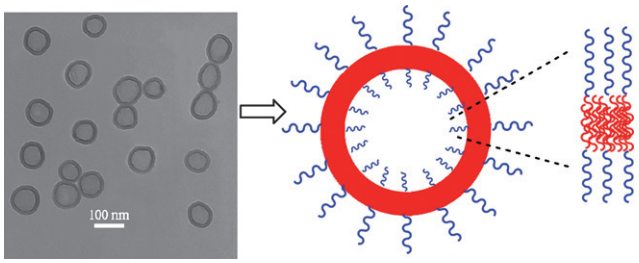

Fig. 1 Transmission electron microscopy (TEM) image (left)[24] and the corresponding schematic representation of a polymer vesicle (middle and right). Red: hydrophobic chains forming the compact vesicle membrane (corresponding to the dark ring in the TEM image); blue: hydrophilic chains forming solvated vesicle coronas (not visible in the TEM image).

polymer micelles which consist of a solid hydrophobic core and instead has a bilayer type structure. Compared to lipid-based vesicles,[3] polymer vesicles are more stable, robust and have potential for more advanced chemical functionalization and physiological application.[4] This has resulted in significant interest in the design, synthesis and modification of polymer vesicles for applications in such wide fields as materials science, biology and biomedical science.[5] Perhaps the most promising potential application of these materials is in the utilization of their interior cavities for the encapsulation of water and other hydrophilic guest molecules such as therapeutics. In addition, the hydrophobic bilayer membrane (also called the vesicle wall) can sequester and trap hydrophobic moieties such as dye molecules to facilitate the tracking and imaging of these materials in applications such as delivery vehicles.[4,6,7] It has also been demonstrated that the hydrophilic corona can be utilized to conjugate biologically active molecules, which are selectively expressed at the exterior of the vesicle, hence altering the vesicle's interaction with the surrounding environment.[8] Although numerous polymers have been used in the preparation of polymer vesicles, amphiphilic block copolymers are the most commonly reported building blocks for the preparation of vesicles by self-assembly techniques.[9,10] However, amphiphilic alternating copolymers[11] and hydrophilically modified comb-like homopolymers have also been explored for the synthesis of vesicle-type structures.[12,13]

Amphiphilic block copolymers can self-assemble into a range of morphologies in solution, including spherical micelles,[14,15] rods,[16] simple vesicles,[17] disc-like micelles,[18] Janus micelles with segregated faces,[19] toroids[20] and other complex polymer aggregates such as large compound micelles with an inverted core–shell structure surrounded by a hydrophilic surface.[17] The vesicle family also has a wide range of morphological variations such as simple vesicles with a hydrophobic membrane and hydrophilic corona as shown in Fig. 1, onion-like vesicles, elongated tubular vesicles,[21] large compound vesicles,[21] genus vesicles, perforated vesicles with a highly folded membrane[22] and flower-like vesicles.[23]

Classically, a dimensionless 'packing parameter', p, is used to define the morphology of the resultant self-assembled structure.[25] Here, $p = v/(al)$ where v is the volume of the hydrophobic polymer chain, a the optimal interfacial area per molecule and l the hydrophobic length normal to the interface. Different packing parameters usually correspond to different shapes of self-assemblies: spheres ($p \leq 1/3$), cylinder ($1/3 \leq p \leq 1/2$), and bilayers ($1/2 \leq p \leq 1$). Unsurprisingly a given molecule does not have a constant packing parameter as all three factors, v, a, and l, depend on the self-assembly conditions, which influence the state of the polymer chains. Factors which control the morphology of the resultant nanostructure include the chemical structure of the copolymer, the hydrophilic/hydrophobic ratio, copolymer concentration in solution and the solvent properties such as the type of organic solvent, the ratio of organic solvent/water, salt concentration, solution pH, and temperature.[9] Among these factors, the volume ratio of the hydrophilic to hydrophobic block is proposed to be an important parameter in the self-assembly process. As a general rule, copolymers with hydrophilic to hydrophobic ratios greater than 1:1 usually form micelles; copolymers with a ratio less than 1:2 usually favor vesicle formation; those with ratios less than 1:3 may form vesicles, inverted micro structures, other complex structures, and finally macroscopic precipitates. However, these ratios are not definitive design features and often exceptions to these guidelines are observed as are multiple morphologies. It should be stressed that it is the balance between all the free energy contributions to the self-assembly and also kinetic factors that determine the morphology of the final nanostructure. The hydrophilic/hydrophobic ratio is an important factor but never the only determinative parameter. For example, spherical micelles, vesicles, lamellae, flower-like vesicles, large compound vesicles and perforated genus vesicles can be made from the same block copolymer assembled under different conditions.

There is significant versatility in the design and synthesis of polymer vesicles. The membrane fluidity, permeability and the corona functionalization of vesicles can be adjusted by tailoring the chemical structure of the block copolymers. The size of vesicles can be readily controlled by altering the polymer block lengths or by changing the solvent ratios.[9] The vesicle membrane thickness usually ranges from several nanometers to tens of nanometers and directly relates to the hydrophobic length (also the hydrophilic/hydrophobic ratio can be important)[26] but is not always directly proportional to it. In principal, the fully stretched hydrophobic chains in the membrane lead to the thickest membrane and ideal random coils of chains form the thinnest membrane. In practice, for example, membrane thicknesses increase up to ~22 nm with the length of the hydrophobic block.[27,28] Quite recently the membrane thickness was reported to be dependent on the vesicle size, with bigger vesicles having thicker membranes than smaller ones.[29] Contrary to this, the membrane thickness has not reported to change with vesicle size according to the majority of polymer vesicle papers.[29] It seems that this rule is only suitable for a very limited class of vesicles with polyelectrolyte coronas. It is usual to observe vesicle wall thicknesses

ranging from 5 to 30 nm however thicknesses beyond 50 nm are rarely seen regardless of the length of the hydrophobic block. A pure phospholipid-like bilayer structure is not entropically favourable in polymer vesicles due to the longer hydrophobic chains than in lipids. Instead, the vesicle membrane is usually composed of an interdigitated hydrophobic block,[30] despite this the term 'bilayer' is still often used to describe the structure of the vesicle membrane.

There are many other classes of nanometer sized self-assembled polymeric hollow particles which do not have a bilayer membrane structure. The most common of these are derived from the modification of robust nanoparticle scaffolds. For example, Wooley and coworkers successfully removed the core domain of polymer nanoparticles by ozonolysis[31,32] or hydrolytic[33] degradation. Jiang and coworkers reported the synthesis of non-covalently connected polymer micelles (NCCMs),[34,35] which could be used to make hollow particles by reversibly breaking the hydrogen bonding interaction between the two polymers,[36] or by *in situ* polymerization following the core removing.[37] O'Reilly and coworkers also reported the synthesis of metal functionalized nanocages by removing the core domain of a preformed, metal connected polymer nanoparticle.[38,39] Other techniques such as by layer-by-layer colloid-templated assembly of polyelectrolytes can also be used for hollow particle preparation.[40,41] Although their preparation is more complex and time consuming than polymer vesicle formation by the self-assembly of macromolecules, these hollow nanoparticles, represent a whole different class of materials which may have interesting properties. However, these materials will not be covered in this Highlight and instead readers are directed to the above publications.

Pioneering work in the area of polymer vesicles has been extensively performed and excellently reviewed in recent years by Eisenberg,[9] Discher,[1,42] Meier,[7,10] Antonetti,[2] Armes and Ryan,[43] Liu,[44] Chen[45] and their coworkers, as well as many other groups. For example, very recently Li and Keller have reviewed the area of stimuli-responsive polymer vesicles.[46] In addition, Zhong and coworkers classified the application of stimuli-responsive polymer vesicles as drug delivery vehicles.[4] This highlights the very active and growing research activities in the field of responsive polymer vesicles. In this Highlight we aim to focus on the recent advances in the development of new 'smart' vesicles which can respond to the application of external stimuli, as well as the post-functionalization of polymer vesicles to afford novel nanomaterials. We also aim to highlight a number of current challenges in the preparation, characterization and real-world application of polymer vesicles.

2. Vesicle formation

2.1 Methods for vesicle preparation

There are two general methods for the preparation of polymer vesicles using the self-assembly of block copolymers. One is the 'solvent-switch' method, where an organic solvent is required to dissolve the copolymer prior to self-assembly.[9] The second is an organic-solvent-free method, where only water is needed for the dissolution of the block to allow for self-assembly.[10]

Solvent-switch techniques have been widely used in the preparation of polymer vesicles (and indeed for other morphologies) as most amphiphilic block copolymers are not directly soluble in water. Initially the amphiphilic copolymer is dissolved in a common organic solvent for both copolymer blocks and then water is added gradually to the copolymer solution. The hydrophobic blocks tend to associate together in the polar environment to form a vesicle membrane whereas the hydrophilic blocks are solvated to form the vesicle corona which colloidally stabilizes the vesicle. It is worth noting that sometimes the final organic solvent/water mixture ratio can strongly affect the resultant self-assembly morphology and also the permeability of the vesicle membrane. Using this technique the removal of the organic cosolvent, by dialysis, is often necessary, this however can be time-consuming and economically unfavourable. Furthermore, as the solvent properties change gradually during dialysis, this can sometimes lead to a change in the morphology of the polymer self-assembles upon cosolvent removal.

The organic-solvent-free preparation method usually involves the rehydration of the copolymer in pure water and includes a film or bulk swelling step. In film swelling, copolymers are firstly dissolved in an organic solvent such as chloroform, then a thin film is formed after evaporation of organic solvent. The subsequent addition of water or aqueous buffer solution hydrates the block copolymer to form vesicles.[47–50] This method can also be applied for the formation of polymer films on the surface of electrodes (electroformation).[51] This can be achieved by the hydration of the polymer film by an electric current to afford giant polymer vesicles. Strictly speaking, film swelling is not a pure solvent-free method because of the involvement of an organic solvent during the film preparation, prior to the vesicle formation.

Compared to film swelling, the bulk swelling approach does not involve any organic solvents during the vesicle preparation as the copolymer can be directly dissolved in water to form nanostructures. However, longer and more vigorous agitation is usually required to fully hydrate the polymer sample and this usually results in structure defects and/or broad particle size distributions.[52–54] However, a recent report by Du and Armes involves the direct dissolution of poly(ε-caprolactone)-*b*-poly(2-aminoethyl methacrylate) [PCL-*b*-PAMA][55]or poly(ε-caprolactone)-*b*-poly[2-(methacryloyloxy) ethyl phosphorylcholine] [PCL-*b*-PMPC][56] block copolymers in pure water without the need of organic solvents, film formation or agitation, to afford well-defined vesicles.

Other solvent-free methods for vesicle preparation which are different from film swelling have also been reported recently. For example, Armes and coworkers reported that by directly dissolving a copolymer in water at low pH, then increasing the pH by the addition of NaOH aqueous solution, lead to the formation of well-defined pH-responsive block copolymer vesicles.[57] Also, Kataoka and coworkers have reported that the mixing of two oppositely charged block copolymers in aqueous solution, afforded the formation of polymer vesicles with a semipermeable polyion complex membrane (PICsome).[58,59] These approaches provide a simplified procedure for the preparation of well-defined polymer vesicles, although these routes cannot be considered generic and are only

applicable to pH-responsive or charged polymers.

2.2 Mechanism of polymer vesicle formation

Polymer vesicles are considered to form in a two-step process. First, the polymer chains form a bilayer-type membrane, which then subsequently closes to form a hollow structure.[2] This process involves an interfacial curvature change, which can correspond to a change in the packing parameter for the polymer and hence a change in the resultant morphology.

However, theoretical calculations have revealed that some vesicle formation process may be more complicated than the above 'two-step' procedure. There are several theoretical simulations which model and study lipid and polymer vesicle formation based on particle models, such as, Brownian dynamics,[60] dissipative particle dynamics,[61] and molecular dynamics.[62] The results of these simulations can be summarized in two different proposed mechanisms for the spontaneous formation of vesicles from the homogeneous state.[63] These are shown in Fig. 2, where (a) mechanism I: the homogenous amphiphilic block copolymers (grey square in Fig. 2) self-assemble into small spherical micelles rapidly, which slowly (relative to the small spheres formation) evolve into larger micelles such as cylindrical micelles, open disc-like micelles by collision. The large disc-like micelles slowly (relative to the first step) close up to form vesicles. The second mechanism shown in (b) has a similar initial stage to mechanism I. Small spherical micelles are formed rapidly. The spherical micelles then grow up to large spherical micelles by an evaporation-condensation-like process. The large micelles take the solvents into them to lower the energy because they are energetically unfavourable.

Mechanism I can explain simple vesicle formation but it does not fully explain more complex vesicle morphologies such as large compound vesicles, multi-layer vesicles, *etc.* In 2006, He and Schmid calculated the dynamical simulation[64] of vesicle formation using external potential dynamics.[65] Their proposed formation process from these calculations was similar to mechanism II. Their results, however do not agree with previous particle simulations but were recently supported by vesicle encapsulation experiments reported by Adams and coworkers.[66] More recently, Uneyama applied the concept of block copolymers density functional theory to the dynamics of self-assembly.[63] Numerical simulations for amphiphilic diblock copolymer solution self-assembly were carried out in three dimensions and by using the continuous field model, it was shown that a vesicle is spontaneously formed from a disordered uniform phase consistent with mechanism I.

In 2004, Du and Chen experimentally trapped the transition states in the evolution of spherical micelles to vesicles by gradually increasing the polarity of the solvent mixture (Fig. 3).[28] First, poly-(ethylene oxide)-*b*-poly(3-(trimethoxysilyl) propyl methacrylate) (PEO-*b*-PTMSPMA) block copolymer was dissolved in methanol, then water was gradually added into this solution. Initially, spherical micelles were the dominant morphology with some rod-like micelles, however upon increasing the water content lamellae with protruding rods formed along with a few vesicles. Further increasing the water content afforded a majority of vesicular structures with some lamellae-type structures evident, and then at greater than 50% water content exclusively vesicles were observed by transmission electron microscopy (TEM) analysis. It was proposed that the experimental results observed can be seen to support mechanism I as the mechanism of vesicle formation.

It has been proposed by Adams that the vesicle formation mechanism may affect the loading efficiency of hydrophilic actives.[66] Mechanism I involves the closure of disc-like micelles, which allows hydrophilic molecules to be trapped during this process with a relatively high loading efficiency. On the contrary, in

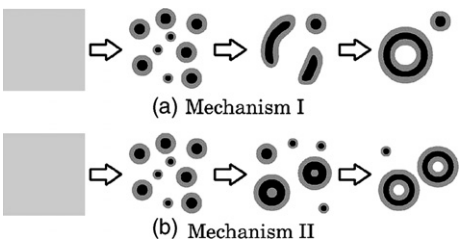

Fig. 2 A schematic representation of two vesicle formation mechanisms ((a) mechanism I and (b) mechanism II) from the initial homogeneous state (grey squares on the left). Black and grey colours in the particles correspond to hydrophobic and hydrophilic chains, respectively.[63]

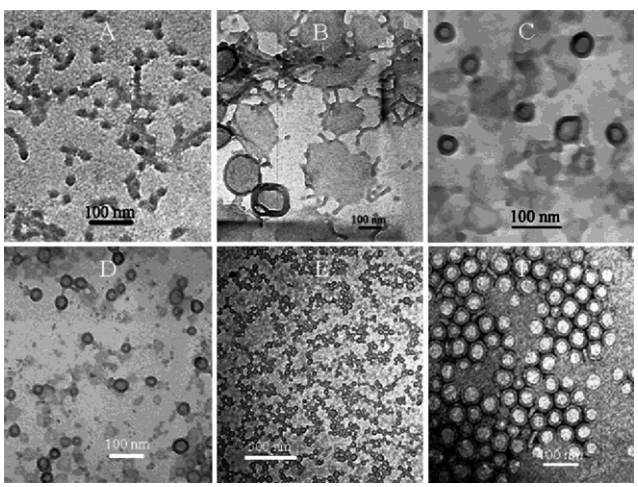

Fig. 3 TEM images of the gelated polymer self-assemblies from PEO_{45}-*b*-$PTMSPMA_{42}$ at different water contents: (A) spheres, coexisted with few rods, 31.3 wt%; (B) lamellae with protruding rods and a few vesicles, 33.6 wt%; (C) vesicles and lamellae, 38.7 wt%; (D) vesicles, 48.7 wt%; (E) vesicles, 55.8 wt%; (F) vesicles in (E) stained with uranyl acetate.[28]

mechanism II vesicles are evolved from the growth of simple micelles without the potential to trap hydrophilic species. This in turn would lead to a very poor loading efficiencies which has indeed been observed.[66]

3. Smart polymer vesicles

Polymer vesicles have been suggested to have promise as delivery vehicles for controlled encapsulation and release. To effectively achieve this, it is important that the polymer vesicles respond to external stimuli such as a change in pH, temperature, oxidation/reduction, light, *etc.* This allows for the triggered release of a payload or the selective uptake of a payload upon introduction of the stimuli which modifies the vesicle. In this Highlight 'smart' copolymer vesicles have been classified according to the stimuli to which they respond.

3.1 pH-responsive vesicles

Given the wide range of pH gradients present in biological and physiological systems the application of pH responsive nanostructures for controlled release/encapsulation is of great interest. In fact, pH tuning or response has been proposed to be one of most effective ways to control the encapsulation or release of small molecules *in vivo*.[4,7] In general, the pH-responsiveness of a polymer is obtained *via* the protonation and deprotonation cycle of a weak polybase and/or weak polyacid in the block copolymers at different pH, or by pH-induced conformation changes of the copolymers. Both block copolymers and synthetic block copolypeptides have been used to make pH-responsive polymer vesicles (see below).

In 2003, Liu and Eisenberg reported a pH-induced vesicle corona switching based on a triblock, poly(acrylic acid)-*b*-polystyrene-*b*-poly(4-vinyl pyridine) (PAA_{26}-*b*-PS_{890}-*b*-$P4VP_{40}$) in DMF/THF/H_2O mixtures (Fig. 4).[67] It was possible to determine whether the PAA or P4VP or both make up the outside surface by measuring the zeta potential (ζ) of aqueous vesicle solution. Starting at an apparent pH (pH*) of 1, and increasing gradually to pH* 14, the nanoparticle morphologies of this triblock change progressively from vesicles at pH* 1, to solid spherical or ellipsoidal aggregates (pH* 3–11), and finally back to vesicles at pH* 14. Vesicles prepared at pH* 1 contain P4VP chains (positively charged, water soluble) as the exterior corona layer and PAA chains (protonated, not water soluble) on the inside, while those prepared from the same triblock at pH* 14 contain PAA chains (negatively charged, water soluble) as the external corona and P4VP chains (deprotonated, not soluble in water) in the interior domain. This segregation is believed to be based on the difference in repulsive interactions within the PAA or P4VP corona at different pH conditions. At low pH, the curvature is stabilized through increased repulsive interactions between the P4VP chains on the outside relative to the lower repulsive interactions between the PAA chains on the inside and *vice versa* at pH* 14. Most importantly, vesicles with PAA on the outside can readily be inverted to present P4VP on the outside by changing the pH and under dynamic conditions. The conversion mechanism is suggested to involve a whole vesicle because the critical micelle concentration (CMC) is far too low for single chain reassembly to be involved. It is likely that the inversion processes for vesicles induced by an external chemical trigger, as described here, might find applications in various controlled release or delivery processes.

However, this pH-triggered inversion process may be difficult to achieve without the aid of organic solvents. That is to say, in pure water, due to the high T_g of polystyrene and the insolubility of PAA in water at low pH and P4VP at high pH, this inversion may not be readily achieved. This may limit its potential utilization in biomedical applications. However, the pH-triggered inversion of polypeptide vesicles in pure water has been reported and will be discussed later in this section.[68]

Du and Armes in 2005 reported a pH-responsive block copolymer vesicle with tunable membrane permeability at different pH. The vesicles were made from poly(ethylene oxide)-*b*-poly[2-(diethylamino)ethyl methacrylate-*s*-3-(trimethoxysilyl)propyl methacrylate], [PEO-*b*-P(DEA-*s*-TMSPMA)], in THF/water mixtures (see Fig. 5A).[69] The amphiphilic copolymer was synthesized by atom transfer radical polymerization (ATRP) from a PEO macroinitiator in methanol at room temperature. The hydrophobic P(DEA-*s*-TMSPMA) block forms the vesicle membrane, which can be subsequently cross-linked by the *in situ* sol-gel reaction of TMSPMA in the membrane in aqueous solution. Fig. 5A

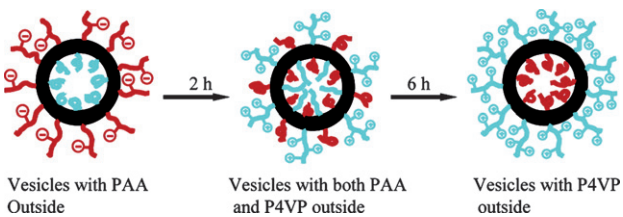

Fig. 4 A schematic of the inversion of vesicles from a negatively charged PAA external corona to a positively P4VP corona. Note that the charge is from the polymer chains, not the counterions.[67]

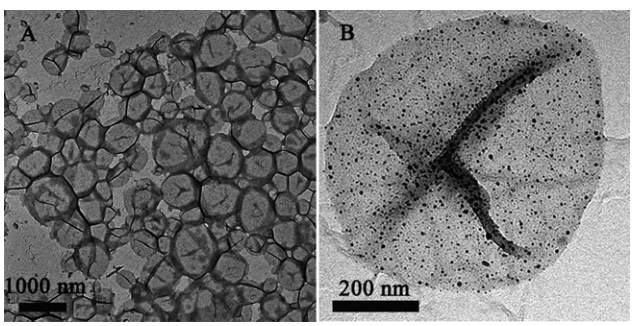

Fig. 5 (A) TEM images of vesicles prepared in 1:2 v/v THF/water and (B) the same vesicles decorated with gold nanoparticles which are located solely within the vesicle walls.[69]

shows a typical TEM image of the polymer vesicles after the cross-linking sol-gel reaction. The PDEA unit was incorporated into the vesicle membrane due to its potentially pH responsive nature. At low pH, the PDEA is protonated and becomes hydrophilic, which produces some leaky sites in the vesicle membrane in the aqueous solution. As a result the gates in the membrane are opened and the permeability of vesicle membrane increases. Conversely, if the pH of the solution is increased the PDEA is deprotonated and becomes hydrophobic, which seals the leaky sites in the membrane. As a result the gates in the membrane are gradually closed and the permeability of vesicle membrane decreases. Thus, the 'gate' size in the vesicle membrane can be readily fine tuned by altering the solution pH, this in turn leads to tunable membrane permeability and hence controlled release and encapsulation. A key advance in this work was the incorporation of chemical cross-linking by the *in situ* sol-gel chemistry in the membrane, this ensures the vesicular morphology is maintained, but the membrane permeability can be modified by changing the solution pH. This amino functionalized membrane can be decorated with gold-nanoparticles as a result of the interaction between cationic PDEA at low pH and anionic $AuCl_4^-$, as shown in Fig. 5B.

In 2008, Chiu *et al.* also reported a pH-responsive vesicle with tunable membrane permeability, by a double emulsion technique in a water/oil/water ($w_1/o/w_2$) system. In this report the copolymer was dissolved in the organic phase prior to emulsification to afford vesicles of *ca.* 1–15 microns.[70] By partially transesterifying poly(*N*-acryloxysuccinimide) (PNAS) with distearin followed by hydrolysis of the unreacted NAS to acrylic acid (AA) units, they prepared copolymers containing AA and acrylate of 1,2-distearoyl-*rac*-glycerol (distearin acrylate, DSA). This copolymer was then dissolved in an organic cosolvent (THF/$CHCl_3$ with various ratios to vary the target size) prior to emulsification. Either water or buffer was used as both the inner (w_1) and outer (w_2) aqueous phases. The evaporation of organic solvents in $w_1/o/w_2$ emulsions induces the self-assembly with different morphologies at different pH, such as micelles (pH > 5.5), vesicles (pH = 4.0–5.5), or large precipitates (pH < 4.0). A change in permeability of the vesicles was achieved by the ionization/deionization of AA residues by tuning the pH between 5.0 (where the membrane gate is closed) and 8.0 (where the membrane gate is open).

However, it can be time-consuming and problematic to remove the organic solvent after vesicle formation, hence solvent-free preparation methods have attracted attention recently. For example, Armes and coworkers recently developed a new pH-sensitive block copolymer vesicle based on a highly biocompatible monomer, 2-(methacryloyloxy)ethyl phosphorylcholine (MPC) and a second monomer, 2-(diisopropylamino)ethyl methacrylate (DPA), which confers pH-sensitivity to the membrane (pK_a of PDPA is ~5.8–6.6).[57] When the solution pH is less than the pK_a, the PDPA block is protonated and can be molecularly dissolved in water (Fig. 6). When the solution pH is greater than the pK_a, the PDPA block becomes hydrophobic due to the deprotonation of the tertiary amine groups. Hence, the vesicles can be easily prepared in solely aqueous media by simply adjusting the solution pH.

Due to the unique structure of polymer vesicles, they are capable of encapsulating hydrophilic moieties such as drugs in their interior water pool. Controlled release of these encapsulates can be locally triggered in tumor and inflammatory tissues (where the pH ~6.8) and in the endosomal and lysosomal compartments of cells (pH ~5–6). Given the requirement of a relatively narrow pH range, the long retention time of encapsulants within vesicles and the opportunity for targeted release, the above PMPC-*b*-PDPA diblock copolymer vesicles have been shown to be good delivery vehicles for water-soluble doxorubicin (Dox), an anti-tumor medicine.[57] In a control experiment utilizing an aqueous solution of 0.556 g/L Dox in the absence of any vesicles, rapid drug elution was found ($\tau_{1/2}$ ~2.5 h). Similar data was obtained after mixing two aqueous solutions comprising of Dox and 1.0 g/L of preformed vesicles for 24 h, followed by dialysis against a pH 7.5 saline buffer for 15 h. Using this protocol, the initial Dox concentration immediately after dialysis was 0.081 g/L, and the maximum possible Dox loading within the preformed vesicles was around 16%. However, the release profile suggests that little or no loading occurred. In contrast, an aqueous solution of Dox-loaded vesicles prepared by mixing 18.25 g/L Dox with 1.0 g/L $PMPC_{25}$-*b*-$PDPA_{120}$ copolymer at pH 2, followed by adjustment to pH 7.4, produced a much slower drug elution profile ($\tau_{1/2}$ > 25 h). For these latter drug-loaded vesicles, the Dox concentration was 0.136 g/L after dialysis. The loading efficiency is estimated to be ~27%, and the release profile indicates significantly retarded release of the drug due to its loading within the vesicles.

These non-cytotoxic polymer vesicles have also been used for *in vitro* DNA encapsulation delivery[71] and intracellular delivery.[72] At pH 7.5, *ca.* 20% DNA can be encapsulated into the vesicle's interior. At lower pH, a DNA–copolymer complex is formed due to the electrostatic interactions between DNA and PDPA. The DNA loading at neutral pH is based on a physical encapsulation approach rather than charge-compensated condensation and hence the vesicles in this work can be considered to be stealth vectors as a consequence of the highly biocompatible PMPC chains on the vesicles exterior surface. In addition, the pH-induced release of hydrophilic dyes from poly(2-vinylpyridine)-*b*-poly(ethylene oxide) (P2VP-*b*-PEO) vesicles has been reported by Förster and coworkers.[73] Similar to the pH-responsive behavior of PDPA, the

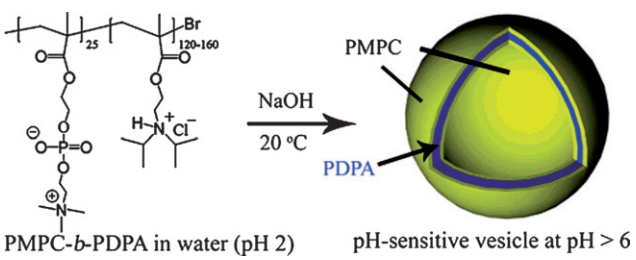

Fig. 6 Formation of PMPC-*b*-PDPA block copolymer vesicles by pH adjustment.[57]

P2VP membrane is protonated and dissolved below pH 5, which leads to rupture of the vesicle and triggered release of the encapsulated hydrophilic dyes.

The pH-dependent permeability and reversible structural transition of polyion complex vesicles in aqueous media was also recently reported by Kataoka and coworkers.[74] At first, the aqueous solution properties of PEG_{45}-$P(Asp\text{-}AP)_{75}$ and PEG_{45}-$PAsp_{75}$, where PEG stands for poly(ethylene glycol), P(Asp-AP) for poly-[(5-aminopentyl)-α,β-aspartamide] and PAsp for poly(α,β-aspartic acid), were analyzed by potentiometric titration. The pK_a values of PEG-P(Asp-AP) and PEG-PAsp were calculated to be 10.47 and 4.88, respectively. These titration results revealed that both block copolymers are equally charged at around physiological pH (pH 7.8, ionization degree = 96%). After mixing the two copolymers, PICsomes form with a mean diameter of ~2 μm, these vesicle structures maintain their structure at pH 7.4 for more than 48 h and only dissociate into small particles upon lowering the pH to 5.7. Interestingly, guest molecules can be trapped through this process which suggests that PICsomes can deliver, release and also trap their cargoes by sensing acidic conditions of the intracellular endosomal compartments.

Another type of pH-responsive vesicles are based on the self-assembly of polypeptides. Deming and coworkers prepared charged block copolypeptide vesicles by the self-assembly of poly(L-lysine)-*b*-poly(L-leucine) and poly(L-glutamatic acid)-*b*-poly(L-leucine) block copolypeptides.[75,76] The morphology and dimension of the self-assemblies are responsive to changes in the solution pH as well as to changes in the conformation of the peptide blocks. For example, copolymer $K^P_{160}(L_{0.3}/K_{0.7})_{40}$ was prepared such that 70% of the L-leucine residues of the hydrophobic domain of a $K^P_{160}L_{40}$ block copolymer were replaced in a statistical sequence with L-lysine (K).[75] At high pH, uncharged poly(L-lysine) is not water soluble, and preferentially adopts the α-helical conformation. Under these conditions, incorporation of lysine residues into this copolymer should neither disrupt the hydrophobicity or helicity of the leucine-rich domain, nor should they greatly disturb the higher-order assembly of the chains. Accordingly, aqueous suspensions of $K^P_{160}(L_{0.3}/K_{0.7})_{40}$ at pH > 9 were found to form vesicles similar to those formed by $K^P_{100}L_{20}$. However, upon lowering the pH and protonation of the amino side-chains on the lysine residues their $K^P_{160}(L_{0.3}/K_{0.7})_{40}$ hydrophilicity is considerably enhanced and this destabilizes the α-helical structure of the leucine-rich domain due to the electrostatic repulsion of the like charges. This results in a helix-to-coil conformational transition in this domain, similar in concept to the mechanism used by some viral capsid proteins to affect endosomal release. The change in pH also destabilizes the vesicular assembly, leading to an increase in permeability of the membrane or even complete dissociation of the structures. This was demonstrated by formation of vesicles of $K^P_{160}(L_{0.3}/K_{0.7})_{40}$ in the presence of Fura-2 dye at pH 10.6. Under these conditions, the excitation maximum of vesicle-encapsulated dye in the presence of external calcium solution was found to be constant for several days, indicating no dye or calcium transport across the membrane barrier had occurred. However, when the pH was lowered by addition of HCl, the excitation maximum of the dye was shifted within seconds, indicating near-instantaneous disruption of the vesicle membranes and complexation of the calcium by Fura-2. In these samples, the membranes were observed to completely dissolve on acidification. These results demonstrate the ease with which functionality, and thus environmental response, can be incorporated into block copolypeptides. Although the pH of the transition for $K^P_{160}(L_{0.3}/K_{0.7})_{40}$ is not optimal for drug delivery *in vivo*, other amino acids (*e.g.*, histidine, $pK_a = 6.0$) can readily be substituted for lysine for potential application in this range.

In 2005, Rodríguez-Hernández and Lecommandoux prepared vesicles by the simple self-assembly of a peptide-based zwitterionic diblock copolymer, poly(L-glutamic acid)-*b*-poly(L-lysine) (PGA-*b*-PLys) in pure water.[68] At pH 5 to 9, both blocks have a charged coil structure and are soluble in water. At pH < 4, PLys is still in a coil confirmation while the PGA block is neutralized, and its secondary conformation becomes an α-helical structure, which is more compact and less soluble than the coil conformation. This hydrophobicity increase induces vesicle formation, with insoluble PGA as membrane layer while the PLys block forms the corona. At pH > 10, PGA is in a coil confirmation and PLys becomes α-helical, forming vesicles with PGA corona and PLys membrane. These biocompatible schizophrenic vesicles may be good candidates for controlled encapsulation and release.

In 2007, Schlaad *et al.* studied the aggregation behavior of polybutadiene$_{165}$-*b*-poly(L-lysine)$_{88}$ (PB_{165}-*b*-$PLys_{88}$) in saline solution and the effect of changing the solution pH on the higher order assembly of the polypeptide blocks (Fig. 7).[77] Block copolymer vesicles with a polypeptide corona were formed when the polypeptide segment was in a 100% coil conformation at pH 7.0. However when the peptide segment was in an 80% R-helical conformation, which occurs at

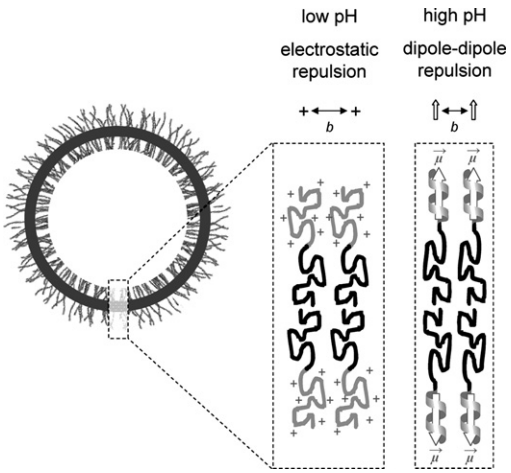

Fig. 7 Tentative structures of the bilayered membrane of PB_{165}-*b*-$PLys_{88}$ vesicles at different pHs; *b* denotes the average distance of chains at the core-corona interface.[77]

pH 10.3, the vesicle size was smaller at the higher pH than that at lower pH (hydrodynamic radius: 364 nm *versus* 215 nm) as a result of a more densely packed interface between core and corona (interchain distance: 3.2 nm *versus* 2.4 nm).

All of the structures are pH-responsive vesicles which have been prepared from linear amphiphilic block copolymers and copolypeptides. Recently however, dendrimers and hyperbranched polymers have been utilized to make pH-responsive vesicles. For example, Tsuda *et al.* reported the synthesis of large polymer vesicles by the self-assembly of three 5th generation poly(propylene imine) dendrimers, which were derivatized to contain 64 palmitoyl (**1**), 32 palmitoyl and 32 azobenzene (**2**), and 64 azobenzene groups (**3**), on their exterior, respectively, in aqueous solution below pH 8.[78] The size of the vesicles was reported to be dependent on the solution pH. For example, for **2**, the number-averaged size changed from 2.2 μm at pH 1 to 1.5 μm at pH 5.5. Yan and coworkers have also reported the preparation of pH-responsive vesicles *via* the self-assembly of a commercially available hyperbranched polyester.[79] The vesicle size can be readily controlled from 200 nm to 10 μm by simply adjusting the solution pH or the degree of branching.

Overall, pH-responsive polymer vesicles can be readily prepared from copolymers containing either polybases, polyacids, polypeptides, or a combination of these. These vesicles have been shown to readily encapsulate and release small molecules and DNA. They also show interesting morphology changes upon the application of stimuli, which can be utilized to trigger a release event. Overall, these responsive materials have shown good promise as smart nano-carriers in biomedical applications yet there still require further work to ensure their potential utility is fully realised.

3.2 Thermo-responsive vesicles

Unlike the pH-responsive hydrophobicity of polybases or polyacids, block copolymer chains may self-assemble into vesicles in aqueous solution if the hydrophilicity or hydrophobicity of one segment of the copolymer can be modified by a change in temperature. It is well established that *N*-isopropylacrylamide (NIPAM) is a thermo-responsive monomer and its polymer (PNIPAM) has a lower critical solution temperature (LCST), which depends on the polymer molecular weight, end group, and the overall composition of the block copolymer. This means that PNIPAM is molecularly soluble in water below its LCST (*ca.* 32 °C) but insoluble in aqueous solution above this temperature. Thus, an amphiphilic block copolymer with a PNIPAM block may be used to make polymer vesicles and other morphologies in pure water simply by varying the solution temperature.

For example, Li *et al.* reported in 2006 the synthesis of thermally-induced polymer vesicles based on poly[*N*-(3-aminopropyl)-methacrylamide hydrochloride]-*b*-PNIPAM (PAMPA-*b*-PNIPAM), which was synthesized by reversible addition–fragmentation chain-transfer (RAFT) polymerization (Fig. 8).[80] This polymer is molecularly dissolved in water when the solution temperature is below the LCST of the PNIPAM block. However, when the temperature is raised above the LCST, vesicles formed spontaneously with PNIPAM forming the vesicle membrane. The vesicles could then be structurally "locked" by ionic cross-linking of the PAMPA coronas upon reaction with poly(sodium 2-acrylamido-2-methylpropanesulfonate) (PAMPS). This ionic cross-linking is reversible and hence perhaps can be utilized to provide stability to the vesicles for delivery applications and also allow for the triggered removal of cross-links to facilitate the rapid removal or degradation of the vesicles.

Using an alternative hydrophilic block, PEO, Discher and coworkers reported a thermo-responsive vesicle based on a PEO-*b*-PNIPAM block copolymer above 32 °C in water.[81] These vesicles can sequester a hydrophobic fluorescent dye into their membranes and also encapsulate a hydrophilic anticancer drug doxorubicin (Dox), into their interior water pool. These small molecules can be readily released at temperatures below 32 °C, upon dissociation of the vesicle structure.[54]

In the above examples PNIPAM always constituted the hydrophobic vesicle membrane at temperatures above its LCST, however it can also form the vesicle corona even in its hydrophobic state. For example, by introducing a permanently hydrophobic block, Ding and coworkers prepared thermo-sensitive polymer vesicles by the self-assembly of poly(2-cinnamoylethyl methacrylate)-*b*-poly(*N*-isopropylacrylamide) ($PCEMA_{61}$-*b*-$PNIPAM_{22}$) in acetone or THF/water mixtures (50:50) at room temperature.[82] The hydrophobic PCEMA membrane can be subsequently cross-linked by UV light irradiation. Upon raising the temperature of the solution above the LCST of the PNIAPM block, surprisingly no precipitate was observed, instead a change in the particle size was reported (from *ca.* 190 to *ca.* 120 nm). The authors also demonstrated that these polymer vesicles could be utilized to load a large amount of 4-aminopyridine, which could be released into the surrounding solution at a tunable rate by modifying the solution temperature.

Other smart materials have also been employed to make thermo-responsive

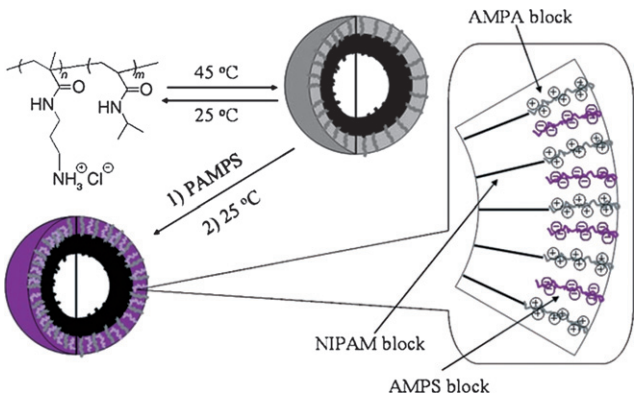

Fig. 8 A schematic illustration of the formation of vesicles from PAMPA-*b*-PNIPAM diblock copolymers and their subsequent ionic cross-linking.[80]

polymer vesicles. For example, Pasparakis and Alexander reported 'sweet-talking' thermo-responsive diblock copolymer vesicles which displayed glucose functionality at their surface.[83] They utilized poly(2-glucosyloxyethyl methacrylate) (PGEMA) as the hydrophilic block and poly(diethyleneglycol methacrylate) (PDEGMA), which has an LCST of 28 °C, as the temperature-sensitive block. They reported vesicle sizes in the range 250–180 nm or from 300–500 nm for two copolymers by varying the comonomer content and hence the LCST. The authors proposed that these materials are of great interest in delivery applications given their glycosylated surfaces which can be utilized to achieve information transfer and targeting to biological cells.

We anticipate that a range of other thermo-responsive materials such as poly(propylene oxide) (PPO), poly[2-(*N*-morpholino)ethyl methacrylate] (PMEMA), sulfobetaine-based poly[2-(dimethylamino)ethyl methacrylate] (PDMA),[84] poly[2-hydroxyethyl methacrylate] (PHEMA),[85] poly{*N*-[(2,2-dimethyl-1,3-dioxolane)methyl] acrylamide} (PDMDOMA)[86] (which has a tunable LCST), poly(*N*-vinylcaprolactam),[87] polymers of acryloyl amino acid methyl esters (such as acryloyl-L-phenylalanine methyl ester and acryloyl-L-leucine methyl ester), and poly(2-oxazoline)'s[88] (with tunable LCSTs in the range 25–100 °C) may be good candidates, in the future, for the preparation of new thermo-responsive polymer vesicles with different properties.

3.3 Redox-responsive vesicles

There is significant interest in the preparation of nanostructures which respond to a change in redox environment. For example, Hubbell and coworkers prepared oxidation-responsive vesicles in pure water from a PEO-*b*-PPS-*b*-PEO triblock copolymer to address the oxidative environment present in inflammation sites (where PPS stands for poly(propylene sulfide)).[89,90] The copolymer was synthesized by a one-pot method by episulfide anionic polymerization.[91] The hydrophobic PPS was converted into hydrophilic poly(propylene sulfoxide) and poly(propylene sulfone) upon exposure to an oxidative environment such as H_2O_2, as shown in Fig. 9. This destabilizes the vesicle membrane and leads to a molecularly dissolved oxidized copolymer which was confirmed using turbidity measurements. In this work the polymer vesicles (with a high turbidity in aqueous solution) dissociated into polymer chains (with almost zero turbidity) after ten hours of oxidation. It has been proposed that these oxidation-responsive vesicles may find applications as nanocontainers in sensing devices or as drug delivery systems.

Hubbell and coworkers subsequently reported a reductive-responsive PEO_{17}-SS-PPS_{30} disulfide vesicle, which can protect biomolecules within its structure in the extracellular environment. These vesicles are sensitive to the endosomal microenvironment because they are ruptured (by reduction of the disulfide linkage) in the presence of intracellular concentrations of cysteine within 10 min of cell exposure.[92]

Abe and coworkers reported that low-molecular-weight *bis*-(11-ferrocenyl(undecyl))dimethylammonium bromide, a ferrocene-modified double-chain-type cationic surfactant, can spontaneously form vesicles which undergo disruption and change to smaller molecular aggregates upon oxidation of ferrocenyl groups of the surfactant.[93] Furthermore, Manners and coworkers reported the redox-active organometallic vesicles which were formed directly in aqueous solution based on a diblock copolymer with a hydrophilic polyferrocenylsilane (PFS) polyelectrolyte and hydrophobic polydimethylsiloxane (PDMS).[94] The PDMS-*b*-PFS vesicles have redox-tunable encapsulation properties. The authors examined the redox chemistry of the vesicles, using extensive cyclic voltammetric studies and reported that there is a reversible oxidation cycle of the ferrocene units in the PFS.

Kataoka and coworkers recently reported the synthesis of polymer vesicles by spontaneous evolution from reducible hetero-PEG PICmicelles by controlled degradation,[95] as shown in Fig. 10. The micelles can rapidly detach a portion of

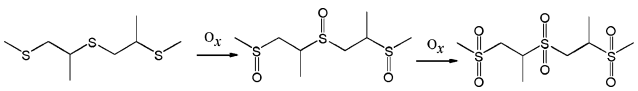

Fig. 9 Redox reaction of PEG-*b*-PPS-*b*-PEG polymer vesicle suspension during oxidation in excess H_2O_2.[89]

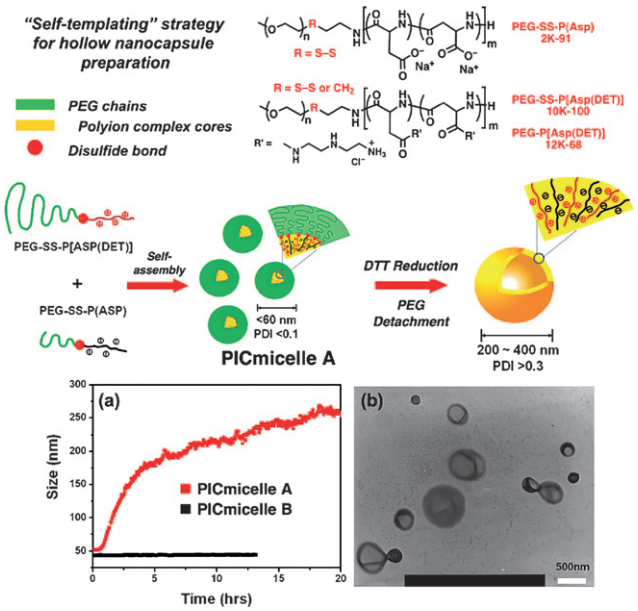

Fig. 10 A schematic illustration of the preparation of hollow nanocapsules by a self-templating strategy. Upon addition of reducing agent DTT into hetero-PEG-detachable PICmicelle solution A, a morphology transition occurs. (a) The change in cumulant average diameter of PICmicelles A and B after the addition of dithiolthreitol (DTT) as a function of time. (b) A TEM image of PICmicelle A deposited on copper grids 12 h after reduction.[95]

the PEG chain in response to thiol-reducing conditions *via* cleavage of disulfide bonds. Thus, these micelles were utilized as "sacrificed templates" for vesicle formation. After reduction agents were injected into PICmicelle solution, the weight fraction of the PEG coronas decreased due to the release of PEG chains. Consequently, spontaneous morphology evolution from micelle to vesicle structures occurred when the PEG weight fraction decreases to a certain value. This micelle to vesicle evolution could be applied to encapsulate hydrophilic actives.

3.4 Light-responsive polymer vesicles

Compared to pH- or redox- responsive vesicles, light responsive vesicles offer the advantage, in the controlled release of encapsulated molecules, that no extra chemical additives are needed to induce the response. Zhao recently reviewed the area of light-responsive amphiphilic copolymers, whose micellar aggregates can be disrupted by light exposure, and their application as delivery vehicles.[96] The dissociation of these structures can be reversibly and irreversibly achieved upon illumination with UV/visible or near IR light.

The basic concept for the preparation of light-responsive polymer vesicles is to incorporate a chromophore into the structure of the hydrophobic block, whose photoreaction can result in a conformational or structural change (*trans* ↔ *cis*) that shifts the hydrophilic/hydrophobic balance toward the destabilization of vesicles structure. The most commonly used chromophore is liquid crystalline azobenzenes (LC-Azo). Its rod-like *trans* configuration makes it more stable than its *cis* form, which stabilizes the structure of the LC phase, whereas its *cis* isomer is bent and tends to destabilize the phase structure of the mixture.[97] Upon UV irradiation, the *trans* configuration isomerizes into its *cis* form. The *cis* form is not thermodynamically stable and usually goes back to its *trans* form within several hours. This transition rate significantly increases (to several minutes) upon visible light irradiation.

For example, diblock copolymers consisting of a side-chain azobenzene polymethacrylate-*b*-poly(acrylic acid) (PAzoMA-*b*-PAA) can form vesicles with the hydrophobic PAzoMA domain forming the membrane and the hydrophilic PAA domain forming corona. Under alternating UV and visible light, reversible changes between micelles and vesicles take place as a result of the reversible *trans-cis* photoisomerization of the azobenzene mesogens in PAzoMA.[98] However, these azobenzene-based polymer self-assemblies cannot be completely disrupted or destroyed by light. [99]

The principle of light-changeable or light-switchable amphiphilicity may be extended to many polymer/chromophore combinations. That is to say other photoactive species may be incorporated into polymer chains to achieve reversible hydrophilic/hydrophobic alternation. For example, spyrobenzopyran[100] can form a zwitterionic species and triphenylmethane leucohydroxide[101] can produce charges upon light irradiation to afford a hydrophobicity change.

3.5 Vesicle morphological changes induced by stimuli

As mentioned above, incorporation of a stimuli-responsive segment into an amphiphilic block copolymer can result in a change in the repulsive interaction between coronal chains or in the hydrophilic/hydrophobic ratio of the block copolymers under different conditions. This in turn may lead to the switching between small (usually micelles) and large (usually vesicles) self-assemblies as a function of stimuli (temperature, pH, polarity of solvent, *etc.*). This has been proposed to be useful in controlled release and sensing applications.

In 1999, Eisenberg and coworkers reported morphological transitions induced by a pH change in the self-assemblies of PS-*b*-P4VP in DMF/H_2O mixtures.[102] As the pH* increases from 7 to 12.3, the aggregate morphologies change from large compound micelles (LCMs) to a mixture of spheres, rods, and vesicles (at pH* = 8), to spheres (at pH* = 8.4), to rods (at pH* = 11.8), and then back to spheres (at pH* = 12.3). In the presence of base, as the pH* increases from 12.3 to 18, the morphology changes to rods (at pH* = 12.6), then back to spheres again (at pH* = 17.5), and finally to a mixture of spheres, rods, lamellae, and vesicles. The reason for this behavior is due to the amphiprotic nature of P4VP in DMF, where additional acid or base introduces ionic groups into the corona chains. Thus electrostatic repulsions are introduced and the aggregate morphology changes from bilayers to spheres. On the other hand, the decrease in the corona repulsion tends to decrease the coil dimensions in the corona hence the morphology is driven from spheres to bilayers. Therefore, a competition between unshielded electrostatic repulsion and shielding coupled with a decrease of the steric stabilization is induced. At relatively low concentrations, the decrease of the steric stabilization dominates, while at high concentrations shielding dominates and at intermediate concentrations, the unshielded repulsion dominants.

More recently, Grubbs and coworkers reported that a PEO-*b*-PNIPAM-*b*-PI, where PI is poly(isoprene), triblock copolymer forms spherical polymer micelles (*ca.* 24 nm) at room temperature, when the central PNIPAM contributes to form a large hydrophilic domain. The authors reported that these micelles converted into well-defined polymer vesicles (*ca.* 128 nm) when the solution temperature was raised slightly above the LCST of PNIPAM. This was attributed to the increased size of the hydrophobic domain which results in a vesicle morphology now being favored.[103] This simple stimuli induced morphology change highlights the potential to design and tailor polymeric morphologies to allow for triggered changes in properties and hence applications.

In 2009, Ulrich Schubert and coworkers also demonstrated nanostructure morphological evolution (spherical micelles, wormlike micelles, vesicles and hollow tubes) by varying the polarity of solvents (which provokes a change in hydrophilic to hydrophobic ratio) based on an amphiphilic metallo-supramolecular triblock copolymer, poly(styrene)-*b*-poly(*p*-trifluoromethylstyrene)-[Ru]-poly(ethylene glycol) (PS–*b*–PTFMS–[Ru]–PEG). This polymer contains a *bis*(2,2′:6′,2-terpyridine)ruthenium(II) complex (–[Ru]–) as a connection between the PS–*b*–PTFMS and the PEG segment.[104] The PTFMS block has markedly different solubility in a range of different alcohols, which allows for subtle changes in the stability of polymer assemblies. In addition, this PTFMS block is also thermally responsive, which allows

for reversible temperature control over the size and morphology of the resultant nanostructure. This work provides an elegant example of orthogonal tunability of the morphology of the resultant nanostructures.

It is well known that P4VP is a pH-responsive polymer, however, Shi and coworkers reported in 2005 that PS_{80}-*b*-$P4VP_{110}$ block copolymer vesicles formed in 2-propanol solution, appeared to thermally re-self-assemble (*via* coalescence and fusion of vesicles) into dumbbell-like aggregates and further into giant elongated, tubular vesicles upon heating the deposited vesicles on the TEM grid (Fig. 11).[105]

In contrast to the above transition in bulk, Rolf Schubert and coworkers recently reported that similar polymer vesicles made from P2VP-*b*-PEO diblock copolymers and traditional film swelling methods underwent a cylinder to vesicle shape transition upon subjecting them to a specific cooling/warming process in solution.[106] Upon cooling the vesicles to 4 °C, the vesicles turn from basket-like aggregates, to wormlike micelles and after rewarming of the dispersion the vesicles reform *via* intermediate discoid and octopus-like structures. Also, the morphology and size of vesicles are dependent on the incubation time at 4 and 25 °C, heating rate, polymer concentration, and ionic strength of the solution.

Rotello and coworkers have reported the transition from spheres to vesicles induced by selective recognition events (Fig. 12).[107] In this work polystyrene functionalized with diamidopyridine (DAP) recognition units self-assembles in nonpolar media to form thermally reversible micro-spheres (of between *ca.* 3 and 9 μm). Upon the addition of a thymine functionalized PS to these self-assembled microspheres a conversion into vesicular aggregates occurs. The morphology change was shown to be reversible by recognition-specific interactions: the addition of DAP-functionalized polymer converted the vesicles back to the original microspheres.

In 2006 Liu and Jiang reported a micelle-vesicle transition switched by light (Fig. 13).[108] The light-sensitive self-assembly contained a poly(4-phenylazomaleinanil-*co*-4-vinylpyridine) P(AzoMI-VP) and polybutadiene with a terminal carboxy group (PCPB). The P(AzoMI-VP)/PCPB forms a *graftlike* interpolymer complex in toluene as a result of hydrogen bonding between the carboxylic acid and pyridine groups. This polymer complex is soluble in toluene when the azobenzene units of AzoMI-VP are in the *trans* configuration. However, upon UV irradiation, the azobenzene units are transformed into the polar *cis* configuration and consequently the P(AzoMI-VP) chains self-assemble into core–shell micelles (*ca.* 250 nm). Upon irradiation with visible light, the micelles are quickly disassociated as the azo *cis* form returns to the *trans* form. After stabilization by reaction of the pyridyl units with 1,4-diiodobutene, the micelles have a reversible morphological change upon light irradiation: visible light causes the formation of hollow spheres, while UV light causes the hollow spheres to return to micelles as a result of isomerization in the opposite direction.

4. Applications of functional polymer vesicles

There are numerous potential applications for polymer vesicles, however perhaps the most promising application is in the area of biomedical delivery. Indeed, polymer vesicles have recently being explored as *in vivo* delivery vehicles.[7,109] To ensure the vesicles reach the targeted tumor tissue they must be able to survive

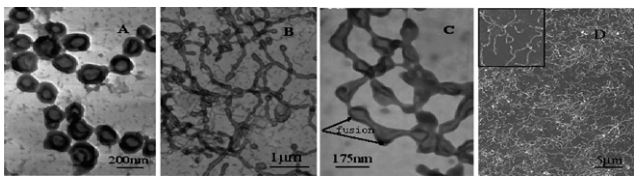

Fig. 11 TEM images of the spherical PS_{80}-*b*-$P4VP_{110}$ vesicles (A), the resultant tubular vesicles formed on a carbon surface (B), an enlarged TEM image of the tubular vesicles shown in B (C), and an SEM image of the tubular vesicles formed on a piece of glass slide (D).[105]

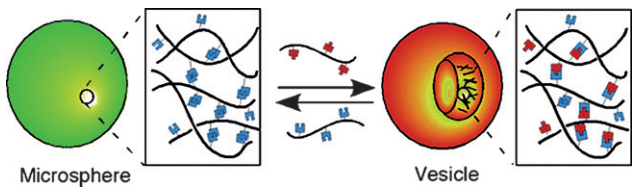

Fig. 12 Polystyrene functionalized with diamidopyridine (DAP) recognition units self-assembles in nonpolar media to form thermally reversible micrometer-scale spherical aggregates.[107]

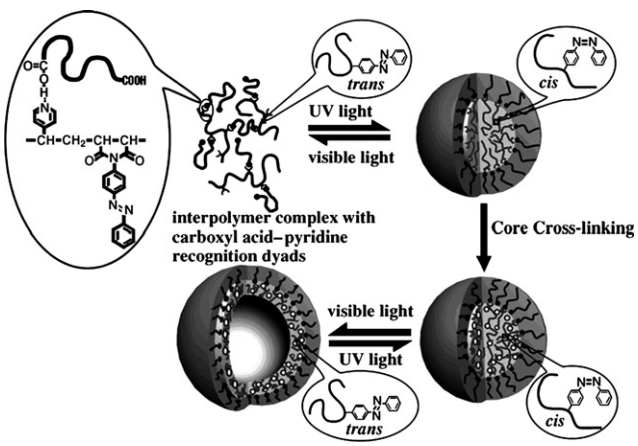

Fig. 13 An illustration of the reversible photo-induced micellization and micelle–vesicle transition of hydrogen-bonded polymers.[108]

in the bloodstream for a considerable time without being eliminated by the native immune system. Nanoparticles or vesicles without surface modification are usually caught by the mononuclear phagocytic system (MPS, also called the reticuloendothelial system), resulting in their rapid clearance from circulation.[110] To solve this problem, the surface coating of nanoparticles with biocompatible hydrophilic polymers has been employed as an efficient way to protect the structures from being captured by macrophages and hence allow for increased circulation times.[111] Compared with the surface coating technique, it is relatively easy to prepare water soluble block copolymer vesicles with hydrophilic biocompatible coronas such as PEO and PMPC.

It is well known that polymer micelles (without the interior water pool) can be used to deliver extremely toxic and hydrophobic therapeutic agents by sequestration within their hydrophobic core.[112,113] However, the main limitation of these materials is that only hydrophobic molecules can be effectively loaded in the micellar core unless additional binding sites, specifically for hydrophilic molecules, are introduced into the coronal domain. This has been achieved by Wooley and coworkers for the selective introduction of bioactive hydrophilic moieties such as folic acid,[114] mannose[115] and biotin[116] using a functional initiator approach or a post-assembly modification of the shell domain. On the contrary, polymer vesicles (with an interior hydrophilic cavity) have been suggested to be more versatile delivery vehicles as they can sequester hydrophobic molecules in their membrane and encapsulate hydrophilic species in their aqueous interior cavity, as well as simultaneously presenting functionality on their surface. These unique properties of polymer vesicles make them excellent candidates for combinational therapies when both hydrophilic and hydrophobic pharmaceuticals are needed simultaneously, along with selective surface functionalization for targeting applications.

The schematic representation of polymer vesicles in Fig. 14 illustrates three routes of possible functionalization and hence potential applications: (1) hydrophobic drug delivery and optical imaging (similar to polymer micelles) and post-functionalization in the vesicle membrane such as silicification; (2) encapsulation and delivery of hydrophilic species (drugs, genes, DNAs, *etc.*); (3) chemical modification in the corona for targeted-therapy. Due to their specific functions and properties, the hydrophobic membrane, hydrophilic corona and the interior water pool can each play a different role in potential delivery applications.

4.1 Hydrophobic membrane functionalization

Polymer vesicles are colloidally stable in aqueous solution due to the stabilization of the hydrophobic bilayer by the hydrophilic corona. They can also effectively load hydrophobic molecules into their membrane, for controlled delivery and release. For example in 2006, Hammer and coworkers reported that hydrophobic dye molecules such as Nile Red can be loaded into PEO-*b*-PCL vesicle membranes (with *ca.* 2 mol% loading) and hydrophilic dyes such as calcein can be loaded into the vesicle interior cavity, as confirmed by fluorescence microscopy.[132] They also reported the synthesis of a near-infrared (NIR) emissive polymer vesicle by incorporating multiporphyrin-based fluorophores within the vesicle membranes for proposed use in biomedical imaging and phototherapy, for example, in deep-tissue fluorescence-based imaging.[117] They further reported that the encapsulation of zinc(II)-based supramolecular fluorophores into poly(1,2-butadiene)-*b*-poly(ethylene oxide) (PB_{46}-*b*-PEO_{30}) vesicle membrane enables emission energy modulation over a broad spectral domain (650–900 nm). The authors reported that the bulk photophysical properties of these soft, supramolecular, optical materials can be finely tuned by varying the polymer-to-fluorophore noncovalent interactions.[118,119]

The incorporation of functional particles such as magnetic nanoparticles into block copolymer vesicles has recently been reported by Lecommandoux and coworkers who have introduced hydrophobic iron oxide nanoparticles into polybutadiene-*b*-poly-(glutamic acid) block copolymer vesicles.[120–122] More recently, Förster and coworkers have also reported the incorporation of magnetic nanoparticles into the bilayers of vesicle membranes with a biocompatible PEO corona. The above magnetic nanoparticle-decorated vesicles have potential applications as *in vivo* magnetic resonance imaging (MRI) contrast agents, or as devices for the magnetothermal treatment of cancer by inductive heating of the nanoparticles coupled to a thermally triggered release of encapsulated hydrophilic or hydrophobic drugs.[123]

The incorporation of silica into the vesicle membrane has been recently reported by Du and Armes. In this work hydrophobic tetramethyl orthosilicate (TMOS) molecules have been successfully encapsulated into the hydrophobic PCL membrane of PCL-*b*-PAMA block copolymer vesicles in aqueous solution.[55] The encapsulated TMOS in the membrane was hydrolyzed and polycondensed into silica, to make the membrane more stable in acidic environments. Moreover, the rate of hydrolysis of the PCL ester groups could be tuned by controlling this degree of silicification of the PCL membrane and this lead to reported tunable release rates for encapsulates.

4.2 Hydrophilic corona functionalization

Besides stabilizing the vesicles in aqueous solution, the hydrophilic corona can also be used to target specific sites such as

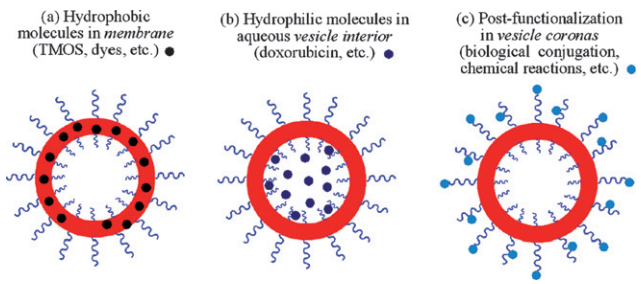

Fig. 14 Possible functionalization strategies for polymer vesicles in (a) the hydrophobic membrane, (b) the water pool and (c) the hydrophilic corona.

abnormal tumor tissues, or be post-functionalized to introduce reactive groups or handles into the vesicle corona. One approach is to use a functional polymer as the corona domain. For example, Du and Armes reported a polymer vesicle in which the corona was composed of many reactive amine groups, *via* the use of a poly(2-aminoethyl methacrylate) (PAMA) hydrophilic block.[55] The primary amines, throughout the corona were shown to be ideal functional handles for post derivatization with functionality of biological interest, *i.e.* for the incorporation of chromophores or for utilization as functional nanoparticle templates. In this work the amino based vesicles were used as templates to direct the formation of gold nanoparticles (5–10 nm) inside the external coronal domain of the vesicles (~190 nm) in aqueous solution.[55] It is proposed given the versatility in coronal functionalization that this approach may be extended to prepare other functional inorganic nanoparticles within hollow polymeric templates.

The second approach is the chain end functionalization of a block copolymer as reported in 2007 by Hest and coworkers for the synthesis of 'clickable' polymer vesicles.[124] In this work styrene and *tert*-butyl acrylate (*t*BA) were polymerized by ATRP to give PS-*b*-P*t*BA-Br block copolymers. The bromide polymer end groups were subsequently modified with azidotrimethylsilane ($Me_3Si–N_3$) and tetrabutylammonium fluoride (TBAF) to afford PS-*b*-PtBA-N_3 copolymers which could be selectively deprotected to afford PS-*b*-PAA-N_3 block copolymers. Azido functionalized polymer vesicles were formed in water/dioxane solution followed by dialysis to remove the organic solvent. These vesicles are capable of conjugation at their periphery using 'click' coupling chemistry to introduce (bio)active moieties however it was proposed that azido groups within the vesicle interior would be unavailable for conjugation. The surface functionalization was achieved using a Cu(II) source, a reducing agent and tris-(benzyltriazolylmethyl)amine ligand (TBTA), as shown in Fig. 15. After 24 h, any unreacted alkynyl probe molecules and catalyst were removed by extensive dialysis against a 0.55 mM solution of ethylenediamine-tetraacetic acid tetrasodium salt tetrahydrate (EDTA). This route allows for the effective surface functionalization (*ca.* 40–50% consumption of the periphery azido groups) of polymer vesicles with dye molecules and also biomolecules, such as biotin.

In 2007, Gillies and coworkers also reported a polymer vesicle whose corona can be readily 'click' conjugated with multivalent dendritic groups.[125] In this case, the commercially available amphiphilic diblock copolymer, PB–*b*–PEO, was derivatized to form an azido chain end functionalized polymer. Following rehydration, the PBD block forms the vesicle membrane and the PEO block, with the terminal azide group, forms the interior and exterior vesicle corona. The azide groups located on the exterior surface of the vesicle can be facilely conjugated with a dendron with an alkyne focal point using Cu(I) catalyzed 3 + 2 'click' cycloaddition chemistries. This approach allows for the conjugation of multi-valent functionality onto the vesicle surfaces for improved biodistribution, cell uptake, and target specificity. This approach is a very versatile functionalization strategy as the peripheral amine groups of the dendron can be easily modified with carboxylic acid, *N*-hydroxysuccinimide (NHS) ester (a common amine-reactive group used in biotinylation and cross-linking reactions), or isothiocyanate derivatives of biological ligands either prior to or after the click reactions. This functionalization approach may significantly expand potential biomedical applications of polymer vesicles by providing a facile means to control their properties.

Macromolecules or polymers can be delivered to *in vivo* pathophysiological abnormalities such as tumor tissue in the body given the unique properties of these angiogenic sites.[126] Compared to normal vasculatures, the tumour vasculature, as well as the blood vessels in other pathological tissues, has a higher permeability due to its discontinuous endothelium. In addition, it has been shown that lymphatic drainage is not fully developed in tumours. These features result in the observation that colloidal particles such as polymer micelles and vesicles extravasate through the "leaky" endothelial layer in tumour and other inflamed tissues and are subsequently retained in these locations.[112] This phenomenon is the so-called *enhanced permeability and retention* (EPR) effect and was proposed by Matsumura and Maeda in 1986.[126] Hence, biologically active molecules conjugated to the corona of vesicles can readily reach targeted sites and due to the EPR effect can remain in these locations for extended periods.

It should be noted that the conjugation of ligands to the corona of vesicles may change the hydrophilic/hydrophobic balance, which may alter the nanostructure morphology. Thus, the ability to stabilize the initial morphology of the vesicle prior to functionalization is important. A few successful cross-linking reactions within the vesicle membrane have been reported, such as

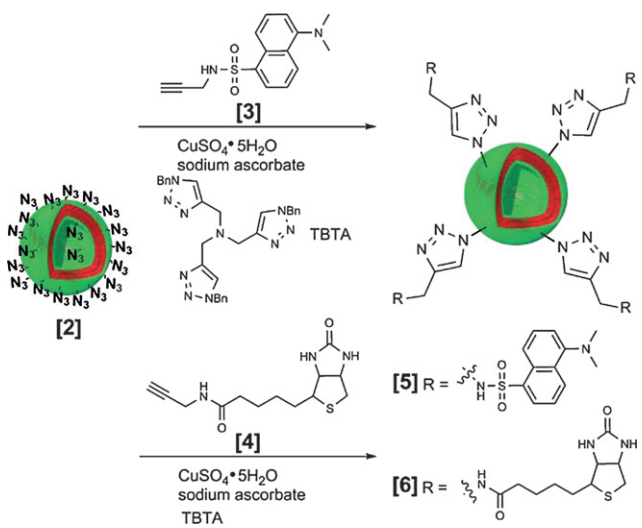

Fig. 15 Click modification of the vesicle corona with both alkynyl bearing fluorescent dansyl probe [3] and biotin [4].[124]

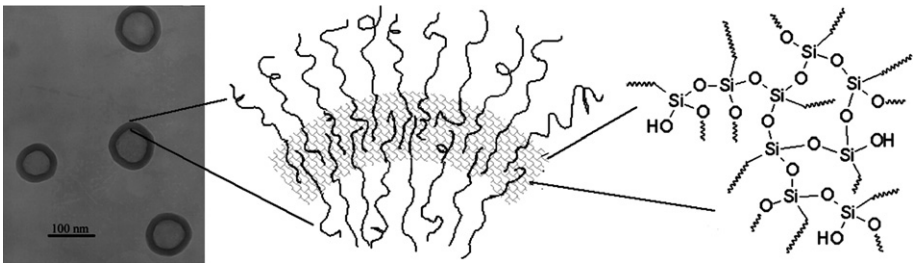

Fig. 16 *In situ* sol-gel chemistry in the vesicle membrane to stabilize polymer vesicles.[24]

photo-dimerization[127] and polymerization reactions.[128] However, it is often difficult to cross-link polymer vesicles in aqueous environment by the above methods. Progress in this area was recently reported by Chen in which PEO-*b*-poly(3-(trimethoxysilyl) propyl methacrylate) (PEO-*b*-PTMSPMA) block copolymer was synthesized and then vesicles were formed in methanol/water solution with PEO as the vesicle corona and PTMSPMA as the membrane. They cross-linked the vesicle by *in situ* sol-gel reactions in the membrane in aqueous solution.[24,28] The R-Si$(OCH_3)_3$ groups in the PTMSPMA block in the vesicle membrane can be easily hydrolyzed into –Si$(OH)_3$, which are subsequently turned into polysilsesquioxane by a polycondensation reaction (Fig. 16). The vesicle membrane is thus 'locked' and the morphology is stabilized in aqueous solution even if these vesicles are utilized to conjugate biomolecules or reactive functionality. The biomimetic PEO vesicle surface, together with the cross-linked membrane, is expected to afford long vesicle circulation times in the body.

4.3 Interior water pool

Kataoka and coworkers have reported the loading of proteins into PICsomes in physiological environments[59] and demonstrated their increased tolerance against protease attack, which is often an issue when using fragile proteins in biomedical applications (Fig. 17). Myoglobin (metMb) was selected as a compartmentalized protein for loading into the PICsome cavity as its biological function can be monitored quantitatively by UV/Vis spectroscopy. The Mb loaded in the PICsome may have potential as an oxygen carrier in the future, because of the inherent blood compatibility of the PEG-shell layer and the stability of the inner PIC layer even at physiological salt concentrations. Vesicle loaded metMb was smoothly reduced to deoxyMb by $S_2O_4^{2-}$ that had permeated through the PIC membrane, and reversible oxygenation/deoxygenation of the Mb in the PICsome was observed even in the presence of trypsin in the outer medium. However, the Mb concentration before encapsulation was *ca.* 5 mg/mL (the actual concentration was lower than this due to the dilution during the mixing of two solutions) and the final concentration of encapsulated Mb was only 0.88 μg/mL. No further information on the loading efficiency of these vesicles was reported in this paper although this report does highlight the potential to encapsulate an active biomolecule within the interior domain of a polymer vesicle.

In 2007 Eisenberg and coworkers reported that vesicles made from PEO-*b*-PCL-*b*-PAA triblock copolymer effectively entrapped a hydrophilic model protein, fluorescently-labelled bovine serum albumin (FITC-BSA).[52] Hydrophobic PCL forms the vesicle membrane and can be easily degraded by hydrolysis of the ester linkages in physiological conditions to allow for the controlled release of the encapsulate. They also found that proteins can be preferentially adsorbed on the inner or outer interfaces, depending on the nature of the different PEO and PAA coronas.

5. Current limitations of polymer vesicles

Several general issues have arisen given the rapid progress in the preparation of smart and functional polymer vesicles in recent years and these will be highlighted in this section.

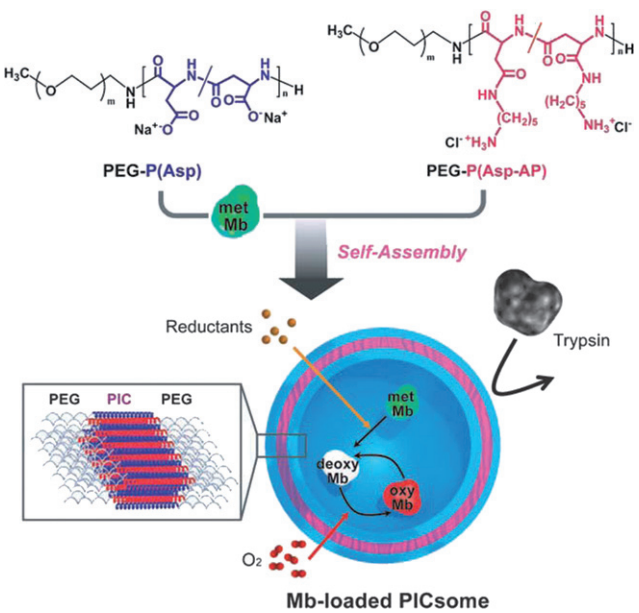

Fig. 17 Reversible Mb oxygenation inside the PICsome self-assembled from a pair of oppositely charged block ionomers.[59]

5.1 Reproducibility and stability of vesicles

Generally, polymer vesicles have been prepared *via* solvent switch or rehydration methods from a range of amphiphilic polymers. However, the removal of organic solvent by dialysis or evaporation can be problematic and time consuming. Therefore, rehydration or direct dissolution of polymers in water which avoids the use of organic cosolvents is a very promising future direction for vesicle preparation. However, available polymer candidates are restricted to a limited class of polymers, usually those with a relatively low T_g. Sometimes heating can aid the dissolution of some polymers in pure water to form vesicles directly,[55] but this does not work for all polymer types. In addition, the size and properties of the vesicles formed using all these techniques is highly condition dependent and this is certainly not ideal for the general preparation and application of these materials.

pH-responsive vesicles usually involve charged species due to the polyelectrolyte nature of the polyamine or polyacid block and consequently vesicles tend to precipitate close to the isoelectric point (IEP) of the pH-responsive block. This is strongly pH-dependent and also affected by the ionic strength and vesicle concentration. In practice, high polymer concentrations are not ideal for preparing vesicles with polyacid or polybase block forming vesicle membranes, which may somewhat limit their commercial application.[57] Nevertheless, copolymers with neutral and long corona forming blocks at low initial copolymer concentrations, along with slow acid/base dilution can be used to prepare pH-responsive vesicles without precipitation problems.[129] If the IEP is close to the physiological pH range the problem of precipitation must be considered if the materials are targeted towards biomedical application. Another restriction of pH-responsive polymer vesicles is the relatively narrow pH range available for the controlled release of the encapsulants *in vivo*. Polymer vesicles should be stable in the blood stream before they reach the targeted sites such as tumors and as these sites are acidic (pH < 6.8) the pK_a of the membrane-forming block must be slightly above the pH of the targeted sites.

Similar to the narrow pH range, the available temperature window for the controlled release of encapsulants from thermo-responsive vesicles is also narrow, especially in biomedical applications. In principle the local temperature in the tumor and inflammatory tissues should be slightly higher than normal body temperature. However, in practice, the exact local temperature strongly depends on the individual and varies with time. This makes the accurate design of polymers with LCSTs for thermo-responsive vesicle formation difficult.

5.2 Loading efficiency of vesicles

High encapsulation efficiencies for polymer vesicles are required if they are to be used as delivery vehicles. However, the encapsulation efficiency of the vesicle may depend on a number of factors such as the preparation method (solvent switch, rehydration and pH, thermo or ionic switches), polymer structure (hydrophobicity, rigidity, charge, *etc.*), encapsulation procedure (*in situ* or post loading), as well as the vesicle formation mechanism. For example, a maximum loading efficiency of ~27% Dox can be reached when pH-responsive PMPC-*b*-PDPA block copolymer vesicles were formed in the presence of Dox.[57] Whereas nearly no loading was found when the preformed vesicles were mixed with Dox.[57] The relatively high loading efficiency achieved by *in situ* loading during vesicle self-assembly is consistent with vesicle formation mechanism I as shown in Fig. 2.

However, based on a different pH-responsive diblock copolymer, PEO-*b*-PDEA, Adams *et al.* showed that vesicles prepared by a pH switch in the presence of a water soluble fluorescent dye had a very poor loading efficiency (~0).[66] They proposed that this was due to the vesicle formation following a different mechanism, mechanism II. During the initial bulk phase separation into polymer rich droplets within the continuous aqueous environment, incorporation of high levels of hydrophilic encapsulate would be precluded. The subsequent re-structuring into a vesicle occurs without opening the structure to the external environment, similar to mixing the preformed vesicles and dye.[57] Based on this recent report it is important that further research explores the loading efficiencies from different sources, irrespective of polymer structure and vesicle formation mechanism.

5.3 Cytotoxicity of vesicles

To enable real world applications of polymer vesicles *in vivo* non-toxic polymeric materials are required. In some cases, polymer vesicles with biocompatible coronas such as PMPC and PEO can be considered invisible or stealthy to the immune system. However, the polymer vesicle after dissociation in the body can become toxic depending on the nature of the membrane-forming block. As a result there is significant research in the application of biocompatible and biodegradable materials such as PCL or PLA to form the vesicle membrane. A key advantage of these materials is that they can be degraded by the action of an enzyme. The realisation of responsive and biocompatible vesicles is a key challenge in their application as biomedical delivery vehicles.

There are only a few examples of PCL- and polylactide (PLA)-based biocompatible/biodegradable block copolymer vesicles whose PCL or PLA membranes are degraded and hence 'respond' to the presence of an enzyme.[54,130–133] However, biodegradable homopolymers are seldom used to make polymer vesicles and instead biocompatible but not biodegradable blocks such as PEO and PMPC, are often used to afford amphiphilic block copolymers for vesicle formation. Several examples are given below, although few *in vivo* tests have been reported to date and will be key in the realisation of the full potential of these materials.

Weitz and coworkers recently described the fabrication of block copolymer vesicles with biocompatible and partly biodegradable diblock copolymers, poly (ethylene glycol)-*b*-poly(lactic acid), by a double emulsion templating technique.[134] These polymer vesicles were shown to be capable of encapsulating small hydrophilic solutes (such as fluorescent HPTS dye). When exposed to osmotic shock, the vesicles respond by breaking and releasing the solutes, providing a simple and effective release mechanism. Similar PEO-*b*-PLA vesicles have showed the ability to release active dyes and anti-cancer drugs over a two week period.[54]

Another interesting approach in the design of biocompatible vesicles is the utilisation of natural scaffolds in combination with synthetic polymers. An excellent example of this was reported in 2006, by Nolte and coworkers who reported a 'biohybrid' polymer vesicle made from a *giant amphiphile* with a protein or enzyme as the polar head group and a synthetic polymer as apolar tail.[135] Horseradish peroxidase (HRP) and myoglobin (Mb) with a polystyrene (PS) chain were synthesized using the cofactor reconstitution method[136] to yield giant amphiphiles, which form functional vesicles in aqueous solution. They found that both HRP and Mb retain their functionality in the vesicle morphology but the reconstitution has appreciable effect on their activity. This is perhaps because of the unfavorable interaction of the protein with the polystyrene chain, or the disturbance of the protein's three-dimensional structure in the vesicle, or the shielding of channels through which the proteins access.

5.4 The next generation of polymer vesicles

Ideally, the synthesis of polymer vesicles with multiple functionalities is required for the design of the next generation of effective nanocarriers for biomedical applications. These multifunctional materials would be ideal biomedical vehicles if they were biocompatible and biodegradable, and could respond to pH, temperature or redox, and/or be capable of carrying other functional particles such as imaging probes or reactive functionality. Yet, combining these key features for the synthesis of polymer vesicles has not yet been achieved.

Indeed, there has been recent interest in this area. For example, Murali and coworkers explored how polymer vesicles could be used as a multi-functional tools for cancer diagnosis and therapy.[119] Sanvicens and Marco also summarized the development of novel strategies for controlled released of drugs by multifunctional nanoparticles, see Fig. 18.[137] Kwon and coworkers focused on the advances in the application of multifunctional particles in cancer imaging and therapy.[138] Whilst rapid advances have been made in the synthesis of polymer vesicles the incorporation of biocompatible polymers in combination with 'smart' properties is not yet been fully explored.

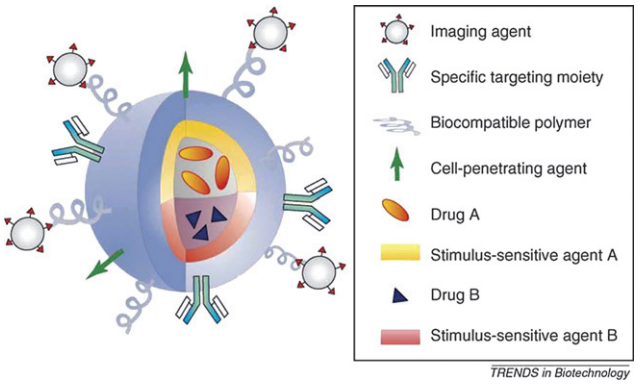

Fig. 18 Multifunctional nanoparticles for drug delivery combine a specific targeting agent (usually an antibody or peptide) with nanoparticles for imaging (such as quantum dots or magnetic nanoparticles), a cell-penetrating agent (*e.g.* the polyArg peptide TAT), a stimulus-sensitive element for drug release, a stabilising polymer to ensure biocompatibility and the therapeutic compound.[137]

Indeed, if polymer vesicles are to be utilized as mimics of nature's delivery vehicles then the issue of functionalisation and compartmentalisation must be addressed along with the responsive nature and compatibility of the vehicles. Compartmentalization (as exemplified by the structure of cells) is one of the most important features to ensure reaction integrity as it isolates the catalytic cycles, prevents interference from other compounds and regulates the flux of molecules passing through the microenvironment. van Hest and coworkers very effectively mimicked this process by the positional assembly of enzymes into polymer vesicles to afford polymeric nanoreactors for application in cascade reactions.[139,140] Three different enzymes can be selectively encapsulated in the water pool or membrane of polymer vesicles by lyophilization, depending on the encapsulation procedures. Water and small molecules can freely penetrate the vesicle membrane whereas the sequestered enzyme remains inside the vesicle interior without leakage. Each of the enzymes can catalyze, in sequence, a biochemical reaction in the vesicle. This allows for a cascade reaction to occur throughout the distinct domains of the vesicle. This elegant combination of reactive functionalities within a single nanostructure highlights the current state-of-the-art in the development of polymer vesicles as functional materials. However the next step needs to be the merging of the aspects of functionalization, responsiveness and biocompatibility to allow for the next generation of polymer vesicles.

6. Summary

Polymer vesicles are diverse materials which can be used in many fields such as templates for nanoparticle preparation, in diagnosis and as a delivery vehicles for therapies. In this Highlight, we have summarized the recent progress and challenges in the area of smart and functional polymer vesicles, focusing especially on stimuli-responsive polymer vesicles which have obtained much attention in recent years due to their potentially excellent properties in controlled encapsulation and release. The diverse stimuli for vesicle modification discussed in this review includes pH, temperature, redox, light and enzymes, which all act by different mechanisms and thus provided a range of materials for a host of different controlled release applications. However, to achieve the full potential of these materials *in vivo* the utilization of biocompatible polymers needs to be explored in combination with controlled release approaches.

The stability of polymer vesicles is an important aspect to consider when these materials are applied in diagnosis and/or therapy. To ensure the vesicle morphology is maintained and to prolong the circulation time in the body, the vesicle membrane is required to be as stable as possible. A variety of chemical cross-linking techniques for application in vesicle membranes can afford stable

nanostructures. However, to release the encapsulated species, suitable and tunable permeability of the membrane is required. Thus, stimuli such as pH, temperature, redox, and light which can be utilized to control the membrane permeability are certainly promising research directions for the future. Also of importance is the ability to selectively functionalize a specific domain or domains within the nanostructure. This allows for the most effective utilization of the phase separated nature of the vesicle to allow for access to multi-functional materials. Overall, the unique combination of multiple functional sites, responsive and biocompatible materials will enable the design and synthesis of complex, compartmentalized structures for advanced delivery vehicles.

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