Stimuli-Responsive Polymersomes

Min-Hui Li

Abstract Polymer vesicles, commonly called polymersomes, are spherical shell structures in which an aqueous compartment is enclosed by a bilayer membrane made from amphiphilic block copolymers. Compared to liposomes, their low molecular weight analogues, polymersomes have many superior properties (higher toughness, better stability, tailorable membrane properties), which make them attractive candidates for applications including drug delivery, diagnosis, nanoreactors and templates for micro- or nano-structured materials. Many potential applications require the ability to control the release of substances encapsulated in the interior compartment and /or in the hydrophobic core of membrane. To address this goal, polymersomes have been developed in which a specific stimulus destabilises the vesicular structure. The responsiveness is mainly achieved via proper hydrophobic block design. In this chapter we review the most promising approaches to make stimuli-responsive polymersomes that permit the controlled release of encapsulated contents. Chemical stimuli including hydrolysis, oxidation, reduction and pH change, and physical stimuli including temperature, light, magnetic field, electric field, osmotic shock and ultrasonic wave are discussed, on emphasizing their effects on the chemical and physical structure of the amphiphilic copolymers.

Keywords Polymersomes • Responsive • Amphiphilic block copolymers • Chemical stimuli • Physical stimuli

1 Introduction

Polymersomes, or polymer vesicles (Discher and Eisenberg 2002a; Hammer and Discher 2001), are macromolecular homologues of liposomes, or lipid vesicles. They have spherical shell structures in which an aqueous compartment is enclosed

M.-H. Li[1, 2, 3] (✉)
[1]Institut Curie, Centre de recherche; [2]CNRS, UMR 168; [3]Université Pierre et Marie Curie, 26 rue d'Ulm, F-75248 Paris, France
e-mail: min-hui.li@curie.fr

A. Prokop (ed.), *Intracellular Delivery: Fundamentals and Applications*, Fundamental Biomedical Technologies 5, DOI 10.1007/978-94-007-1248-5_11,

by a bilayer membrane made of amphiphilic block copolymers. Because of the high molecular weight of the block copolymers, the polymer bilayer has a higher thickness (10–30 nm) than that of the lipid blilayer (3–5 nm). Moreover, the critical micelle concentrations C_{CMC} as well as the amphiphile exchange rates between aggregates (generally proportional to C_{CMC}) are lower for polymersomes than those for liposomes. These confer polymersomes greater toughness and superior stability than liposomes. Polymersomes are therefore excellent candidates for drug carriers. Their interior cavity can be used for the encapsulation of hydrophilic substances such as hydrophilic therapeutics, while the hydrophobic bilayer membrane can trap hydrophobic moieties such as hydrophobic therapeutics or dye molecules for biomedical imaging (Discher and Ahmed 2006). More interestingly, polymer chemistry enables almost unlimited molecular design of polymersomes (Taton and Gnanou 2006). Their membrane properties can be extensively tailored by variation of chemical structures of each polymer component (Napoli et al. 2006; Mabrouk et al. 2009b; Battaglia and Ryan 2005). Targeted transport can be achieved by taking advantage of the many possibilities to end-functionalize the copolymers (Lin et al. 2004; Meng et al. 2005; Broz et al. 2005; Ben-Haim et al. 2008; Christian et al. 2007; Hammer et al. 2008; Zupancich et al. 2009; Robbins et al. 2010; Nehring et al. 2009; Geng et al. 2006; Demirgoz et al. 2009; van Dongen et al. 2008; Opsteen et al. 2007; Sun et al. 2009). The controlled release of therapeutic substances can be integrated through the use of copolymers with blocks that respond to external or internal stimuli in the treated disease sites (Meng et al. 2009; Li and Keller 2009; Du and O'Reilly 2009; Onaca et al. 2009).

In order to achieve effective intracellular drug delivery, the polymersomes should be engineered to (1) survive in biological fluids and extracellular space, (2) bind to cell surface, (3) escape or survive in the endocytic pathway, (4) release drug when the cytosol or another desired intracellular compartment is reached. Development of "smart" polymersomes, i.e., stimuli-responsive polymersomes bearing a protective coat, site-specific targeting ligands and a cell-penetrating function, is the current research trend in this field. In this review, we will focus on the stimuli-responsive properties of polymersomes made from amphiphilic block copolymers. The responsiveness is mainly achieved via proper hydrophobic block design so that the hydrophobic core of the membrane can be altered or destroyed under the action of chemical and physical stimuli. Before the detail discussion of this issue, we first briefly discuss the choice of hydrophilic block, the structural requirement of block copolymer for polymersome formation, and the techniques employed for polymersome preparation, and strategies to get controlled release.

1.1 Hydrophilic Block Choice

Biocompatible and flexible hydrophilic polymers are usually utilized as the hydrophilic block of the amphiphilic block copolymers in order to design non-fouling

and non-antigenic (stealth) polymersomes for a long circulation time in the bloodstream (Knop et al. 2010). The most used hydrophilic polymer is polyethylene glycol (PEG, also known as polyethylene oxide, PEO), which is a neutral polymer and confer the steric stabilization to polymersomes (Lasic and Papahadjopoulos 1998; Semple et al. 1998). Other neutral polymers have also been developed, such as poly(2-methyloxazoline) (PMOXA) (Nardin et al. 2000), which also shows low non-specific protein binding ability (Woodle et al. 1994) and poly[2-(methacryloyloxy)ethyl phosphorylcholine] (PMPC), a promising hydrophilic block reported recently (Du et al. 2005; Du and Armes 2009). Several hydrophilic polymers attempted first for stealth liposomes, including poly[N-(2-hydroxypropyl)methacrylamide] (Whiteman et al. 2001), poly-N-vinylpyrrolidones (Torchilin et al. 2001), polyvinyl alcohol (Takeuchi et al. 2001) and amino-acid-based biodegradable polymer (Romberg et al. 2007), can also be used to design the hydrophilic block of polymersomes. Hydrophilic peptide-based polymers have attracted considerable interests for their high biocompatibility (Kukula et al. 2002; Bertin et al. 2010).

The polyacrylic acid (PAA) is a typical non-neutral hydrophilic block for polymersome preparation (Zhang and Eisenberg 1995b), which is anionic in physiological pH (=7.4). Kataoka's group has shown that anionic polymer spherical micelles had significant promise over neutral spherical micelles in evading the mononuclear phagocytic system of liver and spleen that is primarily responsible for the clearance of foreign particles (Yamamoto et al. 2001). A recent research in Discher's group (Christian et al. 2010) has taken advantage of this anionic profile to make very long-circulating polymersomes with a red cell-like surface charge. As a matter of fact, the life span of healthy human red blood cells is of 100 days, while the polymersomes with neutral PEG hydrophilic shell circulate for 1–2 days. The red blood cells' external "glycocalyx" contains sialic acid which imparts a negative surface charge of approximately −15 mV. Discher's group has achieved this surface charge of polymersomes by blending an anionic diblock copolymer, poly(acrylic acid)-*b*-polybutadiene (PAA-*b*-PBD), with a neutral diblock copolymer (PEG-*b*-PBD) under physiological conditions.

1.2 Structural Consideration of Block Copolymers for Polymersome Formation

The unfavourable contact between water and the hydrophobic part of amphiphilic molecules leads to their self-assembly into small aggregates in water with a hydrophobic core and a hydrophilic shell. The aggregates display rich polymorphism, the simplest structure being spherical micelles. If only isotropic fluidlike arrangements are considered in the hydrophobic core and in the hydrophilic shell, the self-organizing structures can be partially predicted on the basis of concepts developed for small amphiphilic molecules, such as the 'spontaneous curvature' or the 'critical packing parameter', defined as $p = v/al$, where v is the hydrophobic volume, a is the

optimal interfacial area and l is the length of the hydrophobic block normal to the interface.(Israelachvili 2005) For $p \leq 1/3$, surfactants are predicted to assemble into spherical micelles; for $1/3 \leq p \leq 1/2$, cylindrical micelles are expected; and for $1/2 \leq p \leq 1$, lamellar aggregates or vesicles should form spontaneously. The packing parameter is not strictly a geometrical parameter, since it is dependent on a number of other factors such as thermodynamics and interactions. In the case of coil-coil polymer amphiphiles, for example, the consideration of the packing parameter is more complicated and should be connected with the macromolecular character of each block, including the entropy contribution and the interaction between water and the hydrophilic polymer block. It is often noted that the morphology of self-assembled block copolymer aggregates is governed primarily by three components of the free energy of aggregation: (*i*) core–chain stretching, (*ii*) interfacial energy, and (*iii*) intercoronal chain interactions (Gennes 1978; Zhang and Eisenberg 1998; Halperin et al. 1992). These components depend on the chemical structure of the copolymer, the hydrophilic/hydrophobic ratio, the copolymer concentration in solution, the solvent properties such as the type of organic solvent and the ratio of organic solvent/water, salt concentration, solution pH, temperature or shear rate. Extensive studies have been made on prototypical amphiphilic block copolymers, such as poly(acrylic acid)-*b*-polystyrene (PAA-*b*-PS) (Zhang and Eisenberg 1995a, 1996), PEG-*b*-PS (Yu and Eisenberg 1998; Bhargava et al. 2006), PEO-*b*-PBD (Won et al. 1999; Jain and Bates 2003; Discher and Eisenberg 2002b), polyethyleneglycol-*b*-polycaprolactone (PEG-*b*-PCL) (Meng et al. 2003; Ghoroghchian et al. 2006; Ahmed and Discher 2004), poly(methacrylic acid)-*b*-polybutadiene (PMAA-*b*-PBD) (Fernyhough et al. 2009). Spherical micelles, cylindrical micelles and bilayer vesicles have all been observed in these coil-coil polymer systems. An appropriate phospholipids-like hydrophilic/hydrophobic ratio is often used as a practical guide to prepare block copolymers which form vesicles. But this is not universal to all systems and should be taken with care. For example, vesicles were formed directly in water by PEO-*b*-PBD as its hydrophilic to total mass ratio f was around 35% (a phospholipids-like ratio), while vesicles were also obtained by PAA-*b*-PS with a very short hydrophilic block (e.g. $f < 20\%$, even at 4%) in the mixture of water and dioxane (Eisenberg 1999).

If the organization in the hydrophobic core and/or in the hydrophilic shell is not isotropic fluidlike, but crystalline or strongly bound by hydrogen bonding or $\pi - \pi$ stacking etc, considerable changes will take place in the micellar aggregates (Antonietti and Forster 2003). Rigid rods and tubules instead of fluid cylindrical micelles and planar bilayers are, for example, obtained in these kinds of small amphiphilic molecules. In the case of coil-rod block copolymers, the shape anisotropy and additional order (introduced by liquid crystalline, crystalline structures and secondary peptide structures such as α helices or β sheets) in the rod-like block also influences considerably the self-assembly (Pinol et al. 2007; Jia et al. 2009; Jenekhe and Chen 1998; Massey et al. 2000; Kukula et al. 2002; Bellomo et al. 2004; Burkoth et al. 2000; Jiang et al. 2007a). In conclusion, the polymersome formation depends on the chemical and physical structure of the block copolymers.

1.3 Polymersome Preparation

Polymersome preparation requires the mutual diffusion of water into the bulk block copolymer and vice versa (Battaglia and Ryan 2006). In general, all reported methods for liposome preparation can also be used for polymersome preparation (Szoka and Papahadjopoulos 1978; Gregoriadis 1992; Angelova and Dimitrov 1986; Pautot et al. 2003a). In preparation protocols, the contact between water and polymer can be achieved directly or with the aid of organic solvent if the hydrophobic block is glassy at the preparation temperature. For block copolymers with hydrophobic blocks with a low Tg, such as PEO-*b*-PBD ($T_{g,PBD}$ ~ −90°C to −8°C according to their relative 1,4- and 1,2-content), vesicles can be formed by direct hydration techniques assisted by sonication or electrical field (Dimova et al. 2002). In contrast, block copolymers with a glassy hydrophobic block, such as PAA-*b*-PS ($T_{g,PS}$ ~ 100°C), often require an organic co-solvent to fluidize the polymer layers. Typically, a polymer solution is first prepared in an organic solvent and the solvent is then gradually exchanged with water (Zhang and Eisenberg 1995b). It belongs to a more general nanoprecipitation method based on the interfacial deposition due to the displacement of a solvent with the non-solvent. Recently, microfluidic and micro-patterning technology have opened some fascinating ways to prepare polymersomes with controlled size and efficient encapsulation (Hauschild et al. 2005; Shum et al. 2008; Stachowiak et al. 2009; Howse et al. 2009).

Polymersome preparation methods discussed above naturally lead to a symmetric copolymer distribution between both leaflets of bilayer membrane. One special method, called inverted emulsion which is first developed by Weitz' group for liposomes (Pautot et al. 2003c), permits to obtain asymmetric vesicles by independent assembly of the inner and outer leaflets of the vesicle (Pautot et al. 2003b). Briefly, the inner monolayer is first formed via the emulsification of water droplets in oil containing the first amphiphile of interest. The outer monolayer is then formed by the centrifugation of the water droplets stabilized by the first amphiphile through the monolayer of the second amphiphile at the interface between a second oil solution (containing the second amphiphile of interest) and a water solution.

1.4 Strategies for Controlled release

The major advantage of polymersomes is that they can be used as carriers of hydrophilic substances (in the interior compartment) as well as hydrophobic substances (in the membrane) offering a cocktail-treatment or a diagnostic-therapy combination in biomedical applications. A crucial question here is how to release the active substances when and where they are needed. In general, the continuous loss of encapsulated substance via diffusion is very slow as a result of the considerable thickness of the polymer membrane. In some cases, more selective permeability has been achieved through the use of special chemical structures for the block copolymer (Battaglia et al. 2006), (Vriezema et al. 2007), or by incorporating

channel proteins into the polymersome membranes (Choi and Montemagno 2005). In most applications it is highly desirable to be able to control the release of encapsulated substance by triggering a change in membrane properties of polymersomes via the action of a stimulus. The present chapter focuses on this issue of controlled release and reviews the most promising approaches to create stimuli-responsive polymersomes. So far, two strategies have been followed to achieve the controlled disassembly of polymersomes. The first one exploits the vast possibilities of chemical synthesis to develop polymer membranes sensitive to the chemical stimuli, including hydrolysis, oxidation reaction, reduction reaction and pH change. The second strategy, which also takes advantage of the chemical diversity of polymers, uses physical stimuli, such as temperature variation, light, magnetic fields, electric field, osmotic shock or ultrasonic wave, to remotely destroy the polymersomes. Stimuli-responsive polymersomes are then reviewed in detail according to these two strategies by dividing the stimuli into chemical and physical stimuli.

2 Polymersomes Responsive to Chemical Stimuli

2.1 Response to Hydrolytic Degradation

Biodegradable polyester-based polymersomes have been made from polyethyleneglycol-*b*-polylactic acid (PEG-*b*-PLA) (Ahmed and Discher 2004), (Meng et al. 2003), polyethyleneglycol-*b*-polycaprolactone (PEG-*b*-PCL) (Ghoroghchian et al. 2006), and polyethyleneglycol-*b*-poly(γ-methyl-ε-caprolactone) (PEG-*b*-PMCL) (Zupancich et al. 2006). Under physiological conditions (pH = 7.4), these polyesters degrade by hydrolysis. However, polyester hydrolysis is accelerated by low pH, which may be useful given the acidic environment in tumors and endolysosomes. To provide controllable degradation and adjustable release times ranging from hours to weeks, polymersomes were formed by blending PEG-*b*-PLA and PEG-*b*-PCL with inert PEO-*b*-PBD (Ahmed and Discher 2004). These polymersomes were loaded with two anticancer drugs. Doxorubicin (water soluble) was loaded in the aqueous interior of the polymersomes while paclitaxel (water insoluble) was included in the hydrophobic layer of the membrane. The loaded polymersomes were degraded in vivo and drug release occurred with a time scale of a day. This degradation and release was shown to be coupled with the phase transition behaviour of the block copolymer amphiphiles (see Fig. 1). Polyester hydrolysis occurs preferentially at the chain end, thereby increasing the hydrophilic/hydrophobic ratio of PEG-polyester chains and preferred curvature of the self-assembly. If we discuss with the packing parameter *p,* the hydrophobic chain shortening induces a decrease of *p* value from 1 to 1/2 to 1/3. The comparatively short hydrophobic blocks of the degraded chains are unstable in a bilayer. Instead, they tend to segregate, congregate, and ultimately induce hydrophilic (i.e., PEG-lined) pores and eventually the vesicular carriers disintegrate into mixed micellar assemblies. These polymersomes are a promising method for multi-drug delivery.

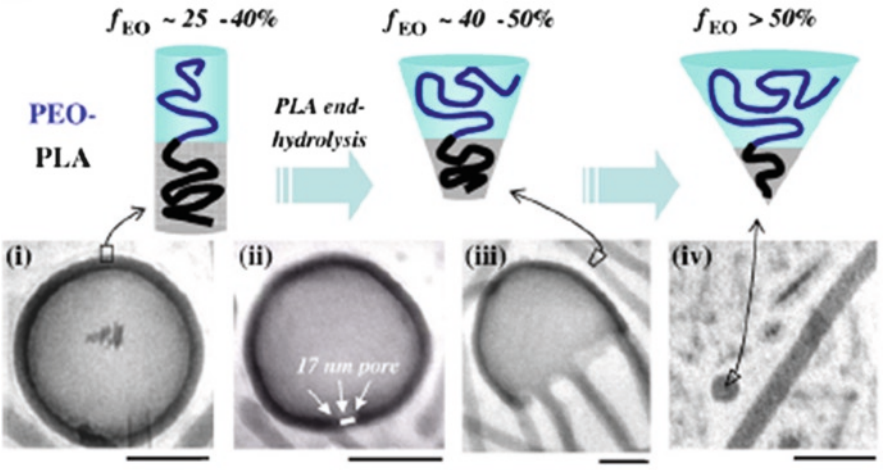


Fig. 1 Representative time series showing the breakdown structures formed as PEG-*b*-PLA polymersomes transform from vesicles to worm-like micelles. The scale bars in the cryo-TEM images are 100 nm. (Reproduced from ref. Ahmed et al. 2006)

2.2 Response to Oxidation Reaction

Instead of hydrolyzing the hydrophobic polymer block, Hubbell and co-workers changed the value of hydrophilic/hydrophobic ratio via oxidation of the hydrophobic block. This strategy could potentially exploit the oxidative environment present in sites of inflammation as well as within endolysosomes. They developed oxidation-responsive polymersomes from PEO-*b*-PPS-*b*-PEO (PPS: polypropylene sulphide) triblock copolymers (Napoli et al. 2004a, b, 2005), and showed that exposure to either aqueous H_2O_2 or H_2O_2 from glucose-oxidase/glucose/oxygen system oxidized the hydrophobic polypropylene sulphide layer, thereby transforming it into hydrophilic poly(propylene sulphoxide) and poly(propylene sulphone). Oxidation thus increased the hydrophilic percentage in the amphiphilic system, thereby causing a transition from polymersomes to wormlike micelles, spherical micelles, and eventually soluble oxidized copolymers. This transition took about 10 h in 10-vol%H_2O_2 aqueous solution and around 300 h in 0.03-vol% H_2O_2. These oxidation-responsive polymersomes may find applications as nanocontainers in drug delivery, biosensing and biodetection.

2.3 Response to Reduction Reaction

Reduction-sensitive materials containing disulphide bonds have been used to produce reduction-responsive polymersomes. Hubbell and co-workers recently reported polymersomes based on diblock copolymers PEG-SS-PPS, where a

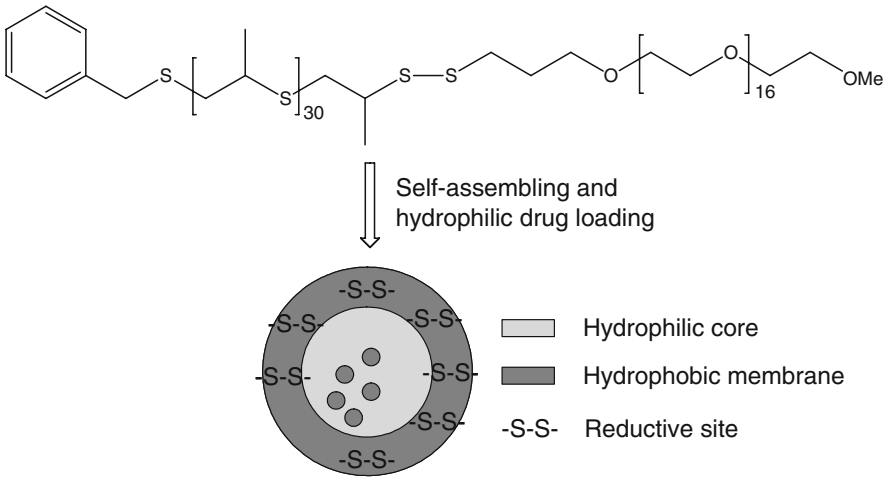


Fig. 2 Polymersomes formed from the block copolymer, PEG_{17}-SS-PPS_{30}, where the hydrophilic poly(ethylene glycol) (PEG) and the hydrophobic poly(propylene sulfide) (PPS) are connected with a reductive disulfide group. (Reproduced from ref. Cerritelli et al. 2007)

reduction-sensitive disulphide link (-S-S-) was placed between the two blocks (see Fig. 2) (Cerritelli et al. 2007). Intracellular glutathione or cysteine can reductively cleave these links and thereby destabilise the system. Other reductive compounds such as dithiothreitol and tris(2-carboxyethyl)phosphine can also cleave the disulfide bonds (Li et al. 2006b).

Polymersomes formed from the block copolymer PEG-SS-PPS were demonstrated to break down in the presence of intracellular concentrations of cysteine. In cellular experiments, uptake, disruption, and release were observed within 10 min of exposure to cells, well within the time frame of early endosome and endolysosomal processing. This system presents obvious advantages over the hydolysis and the oxidation responsive polymersomes reviewed above. The hydrolysis process is not nearly as fast (>hours) and the low pH needed to accelerate the hydrolysis is encountered only within the lysosomal compartment of the endosomal-lysosomal processing pathway. Oxidative conditions are also not encountered until the vesicles are processed within the lysosome. In both cases, the release can be too late for sensitive biological macromolecules, i.e. within the less desirable lysosome, where biomolecular drugs are exposed to very harsh conditions, rather than the more desirable endosome. In contrast, reduction-responsive polymersomes can rapidly burst within the early endosome, releasing their contents prior to exposure to the harsh conditions encountered after lysosomal fusion. This system may be useful in cytoplasmic delivery of biomolecular drugs such as peptides, proteins, oligonucleotides, and DNA.

2.4 pH Response

2.4.1 pH Responsive Polymersomes Formed from Synthetic Block Copolymers

Vesicles containing polymer blocks with solubility responding to changes in pH present additional opportunities for controlled release. A pH-response can be obtained either by using polyacid blocks (e.g. PAA) whose ionization status can be changed by a pH variation, or by using polybase blocks (e.g. PVP: polyvinyl pyridine) that can be rendered water soluble by protonation at low pH. One distinct advantage of pH-triggered release is the fast response of the system. For example, the acid-induced change for the polybase blocks solubility occur almost instantaneously, whereas the hydrolysis of PLA or PCL, occurs over time scales ranging from minutes to days, even when it is acid-catalyzed.

We will first discuss systems based on polyacid. Since the early work of Eisenberg's group (Zhang and Eisenberg 1995b) on mapping the phase diagram of "crew-cut" PAA-*b*-PS in dilute organic/aqueous solution, Liu & Eisenberg (Liu and Eisenberg 2003) have shown rapid pH-triggered inversion of amphiphilic triblock copolymer vesicles of PAA-*b*-PS-*b*-P4VP in organic/aqueous solution mixtures. For vesicles formed from a PAA-*b*-PBD diblock copolymer in water, Discher's group (Geng et al. 2005) observed that a sudden increase in pH induced the rapid (~minutes) transition of vesicles into worms and spheres. Chiu's group has recently reported an interesting work on polymersomes with pH-responsive transmembrane channels (Chiu et al. 2008). The copolymers used to form vesicles were not block copolymers but rather random copolymers of acrylic acid (AAc) and distearin acrylate (DSA), that were obtained from partial transesterification of poly(N-acryloxysuccinimide) (poly(NAS)) with distearin (a lipid graft) followed by thorough hydrolysis of the un-reacted NAS to AAc units. Using a double emulsion technique in a water/oil/water system and a copolymer with an average molecular weight of 2.97×10^5 g mol^{-1} and a composition of 9.1 mol% DSA, they prepared large polymersomes that contained small polymersomes and had a pH of 4.0–5.5 within the interior aqueous compartment (see Fig. 3). When the pH was increased to 6.5, the vesicles became permeable to hydrophilic solutes. The authors suggested that un-ionized AAc-rich regions in the hydrophobic bilayer regions of distearate grafts (parallel to the aligned lipid chains) could act as pH-responsive channels. When the pH was increased to 6.5, AAc ionization would occur and the resulting abrupt disruption of hydrogen bonds and hydrophobic association of un-ionized AAc would create permeable channels. This system is an elegant example of vesicles that were equipped with transmembrane channels without requiring the incorporation of channel-forming proteins. Similarly, Eisenberg's group (Yu et al. 2009) has also reported polymersomes with pH-induced reversible change of permeability to water and to proton. They call them "breathing" polymerosmes, which are prepared from triblock copolymer poly(ethylene oxide)$_{45}$-*b*-polystyrene

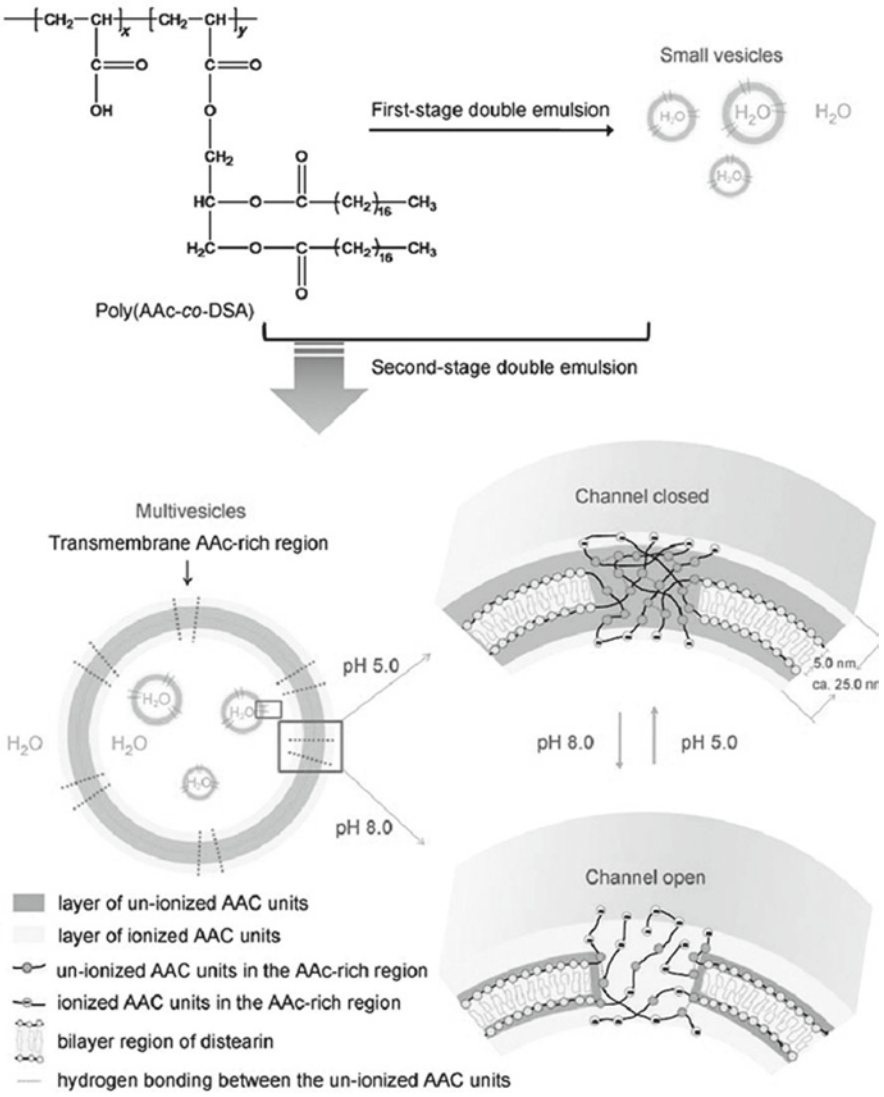


Fig. 3 Illustration of multi-vesicular assemblies produced via a two-stage double emulsion of poly(AAc-*co*-DSA). The resulting vesicles are equipped with pH-responsive transmembrane channels. (Reproduced from ref. Chiu et al. 2008)

$_{130}$-*b*-poly(2-diethylaminoethyl methacrylate)$_{120}$ (PEO$_{45}$-*b*-PS$_{130}$-*b*-PDEA$_{120}$). Self-assembly into vesicles was carried out at a pH of ca.10.4. The vesicle wall was shown by cryo-TEM to consist of a sandwich of two external ca. 4 nm thick continuous PS layers and one ca. 17 nm thick PDEA layer in the middle (Fig. 4). As the pH decreases, both the vesicle size and the thickness of all three layers increase. The increase of the thickness of the intermediate PDEA layer arises from the protonation and hydration, but the swelling is constrained by the PS layers.

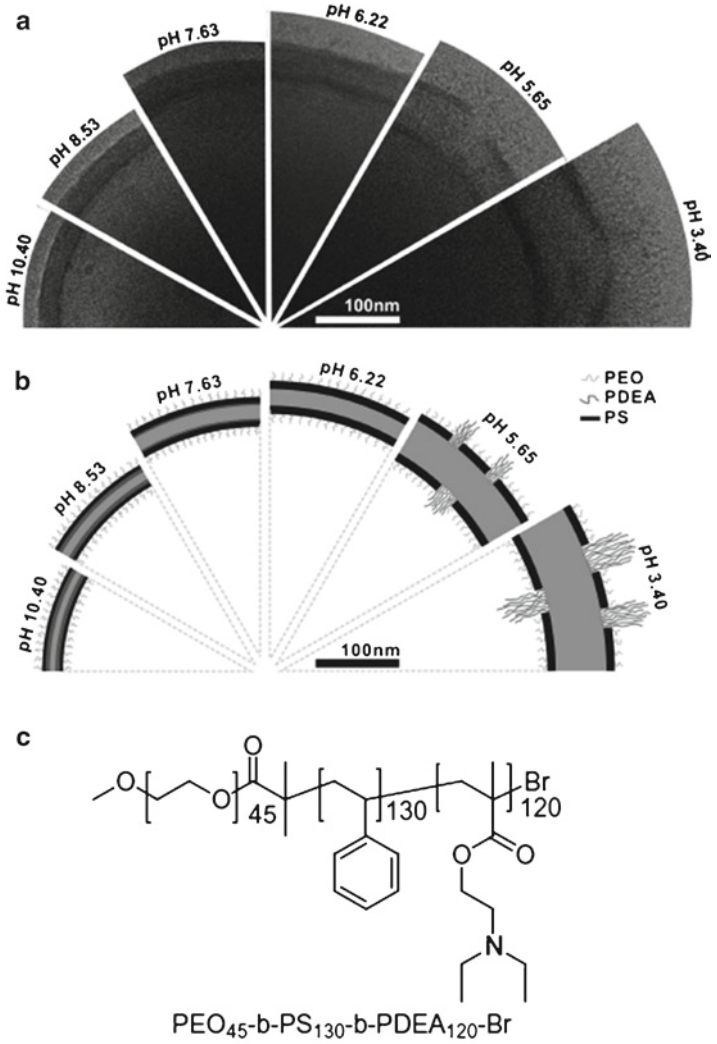


Fig. 4 (**a**) cryo-TEM images of vesicle wall structures at pH 10.4, 8.53, 7.63, 6.22, 5.65, and 3.40, respectively; (**b**) schematic illustrations of the three-layered wall structures at corresponding pH values; (**c**) the chemical structure of the triblock copolymer used to form the breathing polymersomes. (Reproduced from ref Yu et al. 2009)

The increase of the thickness of the two PS layers is a result of an increasing incompatibility and an accompanying sharpening of the interface between the PS layers and the PDEA layer. Starting at a pH slightly below 6, progressive swelling of the PDEA layer with decreasing pH induces a cracking of the two PS layers and also a sharp increase of the vesicle size and the wall thickness.

For applications in chemical and gene therapy, pH-induced release based on polybase blocks may permit the delivery of drugs and genes into the cytosol via endolysosomal acidification and escape. Battaglia et al. recently prepared biomimetic pH

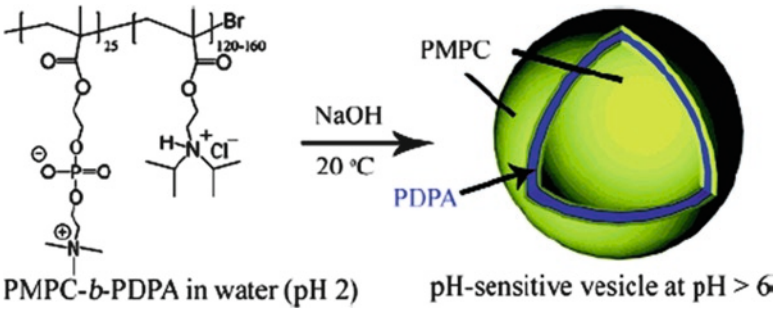


Fig. 5 Formation of PMPC-*b*-PDPA block copolymer vesicles. (Reproduced from ref. Du et al. 2005)

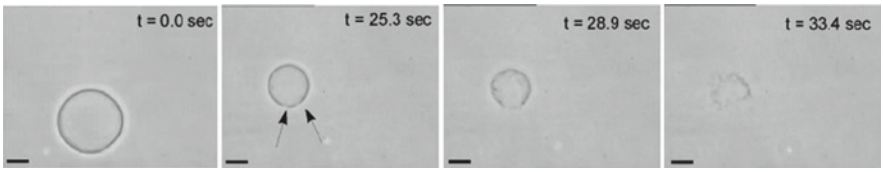


Fig. 6 Sequence of microscopy images showing the dissolution of a giant polymersome of P2VP-*b*-PEO upon addition of dilute acetic acid. (Reproduced from ref. Borchert et al. 2006)

sensitive polymersomes for efficient DNA encapsulation (Lomas et al. 2007) using pH-sensitive diblock copolymers, poly(2-(methacryloyloxy)ethyl-phosphorylcholine)-*b*-poly(2-diisopropylamino)ethyl methacrylate) (PMPC-*b*-PDPA, see Fig. 5), developed in Armes' group (Du et al. 2005).

PMPC is a phospholipid-like, biocompatible, and stealthy hydrophilic polymer, while PDPA is a polybase that has a pH-dependent solubility in water (pK_a 5.8–6.6). Such diblock copolymers form stable vesicles at physiological pH but rapidly dissociate at around pH 5–6. DNA was encapsulated within these PMPC-*b*-PDPA polymersomes at neutral pH, and lowering the solution pH then caused the disruption of the polymersomes and the formation of DNA-copolymer complexes.

Förster's group has used poly(2-vinylpyridine)-*b*-polyethylene oxide (P2VP-*b*-PEO) copolymers to produce another example of fast, complete release of polymersome contents via pH-induced dissolution (Borchert et al. 2006). P2VP is also a polybase that is insoluble in water under neutral and alkaline conditions. However, when the pH is below 5, P2VP is protonated and becomes water-soluble. Fig. 6 shows the rapid dissolution of a P2VP-*b*-PEO giant polymersome within around 30 sec resulting from the addition of dilute acetic acid.

Du and Armes have reported that variation of pH could be used to tune the membrane permeability of cross-linked polymer vesicles in THF/water mixtures (Du and Armes 2005). These vesicles are formed by a pH-responsive, hydrolytically self-crosslinkable copolymer, poly(ethylene oxide)-*b*-poly(2-(diethylamino) ethyl methacrylate-*stat*-3-(trimethoxysilyl)propyl methacrylate) (PEO-*b*-P(DEA-*stat*-TMSPMA)). DEA homopolymer is water soluble at low pH and becomes

insoluble above pH 7. In the cross-linked hydrophobic membranes of polymersomes, a decrease in solution pH protonates the DEA residues which causes in turn the membrane swelling and the permeability increasing.

2.4.2 pH Responsive Polymersomes Formed from Peptide-Based Polymersomes

Polypeptides are a special class of building blocks for vesicle-forming amphiphilic block copolymers because of their stimuli-responsiveness (to pH or temperature), secondary structures, functionalities, and biocompatibility (Schlaad 2006), (Carlsen and Lecommandoux 2009). Nevertheless, there are only a limited number of examples of polymersomes made from polypeptide-containing block copolymers and these examples can be divided into two main families. The first family is composed of hybrid block copolymers, where the polypeptide is the hydrophilic block and a classical synthetic polymer, such as polybutadiene (PBD) or polyisoprene (PI), is the hydrophobic block. The second family includes co-polypeptides in which both the hydrophilic and hydrophobic blocks are polypeptides. Polymersomes formed by hybrid block copolymers with a polypeptide as the hydrophobic block and a synthetic polymer as the hydrophilic one have not yet been reported.

Polymersomes have been formed in aqueous solutions using polybutadiene-*b*-poly(L-glutamic acid) (PBD-*b*-PGlu, 17–54 mol% glutamate) (Kukula et al. 2002; Checot et al. 2002, 2005), polybutadiene-*b*-poly(L-lysine) (PBD_{165}-*b*-$PLys_{88}$ and PBD_{107}-*b*-$PLys_{27}$) (Sigel et al. 2007; Gebhardt et al. 2008) and polyisoprene-*b*-poly(L-lysine). In these systems, a change in pH or temperature can induce a change in the secondary structure of the hydrophilic polypeptide corona (charged coil, α-helix or β-sheet). The secondary structure of poly(L-glutamic acid) (a polypeptide with –COOH side groups) changes from a charged coil at high pH (pH > 6) to an α-helix at low pH (pH < 5), while that of poly(L-lysine) (a polypeptide with $–NH_2$ side groups) changes from an α-helix at high pH (pH = 11) to a charged coil at low pH (pH = 6). It has been reported that the transition from a charged coil to a α-helix conformation in the hydrophilic corona did not change the morphology of the polymersomes, but did cause a large decrease in their size (hydrodynamic radius, by 20–50%). Savin's group has also reported that in basic conditions (pH = 10.9), a temperature increase induced a transition from an α-helix to a β-sheet conformation in the poly(L-lysine) corona of polymersomes made from PBD_{107}-*b*-$PLys_{27}$ (Gebhardt et al. 2008). Consequently, the hydrodynamic radius of the polymersomes increased by a factor of two (from 70 nm to 140 nm) when the temperature was raised from 40°C to 63°C. This block copolymer is a good example of a dual-responsive system sensitive to both pH and temperature changes.

Similar pH and temperature dual-responsive polymersomes were also formed from a series of triblock ABA copolypeptides (poly(L-lysine)-*b*-poly(γ-benzyl-L-glutamate)-*b*-poly(L-lysine) (PLys-*b*-PBGlu-*b*-PLys)) in which the block ratios ranged widely (Iatrou et al. 2007). For example, the hydrodynamic radius of polymersomes made by $PLys_{134}$-*b*-$PBGlu_{64}$-*b*-$PLys_{134}$ was of 129 nm at pH = 7.4 and T = 25°C (PLys in charged coil); this value decreased to 92.5 nm at pH = 11.7 and

T = 25°C (PLys in α-helix); it increased further to 148 nm at the same pH = 11.7 but at higher temperature T = 37°C (PLys in β-sheet). In all these conditions, the neutral hydrophobic central block PBGlu retained an α-helix conformation.

While the secondary structure change in the polypeptide hydrophilic corona induces a change in polymersome size, the mechanism has not been resolved and is the subject of vigorous discussion (Checot et al. 2002; Sigel et al. 2007). The release process seems not to be directly connected with these size changes. Conversely, the effects of secondary structure on polymersome assemblies should be pronounced when the stimuli-responsive polypeptide constitutes the hydrophobic layer of the membrane. These effects are well illustrated by the following two examples.

Deming's group has introduced non-ionic block copolypeptides of L-leucine and ethylene glycol-modified L-lysine residues, PELys-*b*-PLeu [poly(N_ε-2-(2-(2-methoxyethoxy)ethoxy)acetyl –L-lysine)-*b*-poly(L-leucine)] (Bellomo et al. 2004). These copolypeptides have been shown to self-assemble into bilayer vesicles whose size and structures are dictated primarily by the ordered conformations (rod-like or α-helical) of the peptides segments. pH sensitivity can be achieved by replacing 70% of the L-leucine in the hydrophobic domain with L-lysine. At pH = 9, the conformation of the hydrophobic block remains α-helical and vesicles form. However, at pH = 3, protonation of the lysine residues enhances their hydrophilicity and simultaneously destabilizes the α-helical structure of the leucine-rich domain because of electrostatic repulsion. This helix-to-coil transition destabilises the vesicular assembly, leading to porous membranes or complete dissociation of the structures (see Fig. 7).

Rodriguez-Hernandez and Lecommandoux have used a zwitterionic block copolypeptide, poly(L-glutamic acid)-*b*-poly(L-lysine) ($PGlu_{15}$-*b*-$PLys_{15}$), to form

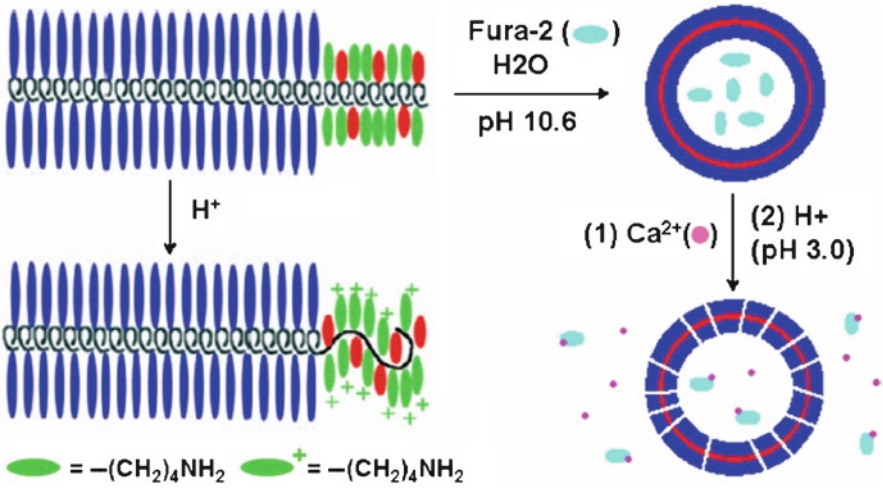


Fig. 7 pH-responsive polymer vesicles formed from PELys-*b*-P($Leu_{0.3}$-*co*-$Lys_{0.7}$)$_{40}$. When the pH was lowered by addition of HCl, the release of entrapped Fura-2 dye took place within seconds. (Reproduced from ref. Bellomo et al. 2004)

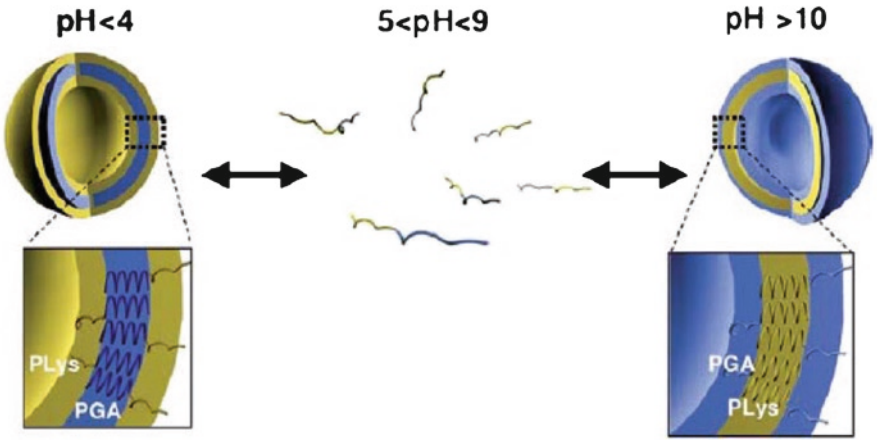


Fig. 8 Schematic representation of the self-assembly of vesicles from the diblock copolymer, $PGlu_{15}$-*b*-$PLys_{15}$ (PGlu is noted as PGA in the sketch). (Reproduced from ref. Rodriguez-Hernandez and Lecommandoux 2005)

schizophrenic vesicles (see Fig. 8) (Rodriguez-Hernandez and Lecommandoux 2005). Using this polyacid-b-polybase, the vesicles can be reversibly produced as a function of pH. At low pH, the poly(L-glutamic acid) with helical structure constitutes the hydrophobic part of the membrane. At high pH, this hydrophobic part is destabilized and becomes hydrophilic because of its transition to the charged coil conformation; instead, the deprotonated poly(L-lysine) takes its place to form the hydrophobic part with its rodlike α-helical structure.

These last two copolypeptide systems are very promising candidates for macromolecular nanobiotechnologies. For drug delivery *in vivo*, the pH of transition (around 3) for lysine is not optimal but other aminoacids (for example, histidine, $pK_a = 6.0$) could be substituted to the lysine to adjust the pH range.

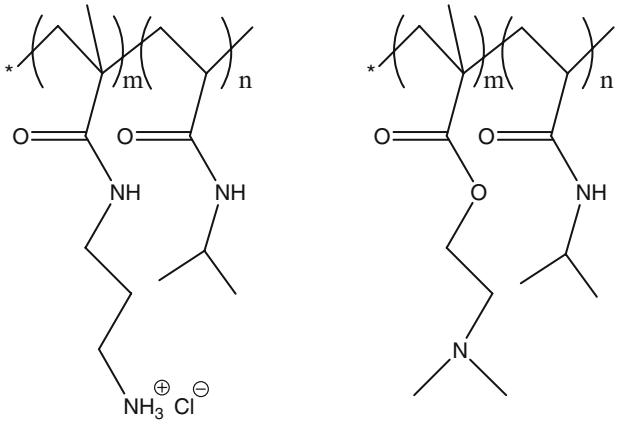
The pH-dependent permeability and reversible structural transition of polyion complex vesicles (PICsomes) in aqueous media was also recently reported by Kataoka and coworkers (Kishimura et al. 2009). At first, the aqueous solution properties of PEG_{45}-$P(Asp\text{-}AP)_{75}$ and PEG_{45}-$PAsp_{75}$, where, P(Asp-AP) stands for poly-[(5-aminopentyl)-α,β-aspartamide] and PAsp for poly(α,β-aspartic acid), were analyzed by potentiometric titration. The pKa 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.

3 Polymersomes Responsive to Physical Stimuli

3.1 Temperature Responsive Polymersomes

In the previous section, we mentioned that polymersomes made from PBD-*b*-PLys (Gebhardt et al. 2008) and from PLys-*b*-PBGlu-*b*-PLys (Iatrou et al. 2007) are temperature responsive in basic solutions. With a temperature increase, polymersome size increased and the poly(L-lysine) block underwent a conformational transition from an α-helix to a β-sheet structure. In the following examples (Li et al. 2006a, 2007; Qin et al. 2006), a well-known thermo-responsive polymer, poly(*N*-isopropylacrylamide) (PNIPAM), was used to produce temperature responsive polymersomes. In aqueous solution, PNIPAM undergoes a reversible phase transition from hydrophilic to hydrophobic at a lower critical solution temperature (LCST, typically around 32°C). Hydrophilic-hydrophilic block copolymers can then be designed using this kind of thermo-responsive block. When the solution temperature is higher than the LCST, the thermo-responsive block becomes hydrophobic and the block copolymer amphiphilic, which can self-assemble to form polymersomes. Below the LCST, the thermo-responsive block is turned hydrophilic again and the polymersomes dissociate to rapidly release of encapsulated substances.

McCormick and co-workers (Li et al. 2006a, 2007) have recently prepared hydrophilic-hydrophilic block copolymers, poly(*N*-(3-aminopropyl)methacrylamide hydrochloride)-*b*-poly(*N*-isopropylacrylamide) (PAMPA-*b*-PNIPAM) and poly(2-(dimethylamino)ethyl methacrylate-*b*-poly(*N*-isopropylacrylamide) (PDMAEMA-*b*-PNIPAM) (see Fig. 9), by reversible addition-fragmentation chain-transfer (RAFT) polymerization. These researchers reported that these water-soluble block copolymers could directly from vesicular aggregates in water and that aggregate



PAMPA-*b*-PNIPAM PDMAEMA-*b*-PNIPAM

Fig. 9 Hydrophilic-hydrophilic block copolymers with a temperature responsive block PNIPAM

formation could be reversed by changing the solution temperature with respect to the LCST of PNIPAM (32°C or higher, depending on the molecular weight of both blocks). The aggregates could also be trapped by ionic cross-linking of the hydrophilic PAMPA block, either through the addition of an oppositely charged polyelectrolyte that causes inter-polyelectrolyte complexation or by forming gold nanoparticles from the reduction of $NaAuCl_4$ complexed to amine groups in the PDMAEMA.

Qin et al. described polymersome formation using a well-defined and monodisperse ($M_w/M_n < 1.2$) block copolymer PEO-*b*-PNIPAM (Qin et al. 2006). The block copolymer was synthesized by RAFT polymerization and polymersomes were grown in an aqueous solution above the LCST of PNIPAM. This family of biocompatible block copolymers is a promising system for drug delivery because the LCST can be adjusted to a value near or slightly above physiological temperatures.

Our group developed a new class of polymersomes in which the hydrophobic part is a liquid crystal (LC) polymer (Yang et al. 2005, 2006). It is well known that liquid crystal systems excel as responsive systems and can respond to multiple stimuli including temperature, light, electric and magnetic fields. If this responsiveness could be retained in the liquid crystal membrane, we speculated that liquid crystal polymersomes would have potential as multi-responsive, smart polymersomes. Recently, we studied the structural changes in liquid crystal polymersomes triggered by changes in temperature using small angle neutron scattering (SANS), cryo-TEM, SEM and high sensitivity DSC (Hocine et al. 2011). PEG-*b*-PA444 and PEG-*b*-PMAazo444 (Fig. 10), two block copolymers with side-on nematic hydrophobic blocks, were used to form vesicles with a bilayer membrane thickness of 10–15 nm at room temperature (Fig. 11a) . Upon heating the membrane thickness, d, started to increase dramatically from a temperature (~55°C) above T_g but below T_{NI} of the LC polymer block, and reached up to 120 nm at $T > T_{NI}$ ($T_{NI} \sim 80$–85°C) (Fig. 11b). The vesicles transformed into thick-walled capsules. The thickness of the membrane was inconsistent with a bilayer structure and surprisingly the capsules were stable even for temperatures above T_{NI}. As the PEG chains should partially dehydrate with temperature, we propose that the membrane reorganized into a structure consisting of microphase separated LC and PEG domains. Analysis of changes in structural parameters such as the internal aqueous volume and the polymer membrane volume suggest that capsule scission and fusion also occurred during this transition. Substance release would be accompanied by capsule scission.

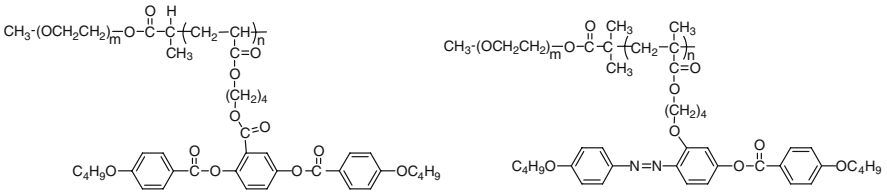


Fig. 10 Molecular structures of liquid crystal copolymers PEG-*b*-PA444 and PEG-*b*-PMAazo444

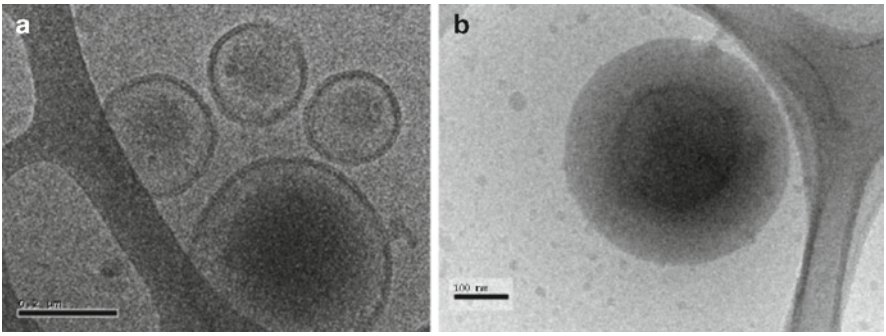


Fig. 11 (**a**) Cryo-TEM image of PEG-*b*-PMAazo444 vesicles at room temperature. Scale: 200 nm. (**b**) Cryo-TEM image of PEG-*b*-PMAazo444 vesicles heated for 1 h at 90°C. Scale: 100 nm

3.2 Light Responsive Polymersomes

Stable, light-responsive polymersomes are attractive because the release of entrapped species can be rapidly induced at specific time and location via exposure to light. While pH, oxidation and reduction-responsive systems can also respond quickly, they require that the chemical environment be modified by the addition of acids or bases or other reagents. These environment changes may not necessarily compatible with the drug targeting sites (in the case of applications in drug delivery) or with other applications such as controlled chemical reactions in microfluidics. In contrast, light is a remote stimulus that does not require any chemical environmental change and can be applied locally.

The first examples of light responsive polymersomes are built by incorporating photo-responsive protein channels (such as Bacteriorhodopsin) in the membrane of polymersomes (Choi and Montemagno 2005). However, this case will not be further discussed in this review because the responsiveness does not result from a change of the membrane properties.

3.2.1 Systems with Photoactive Groups in Their Polymer Structures

The exposure of some photoactive groups to light can generate reversible structural changes, thereby directly changing the hydrophilic-hydrophobic balance without addition of other reagents. Typical groups that display photochemically-induced transitions include azobenzenes (change of dipole moment, size and shape), triphenylmethane leucohydroxide or triphenylmethane nitrile (generation of charges), spyrobenzopyran (formation of zwitterionic species) and cinnamoyl (photodimerization) (see Fig. 12). These transitions can further induce changes in the optical, mechanical and chemical properties of the system containing the chromophore. Currently, these groups, especially azo derivatives, are intensively investigated to implement light sensitivity in polymers (Pieroni et al. 1998; Ikeda et al. 2007).

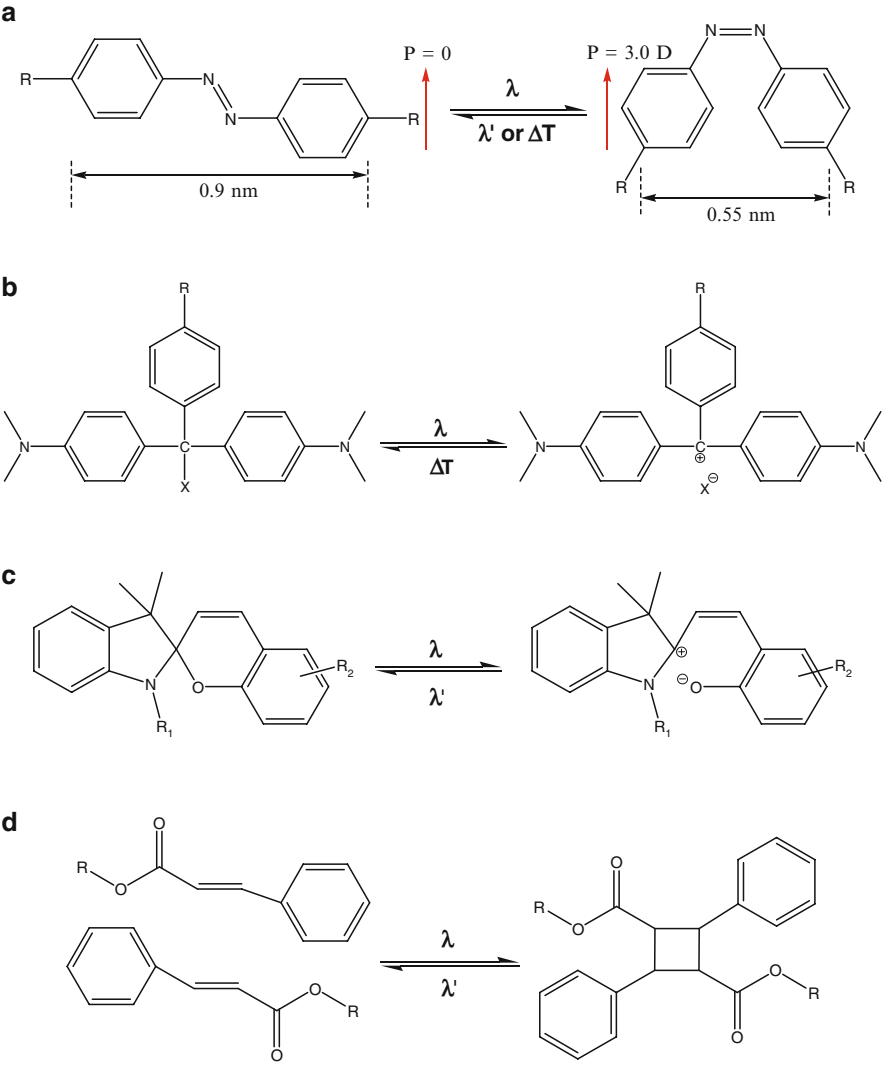


Fig. 12 Examples of chromophores that display photochemically- induced transitions. (**a**) Reversible *trans* (*left*) and *cis* (*right*) photo-isomerization of azobenzene. (**b**) Dissociation of triphenylmethane leucoderivatives into an ion pair under ultraviolet irradiation. (**c**) Reversible photo-isomerization of spirobenzopyran derivatives. (**d**) Reversible photodimerization of cinnamoyl group

These light sensitive triggers could, in principle, be incorporated into amphiphilic copolymers suitable for polymer vesicle formation.

Azobenzene derivatives are the most studied photoactive groups. The azo group can undergo reversible isomerization between the *trans* and *cis* configurations by light and heat (see Fig. 12a). The *trans* isomer is thermodynamically more stable in common conditions. The *cis* isomer obtained by light (usually UV) irradiation is

thermodynamically less stable and therefore it isomerises slowly back to *trans* form (typically over several hours). However, irradiation (usually by visible light) reduces the back reaction time to minutes. The photoisomerization of azobenzene chromophores has no side reactions and the wavelength to induce the transformation can be tuned by incorporating substituents in the chromophores.

The polarity, shape and size changes that azobenzene undergoes after isomerization modify significantly the structures and properties of azobenzene-containing polymer blocks.

Zhao's group (Wang et al. 2004), (Tong et al. 2005) prepared amphiphilic diblock copolymers composed of hydrophilic poly(acrylic acid) and hydrophobic polymethacylate with azobenzene side groups. They showed that these copolymers self-assembled in dioxane/water mixtures to form photoresponsive polymer vesicles of 100–200 nm. Upon UV irradiation, the majority of the vesicular aggregates disappeared. The disruption of these morphological structures appeared to be fully reversible as the aggregates reformed following subsequent illumination with visible light. Solubilization of the hydrophobic block in dioxane/water mixtures caused by the polarity change of azobenzene after *trans*-to-*cis* isomerization is claimed to be responsible for vesicle disruption.

We have recently reported photo-responsive polymersomes (del Barrio et al. 2010) base on amphiphilic PEG-*b*-azodendron block copolymer with a hydrophobic block based on the fourth generation of aliphatic polyester dendrons functionalized at the periphery with cyanoazobenzene mesogens (**PEG45-AZO16)**. The polymersomes in water were prepared by nanoprecipitation with dioxane/water co-solvents followed by extensive dialyse against water. Wrinkles, and even rupture, in the membrane of the polymersome were detected upon UV irradiation of polymersome dispersion in water (Fig. 13).

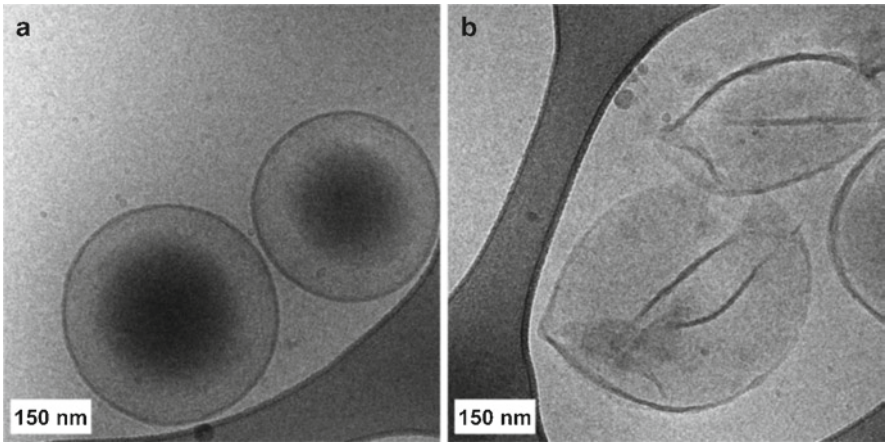


Fig. 13 Cryo-transmission electron micrograph of the polymersomes formed in water by the PEG-*b*-azodendron block copolymer **PEG45-AZO16** (**a**) before and (**b**) after illumination with 360 nm unpolarized light (150 mW/cm^2) for 35 min

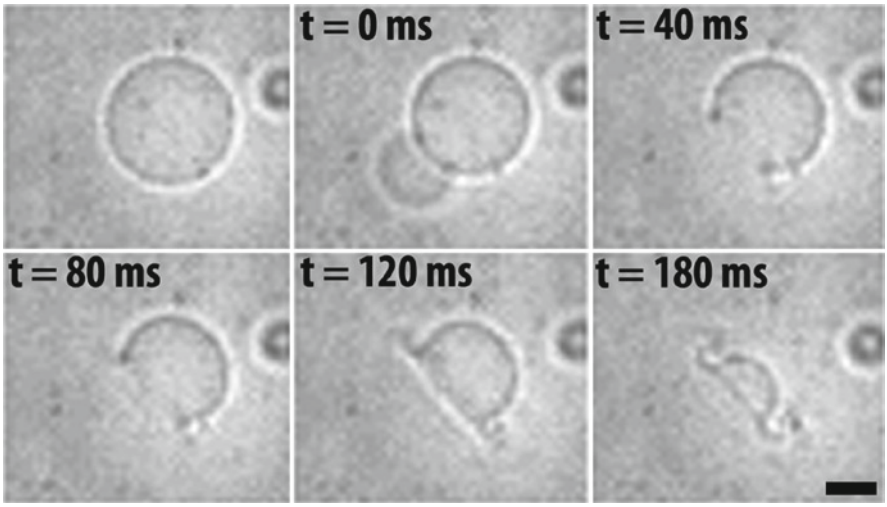


Fig. 14 Snapshots of an ePBD-iPAzo polymersome bursting under UV illumination. Bright-field images were taken using a high-speed digital camera. The first image shows the vesicle prior to illumination. Time t = 0 corresponds to pore nucleation. The expulsion of sucrose solution is visible as the pore nucleated. The other images correspond to pore growth and clearly show outward spirals (scale bar = 5 μm)

Our group has further achieved the polymersome bursting induced by UV light (Mabrouk et al. 2009a). Exposure to UV illumination around 360 nm caused vesicle rupture which was completed in less than a few hundreds of milliseconds (Fig. 14). The basic principle to rapidly burst the polymersome is to induce frustration in the membrane via a remote stimulus. To implement this approach, we prepared asymmetric polymersomes in which each leaflet consisted of a different type of diblock copolymer: one copolymer was insensitive to any remote stimulus (PEG-*b*-PBD, or PBD for simplicity) while the hydrophobic moiety of the second copolymer was a light sensitive liquid crystalline polymer PEG-*b*-PMAazo444 (PAzo). The mesogenic unit in this side-on LC polymer block contains an azobenzene group, which undergoes a *trans*-to-*cis* configurational transition upon UV exposure. The azobenzene shape change after isomerization induces a nematic (N) to isotropic (I) transition in the LC polymer (Li et al. 2000) causing a conformational change of the chain from a rod to a coil (Cotton and Hardouin 1997; Li et al. 2003). Figure 15a, b shows the chemical structures of the two selected copolymers and a cartoon of the LC copolymer conformation in the membrane both in the absence and in the presence of UV light for polymersomes ePBD-iPAzo (external leaflet = PBD, inner leaflet = PAzo). Starting from a thin, cigar-like shape corresponding to N state (Yang et al. 2005), UV irradiation transforms the LC hydrophobic block to a coil characterized by an increased molecular area. The induced area difference between the two polymer monolayers, i.e., the creation of spontaneous curvature, triggers membrane rupture and

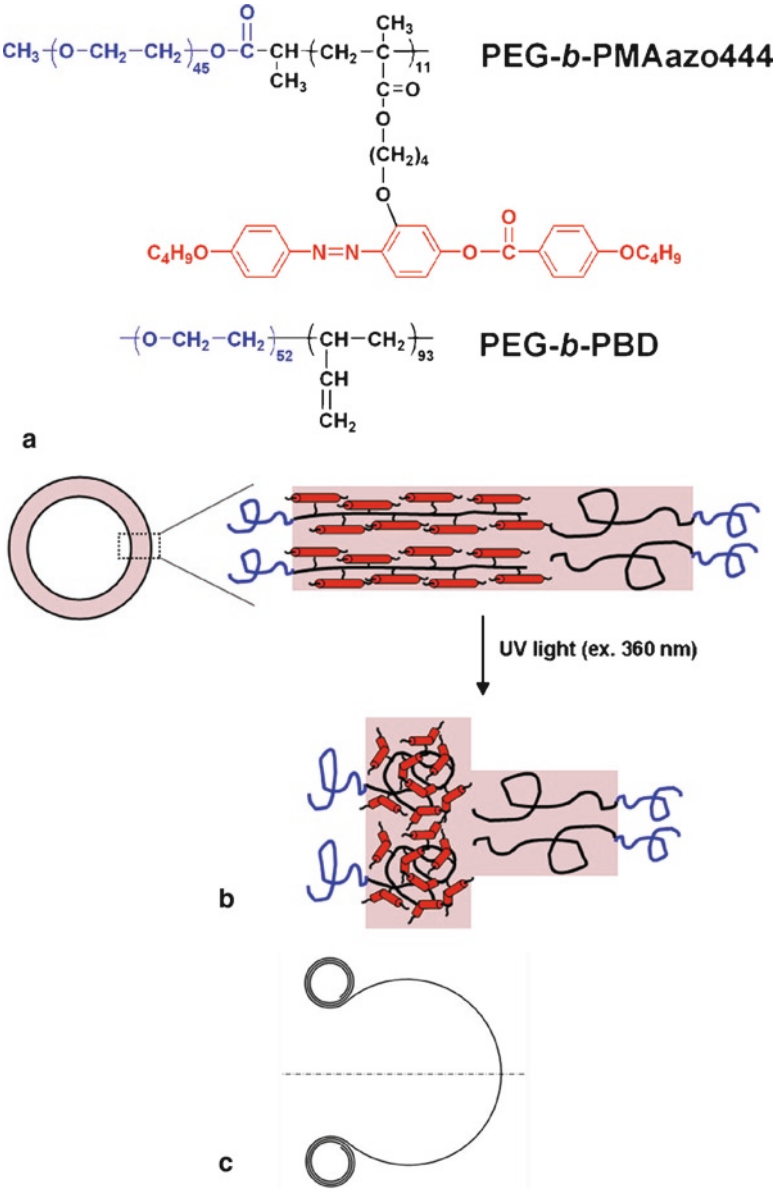


Fig. 15 Copolymers and bilayer conformation. (**a**) Chemical structures of the two selected copolymers, PEG-*b*-PBD and PEG-*b*-PMAzo444. (**b**) Cartoon of a polymersome and cartoon depicting the conformation of both copolymers within the bilayer for an ePBD-iPAzo vesicle. The PEG-*b*-PBD copolymer is always in a coil-coil state. In the absence of UV light, the hydrophobic LC block of the PEG-*b*-PMAzo444 copolymer has a rod-like conformation (corresponding to a nematic state). Under UV illumination, isomerization of the mesogenic groups induces a conformational change of the polymer backbone to a disordered, isotropic state. The net effect of UV exposure is two-fold: at the molecular scale, the projected area of the LC block is increased; at the mesoscopic scale, the spontaneous curvature of the bilayer is increased. (**c**) Schematic representation of pore opening driven by outward curling (for ePBD-iPAzo)

polymersomes bursting. Figure 15c shows the outward curling rim expected to be generated by the change of spontaneous curvature in the membrane where the inner leaflet is light-responsive. This is actually observed in the giant asymmetric polymersomes as shown in Fig. 14. The giant asymmetric polymersomes (>few microns in diameter) were prepared by the method of inverted emulsion. The polymersome bursting takes place also if the inner leaflet is inert and the external leaflet is light-responsive, but with inward curling rim during the vesicle opening. These results highlight a new general strategy to create stimuli-responsive polymersomes based on the fabrication of asymmetric membranes, and driven by a change in membrane spontaneous curvature. While UV light was the stimulus used for this study, temperature, electric or magnetic fields could also act as remote stimuli provided that one of the two leaflets of the membrane is composed of suitably designed copolymers sensitive to these physical stimuli. This flexibility, combined with the low permeability of polymer bilayers, ensures a wide range of potential applications in the fields of drug delivery, cosmetics and material chemistry.

Trans-cis isomerization of azobenzene was also used to modify (normally increase) the LCST of thermosensitive polymers because the *cis* conformers is more polar and hydrophilic (Sugiyama and Sono 2001; Desponds and Freitag 2003; Ravi et al. 2005; Sin et al. 2005). These polymers could potentially be used to produce temperature and light dual-responsive polymersomes.

An interesting system containing triphenylmethane nitrile (Fig. 12b) was described by the group of X. Zhang (Jiang et al. 2007b), as illustrated Fig. 16, which consists of a PEG chain linked to a group of hydrophobic Malachite green. This PEG-*b*-bis(4-dimethylaminophenyl)phenyl methyl nitrile is able to self assemble into vesicles in water. Under UV irradiation, the photochromic group ionizes in its cationic form and the molecules become soluble in water. Consequently vesicles are disassembled. The molecules can recover their neutral form by heat treatment and free chains are reassembled again into vesicles.

Photoactive polymers bearing triphenylmethane leucohydroxide (Kono et al. 1995) and nitrocinnamate (Yuan et al. 2005) have been used to prepare light responsive microcapsules for controlled encapsulation and release of substance. Spirobenzopyran derivatives (Fig. 12c) have been used to create water-soluble polymers that associate into aggregates under UV irradiation (Konak et al. 1997). We foresee that these photoactive groups are also potentially useful for light-responsive polymersome formation providing that proper chemical design is made for block copolymers.

The last example, described as follows, with photoactive groups incorporated in their polymer structures is rather different from the systems discussed above. It is a polymersome susceptible to UV-induced degradation (Katz et al. 2010). Photolabile 2-nitrophenylalanine (2NPA) was used to join the hydrophilic PEG block and hydrophobic PCL block (Fig. 17a). Exposure of the polymersomes formed by this 2NPA linked PEG-*b*-PCL to 365 nm light enable the cleavage between PEG and

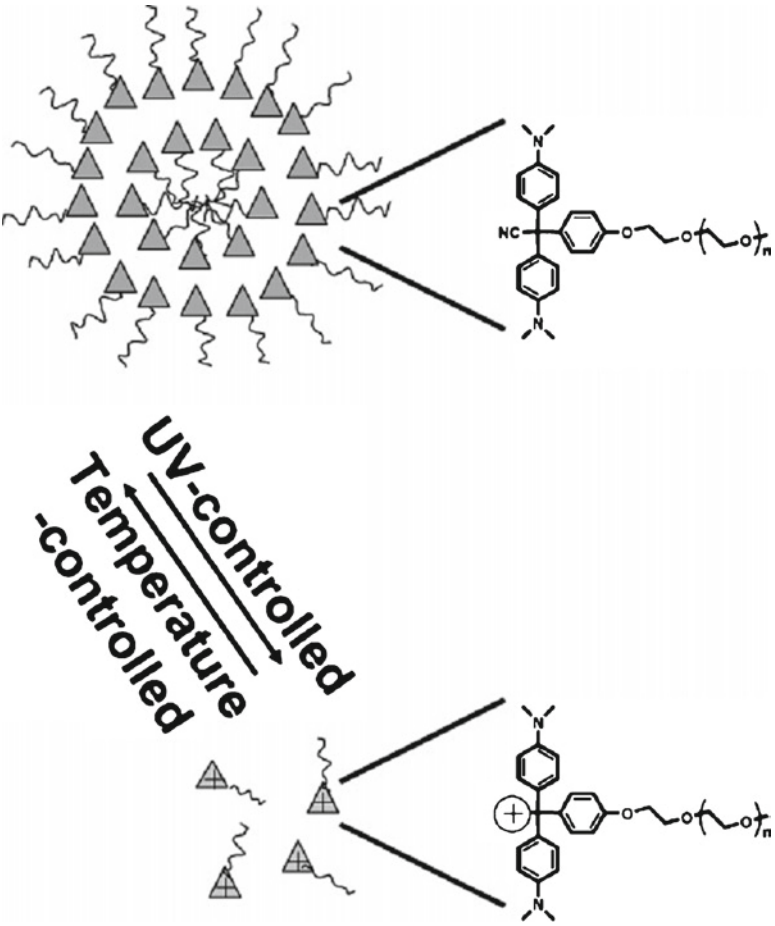


Fig. 16 Schematic illustration of vesicles made from PEG and malachite green and their disassembly under UV illumination. (Reproduced from ref. Jiang et al. 2007b)

PCL blocks. As PEG is liberated from the membrane by UV light, there is excess hydrophobic material that must be stabilized by the remaining PEG shell. This results in membrane thickening, decreasing overall size of the polymersome, and flux of aqueous contents out of the polymersome (Fig. 17b, c). Ultimately, there is insufficient PEG surface-coverage to stabilize the PCL shell and the insoluble PCL crashes out of solution and aggregates.

The above system is an example that light (a physical stimuli) induce irreversible chemical structure changes in light-responsive polymers. The chemical structure changes can also be produced by light in photo-inert polymers with the aid of photosensitizers as discussed below.

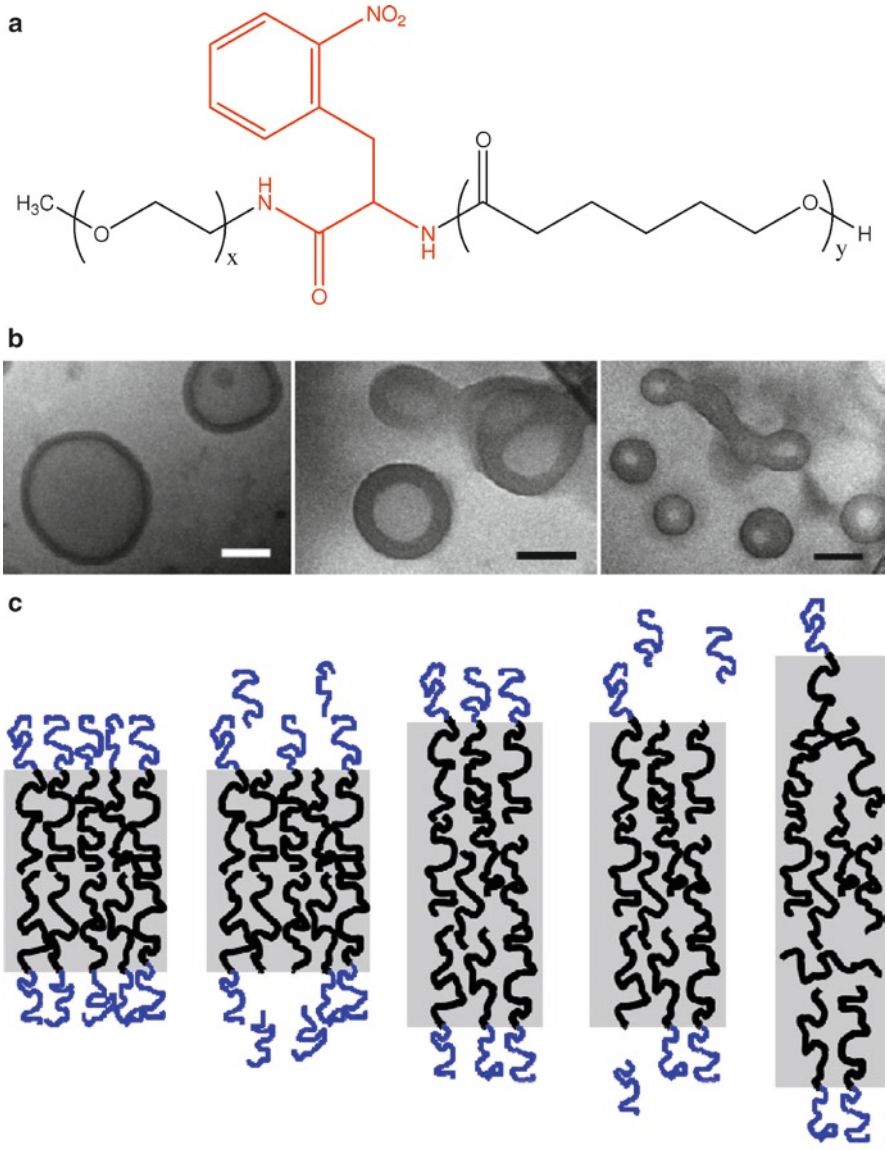


Fig. 17 (**a**) Chemical structure of 2NPA linked PEG-*b*-PCL. (**b**) Cryo-TEM images of 2NPA polymersomes (*left to right*) before, during and after 6 h of UV exposure. Scale bars = 100 nm. (**c**) Schematic representation of membrane structures to explain the Cryo-TEM images. (Reproduced from Katz et al. 2010)

3.2.2 Composite Systems with Classical Polymersomes Charged with Photosensitizers

The group of Dmochowski has first reported photoinitiated destruction of composite porphyrin-protein polymersomes (Robbins et al. 2009). The polymersomes were formed by incorporating a protein in the aqueous interior and a meso-to-meso ethyn-bridged bis[(porphinato)zinc] (PZn_2) chromophore in the membrane polymersomes made from classical photo-inert PEO-*b*-PBD. Confocal laser scanning microscopy (CLSM) imaging of polymersomes loaded with both ferritin and PZn2 at excitation wavelengths (488, 543, or 633 nm, where PZn2 absorbs strongly) caused many of the vesicles to undergo irreversible morphological changes ranging from formation of new bends or "arms" and budding of smaller vesicles to total polymersome destruction (Fig. 18). Similar results were seen during imaging by widefield fluorescence microscopy using a mercury arc lamp. Even though the mechanism of photodestruction is not elucidated, it may be possible to harness light-activated vesicle destruction for in vivo targeted drug delivery, given the established exceptional NIR absorptivity of PZn2 and closely related chromophores.

Our group has prepared a binary system of polymersome and chlorine e6 (Ce6) (Mabrouk et al. 2010). Ce6 (see Fig. 19) is a classical chlorine photosensitizer,

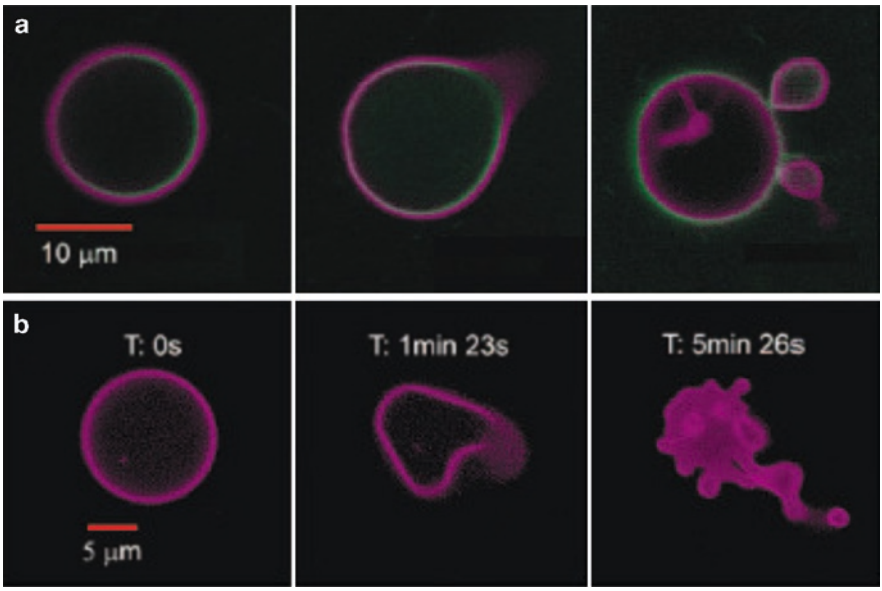


Fig. 18 Confocal micrographs of polymersomes that membrane-disperse PZn2 (purple) and encapsulate HSAF obtained in continuous scanning mode. (**a**) BODIPY-FL-labeled HSAF (green, 3 mg/mL) + PZn2 vesicle, imaged using two lasers simultaneously (488, 543 nm). HSAF is the horse spleen iron-free apoferritin, and BODIPY-FL a neutral dye. Images proceed in time, left to right, over a period of ~ 5 min. (**b**) Unlabeled HSAF (1.5 mg/mL) + PZn2 vesicle. Vesicle imaged using three lasers simultaneously (488,543, 633 nm). (Reproduced from Robbins et al. 2009)

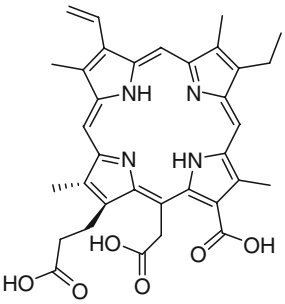


Fig. 19 Chemical structure of the photosensitizer Chlorine e6

which has been used in photodynamic therapy (PDT) because upon light exposure Ce6 generates highly reactive oxygen species (ROS) including free radicals and/or singlet oxygen $^1O_2$. In the core of a lipid bilayer, carbon-carbon double bonds of unsaturated lipids have been shown to be privileged sites for $^1O_2$ and radical reactions that lead to cellular membrane alteration. The selected photo-inert copolymer is again the prototypical PEO-*b*-PBD, which contains a large number of carbon–carbon double bonds that are preferred sites of photo-oxidation. Ce6 is an amphiphilic molecule and can be loaded in the membrane as well as in the aqueous internal compartment. In these polymersomes loaded with Ce6, we have observed a complex sequence of light-induced morphological changes. Using micromechanical assays based on micropipette manipulation, we have quantitatively monitored the different phases of the photo-response, which include membrane area variation, osmotic swelling, membrane cross-linking and vesicle deflation. In addition, these morphological changes can be adjusted by the concentration of Ce6 (see Fig. 20). We have thus gained insight into the complex cascade of chemical reactions involved in photosensitization. Our findings suggest that composite chlorine-copolymer vesicles may be used as a new class of light-sensitive drug carriers.

3.3 Polymersomes Responsive to Magnetic Field

The magnetic field is a very promising stimulus in drug delivery, because it is easy to be applied remotely and permits also to combine together the medical diagnosis and therapy due to the development of MRI (magnetic resonance imaging) technique. The common strategy is to incorporate magnetic nanoparticles (e.g., γ-Fe_2O_3) into the vesicle structures. Iron oxide nanoparticles, namely magnemite (γ-Fe_2O_3) or magnetite (Fe_3O_4), with particle sizes of 4–10 nm have drawn special interest for biomedical applications. They are referred to as superparamagnetic iron oxide nanoparticles (SPIONs) due to their superparamagnetic properties and small size. The resulting superparamagnetic vesicles have potential applications because of the specific properties of magnetic iron oxide in the fields of biomedicine and biotechnology: e.g., manipulation by an external magnetic field gradient, radio-frequency heating for cancer therapy, and labelling of organs in magnetic resonant imaging.

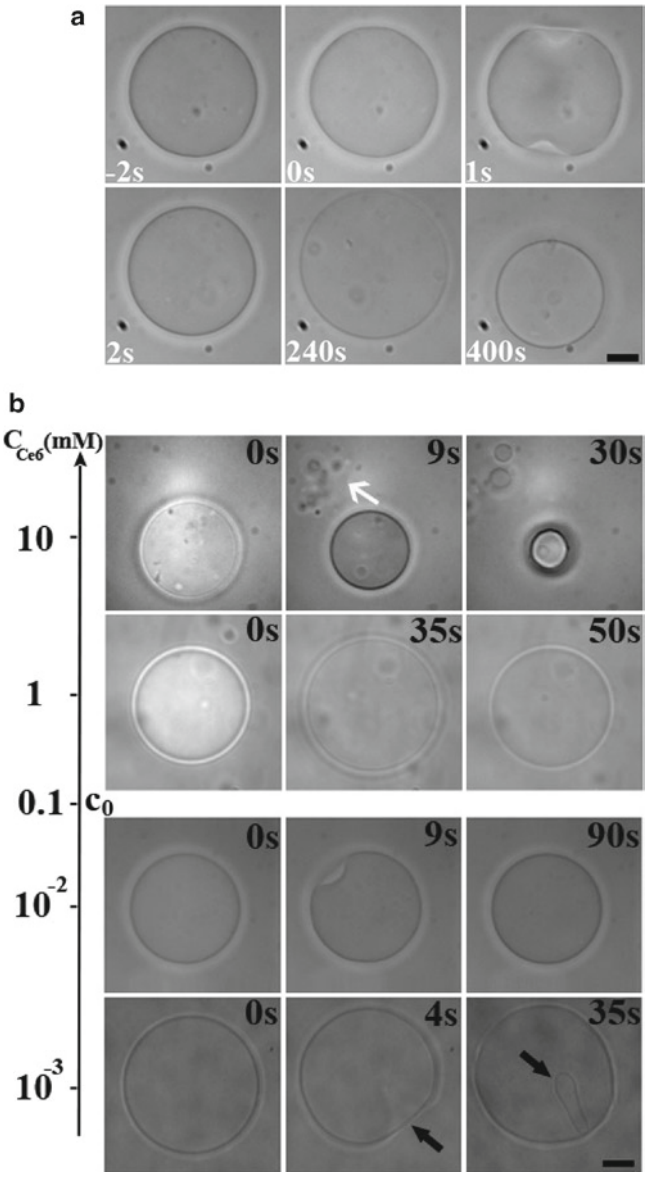


Fig. 20 (**a**) Video sequence showing the morphological changes of Ce6-loaded PEO-*b*-PBD polymersomes under UV exposure. The bulk concentration of Ce6 is $c_0 = 0.1$ mM. Irradiation starts at time t = 0. Scale bar = 5 μm. (**b**) Representative UV-induced morphological changes of Ce6-loaded PEO-*b*-PBD polymersomes for different bulk concentrations of Ce6. The black arrows show the formation of membrane invaginations during UV illumination. Scale bar = 5 μm

Here we will emphasize the vesicle structure changes induced by the magnetic field for the purpose of controlled release.

Magnetic nanoparticles were firstly incorporated in liposomes (small molecular vesicles) either in its aqueous compartment (Sabaté et al. 2008; Zhang et al. 2005) or in its lipid bilayer via magnetized polymers (Kamaly et al. 2007; Leclercq et al. 2003). Such magnetic liposomes can be used to target therapeutic molecules to a specific site when exposed to a magnetic field. Once the drug-loaded magnetic liposomes reach the target, the drug can also be released by radio-frequency heating (hyperthermia) of SPIONs with a thermosensitive lipid bilayer. Temperature-sensitive lipid bilayer frequently include dipalmitoylphosphatidylcholine (DPPC) as the key component, since liposomes usually become leaky at a gel-to-liquid crystalline phase transition that takes place at 41°C (Yatvin et al. 1978; Jeong et al. 2009). Pradhan et al. (2010) developed folate receptor targeted thermosensitive magnetic liposomes (MagFolDox), which are designed to combine features of biological and physical (magnetic) drug targeting for use in magnetic hyperthermia-triggered drug release (Fig. 21). The optimized liposome formulation had

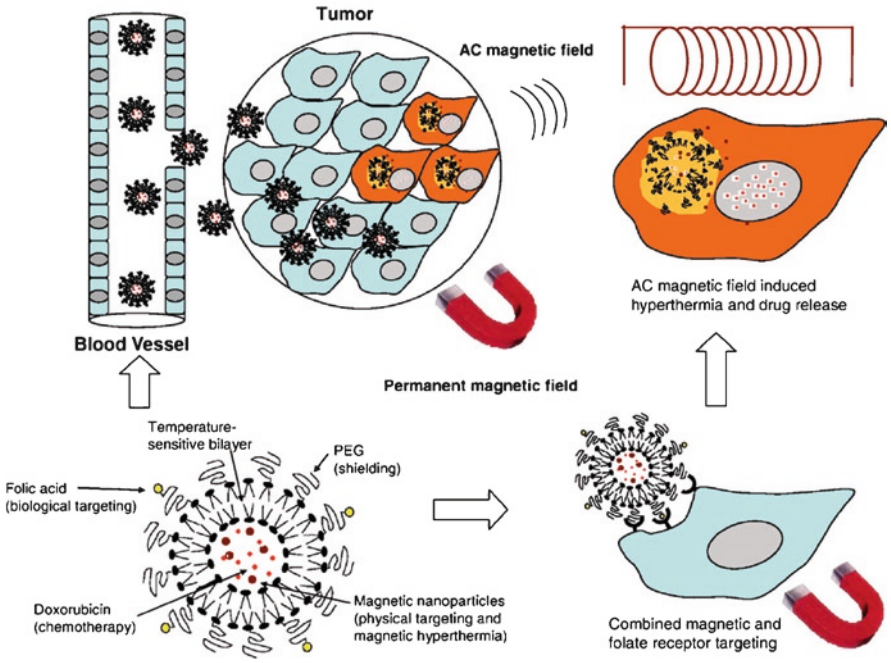


Fig. 21 The figure shows the concept of a multifunctional drug carrier responsive to magnetic field. It is a folate-receptor-targeted and temperature-sensitive magnetic liposome containing doxorubicin, which can be targeted physically by magnetic field and biologically by folic acid to tumor cells. Drug release will be triggered by hyperthermia upon local application of an AC magnetic field on the tumor tissue. (Reproduced from Pradhan et al. 2010)

the composition of DPPC: cholesterol: DSPE-PEG2000: DSPE-PEG2000-folate at 80: 20: 4.5: 0.5M ratio. Magnetic hyperthermia at 42.5°C and 43.5°C synergistically increased the cytotoxicity of MagFolDox. The results suggest that an integrated concept of biological and physical drug targeting, triggered drug release and hyperthermia based on magnetic field influence can be used advantageously for thermo-chemotherapy of cancers.

It is nearly impossible to incorporate inorganic nanoparticles within the membrane of liposomes because of their very thin membrane thickness (3–5 nm). However, this becomes possible, even still challenging, with polymersomes due to the thick membrane (5—30 nm) and toughness resulting from polymer characters. The soft magnetic shells are especially promising for drug delivery, because their internal compartment is available for encapsulation of water-soluble species and the possible toxicity arising from cells directly contacting the iron oxide would not be an issue because it is embedded in the copolymer. Application of a magnetic field could trigger the transient opening of the bilayer and the release of an encapsulated content. The pioneer work on magnetic polymersomes has been made by Lecommandoux & Sandre et al. (Lecommandoux et al. 2005). They have prepared polymersomes of PGA_{56}-*b*-PBD_{48} loaded with surfactant-coated γ-Fe_2O_3 nanoparticles in the layer of PBD blocks, and studied their structural transformation under magnetic field by small angle neutron scattering (SANS). Anisotropic SANS data detected with a 2-dimensional detector provide experimental evidence of the capability to modify the shape of these hybrid membranes in response to a magnetic field of an intensity as low as 290 G. Analyses of the anisotropic SANS patterns (at intermediate wave vector q range) have shown that the portions of membranes mostly affected by the magnetic field are those with their normal vector parallel to the field. The membrane becomes stretched in these portions (decrease of the apparent membrane thickness) or almost equivalently the nanoparticles move away from the magnetic poles.

Our group worked on the LC polymersomes PEG-*b*-PA444 and PEG-*b*-PAazo444 that contain diamagnetic mesogens (Hocine et al. 2011). We hoped to achieve the structural changes triggered by magnetic field using the intrinsic positive diamagnetic response of the LC polymers. Interestingly, for temperatures between 65 and 80°C, the application of a magnetic field of 1.4 T can increase the membrane thickness by up to 50% without significantly changing the inner volume of vesicles. This structural change is consistent with the alignment of LC domains under magnetic field. While thickness changes could happen in polymersomes loaded with magnetic nanoparticles as discussed above, to the best of our knowledge, this is the first time that an increase in membrane thickness has been observed in a pure polymer vesicle system.

We envision that magnetic field triggered drug release from SIONs-loaded magnetic polymersomes would be achieved in the near future provided that the hydrophobic block is composed of suitably designed liquid crystalline polymer or crystalline polymer with proper transition temperature by analogy with the gel-to-liquid crystalline phase transition in lipid DPPC.

3.4 *Responses to Electric Field, Osmotic Shock and Ultrasonic Wave*

The effects of electric field on giant polymersomes of PEO-*b*-PBD (Aranda-Espinoza et al. 2001; Bermudez et al. 2003) and on giant liposomes (Vlahovska et al. 2009; Riske and Dimova 2005, 2006; Dimova et al. 2007) have been studied by the group of Discher and by the group of Dimova, respectively. The stimulation by an electric field is not specific to a given amphiphile. The response of the vesicles to the electric field and the destruction of the membrane are due to an increase tension of the vesicle directly induced by the electric field. The hydrophobic core of the membrane thickness d and dielectric constant ε (low compared to that of the aqueous environment) behaves as a capacitance C (typically in the order of $\mu F.cm^{-2}$). The charging time for the membrane depends on the environmental conductivity inside and outside the vesicle, and typically takes the order of a fraction of μs in saline solution. Consequently, if the pulse duration supply is larger than the charging time of the capacitance, the transmembrane potential V thus generated can be considered constant and only dependent on the applied electric field E, the radius R of the vesicle and the angle θ between the field direction and the normal to the membrane: $V = REcos(\theta)$. This potential creates an electro-compressive stress perpendicular to the plane of the membrane $\sigma = 1/2CV^2$. The increase in electric field applied resulting in a net increase in tension of the membrane. There is a threshold electric potential for which the vesicle tension reaches its lysis value and consequently the vesicle disintegrates. This experiment is illustrated in Fig. 22. In practice, the critical transmembrane potential is of order of $V_c = 1–10$ V. The critical constraints are then in the order of $\sigma_c = 10$ (liposomes) to 30 (polymersomes) $pN.nm^{-1}$. Nevertheless, it should be noticed that the magnitude of the electric field necessary to achieve polymersome lysis is kV/cm. Apply these fields through the human body for drug delivery would be likely accompanied by severe side effects.

The responsive polymersomes to osmotic shock has been reported by the group of Weitz (Shum et al. 2008). Polymersomes encapsulating 1-hydroxypyrene-3,6,8-trisulfonate sodium (HPTS) were produced from PEG-*b*-PLA block copolymer using microfluidic fabrication. Although the membrane is impermeable to the small HPTS salts, water molecules can diffuse in and out of the polymersomes. The osmotic pressure, π_{osm}, is related to the concentration of solutes,

$$\pi_{osm} = cRT$$

where c is the molar concentration of the solutes, R is the gas constant and T is the temperature. Due to osmotic pressure difference, water diffuses from regions with a low salt concentration to regions with a higher concentration. Osmotic pressure can therefore be used to tune the sizes of the polymersomes. If the osmotic pressure change is sudden and large, the resulting shock can break the polymersomes. The kinetics of the polymersomes' response following a large osmotic shock is too fast to visualize; the process is therefore slowed down for visualization by gradually

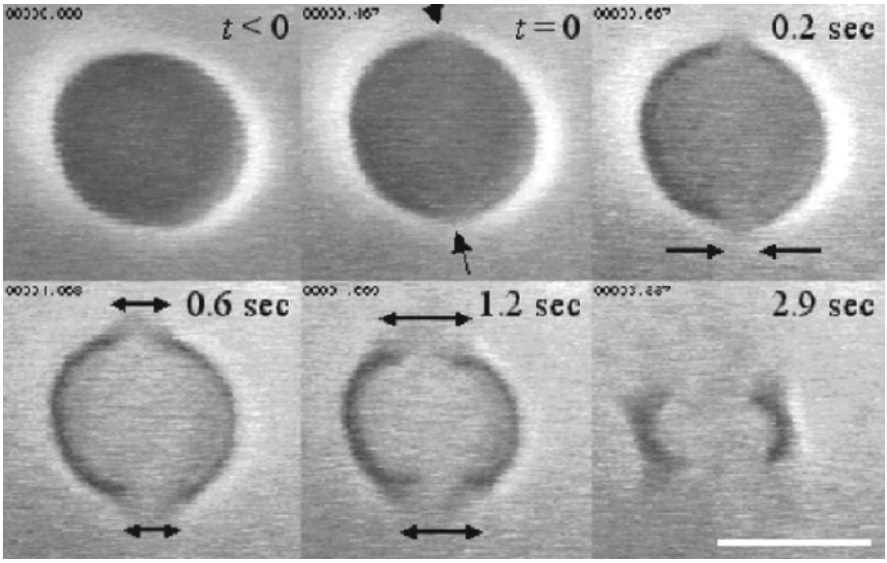


Fig. 22 Phase contrast imaging of pore growth with time during a PEG-*b*-PBD polymersome bursting under electric field. The first image shows the polymersome prior to application of electric field ($t<0$). Time $t=0$ corresponds to the formation of two pores at both vertical poles, indicated by the vertical arrows in the second image (from left to right). The applied electric field is in the vertical direction. Time sequence is $t=0.2$ sec, $t=0.6$ sec, $t=1.2$ sec and $t=2.9$ sec in other images, where the horizontal double arrows show the growth of the pores. Scale bar = 10 μm. (Reproduced from ref. Bermudez et al. 2003)

increasing polyvinyl alcohol (PVA) concentration (c_0 = 10 wt%) in the continuous phase through water evaporation. As water evaporates, the PVA concentration in becomes higher and higher outside of the polymersomes and so water is squeezed out from the inside of the polymersomes. As a result, the polymersome becomes smaller, and its wall buckles, as shown in Fig. 23. This provides a simple trigger for the release of the encapsulated fluorescent HPTS. By tuning the properties of the polymersome wall, it might also be possible to adjust the level of osmotic shock required to break the polymersomes. Alternatively, release can also be triggered by diluting the continuous phase and thus reducing its osmotic pressure. This simple triggered release mechanism makes polymersomes a promising candidate for encapsulation and release of actives.

Ultrasound is gaining attention as a therapeutic tool in addition to its use in diagnostics. Recently, Hammer's group (Pangu et al. 2010) reported ultrasonically induced release from nanosized polymersomes made from PEO-*b*-PBD copolymers under ultrasound at 20 kHz. Leakage of a fluorescent dye from vesicle core was measured to study the permeation. Ultrasound causes significant leakage from the core above threshold intensity, suggesting that leakage is governed by acoustic cavitation. Size measurements and direct visualization of vesicles show that ultrasound does not completely rupture them into fragments but causes transient poration (Fig. 24). The extent of leakage inversely depends on membrane thickness and

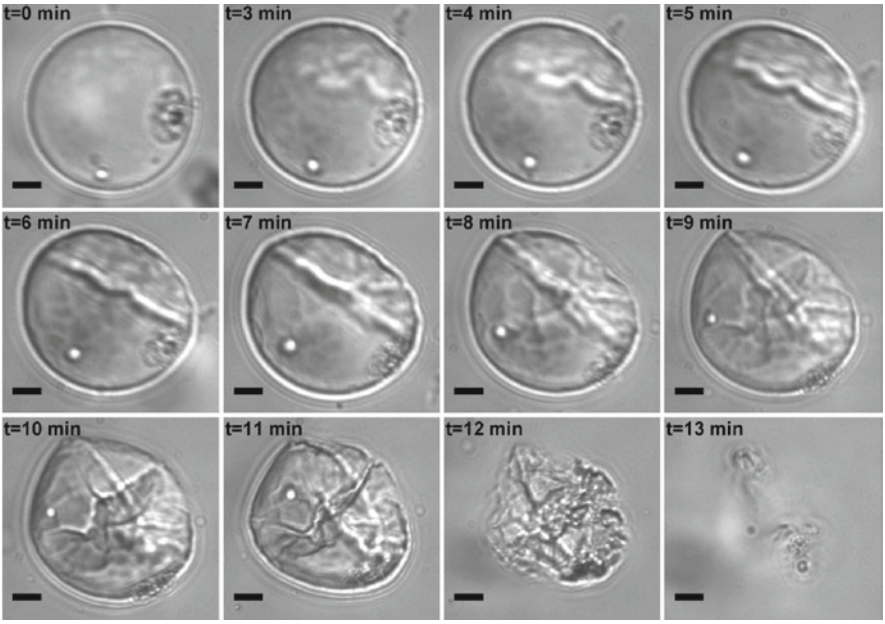


Fig. 23 Bright-field microscope images showing the shrinkage and breakage of a PEG(5,000)-*b*-PLA(5,000) polymersome after an osmotic shock. As a result of water expulsion from its inside, the polymersome shrinks and buckles. Scale bar is 10 μm. (Reproduced from Shum et al. 2008)

directly depends on sonication time and intensity. The strong dependence of membrane permeation on ultrasonic and chemical parameters suggests the possibility of tailoring polymersome features to tune the desired extent of membrane destabilization. Even though the exact mechanism of pore formation and release under an ultrasonic field need to be elucidated, the results indicate that ultrasound has potential as a therapeutic tool for drug delivery from polymer vesicles.

4 Concluding Remarks

In Nature, supra-molecular nano or micro-objects formed by the self-assembly of amphiphilic molecules or macromolecules are omnipresent (e.g. cells, liposomes, viral capsids etc.) and come in a wide range of shapes, sizes and functions. Synthetic amphiphilic copolymers are an attractive biomimetic approach that has been widely used to produce nano or micro assemblies such as polymer vesicles, polymer micelles, nanofibers and nanotubes. Applications in drug delivery have driven much of this research. Other opportunities also exist for these polymer assemblies including use in diagnosis, as nanoreactors and as templates for nanomaterial preparation. In this paper, we have focused on polymer vesicle structures, i.e. polymersomes. Many applications require the ability to selectively and

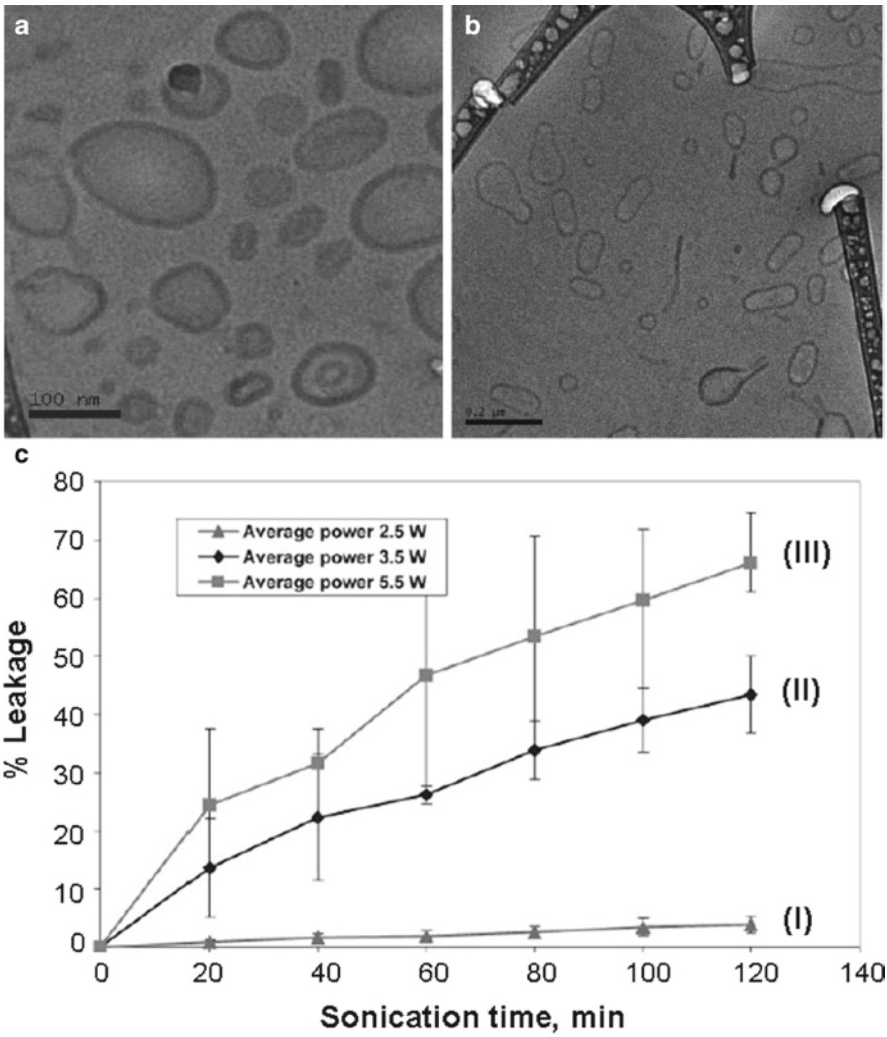


Fig. 24 Cryo-TEM images of polymersomes (**a**) before and (**b**) after sonication. Scale bar = 100 nm in (**a**) and 200 nm in (**b**). Percentage of ANTS (8-Aminonaphthalene-1, 3, 6-trisulfonic acid, disodium salt) release as a function of time where the average sonication power during sonication cycles as recorded on sonic dismembrator dial was (**I**) 2.5 W, (**II**) 3.5 W, and (**III**) 5.5 W. (Reproduced from Pangu et al. 2010)

controllably destabilise the polymer vesicle structures so as to liberate the substances encapsulated in the interior compartment and/or in the membrane. To achieve this goal, stimuli-responsive polymersomes have been developed. In addition, the utilization of biocompatible polymers and biocompatible stimuli is essential for *in vivo* applications.

We have discussed in this review all the most promising approaches to make stimuli-responsive polymersomes, including non-biocompatible ones, for the proof of concept and for the larger applications of polymersomes. Classical chemical stimuli such as pH changes, hydrolysis, oxidation or reduction reaction, are used to trigger a change in the hydrophilic-hydrophobic balance of the amphiphilic copolymers, which in turn destabilises the vesicular structure either by forming leaking pores or causing the vesicle to burst. Physical stimuli such as temperature, light, magnetic field or ultrasonic wave are also of great potential interest, because they don't require any chemical environmental change and can be applied remotely and/or locally. Different mechanisms are involved in the destruction or deformation of polymersomes under physical stimuli. We believe the use of the physical stimuli will provide the impetus for the development of new stimuli-responsive polymer vesicles.

References

Ahmed, F. & Discher, D. (2004) Self-porating polymersomes of PEG-PLA and PEG-PCL: hydrolysis-triggered controlled release vesicles. *Journal of Controlled Release*, 96, 37–53.

Ahmed, F., Pakunlu, R., Brannan, A., Bates, F., Minko, T. & Discher, D. (2006) Biodegradable polymersomes loaded with both paclitaxel and doxorubicin permeate and shrink tumors, inducing apoptosis in proportion to accumulated drug. *Journal of Controlled Release*, 116, 150–158.

Angelova, M. I. & Dimitrov, D. S. (1986) Liposome electroformation. *Faraday Discussions*, 81, 303-+.

Antonietti, M. & Forster, S. (2003) Vesicles and liposomes: A self-assembly principle beyond lipids. *Advanced Materials*, 15, 1323–1333.

Aranda-Espinoza, H., Bermudez, H., Bates, F. & Discher, D. (2001) Electromechanical limits of polymersomes. *Physical Review Letters*, 87, 208301.

Battaglia, G. & Ryan, A. (2005) Bilayers and interdigitation in block copolymer vesicles. *Journal of the American Chemical Society*, 127, 8757–8764.

Battaglia, G., Ryan, A. & Tomas, S. (2006) Polymeric vesicle permeability: A facile chemical assay. *Langmuir*, 22, 4910–4913.

Battaglia, G. & Ryan, A. J. (2006) Pathways of polymeric vesicle formation. *Journal of Physical Chemistry B*, 110, 10272–10279.

Bellomo, E., Wyrsta, M., Pakstis, L., Pochan, D. & Deming, T. (2004) Stimuli-responsive polypeptide vesicles by conformation-specific assembly. *Nature Materials*, 3, 244–248.

Ben-Haim, N., Broz, P., Marsch, S., Meier, W. & Hunziker, P. (2008) Cell-specific integration of artificial organelles based on functionalized polymer vesicles. *Nano Letters*, 8, 1368–1373.

Bermudez, H., Aranda-Espinoza, H., Hammer, D. & Discher, D. (2003) Pore stability and dynamics in polymer membranes. *Europhysics Letters*, 64, 550.

Bertin, A., Hermes, F. & Schlaad, H. (2010) Biohybrid and Peptide-Based Polymer Vesicles. *Polymer Membranes/Biomembranes.* Berlin, Springer-Verlag Berlin.

Bhargava, P., Zheng, J. X., Li, P., Quirk, R. P., Harris, F. W. & Cheng, S. Z. D. (2006) Self-assembled polystyrene-block-poly(ethylene oxide) micelle morphologies in solution. *Macromolecules*, 39, 4880–4888.

Borchert, U., Lipprandt, U., Bilang, M., Kimpfler, A., Rank, A., Peschka-Suss, R., Schubert, R., Lindner, P. & Forster, S. (2006) pH-Induced Release from P2VP PEO Block Copolymer Vesicles. *Langmuir*, 22, 5843–5847.

Broz, P., Benito, S. M., Saw, C., Burger, P., Heider, H., Pfisterer, M., Marsch, S., Meier, W. & Hunziker, P. (2005) Cell targeting by a generic receptor-targeted polymer nanocontainer platform. *Journal of Controlled Release*, 102, 475–488.

Burkoth, T., Benzinger, T., Urban, V., Morgan, D., Gregory, D., Thiyagarajan, P., Botto, R., Meredith, S. & Lynn, D. (2000) Structure of the -amyloid (10–35) fibril. *J. Am. Chem. Soc*, 122, 7883–7889.

Cameron, N. S., Corbierre, M. K. & Eisenberg, A. (1999) Asymmetric amphiphilic block copolymers in solution: a morphological wonderland. *Can. J. Chem.*, 77, 1311–1326.

Carlsen, A. & Lecommandoux, S. (2009) Self-assembly of polypeptide-based block copolymer amphiphiles. *Current Opinion in Colloid & Interface Science*, 14, 329–339.

Cerritelli, S., Velluto, D. & Hubbell, J. (2007) PEG-SS-PPS: reduction-sensitive disulfide block copolymer vesicles for intracellular drug delivery. *Biomacromolecules*, 8, 1966–1972.

Chécot, F., Brûlet, A., Oberdisse, J., Gnanou, Y., Mondain-Monval, O. & Lecommandoux, S. (2005) Structure of polypeptide-based diblock copolymers in solution: Stimuli-responsive vesicles and micelles. *Langmuir*, 21, 4308–4315.

Checot, F., Lecommandoux, S., Gnanou, Y. & Klok, H. A. (2002) Water-soluble stimuli-responsive vesicles from peptide-based diblock copolymers. *Angewandte Chemie-International Edition*, 41, 1339–1343.

Chiu, H., Lin, Y., Huang, Y., Chuang, C. & Chern, C. (2008) Cover Picture: Polymer Vesicles Containing Small Vesicles within Interior Aqueous Compartments and pH-Responsive Transmembrane Channels (Angew. Chem. Int. Ed. 10/2008). *Angewandte Chemie International Edition*, 47.

Choi, H. & Montemagno, C. (2005) Artificial organelle: ATP synthesis from cellular mimetic polymersomes. *Nano Letters*, 5, 2538–2542.

Christian, D. A., Garbuzenko, O. B., Minko, T. & Discher, D. E. (2010) Polymer Vesicles with a Red Cell-like Surface Charge: Microvascular Imaging and in vivo Tracking with Near-Infrared Fluorescence. *Macromolecular Rapid Communications*, 31, 135–141.

Christian, N. A., Milone, M. C., Ranka, S. S., Li, G. Z., Frail, P. R., Davis, K. P., Bates, F. S., Therien, M. J., Ghoroghchian, P. P., June, C. H. & Hammer, D. A. (2007) Tat-functionalized near-infrared emissive polymersomes for dendritic cell labeling. *Bioconjugate Chemistry*, 18, 31–40.

Cotton, J. P. & Hardouin, F. (1997) Chain conformation of liquid-crystalline polymers studied by small-angle neutron scattering. *Progress in Polymer Science*, 22, 795–828.

Del Barrio, J., Oriol, L., Sanchez, C., Serrano, J.-L., Di Cicco, A., Keller, P. & Li, M.-H. (2010) Self-Assembly of Linear- Dendritic Diblock Copolymers: From Nanofibers to Polymersomes. *Journal of the American Chemical Society*, 132, 3762–3769.

Demirgoz, D., Pangburn, T. O., Davis, K. P., Lee, S., Bates, F. S. & Kokkoli, E. (2009) PR_b-targeted delivery of tumor necrosis factor-alpha by polymersomes for the treatment of prostate cancer. *Soft Matter*, 5, 2011–2019.

Desponds, A. & Freitag, R. (2003) Synthesis and characterization of photoresponsive N-isopropylacrylamide cotelomers. *Langmuir*, 19, 6261–6270.

Dimova, R., Riske, K., Aranda, S., Bezlyepkina, N., Knorr, R. & Lipowsky, R. (2007) Giant vesicles in electric fields. *Soft Matter*, 3, 817–827.

Dimova, R., Seifert, U., Pouligny, B., Förster, S. & Döbereiner, H. (2002) Hyperviscous diblock copolymer vesicles. *The European Physical Journal E: Soft Matter and Biological Physics*, 7, 241–250.

Discher, D. & Ahmed, F. (2006) Polymersomes. *The Annual Review of Biomedical Engineering*, 8, 323–341.

Discher, D. & Eisenberg, A. (2002a) Polymer vesicles. *Science*, 297, 967–973.

Discher, D. & Eisenberg, A. (2002b) Polymer vesicles. *Science*, 297, 967.

Du, J. & Armes, S. (2005) pH-responsive vesicles based on a hydrolytically self-cross-linkable copolymer. *J. Am. Chem. Soc*, 127, 12800–12801.

Du, J. & O'reilly, R. (2009) Advances and challenges in smart and functional polymer vesicles. *Soft Matter*, 5, 3544–3561.

Du, J. Z. & Armes, S. P. (2009) Preparation of Biocompatible Zwitterionic Block Copolymer Vesicles by Direct Dissolution in Water and Subsequent Silicification within Their Membranes. *Langmuir*, 25, 9564–9570.

Du, J. Z., Tang, Y. P., Lewis, A. L. & Armes, S. P. (2005) pH-sensitive vesicles based on a biocompatible zwitterionic diblock copolymer. *Journal of the American Chemical Society*, 127, 17982–17983.

Fernyhough, C., Ryan, A. & Battaglia, G. (2009) pH controlled assembly of a polybutadiene–poly (methacrylic acid) copolymer in water: packing considerations and kinetic limitations. *Soft Matter*, 5, 1674–1682.

Gebhardt, K., Ahn, S., Venkatachalam, G. & Savin, D. (2008) Role of secondary structure changes on the morphology of polypeptide-based block copolymer vesicles. *Journal of Colloid And Interface Science*, 317, 70–76.

Geng, Y., Ahmed, F., Bhasin, N. & Discher, D. (2005) Visualizing worm micelle dynamics and phase transitions of a charged diblock copolymer in water. *Journal of Physical Chemistry B-Condensed Phase*, 109, 3772–3779.

Geng, Y., Discher, D. E., Justynska, J. & Schlaad, H. (2006) Grafting short peptides onto polybutadiene-block-poly(ethylene oxide): A platform for self-assembling hybrid amphiphiles. *Angewandte Chemie-International Edition*, 45, 7578–7581.

Gennes, P.-G. D. (1978) Macromolecules and liquid crystals: reflexions on certain lines of research. *Solid State Physics*, Suppl.14, 1–18.

Ghoroghchian, P., Li, G., Levine, D., Davis, K., Bates, F., Hammer, D. & Therien, M. (2006) Bioresorbable vesicles formed through spontaneous self-assembly of amphiphilic poly (ethylene oxide)-block-polycaprolactone. *Macromolecules*, 39, 1673–1675.

Gregoriadis, G. (1992) *Liposome technology*, CRC.

Halperin, A., Tirrell, M. & Lodge, T. P. (1992) Tethered chains in polymer microstructures. *Advances in Polymer Science*, 100, 31–71.

Hammer, D. & Discher, D. (2001) Synthetic cells-self-assembling polymer membranes and bioadhesive colloids. *Annual Review of Materials Research*, 31, 387–404.

Hammer, D. A., Robbins, G. P., Haun, J. B., Lin, J. J., Qi, W., Smith, L. A., Ghoroghchian, P. P., Therien, M. J. & Bates, F. S. (2008) Leuko-polymersomes. *Faraday Discussions*, 139, 129–141.

Hauschild, S., Lipprandt, U., Rumplecker, A., Borchert, U., Rank, A., Schubert, R. & Förster, S. (2005) Direct preparation and loading of lipid and polymer vesicles using inkjets. *Small*, 1, 1177–1180.

Hocine, S., Brûlet, A., Jia, L., Yang, J., Di Cicco, A., Bouteiller, L. & Li, M.-H. (2011) Structural changes in liquid crystal polymer vesicles induced by temperature variation and magnetic fields. *Soft Matter*, in press.

Howse, J., Jones, R., Battaglia, G., Ducker, R., Leggett, G. & Ryan, A. (2009) Templated formation of giant polymer vesicles with controlled size distributions. *Nature Materials*, 8, 507–511.

Iatrou, H., Frielinghaus, H., Hanski, S., Ferderigos, N., Ruokolainen, J., Ikkala, O., Richter, D., Mays, J. & Hadjichristidis, N. (2007) Architecturally induced multiresponsive vesicles from well-defined polypeptides. Formation of gene vehicles. *Biomacromolecules*, 8, 2173–2181.

Ikeda, T., Mamiya, J. & Yu, Y. L. (2007) Photomechanics of liquid-crystalline elastomers and other polymers. *Angewandte Chemie-International Edition*, 46, 506–528.

Israelachvili, J. (2005) Intermolecular and Surface Forces: With Applications to Colloidal and Biological Systems (Colloid Science). *London: Academic Press*.

Jain, S. & Bates, F. S. (2003) On the origins of morphological complexity in block copolymer surfactants. *Science*, 300, 460–464.

Jenekhe, S. & Chen, X. (1998) Self-assembled aggregates of rod-coil block copolymers and their solubilization and encapsulation of fullerenes. *Science*, 279, 1903.

Jeong, S., Yi, S., Lim, S., Park, S., Jung, J., Woo, H., Song, S., Kim, J. & Lee, J. (2009) Enhancement of radiotherapeutic effectiveness by temperature-sensitive liposomal 1-methylxanthine. *International Journal of Pharmaceutics*, 372, 132–139.

Jia, L., Cao, A., Levy, D., Xu, B., Albouy, P. A., Xing, X. J., Bowick, M. J. & Li, M. H. (2009) Smectic polymer vesicles. *Soft Matter*, 5, 3446–3451.

Jiang, H. Z., Guler, M. O. & Stupp, S. I. (2007a) The internal structure of self-assembled peptide amphiphiles nanofibers. *Soft Matter*, 3, 454–462.

Jiang, Y., Wang, Y., Ma, N., Wang, Z., Smet, M. & Zhang, X. (2007b) Reversible self-organization of a UV-responsive PEG-terminated malachite green derivative: Vesicle formation and photo-induced disassembly. *Langmuir*, 23, 4029–4034.

Kamaly, N., Kalber, T., Ahmad, A., Oliver, M., So, P., Herlihy, A., Bell, J., Jorgensen, M. & Miller, A. (2007) Bimodal paramagnetic and fluorescent liposomes for cellular and tumor magnetic resonance imaging. *Bioconjugate Chemistry*, 19, 118–129.

Katz, J. S., Zhong, S., Ricart, B. G., Pochan, D. J., Hammer, D. A. & Burdick, J. A. (2010) Modular Synthesis of Biodegradable Diblock Copolymers for Designing Functional Polymersomes. *Journal of the American Chemical Society*, 132.

Kishimura, A., Liamsuwan, S., Matsuda, H., Dong, W. F., Osada, K., Yamasaki, Y. & Kataoka, K. (2009) pH-dependent permeability change and reversible structural transition of PEGylated polyion complex vesicles (PICsomes) in aqueous media. *Soft Matter*, 5, 529–532.

Knop, K., Hoogenboom, R., Fischer, D. & Schubert, U. (2010) Poly (ethylene glycol) in Drug Delivery: Pros and Cons As Well as Potential Alternatives. *Angewandte Chemie International Edition*, 49, 6288–6308.

Konak, C., Rathi, R., Kopec Kova, P. & Kopecek, J. (1997) Photoregulated association of water-soluble copolymers with spirobenzopyran-containing side chains. *Macromolecules*, 30, 5553–5556.

Kono, K., Nishihara, Y. & Takagishi, T. (1995) Photoresponsive permeability of polyelectrolyte complex capsule membrane containing triphenylmethane leucohydroxide residues. *Journal of Applied Polymer Science*, 56, 707–713.

Kukula, H., Schlaad, H., Antonietti, M. & Forster, S. (2002) The formation of polymer vesicles or "peptosomes" by polybutadiene-block-poly(L-glutamate)s in dilute aqueous solution. *Journal of the American Chemical Society*, 124, 1658–1663.

Lasic, D. & Papahadjopoulos, D. (1998) *Medical applications of liposomes*, Elsevier Science Ltd.

Leclercq, F., Cohen-Ohana, M., Mignet, N., Sbarbati, A., Herscovici, J., Scherman, D. & Byk, G. (2003) Design, synthesis, and evaluation of gadolinium cationic lipids as tools for biodistribution studies of gene delivery complexes. *Bioconjugate Chemistry*, 14, 112–119.

Lecommandoux, S., Sandre, O., Chécot, F., Rodriguez-Hernandez, J. & Perzynski, R. (2005) Magnetic nanocomposite micelles and vesicles. *Advanced Materials*, 17, 712–718.

Li, M.-H., Auroy, P. & Keller, P. (2000) An azobenzene-containing side-on liquid crystal polymer. *Liquid crystals*, 27, 1497–1502.

Li, M.-H. & Keller, P. (2009) Stimuli-responsive polymer vesicles. *Soft Matter*, 5, 927–937.

Li, M.-H., Keller, P., Li, B., Wang, X. & Brunet, M. (2003) Light-Driven Side-On Nematic Elastomer Actuators. *Advanced Materials*, 15, 569--572.

Li, Y., Lokitz, B. & Mccormick, C. (2006a) Thermally responsive vesicles and their structural locking through polyelectrolyte complex formation. *Angewandte Chemie International Edition*, 45, 5792–5795.

Li, Y., Smith, A., Lokitz, B. & Mccormick, C. (2007) In Situ Formation of Gold-"Decorated" Vesicles from a RAFT-Synthesized, Thermally Responsive Block Copolymers. *Macromolecules*, 40, 8524–8526.

Li, Y. T., Lokitz, B. S., Armes, S. P. & Mccormick, C. L. (2006b) Synthesis of reversible shell cross-linked micelles for controlled release of bioactive agents. *Macromolecules*, 39, 2726–2728.

Lin, J. J., Silas, J. A., Bermudez, H., Milam, V. T., Bates, F. S. & Hammer, D. A. (2004) The effect of polymer chain length and surface density on the adhesiveness of functionalized polymersomes. *Langmuir*, 20, 5493–5500.

Liu, F. & Eisenberg, A. (2003) Preparation and pH triggered inversion of vesicles from poly (acrylic acid)-block-polystyrene-block-poly (4-vinyl pyridine). *J. Am. Chem. Soc*, 125, 15059–15064.

Lomas, H., Canton, I., Macneil, S., Du, J., Armes, S., Ryan, A., Lewis, A. & Battaglia, G. (2007) Biomimetic pH sensitive polymersomes for efficient DNA encapsulation and delivery. *Advanced Materials*, 19, 4238–43.

Mabrouk, E., Bonneau, S., Jia, L., Cuvelier, D., Li, M.-H. & Nassoy, P. (2010) *Soft Matter*, 6, 4863–4875.

Mabrouk, E., Cuvelier, D., Brochard-Wyart, F., Nassoy, P. & Li, M.-H. (2009a) Bursting of sensitive polymersomes induced by curling. *Proceedings of the National Academy of Sciences*, 106, 7294–7298.

Mabrouk, E., Cuvelier, D., Pontani, L., Xu, B., Lévy, D., Keller, P., Brochard-Wyart, F., Nassoy, P. & Li, M. (2009b) Formation and material properties of giant liquid crystal polymersomes. *Soft Matter*, 5, 1870–1878.

Massey, J., Temple, K., Cao, L., Rharbi, Y., Raez, J., Winnik, M. & Manners, I. (2000) Self-assembly of organometallic block copolymers: The role of crystallinity of the core-forming polyferrocene block in the micellar morphologies formed by poly (ferrocenylsilane-b-dimethylsiloxane) in n-alkane solvents. *J. Am. Chem. Soc*, 122, 11577–11584.

Meng, F., Hiemstra, C., Engbers, G. & Feijen, J. (2003) Biodegradable polymersomes. *Macromolecules*, 36, 3004–3006.

Meng, F., Zhong, Z. & Feijen, J. (2009) Stimuli-Responsive Polymersomes for Programmed Drug Delivery. *Biomacromolecules*, 10, 197–209.

Meng, F. H., Engbers, G. H. M. & Feijen, J. (2005) Biodegradable polymersomes as a basis for artificial cells: encapsulation, release and targeting. *Journal of Controlled Release*, 101, 187–198.

Napoli, A., Bermudez, H. & Hubbell, J. A. (2005) Interfacial reactivity of block copolymers: Understanding the amphiphile-to-hydrophile transition. *Langmuir*, 21, 9149–9153.

Napoli, A., Boerakker, M., Tirelli, N., Nolte, R., Sommerdijk, N. & Hubbell, J. (2004a) Glucose-oxidase based self-destructing polymeric vesicles. *Langmuir*, 20, 3487–3491.

Napoli, A., Sebok, D., Senti, A. & Meier, W. (2006) Block Copolymer Vesicles. *Block Copolymers in Nanoscience*.

Napoli, A., Valentini, M., Tirelli, N., Müller, M. & Hubbell, J. (2004b) Oxidation-responsive polymeric vesicles. *Nature Materials*, 3, 183–189.

Nardin, C., Hirt, T., Leukel, J. & Meier, W. (2000) Polymerized ABA triblock copolymer vesicles. *Langmuir*, 16, 1035–1041.

Nehring, R., Palivan, C. G., Casse, O., Tanner, P., Tuxen, J. & Meier, W. (2009) Amphiphilic Diblock Copolymers for Molecular Recognition: Metal-Nitrilotriacetic Acid Functionalized Vesicles. *Langmuir*, 25, 1122–1130.

Onaca, O., Enea, R., Hughes, D. W. & Meier, W. (2009) Stimuli-Responsive Polymersomes as Nanocarriers for Drug and Gene Delivery. *Macromolecular Bioscience*, 9, 129–139.

Opsteen, J. A., Brinkhuis, R. P., Teeuwen, R. L. M., Lowik, D. & Van Hest, J. C. M. (2007) "Clickable" polymersomes. *Chemical Communications*, 3136–3138.

Pangu, G., Davis, K., Bates, F. & Hammer, D. (2010) Ultrasonically Induced Release from Nanosized Polymer Vesicles. *Macromolecular Bioscience*, 10, 546–554.

Pautot, S., Frisken, B., Cheng, J., Xie, X. & Weitz, D. (2003a) Spontaneous formation of lipid structures at oil/water/lipid interfaces. *Langmuir*, 19, 10281–10287.

Pautot, S., Frisken, B. & Weitz, D. (2003b) Engineering asymmetric vesicles. *Proceedings of the National Academy of Sciences*, 100, 10718–10721.

Pautot, S., Frisken, B. & Weitz, D. (2003c) Production of unilamellar vesicles using an inverted emulsion. *Langmuir*, 19, 2870–2879.

Pieroni, O., Fissi, A. & Popova, G. (1998) Photochromic polypeptides. *Progress in Polymer Science*, 23, 81–123.

Pinol, R., Jia, L., Gubellini, F., Levy, D., Albouy, P. A., Keller, P., Cao, A. & Li, M. H. (2007) Self-assembly of PEG-b-Liquid crystal polymer: The role of smectic order in the formation of nanofibers. *Macromolecules*, 40, 5625–5627.

Pradhan, P., Giri, J., Rieken, F., Koch, C., Mykhaylyk, O., Döblinger, M., Banerjee, R., Bahadur, D. & Plank, C. (2010) Targeted temperature sensitive magnetic liposomes for thermo-chemotherapy. *Journal of Controlled Release*, 142, 108–121.

Qin, S., Geng, Y., Discher, D. E. & Yang, S. (2006) Temperature-controlled assembly and release from polymer vesicles of poly (ethylene oxide)-block-gpoly (N-isopropylacrylamide). *Advanced materials(Weinheim)*, 18, 2905–2909.

Ravi, P., Sin, S., Gan, L., Gan, Y., Tam, K., Xia, X. & Hu, X. (2005) New water soluble azobenzene-containing diblock copolymers: synthesis and aggregation behavior. *Polymer*, 46, 137–146.

Riske, K. & Dimova, R. (2005) Electro-deformation and poration of giant vesicles viewed with high temporal resolution. *Biophysical Journal*, 88, 1143–1155.

Riske, K. & Dimova, R. (2006) Electric pulses induce cylindrical deformations on giant vesicles in salt solutions. *Biophysical Journal*, 91, 1778–1786.

Robbins, G., Jimbo, M., Swift, J., Therien, M., Hammer, D. & Dmochowski, I. (2009) Photoinitiated Destruction of Composite Porphyrin Protein Polymersomes. *Journal of the American Chemical Society*, 131, 3872–3874.

Robbins, G. P., Saunders, R. L., Haun, J. B., Rawson, J., Therien, M. J. & Hammer, D. A. (2010) Tunable Leuko-polymersomes That Adhere Specifically to Inflammatory Markers. *Langmuir*, 26, 14089–14096.

Rodriguez-Hernandez, J. & Lecommandoux, S. (2005) Reversible Inside Out Micellization of pH-responsive and Water-Soluble Vesicles Based on Polypeptide Diblock Copolymers. *J. Am. Chem. Soc*, 127, 2026–2027.

Romberg, B., Metselaar, J., Baranyi, L., Snel, C., Bünger, R., Hennink, W., Szebeni, J. & Storm, G. (2007) Poly (amino acid) s: promising enzymatically degradable stealth coatings for liposomes. *International Journal of Pharmaceutics*, 331, 186–189.

Sabaté, R., Barnadas-Rodríguez, R., Callejas-Fernández, J., Hidalgo-Álvarez, R. & Estelrich, J. (2008) Preparation and characterization of extruded magnetoliposomes. *International Journal of Pharmaceutics*, 347, 156–162.

Schlaad, H. (2006) Solution properties of polypeptide-based copolymers. *Peptide Hybrid Polymers.* Berlin, Springer-Verlag Berlin.

Semple, S., Chonn, A. & Cullis, P. (1998) Interactions of liposomes and lipid-based carrier systems with blood proteins: Relation to clearance behaviour in vivo. *Advanced Drug Delivery Reviews*, 32, 3–17.

Shum, H., Kim, J. & Weitz, D. (2008) Microfluidic Fabrication of Monodisperse Biocompatible and Biodegradable Polymersomes with Controlled Permeability. *Journal of the American Chemical Society*, 130, 9543–9549.

Sigel, R., Losik, M. & Schlaad, H. (2007) pH responsiveness of block copolymer vesicles with a polypeptide corona. *Langmuir*, 23, 7196–7199.

Sin, S., Gan, L., Hu, X., Tam, K. & Gan, Y. (2005) Photochemical and thermal isomerizations of azobenzene-containing amphiphilic diblock copolymers in aqueous micellar aggregates and in film. *Macromolecules*, 38, 3943–3948.

Stachowiak, J., Richmond, D., Li, T., Brochard-Wyart, F. & Fletcher, D. (2009) Inkjet formation of unilamellar lipid vesicles for cell-like encapsulation. *Lab on a Chip*, 9, 2003–2009.

Sugiyama, K. & Sono, K. (2001) Characterization of photo and thermoresponsible amphiphilic copolymers having azobenzene moieties as side groups. *Journal of Applied Polymer Science*, 81, 3056–3063.

Sun, G., Fang, H., Cheng, C., Lu, P., Zhang, K., Walker, A., Taylor, J. & Wooley, K. (2009) Benzaldehyde-Functionalized Polymer Vesicles.

Szoka, F. & Papahadjopoulos, D. (1978) Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. *Proceedings of the National Academy of Sciences of the United States of America*, 75, 4194.

Takeuchi, H., Kojima, H., Yamamoto, H. & Kawashima, Y. (2001) Evaluation of circulation profiles of liposomes coated with hydrophilic polymers having different molecular weights in rats. *Journal of Controlled Release*, 75, 83–91.

Taton, D. & Gnanou, Y. (2006) Guidelines for Synthesizing Block Copolymers. *Block Copolymers in Nanoscience*, 9–35.

Tong, X., Wang, G., Soldera, A. & Zhao, Y. (2005) How can azobenzene block copolymer vesicles be dissociated and reformed by light? *Journal of Physical Chemistry B*, 109, 20281–20287.

Torchilin, V., Levchenko, T., Whiteman, K., Yaroslavov, A., Tsatsakis, A., Rizos, A., Michailova, E. & Shtilman, M. (2001) Amphiphilic poly-N-vinylpyrrolidones synthesis, properties and liposome surface modification. *Biomaterials*, 22, 3035–3044.

Van Dongen, S. F. M., Nallani, M., Schoffelen, S., Cornelissen, J., Nolte, R. J. M. & Van Hest, J. C. M. (2008) A block copolymer for functionalisation of polymersome surfaces. *Macromolecular Rapid Communications*, 29, 321–325.

Vlahovska, P., Graci, R., Aranda-Espinoza, S. & Dimova, R. (2009) Electrohydrodynamic model of vesicle deformation in alternating electric fields. *Biophysical Journal*, 96, 4789–4803.

Vriezema, D., Garcia, P., Sancho, EMSP14, Oltra, N., Hatzakis, N., Kuiper, S., Nolte, R., Rowan, A., Van & Hest, J. (2007) Positional Assembly of Enzymes in Polymersome Nanoreactors for Cascade Reactions We thank Dr. E. Pierson for guidance with the fluorescence microscope and NWO-ACTS and Synthon BV for financial support. *Angewandte Chemie*, 119.

Wang, G., Tong, X. & Zhao, Y. (2004) Preparation of azobenzene-containing amphiphilic diblock copolymers for light-responsive micellar aggregates. *Macromolecules*, 37, 8911–8917.

Whiteman, K., Subr, V., Ulbrich, K. & Torchilin, V. (2001) Poly (HPMA)-coated liposomes demonstrate prolonged circulation in mice. *Journal of Liposome Research*, 11, 153–164.

Won, Y. Y., Davis, H. T. & Bates, F. S. (1999) Giant wormlike rubber micelles. *Science*, 283, 960–963.

Woodle, M. C., Engbers, C. M. & Zalipsky, S. (1994) New amphipatic polymer lipid conjugates forming long-circulating reticuloendothelial system-evading liposomes. *Bioconjugate Chemistry*, 5, 493–496.

Yamamoto, Y., Nagasaki, Y., Kato, Y., Sugiyama, Y. & Kataoka, K. (2001) Long-circulating poly(ethylene glycol)-poly(D,L-lactide) block copolymer micelles with modulated surface charge. *Journal of Controlled Release*, 77, 27–38.

Yang, J., Lévy, D., Deng, W., Keller, P. & Li, M.-H. (2005) Polymer vesicles formed by amphiphilic diblock copolymers containing a thermotropic liquid crystalline polymer block. *Chemical Communications*, 2005, 4345–4347.

Yang, J., Pinol, R., Gubellini, F., Levy, D., Albouy, P., Keller, P. & Li, M.-H. (2006) Formation of polymer vesicles by liquid crystal amphiphilic block copolymers. *Langmuir*, 22, 7907–7911.

Yatvin, M., Weinstein, J., Dennis, W. & Blumenthal, R. (1978) Design of liposomes for enhanced local release of drugs by hyperthermia. *Science*, 202, 1290.

Yu, K. & Eisenberg, A. (1998) Bilayer morphologies of self-assembled crew-cut aggregates of amphiphilic PS-b-PEO diblock copolymers in solution. *Macromolecules*, 31, 3509–3518.

Yu, S., Azzam, T., Rouiller, I., & Eisenberg, A. (2009) "Breathing" Vesicles, *J. AM. CHEM. SOC*, 131, 10557–10566.

Yuan, X., Fischer, K. & Schärtl, W. (2005) Photocleavable microcapsules built from photoreactive nanospheres. *Langmuir*, 21, 9374–9380.

Zhang, J., Zhang, Z., Yang, H., Tan, Q., Qin, S. & Qiu, X. (2005) Lyophilized paclitaxel magnetoliposomes as a potential drug delivery system for breast carcinoma via parenteral administration: in vitro and in vivo studies. *Pharmaceutical Research*, 22, 573–583.

Zhang, L. & Eisenberg, A. (1995a) Aggregates of Polystyrene-b-poly (acrylic acid) Block Copolymers. *Science*, 268, 23.

Zhang, L. & Eisenberg, A. (1995b) Multiple morphologies of "crew-cut" aggregates of polystyrene-b-poly (acrylic acid) block copolymers. *Science*, 268, 1728–1731.

Zhang, L. & Eisenberg, A. (1996) Multiple morphologies and characteristics of "crew-cut" micelle-like aggregates of polystyrene-b-poly (acrylic acid) diblock copolymers in aqueous solutions. *J. Am. Chem. Soc*, 118, 3168–3181.

Zhang, L. F. & Eisenberg, A. (1998) Formation of crew-cut aggregates of various morphologies from amphiphilic block copolymers in solution. *Polymers for Advanced Technologies*, 9, 677–699.

Zupancich, J., Bates, F. & Hillmyer, M. (2006) Aqueous dispersions of poly (ethylene oxide)-b-poly (−methyl- -caprolactone) block copolymers. *Macromolecules*, 39, 4286–4288.

Zupancich, J. A., Bates, F. S. & Hillmyer, M. A. (2009) Synthesis and Self-Assembly of RGD-Functionalized PEO-PB Amphiphiles. *Biomacromolecules*, 10, 1554–1563.