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Reviews

Stimuli-Responsive Polymersomes for Programmed Drug Delivery

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In the past decade, polymersomes (also referred to as polymeric vesicles) have attracted rapidly growing interest based on their intriguing aggregation phenomena, cell and virus-mimicking dimensions and functions, as well as tremendous potential applications in medicine, pharmacy, and biotechnology. Unlike liposomes self-assembled from low molecular weight lipids, polymersomes are in general prepared from macromolecular amphiphiles of various architectures including amphiphilic diblock, triblock, graft and dendritic copolymers. Polymersomes exhibit very unique features highlighted with high stability, tunable membrane properties, versatility, and capacity of transporting hydrophilic as well as hydrophobic species such as anticancer drugs, genes, proteins, and diagnostic probes. Recently, much effort has been directed to the development of intelligent polymersomes that respond to internal or external stimuli, in particular, pH, temperature, redox potential, light, magnetic field, and ultrasound, either reversibly or nonreversibly. Stimuli-sensitive polymersomes have emerged as novel programmable delivery systems in which the release of the encapsulated contents can be readily modulated by the stimulus. The stimuli-responsive release may result in significantly enhanced therapeutic efficacy and minimized possible side effects. It is also feasible to form and disassemble polymersomes in water simply by applying an appropriate stimulus. In this article, recent advances in stimuli-sensitive polymersomes have been reviewed, and perspectives on future developments have been discussed.

1. Introduction

In the past decade, polymersomes (also referred to as polymeric vesicles) have attracted rapidly growing interest based on their intriguing aggregation phenomena, cell and virus-mimicking dimensions and functions, as well as tremendous potential applications in medicine, pharmacy, and biotechnology. Polymersomes have fluid-filled cores with walls that consist of entangled chains,[1] separating the core from the outside medium. Together with micelles, they are the most common and stable morphological structures of amphiphiles in water. However, unlike micelles, which mostly encapsulate hydrophobic compounds, polymersomes can encapsulate hydrophilic molecules within the aqueous interior and also integrate hydrophobic molecules within the membrane. Compared with liposomes, polymersomes have many distinct properties. The membrane thickness of polymersomes, which can be controlled by the molecular weight of the hydrophobic block of the copolymer, determines polymersome properties such as elasticity, permeability, and mechanical stability.[2] Due to the higher molecular weight of the polymers as compared to lipids, the membrane of polymersomes is generally thicker, stronger, tougher and, thus, are inherently more stable than conventional liposomes. Furthermore, the use of polymeric building blocks for membrane formation also allows greater chemical diversity. In recent years, stimuli-responsive polymersomes have become one of the most fascinating topics and much progress has been made as seen

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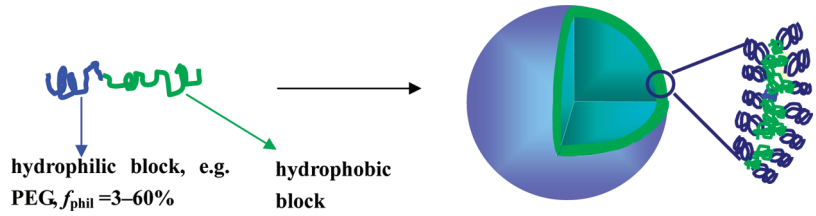

Figure 1. Polymersomes derived from asymmetric block copolymers.

by a rapidly growing number of publications. The most frequently applied stimuli are pH, temperature, redox potential, magnetic field, light, and ultrasound. There are excellent reviews on stimuli-sensitive polymers and micelles,[3−5] as well as polymersome formation and applications,[6,7] however, none has focused on responsive polymersomes. This review presents recent advances in stimuli-sensitive polymersomes, with a particular focus on novel design, preparation, stimuli-responsiveness, and potential medical applications.

1.1. Formation of Polymersomes. Polymersomes are in general prepared from macromolecular amphiphiles of various architectures including diblock copolymers, for example, poly(ethyl ethylene)-*b*-poly(ethylene glycol) (PEE-PEG),[2] poly(lactic acid)-*b*-poly(ethylene glycol) (PLA-PEG), poly(ε-caprolactone)-*b*-poly(ethylene glycol) (PCL-PEG),[8,9] poly(*N*-isopropylacrylamide)-*b*-poly(ethylene glycol) (PNIPAAM-PEG),[10] poly(styrene-*b*-acrylic acid) (PS-PAA),[11] poly(2-vinylpyridine)-*b*-poly(ethylene glycol) (PV2P-PEG),[12] and polybutadiene-*b*-poly(glutamic acid) (PBD-PGA),[13] triblock copolymers, for example, poly(ethylene glycol)-*b*-poly(propylene sulfide)-*b*-poly(ethylene glycol) (PEG-PPS-PEG),[14] graft copolymers,[15] and branched polymers.[16] PEG is a common choice as the hydrophilic block of copolymers that self-assemble into vesicles. PEG is noted for its biocompatibility and resistance to both protein adsorption and cellular adhesion, resulting in a prolonged circulation time of PEG based micelles and polymersomes. For biomedical applications, biodegradable polymers have been used as hydrophobic blocks.[8,9,15] Polymersomes can be formed using organic solvents, organic solvent/water systems, or aqueous media. The most common method involves the use of an organic solvent. Generally, the addition of a block selective solvent to a block-copolymer solution in a good solvent for both blocks leads to polymersome formation. Other methods to produce polymersomes include thin film rehydration,[2,17] w/o/w double emulsions,[18] inkjets, and microfluidic devices,[19,20] as well as electroformation.[2]

Whether polymersomes can be formed or not from a certain block copolymer is mainly determined by the weight fraction of its hydrophilic block (f_{phil}), its molecular weight, and the effective interaction parameter of its hydrophobic block with H_2O (χ). For block copolymers with a high χ, vesicles are favored when $f_{PEG} = 20-40\%$,[21,22] which is the same for natural lipids. At $f_{PEG} = 45-55\%$, cylindrical micelles tend to form, and at $f_{PEG} = 55-70\%$, spherical micelles are predominantly formed.[21,22] On the other hand, it should be noticed that block copolymers like PBD-PEG or PEE-PEG of which the hydrophobic blocks have a low T_g (e.g., PEE and PBD have a T_g of −30 and −21 °C, respectively), could directly form polymersomes in water. In contrast, copolymers with hydrophobic blocks with a high T_g cannot form polymersomes directly in a pure water medium,[23] and an organic solvent has to be used to lower the T_g to provide sufficient chain mobility. In this case, not only the copolymer composition (molecular weight of total copolymer, hydrophobic block (HB) and hydrophilic block (HL) or the molecular weight ratio) but also the concentration of the polymer solution and the water percentage in the final mixture are important factors.[24] Shen et al. showed that for PS-PAA copolymers a long HB and a high water percentage in the mixture favored the formation of polymersomes.[24] The copolymers used there had an f_{PEG} value of 13−17%. Unlike for the formation of polymersomes in water, polymersome formation in water−organic solvent mixtures was possible when f_{PEG} was in the range of 3−60%.[24,25] The length of the HB and HL both influenced polymersome formation but in the opposite way: an increase in the total copolymer length or HB length and a decrease in the HL block length favored the formation of polymersomes. The formation of polymersomes is schematically shown in Figure 1.

1.2. Applications of Polymersomes. There is growing interest in cellular targeting and delivery of biologically relevant molecules such as anticancer drugs, proteins, and genes. To enhance the bioavailability of these molecules they can be administrated after incorporation in polymeric nanocarriers. Owing to the large compartment for carrying hydrophilic cargo and the membrane for hydrophobic substances, polymersomes are very promising in biomedical and biotechnological applications. Moreover, amphiphilic copolymers as building blocks have low critical aggregation concentrations and very slow chain exchange dynamics, implying that they have very slow rates of dissociation. They may allow the retention of the payload for long periods depending on the properties of the hydrophobic block of the copolymers. Therefore, polymersomes are particularly attractive as vehicles for drug and gene encapsulation and delivery[26−30] and for biomedical imaging and diagnostic purposes.[31,32]

Both hydrophobic and hydrophilic molecules can be incorporated into polymersomes. The methods of loading the polymersomes with hydrophilic drugs include (1) direct encapsulation during polymersome formation, followed by column chromatography for separation of the free drug;[29,33] (2) the use of pH[34] or ammonia salt gradients;[26] (3) application of w/o/w emulsions;[18] and (4) using inkjets.[19] For the encapsulation of hydrophobic species, one can adopt (1) cooperative incorporation during the self-assembly process;[32] (2) the formation of w/o/w double emulsions;[18] or (3) diffusion.[26] For instance, near-infrared (NIR) emissive polymersomes (50 nm to 50 μm) of PBD-PEG and PCL-PEG[35] were obtained by loading up to 10 mol/wt % of conjugated multi(porphyrin) based NIR fluorophores (NIRFs), which uniformly distributed in the membrane (Figure 2).[32] With cell permeable peptide Tat present on polymersome surfaces, polymersomes were able to reach dendritic cells intracellularly. These polymersomes could be detected in vivo with NIR imaging at centimeter tissue depths with extremely high sensitivity without disturbing cellular function.[31]

Membranes of polymersomes have been used as model systems to study the permeability of certain substances for fundamental research and cell mimicry.[36] The molecular exchange of tracer molecules through the polymersome membrane of P2VP-PEG[37] and proton diffusion across the membranes of polymersomes of PS-PAA[11] were investigated. Yan

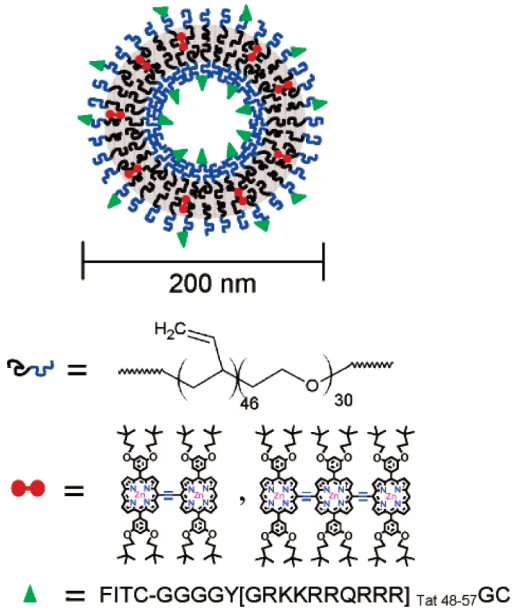

Figure 2. Tat-conjugated NIR emissive polymersomes.[31]

et al. exploited giant polymersomes composed of hyperbranched polymer to mimic cell fusion[38] and fission.[39] Incorporating channel forming proteins like OmpF,[40] water-channel protein Aquaporin Z,[41] and photoactive protein bacteriorhodopsin[42] into the polymersome membranes while fully retaining the protein functions represented an important means of equipping polymersomes with a permeation pathway for hydrophilic molecules, which is usually lacking for nonmodified polymersomes. The compartmentalization of polymersomes makes it possible to study cellular metabolism, for example, biochemical synthesis,[43] to mimic multistep reactions[44] and to investigate enzymatic reactions[45] in vitro. It should be noted that, despite the high expectations for polymersomes in biomedical applications, only a few in vivo studies have been performed.

1.3. Surface Modification of Polymersomes (Targetability of Polymersomes). Surface modification of polymersomes is of crucial importance, especially for being able to attach targeting moieties onto the polymersome surfaces, which is one of the prerequisites for successful drug or gene carriers. Generally, end groups of the hydrophilic block, for example, PEG were used to anchor these moieties,[33] for example, antibodies, antibody fragments, or RGD-containing peptides. Examples are PEG-PLA or PEG-PCL based biodegradable polymersomes with antihuman IgG or antihuman serum attached onto the surface, which showed specific binding to human IgG or human serum coated SPR disks.[33] The targetability of polymersomes of PBD-PEG functionalized with an anti-ICAM-1 to vascular endothelial cells was demonstrated.[46]

Except for PEG, water-soluble polysaccharides were also utilized. Polysaccharides contain many functional groups in the backbone, allowing further functionalization.[47,48] You et al. reported an easy way to prepare polysaccharide-containing polymersomes.[47] Polystyrene-*b*-dextran based polymersomes (ca.100 nm) containing 87 vol % of PS were formed by addition of water to a solution of the polymer in DMSO/THF.[48] In addition, sugar containing molecules or vesicles can have a specific interaction with carbohydrate recognition sites on certain cell surfaces. A very recent study indeed successfully displayed the specific binding of polymersomes, based on poly(2-glucosyloxyethyl methacrylate)-*b*-poly(diethyleneglycol methacrylate) containing a simple glycoligand glucose in the repeating unit of the hydrophilic block, with a mutant *E. coli* strain, which has binding specificity for glucose and mannose.[49]

Polymersomes based on PS-PAA with the periphery being covered with azide groups, could be used for further functionalization using "click" chemistry, offering a new approach for surface modification of polymersomes.[50]

2. Stimuli-Sensitive Polymersomes

It is well acknowledged that for potential drug delivery applications, drug carriers have to combine the properties of targetability and stimuli responsiveness to enhance the bioavailability of the drug. Therefore, designing stimuli-responsive polymersomes for programmed drug delivery, which can release the drug in a controlled manner on arrival at the target site, is highly desired. The reasons are that fast release may lead to premature release causing systemic side effects, while slow release may reduce the efficacy of the drug at the site of action and increase the drug resistance in cells.

The responsiveness or smartness of a system refers to its ability to receive, transmit a stimulus, and respond with a useful effect. Typical stimuli are pH, temperature, light, magnetic field, and concentrations of electrolytes or glucose. The responses can be dissolution/precipitation, swelling/collapsing, hydrophilic/hydrophobic transition, bond cleavage, degradation, drug release, and so on. Considering their potential applications in therapeutics, product scale-up, and cost considerations, internal stimuli-responding systems (i.e., those responding to pH, temperature, redox potential, glucose level, etc.) are more interesting than those responding to external stimuli such as light, magnetic field, and ultrasound. Thus, polymeric nanocarrier systems responsive to changes in temperature and pH, more particularly, in the pH range of 3−7, have been the focus of many studies. Generally, polymers used for carrier systems are designed in such a way that carrier structures can be disrupted, thus triggering the release of encapsulated molecules, either under acidic conditions, with a pH below the physiological pH, or at temperatures higher than normal body temperature (37 °C).

Up to now, studies on stimuli-responsive polymersomes are mostly based on changing the hydrophobicity−hydrophilicity balance as a response to stimuli. Specifically, responsive polymersome systems were designed in the following ways (Figure 3): (A) to facilitate the formation of polymersomes by changing the solubility of one block as response to stimuli, (B) to dissolve/disintegrate the polymersomes to release the cargo, or (C) to create pores in the membrane starting the release of drugs as response to certain stimuli. Some studies were directed to polymersome systems, which collapse due to bond cleavage triggered by a stimulus. An overview of stimuli-sensitive polymersomes are given in Table 1.

2.1. pH-Responsive Polymersomes. The pH-responsive systems have attracted great attention and are one of the most studied stimuli-sensitive systems because of the presence of pH variations within the body. For example, along the GI tract the pH changes from acidic in the stomach (pH = 2) to more basic in the intestine (pH = 5−8),[5] which has to be considered for oral drug delivery. The pH differences within different tissues and cellular compartments are more subtle. For instance, cancerous tissue is slightly acidic extracellularly (pH = 6.5−7.2).[5,51,52] In cytosol, like in normal tissue and blood, the pH is about 7.4, in endosome the pH is about 5.0−6.5, and lysosome has an even lower pH of 4.5−5.0.[5,53,54] This intrinsic characteristic of the body has been used to direct the response to a certain tissue (e.g., tumor tissue) or cellular compartment.

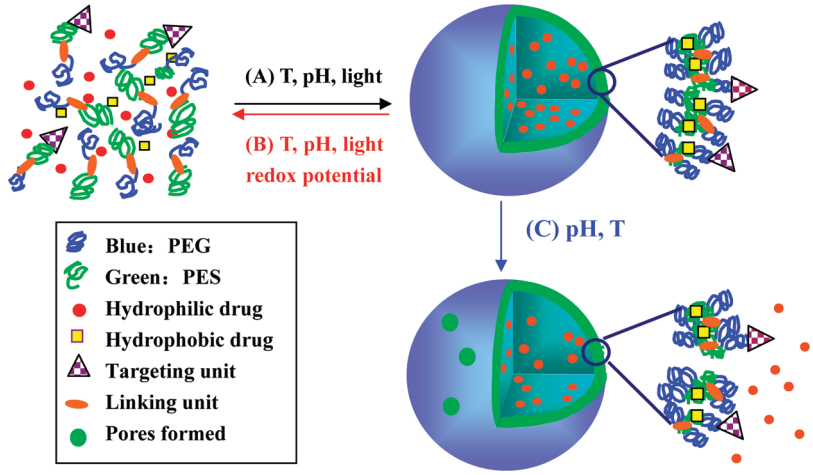

Figure 3. Schematic presentation of the formation and dissociation of stimuli-sensitive polymersomes.

Table 1. Overview of Stimuli-Sensitive Polymersomes

polymers	preparation method	stimulus	degradability	ref.
PBD-PGA	basic solution	pH (polyacid)	no	13
Carboxyl Boltorn	water	pH (polyacid)	yes	16
HEC-g-PAA	aqueous media	pH (polyacid)	no	64
PEG -P2VP	phase transfer from chloroform to water	pH (polybase)	no	12
PLE-PLL	water or THF/ H_2O	pH (polybase)	yes	68
PARG-PLE	THF/ H_2O	pH (polybase)	yes	69
PMPC-PDPA	water, pH from 2 to >6	pH (polybase)	no	62, 29
PLL-PBLG-PLL	water at neutral pH	pH (polybase)	yes	30
PEG-b-P(DEA-s-TMSPMA)	THF/aqueous solution	pH (polybase)	no	63
PGA-PLL	acid (pH < 4) or basic (pH > 10) media	pH (schizophrenic)	yes	61
PEG-b-(PG2MA-IND)	neutral pH, adjusting the pH to 2.0−3.5	pH-triggered hydrolysis	no	65
PEG-PLA, PEG-PCL	THF/water	pH-triggered hydrolysis	yes	8, 9, 26, 66
PAMPA-PNIPAAM	water	temperature	no	78
PLA-PNIPAAM	*N,N*-dimethylacetamide/H_2O	temperature	no	79
PCEMA-PNIPAAM	THF/water, acetone/water	temperature	no	80
PEG-PNIPAAM	water	temperature	no	10
HBPO-PEG	water	temperature	no	81
PEG-PPS-PEG	ethanol/water	oxidation	no	14
PDMS-*b*-PFS	water	oxidation	no	83
PEG-SS-PPS	film rehydration	reduction	no	87
PAzoMA-*b*-PAA	dioxane/water	UV	no	88
PEG-malachite green	water	UV	no	89
polystyrene with DNA-binding motifs	chloroform	hydrogen bonding	no	92
oppositely charged PEG-*b*- poly(amino acid)s	aqueous solutions	electrostatic interaction	yes	94
PBD-PGA with γ-Fe_2O_3 nanoparticles	water	magnetic field	no	95
PI-PEG with Fe_3O_4 nanoparticles	water	magnetic field	no	96
P2VP-PEG with Fe_3O_4 nanoparticles	water	magnetic field	no	96
PLA-PEG with air	THF/water	ultrasound	yes	99

Micelles,[55] liposomes,[56] capsules,[57] hydrogels,[58] and nanoparticles[59] based on pH-sensitive polymers have been designed to deliver drugs or bioactive molecules using the pH as a stimulus. The pH sensitive polymers usually have "titratable" groups (pK_a from 3−11), and typical polymers are polyacids and polybases. Polyacids are generally polymers that have pendant weak acidic groups like carboxylic acids, sulfonic acids, and so on, while polybases are polymers that have pendant weak basic groups like (primary, secondary, or tertiary) amine groups, like poly(β-amino ester).[60] The sensitivity comes from the ionization (protonation or deprotonation) of the pendant groups upon changing the pH, which turns the molecules from soluble to insoluble or vice versa. In this way, the pH can trigger the formation of the polymersomes or the release of the cargo from the polymersomes. Polymers with pH-sensitive linkages (orthoester, hydrazone, and acetal) in the backbone have also been developed. Excellent reviews on pH-sensitive polymers are available.[3−5]

2.1.1. pH-Induced Polymersome Formation (from Soluble to Insoluble). Formation of polymersomes by self-assembly of block copolymers is driven by phase separation of the two blocks, resulting in a structure held together by noncovalent forces. Polymersomes can be formed from block copolymers using organic solvents, organic solvent/water systems, or pure aqueous media. The most common method involves the use of an organic solvent. Generally, the addition of a block selective solvent to a block copolymer solution in a good solvent for both blocks results in polymersome formation. It is, nevertheless, often difficult to thoroughly remove the organic solvent, which may pose toxicity problems. Furthermore, use of organic solvent may damage the encapsulated bioactive molecules such as proteins, oligonucleotides, and DNA.

To overcome these shortcomings, researchers utilized stimuli-responsive block copolymers that self-assemble into polymersomes directly from water in the absence of organic solvents (Figure 4). Polypeptide containing block copolymers were

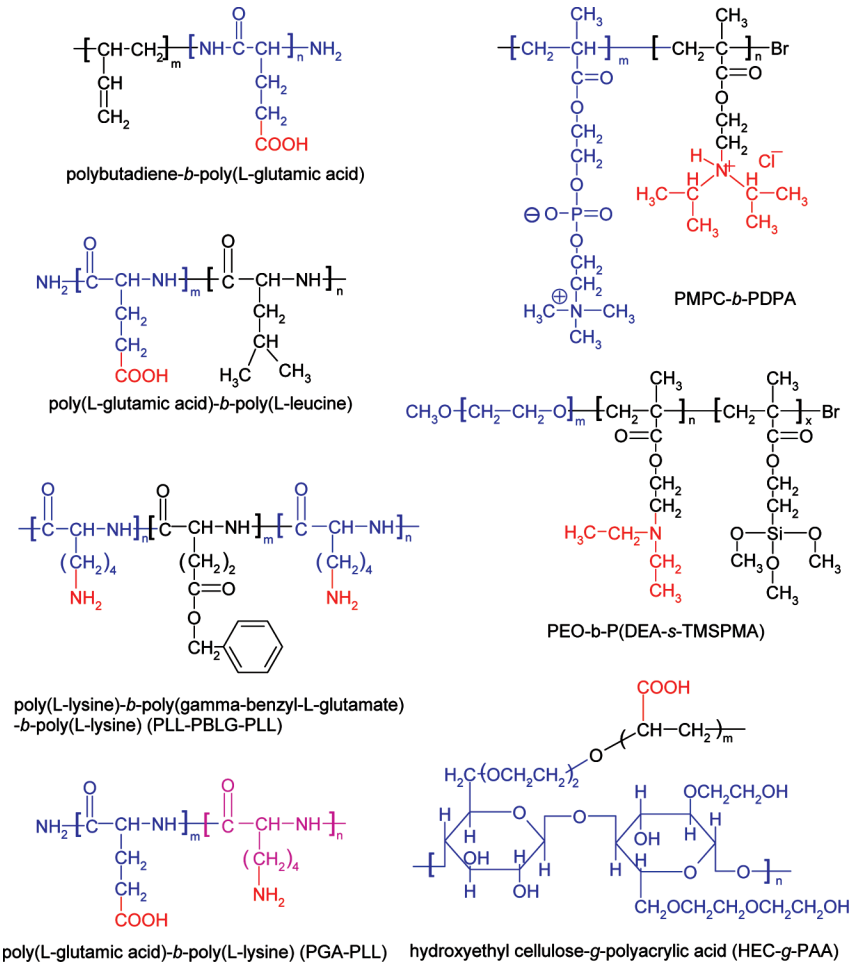

Figure 4. List of polymer structures that undergo a pH-induced solubility change used to construct pH-sensitive polymersomes: blue, hydrophilic block; red, ionizable groups; pink, biszwitterionic; also changeable to hydrophilic.

among the first studied as building blocks. For example, after adding 1 equiv NaOH to an aqueous solution of polybutadiene-*b*-poly(L-glutamic acid) (PBD-PGA) and vigorously stirring for 10 days, well-defined vesicular structures with a corona of deprotonated PGA were formed.[13] The size of the aggregated structure (100−150 nm) was reversibly tunable by changing the pH of the solution and is related to the coil−helix transition. The response to the pH was effective even in media with a high salt concentration. Moreover, pH-responsive "schizophrenic" polymersomes based on poly(L-glutamic acid)-*b*-poly(L-lysine) (PGA-PLL) could be reversibly produced in acidic (pH < 4, protonated PLL forming the hydrophilic corona) or in basic media (pH > 10, deprotonated PGA forms the corona).[61] The transition from one type of vesicle to another type took place over 3 to 4 pH units. These polypeptide-based pH-sensitive nanoparticles are biocompatible and expected to be promising candidates for drug delivery systems and nanobiotechnology.

Amphiphilic triblock copolypeptide poly(L-lysine hydrochloride)-*b*-poly(γ-benzyl-d7-L-glutamate)-*b*-poly(L-lysine hydrochloride) (PLL-PBLG-PLL; monomeric unit of PBLG ranged between 19 and 74%) formed pH and temperature responsive polymersomes in water at neutral pH (size ca. 130 nm). When the copolypeptides were mixed with plasmid DNA (pDNA), large vesicular structures with encapsulated pDNA were formed. pDNA was both partially condensed on the PLL phase and partially encapsulated inside the vesicle.[30] Consequently, the synthesized vectors combine the advantages of the polylysine−DNA systems to condense large amounts of genes, as well as those of the liposome−DNA systems, to better protect encapsulated DNA. Though interesting, the pH response of the copolypeptide is outside the physiological range and no in vitro and in vivo experiments followed.

Copolymers other than polypeptides can also be utilized to construct pH-sensitive polymersomes. For example, upon changing the solution pH from 2 to above 6, poly(2-(methacryloyloxy) ethyl phosphorylcholine)-*b*-poly(2-(diisopropylamino) ethyl methacrylate) (PMPC-PDPA) formed vesicles spontaneously, with the hydrophobic PDPA chains forming the vesicle walls.[62] This transition is due to protonation of the tertiary amine groups on the PDPA chains (pK_a 6.3), which switch from hydrophilic (a weak cationic polyelectrolyte) in acidic solution to hydrophobic at physiological pH (Figure 5). The response to pH was very sharp and rapid: vesicles formed in situ within 10 min after the solution pH was adjusted from 5.5 to 6.

The anticancer drug doxorubicin (DOX, loading efficiency 27%) was encapsulated during vesicle formation. The release of DOX from the vesicles was studied by placing the DOX-loaded vesicles in a dialysis bag and measuring the DOX concentrations outside the bag in time. Concentrations versus time were also measured for free DOX and free DOX in the presence of empty vesicles. Incorporation of DOX in the vesicles led to a retarded release ($\tau_{1/2}$ for free DOX was ca. 2.5 h, and $\tau_{1/2}$ for DOX-loaded polymersomes was longer than 25 h; Figure 5).

PMPC-PDPA polymersomes were explored as gene delivery carriers as well. DNA was encapsulated within the polymer-

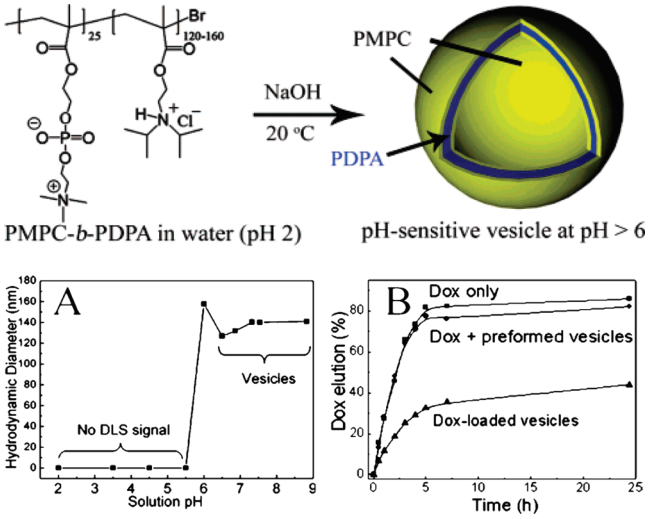

Figure 5. Schematic presentation of the formation of PMPC-PDPA block copolymer vesicles (above). Bottom graphs: (A) particle size in aqueous solution versus solution pH (1.0 g/L) and (B) Dox elution profiles from PMPC-PDPA vesicles.[62]

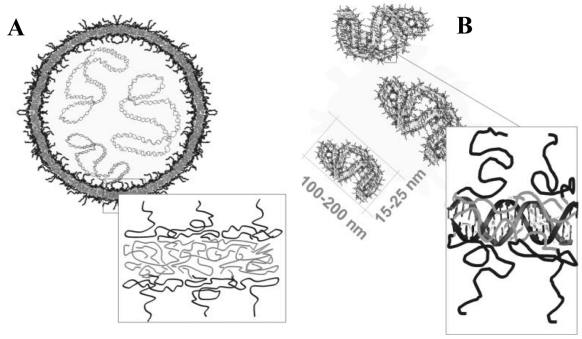

Figure 6. Presentations of copolymer−DNA complex at pH 7 (A) and 6 (B).[29] Lomas, H.; Canton, I.; MacNeil, S.; Du, J.; Armes, S. P.; Ryan, A. J.; Lewis, A. L.; Battaglia, G. Biomimetic pH Sensitive Polymersomes for Efficient DNA Encapsulation and Delivery. *Adv. Mater.* **2007**, *19*, 4238−4243. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

somes during the self-assembly process at neutral pH, whereas lowering the pH of the solution brought about the formation of DNA−copolymer complexes (Figure 6).[29] The polymersome structure ensured protection of plasmid DNA by two different mechanisms: DNA loading at neutral pH was based on physical encapsulation rather than charge-compensated condensation. These polymersomes showed very low cytotoxicity and significantly reduced cellular damage. The delivery of green fluorescent protein (GFP)-encoding DNA plasmid by polymersomes to primary human dermal fibroblast (HDF) cells and Chinese hamster ovary (CHO) cells displayed high levels of GFP expression within the cellular cytosol in living cells after 24 h incubation of the GFP−DNA loaded polymersomes.

Du and co-workers reported pH-responsive self-cross-linked polymersomes from poly(ethylene glycol)-*b*-poly((2-(diethylamino) ethyl methacrylate)-*s*-(3-(trimethoxysilyl) propyl methacrylate)) (PEG-*b*-P(DEA-*s*-TMSPMA)).[63] The vesicles formed spontaneously in aqueous/THF solution (ca. 200−400 nm), with the pH-sensitive P(DEA-*s*-TMSPMA) blocks located in the membrane. The -$Si(OCH_3)_3$ groups in the hydrophobic block hydrolyzed in situ to -$Si(OH)_3$, which subsequently reacted to produce siloxane cross-links. Because the ionization state of the PDEA block can be altered by changing the pH, the swelling of the membrane and therefore the permeability of the vesicle walls can be tuned. This pH sensitivity was higher for polymers with a higher DEA content.

Very recently, Yan et al.[16] demonstrated the spontaneous formation of pH-responsive size-controllable polymersomes (200 nm to 10 μm) of carboxyl-terminated commercial hyperbranched polyesters Boltorn Hx by adjusting the solution pH. At neutral pH, the solution was transparent with unimolecular micelles of about 4−5 nm in diameter. When the pH of the solution was lowered to a mildly acidic, for example, 3 < pH < 5.5, vesicles with a diameter of about 200 nm were formed. But the diameters of the vesicles increased rapidly (to 10 μm) with a further decrease of the pH to 1.8−3. This change was reversible. This system involved a cheap and simple synthesis yielding biodegradable vesicles with abundant surface carboxyl groups and a controllable size (200 nm to 10 μm). Such advantages make these polymer vesicles attractive in applications such as drug release.

Jiang and co-workers have reported pH-responsive polymeric hollow spheres formed by self-assembly of graft copolymers based on hydroxyethyl cellulose-*g*-poly(acrylic acid) (HEC-*g*-PAA) in aqueous media.[64] HEC-*g*-PAA readily dissolved in solutions with pH of 4−13.5. As the pH was decreased to less than 4, core−shell micelles (250−330 nm) formed, caused by the transition from individual molecules to aggregates. When the PAA chains in micelles were cross-linked, the micelles reversibly changed into hollow spheres of about 600 nm upon increasing the pH (pH > 3; Figure 7).[64] Both the micellization and the transition from micelles to hollow spheres are reversible. The hollow spheres showed "on−off" character at a pH of 2−4, which is nevertheless not in a biologically relevant pH range.

2.1.2. pH-Induced Release (Polybase). For many applications, designing drug delivery systems that can release the cargo in a controlled manner upon arrival at the target site is required. With respect to vesicle release properties, two common release modes are (1) a sustained release over a longer period of time and (2) a spontaneous release that is triggered by environmental stimuli such as changes in pH and temperature. The pH-induced spontaneous release mainly relies on solubilization of the polymer leading to disintegration of polymersomes and to instantaneous release of encapsulated drugs. The pH-induced sustained release is usually realized by hydrolysis/ degradation of the block copolymer.

2.1.2.1. Degradation Triggered Release from Polymersomes: Sustained Release. A pH-triggered release can be realized by acid-catalyzed hydrolysis of the vesicle-forming amphiphiles containing hydrolytically sensitive hydrophobic blocks. For instance, pH-dependent release of anti-inflammatory agent indomethacin (IND) from polymersomes of PEG-*b*-poly(glycerol monomethacrylate)-drug conjugates (PEG_{113}-*b*-($PG2MA_{65}$-IND_{28})) was demonstrated.[65] IND was connected with the copolymer via an ester bond. At neutral pH, the amphiphilic copolymer self-assembled into polymersomes. After adjusting pH to 2.0−3.5 for 5 h, large particles (>2 μm) attributable to precipitation of released indomethacin were detected, owing to hydrolysis of ester bonds. Thus, IND was released in a pH-dependent manner and slow sustained release of IND from these systems could be achieved.

The hydrolytic degradation of FDA approved polymers like poly(lactic acid) (PLA) and poly(ϵ-caprolactone) (PCL) containing polymersomes should foster drug release. These polyester-based degradable polymersomes[8,9,15,26,66] are, therefore, of particular interest and have been made from PEG-PLA and PEG-PCL. These block copolymers have relatively low hydrophilic PEG fractions (f_{PEG} < 20%).[8] The release rates of

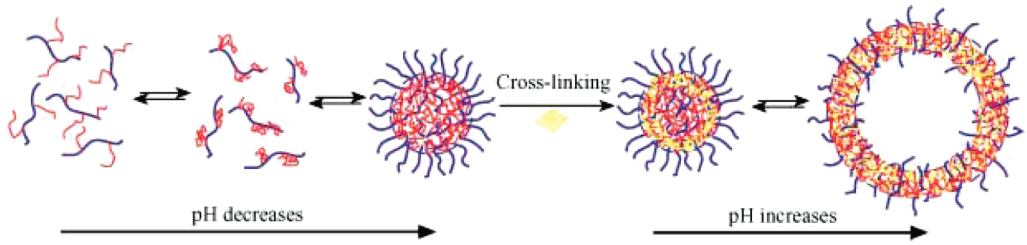

Figure 7. pH-dependent micellization of HEC-*g*-PAA and transition to hollow spheres.[64] Dou, H. J.; Jiang, M.; Peng, H. S.; Chen, D. Y.; Hong, Y.: pH-Dependent Self-Assembly: Micellization and Micelle-Hollow-Sphere Transition of Cellulose-Based Copolymers. *Angew. Chem., Int. Ed.* **2003**, *42*, 1516−1519. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

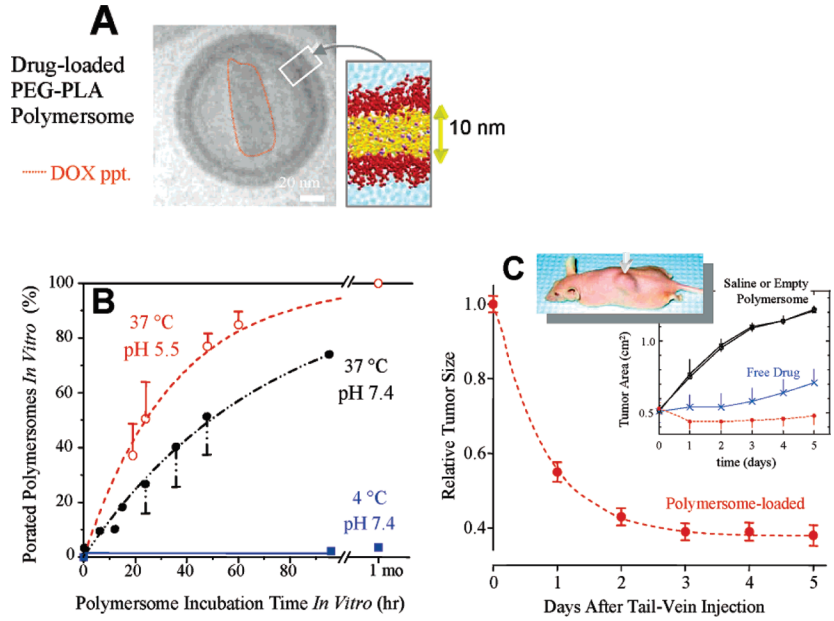

Figure 8. Drug loading, release, and antitumor activity of degradable polymersomes.[67] (A) Cryo-TEM image of DOX- and TAX-loaded polymersome. (B) Degradable giant vesicles visibly porate and release encapsulants in PBS, pH 7.4, at 37 °C, and even faster in HEPES, pH 5.5, at 37 °C, but the vesicles are stable in PBS at 4 °C. (C) Solid tumors shrink after a single injection of (DOX+TAX)-loaded polymersomes.

encapsulated molecules from polymersomes made of blends of PEG-PLA with PEG-PBD increased linearly with the molar ratio of PEG-PLA ($k_{release}$ = constant × [polyester], and $k_{release} = 1/\tau_{release}$) in acidic media.[28] Average release time $\tau_{release}$ ranged from 20 to 200 h. Under acidic conditions, the polyester first underwent hydrolysis, and hours later, pores formed in the membrane followed by final membrane disintegration. Cellular uptake studies showed that these polymersomes hydrolyzed within the endolysosomal compartments and released their contents.[67] In vivo experiments demonstrated growth arrest and shrinkage of rapidly growing tumors after intravenous injection of polymersomes of PEG-polyester (Figure 8).[26,67] Combination therapy with (DOX+TAX)-loaded polymersomes triggered apoptosis in the tumors. Within one day of treatment, fluorescence staining for cell death was strongly positive. Noticeably, apoptosis was enhanced 2-fold with polymersome-delivered drug versus free drug and a 425-fold increase relative to both untreated and empty vesicles. High levels of apoptosis appeared more sustained for polymersome-based delivery than for free drugs.

2.1.2.2. pH-Induced Solubility Change Triggers the Release from Polymersomes: Instantaneous Release. A potential limitation in the use of pH-triggered hydrolysis is that the rate of hydrolysis is rather low: it occurs over time scales of hours to days. As such, release can be somewhat late for sensitive biological macromolecules, that is, within the less desirable lysosome, where biomolecules are exposed to very harsh conditions, rather than the more desirable endosome. Therefore, it would be beneficial to design sensitive polymersome systems with sharp transitions. One approach is to use acid-induced solubility changes that occur almost instantaneously, leading to rapid drug release. For example, polybasic poly(2-vinylpyridine) (P2VP) has a pH-dependent solubility. Under neutral and alkaline conditions, P2VP is insoluble in water. Upon lowering the pH below 5, it gets protonated becoming water-soluble. P2VP-PEG vesicles (1−10 μm), prepared via the phase transfer method from chloroform to water, disappeared at pH = 4.91 by titration with dilute HCl. The study of pH-induced release of encapsulated fluorescein using a gel-electrophoresis cell that was setup in a fluorescence microscope demonstrated the rupture of the vesicles and fluorescein release occurred after 30 s upon the start of the electrophoresis.[12]

Similarly, polypeptide polymersomes of polyleucine-*b*-polylysine (PLE-PLL) showed a pH-dependent release of encapsulated Fura-2 dye. Upon lowering pH, polylysine protonated, leading to the solubilization of the membrane and the instantaneous release of the encapsulated Fura-2.[68] Holowka et al. reported pH-sensitive polymersomes composed of the copolypeptide polyarginine-*b*-polyleucine (PARG-PLE)[69] in which the polyarginine block can drive both vesicular assembly and intracellular delivery. Some polymers used for the pH-induced release from polymersomes are shown in Figure 9. It has to be realized that these systems are not reversible owing to the salt formation during the pH adjustment. In addition, these poly-

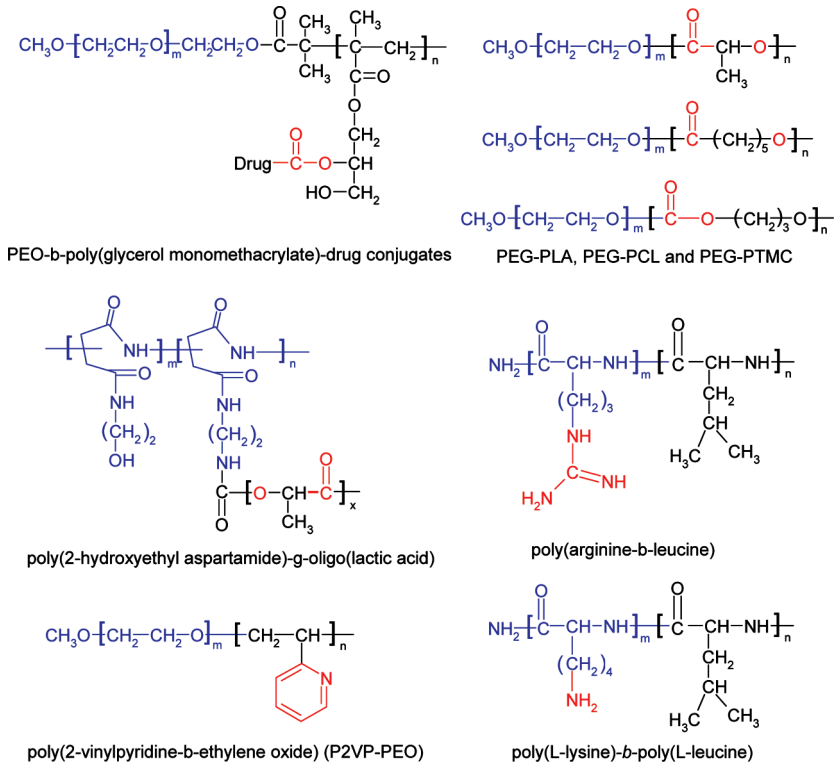

Figure 9. List of polymers used for polymersomes preparation, which will rapidly dissolve upon changing the pH, thereby also providing rapid release of encapsulants: blue, hydrophilic block; red, groups responsible for the sudden dissolution.

mersomes are only stable within certain ranges of concentration, temperature, and pH.

2.1.2.3. Cleavage of pH-Sensitive Bonds Leading to Drug Release. To design sensitive polymersome systems with sharp transitions leading to rapid drug release, one can also utilize amphiphiles (or hydrobic systems) that can be cleaved quickly in the backbone upon changing pH. Examples of such polymers are poly(ortho ester)s,[70] which have been applied to prepare micelles or nanoparticles. In addition, pH-sensitive linkages orthoester, hydrazone, and acetal can be built into polymer backbone, yielding new pH responsive polymers ready for constructing nanocarriers. For instance, acetal or ketal linkages incorporated poly(amidoamine) showed pH sensitivity and biodegradability.[71] Recently, an acid-labile block copolymer of PDMAEMA and PEG connected through a cyclic ortho ester linkage was reported as a potential gene delivery carrier.[72] Moreover, pH-sensitive linkages can also be incorporated into cross-linkers, which then are applied to cross-link drug loaded micelles affording acid-sensitive micelles, as reported by Bulmus et al.[73] However, to our best knowledge, there are no reports on polymersomes prepared from polymers of which the backbone can be cleaved very rapidly.

2.2. Temperature-Responsive Polymersomes. Temperature-responsive polymersomes are generally prepared from thermal sensitive polymers, which exhibit a volume phase transition at a certain temperature. Polymers, which become insoluble in an aqueous environment upon heating, have a lower critical solution temperature (LCST). In contrast, polymers that turn soluble upon heating have an upper critical solution temperature (UCST). The change in the hydration state causes the volume phase transition, which is caused by the coil-to-globule transition. Thermodynamically, these transitions are governed by entropic effects due to the release of ordered water molecules in the vicinity of the polymer and the dissolution process itself, and enthalpic effects due to intra- and intermolecular forces and solvation, for example, hydrogen bonding and hydrophobic interactions. Typical polymers are poly(*N*-alkylacrylamide)s, for example, poly(*N*-isopropylacrylamide) (PNIPAAM),[74,75] poly(methyl vinyl ether) (PMVE), poly(*N*-vinyl caprolactam) (PVCa), and poly(*N*-ethyl oxazoline) (PEtOx). For reviews on thermal-sensitive polymers, see ref 5.

To apply temperature-sensitive polymersomes, which show instantaneous temperature-induced drug release, it is relevant to either combine thermal-sensitive polymersomes with hyperthermia therapy[76] or to take advantage of the slightly higher temperature of tumor tissue (2−5 °C) as compared to healthy tissues. These applications may use polymers with either a UCST or an LCST. Alternatively, a local decrease in tissue temperature realized externally may also be applied and this requires the use of polymers with an LCST. In fact, most of thermal-sensitive polymers so far studied have an LCST. When temperature is raised above LCST, the conformation of polymers changes from a coil (hydrophilic) to a globule (hydrophobic). Therefore, in applications like thermal-sensitive nanocarriers[77] and hydrogels the change from room temperature to body temperature is generally used to induce a change in the physical properties of the polymers, for instance, gelation, in the case of injectable in situ forming biodegradable scaffolds.

Studies on thermal-sensitive polymersomes are so far mainly directed to their formation as well as the temperature-induced release of incorporated compounds. An elegant example of temperature-induced self-assembly of polymersomes from amphiphilic block copolymers in water was reported.[78] These polymersomes were prepared from poly[*N*-(3-aminopropyl)-methacrylamide hydrochloride]-*b*-poly(*N*-isopropylacrylamide) (PAMPA-PNIPAAM). The polymer exists as unimers in aqueous solution at room temperature and self-assembled into vesicles when the solution temperature was raised above the LCST of the PNIPAAM chain (Figure 10). The transition was reversible and occurred in a narrow temperature range (2−3

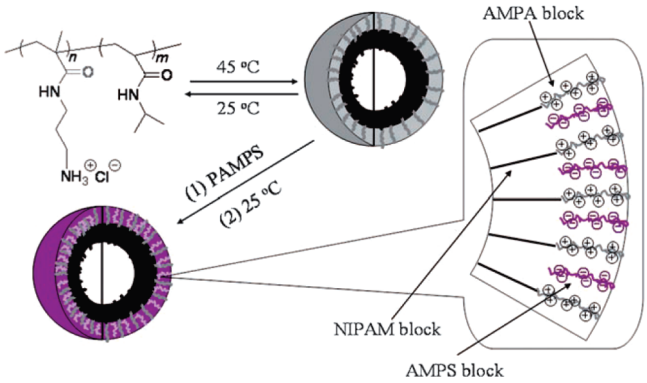

Figure 10. Schematic illustration of the formation of vesicles from diblock copolymers PAMPA-PNIPAAM (poly[*N*-(3-aminopropyl)-methacrylamide hydrochloride]-*b*- poly(N-isopropylacrylamide)) and their subsequent ionic cross-linking.[78] Li, Y.; Lokitz, B. S.; McCormick, C. L. Thermally responsive vesicles and their structural "locking" through polyelectrolyte complex formation. *Angew. Chem., Int. Ed.* **2006**, *45*, 5792−5795. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

°C), between 30 to 40 °C, depending on the composition of the polymer. The vesicles were further "locked" by ionic cross-linking of PAMPA block with an oppositely charged polyelectrolyte poly(sodium 2-acrylamido-2-methylpropanesulfonate) (PAMPS). Thermal-sensitive polymersomes based on PLA-PNIPAAM[79] and poly(2-cinnamoylethyl methacrylate)-*b*-PNIPAAM[80] were also reported.

Temperature was not only exploited as a stimulus for the formation of thermal sensitive polymersomes, but also as a trigger to release encapsulated molecules in a controlled manner upon cooling. Qin et al. reported the temperature-dependent assembly and disassembly of polymersomes,[10] which were fabricated from PEG-PNIPAAM with f_{PEG} ranging from 7.6 to 21 wt %. In water at room temperature the copolymers exist as unimers and self-assemble into vesicles in water above 37 °C with a much broader phase transition window (>10 °C) as compared to 2−3 °C of other PNIPAAM containing systems. Doxorubicin (Dox) was encapsulated into the polymersome interior using an acid gradient method. Upon cooling to room temperature, hydrophobic PNIPAAM core of the vesicles becomes hydrophilic, causing rupture/disassembly, thus, triggering the release of Dox. More than 70% of Dox was released within 2 h upon cooling to room temperature, in contrast to the undetectable release of Dox for up to 7 h, when the polymersomes are kept at 37 °C.[10] These polymersomes are promising for drug delivery as they can release drugs via local cooling by either ice packs or clinically used penetrating cryoprobes. However, PNIPAAM is not biodegradable. There is concern about possible toxicity for in vivo application of PNIPAAM containing polymers.

An interesting system based on hyperbranched polyester poly[3-ethyl-3-(hydroxymethyl)oxetane]-*star*- poly(ethylene glycol) (HBPO-PEG)[81] provides a new trigger mechanism for vesicle destabilization. HBPO-PEG self-assembles into giant vesicles in aqueous media at temperatures depending on copolymer composition. When a clear solution of the polymer was heated up for several minutes it became cloudy. The transition was reversible and could be tuned from 8 to 81 °C. The thermosensitivity of these polymersomes was proposed to result from decreasing water solubility as well as morphological transformations of the vesicles with increasing temperature induced by partial dehydration of PEG corona. A monolayer and bilayer model of the membrane packing were proposed (Figure 11).[81]

Polyphosphazenes, polymers with backbones consisting of alternating nitrogen and phosphorus atoms, can easily be rendered biodegradable by introducing hydrolytically labile substituents with nontoxic degradation products. Amphiphilic poly(organophosphazenes) with varying ratios of ethylene oxide, alkyl chains, and free acid units exhibited a pH-dependent LCST ranging from 32 to 44 °C.[82] Attachment of such copolymers to phospholipids resulted in stimuli-responsive liposomes. An increase in temperature triggered up to 80% release of encapsulated trisodium 8-hydroxypyrene trisulfonate (HPTS) after an 8 min incubation. Lowering pH to 5−6 at 37 °C increased polymer dehydration and triggered, within 1 min, complete release of entrapped HPTS for polymer/lipid mass ratios of 0.05 and 0.1. Therefore, the PPZ systems could provide a valuable biodegradable alternative for drug delivery to endosomal compartments or to tumor sites where external heat can be applied.

As mentioned before, most of the temperature-sensitive polymers have an LCST. Polymersomes with a UCST, though not reported so far, would be extremely interesting for designing site-specific intracellular drug delivery systems.

2.3. Redox-Potential Sensitive Polymersomes. Naturally occurring redox potentials within the body can be utilized as a stimulus to trigger the release of encapsulated molecules from nanocarrier systems. Both oxidative conditions existing physiologically in extracellular fluids and pathophysiologically in, for instance, inflamed or tumor tissues, as well as reductive environments within the cell, can be exploited to destabilize carrier systems. Hubbell and co-workers developed oxidation-responsive polymersomes based on triblock copolymers PEG-poly(propylene sulfide)-PEG (PEG-PPS-PEG).[14] Upon exposure to either aqueous H_2O_2 or H_2O_2 from a glucose-oxidase/glucose/oxygen system, the hydrophobic PPS core was oxidized and transformed within 2 h into hydrophilic poly(sulfoxides) and poly(sulfones), thereby destabilizing the vesicular structure. The metal-containing polydimethylsiloxane-*b*-polyferrocenylsilane (PDMS-*b*-PFS) diblock copolymer was utilized to construct redox-active organometallic vesicles,[83] offering a potential for redox-tunable encapsulation. Upon dissolution in water, this copolymer self-assembled into polymersomes (85 nm, T_g of PDMS is −123 °C) with the hydrophilic electro active PFS polyelectrolyte block as corona. Cyclic voltammetric studies demonstrated that ferrocene units in PFS underwent reversible redox reactions in water, chemically or electrochemically, which altered the number of charges on the polymers, thus changing its solubility. This underscores the potential of these vesicles as intelligent switching materials. However, electrochemical oxidation triggered destabilization of the metal containing polymersomes was not reported, although polymersomes composed of ferrocene containing hydrophobic blocks may be reversibly opened and reformed by sequential oxidation and reduction of the ferrocene units by applying reversible electrochemical oxidation. Redox sensitive/tunable polymersomes may find applications in the controlled release of drugs externally (applying electric current) and internally, for instance, in inflamed tissues and certain tumor tissues where oxygen-reactive species released by activated macrophages may trigger the transformation of redox sensitive vesicles thereby enabling selective release at pathogenic sites.

The existence of a high difference in redox potential between the mildly oxidizing extracellular space and the reducing intracellular space, due to the presence of endogenous thiols, including glutathione (GSH, ca.10 μM in human plasma), free reduced homocysteine (0.1−0.35 μM), and free cysteine (ca. 5

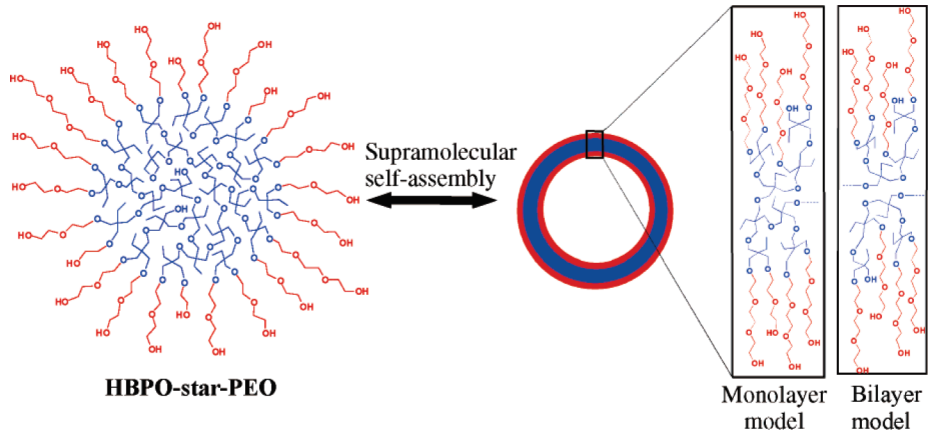

Figure 11. HBPO-*star*-PEG multiarm copolymer and the molecular packing models in the self-assembled polymer vesicles (blue, HBPO core; red, PEG arms).[81]

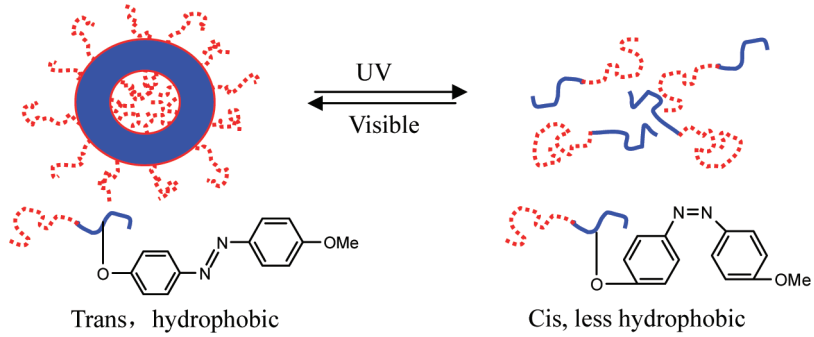

Figure 12. Reversible polymersome formation by UV/visible illumination.[88]

μM), can offer a valuable alternative to design polymeric release systems. Disulfide bonds can respond to this reductive condition via reversible cleavage into free thiols. For example, disulfides have been used in bioreducible poly(amido amine)s-based DNA delivery systems[84] and reversibly cross-linked polymer micelles.[85] It should be noted, nevertheless, that despite the oxidizing environment in the blood, reduction of disulfide bonds may occur during systemic circulation due to the presence of low concentrations of cysteine and glutathione, which may lead to premature release of drugs.[86]

Reducible polymersomes were obtained from polymers with a strategically placed disulfide bond between the two blocks of the vesicle forming diblock copolymer PEG and PPS (PEG-*SS*-PPS).[87] By hydration of a dry thin layer of this polymer, polymersomes spontaneously formed. Unilamellar vesicles (120 nm) with a narrow size distribution were obtained after extrusion through a 100 nm porous membrane. These polymersomes were suddenly disrupted in the presence of intracellular concentrations of cysteine. The response of the polymersomes to thiols was sensitive enough so that a single reduction per polymer chain triggered the cleavage of the macroamphiphile to a hydrophobic and a hydrophilic homopolymer. In cellular experiments, uptake, disruption, and release were observed within 10 min of exposure of the polymersomes to cells within the time frame of the early endosome of endolysosomal processing (ca. 30 min). These reduction sensitive polymersomes can protect biomolecules in the extracellular environment and suddenly burst releasing their contents within the early endosome prior to exposure to the harsh conditions encountered after lysosomal fusion. Therefore, they may be suitable for the cytoplasmic delivery of biomolecular drugs such as peptides, proteins, oligonucleotides, and DNA. Though fascinating and intriguing, this system is not biodegradable.

2.4. Light-Responsive Polymersomes. Light is the most desirable external stimulus to be used for intelligent systems due to the absence of additional substances and a broad range of tunable parameters, which can be modulated for potential applications. A suitable chromophore, such as azobenzene, spiropyran, triphenylmethane, or stilbene has to be incorporated into the amphiphilic system to render it susceptible to light stimuli for photochemical control of vesicles. For example, Zhao et al. reported light-breakable polymer vesicles based on the photolysis of a photolabile group on the hydrophobic block.[88] Diblock copolymers composed of a side-chain azobenzene containing polymethacrylate and poly(acrylic acid) (PAzoMA-*b*-PAA) formed polymersomes when adding water to the copolymer solution in dioxane. Under alternating UV and visible light illumination for about 20 s, reversible changes in the vesicles occurred. UV-induced dissociation of the vesicles resulted from their thermodynamic instability due to a shift of the hydrophilic/hydrophobic balance arising from the *trans*−*cis* photoisomerization of azobenzene mesogens in PAzoMA. Reaggregation took place upon visible light irradiation, shifting the hydrophilic/hydrophobic balance in the opposite direction after the reverse *cis*−*trans* isomerization[88] (Figure 12). It was suggested that to design light-induced reversible change in vesicular aggregation, one should combine a weakly hydrophilic block and a hydrophobic block with an azobenzene structure showing a significant increase in polarity under UV light irradiation.[88]

Recently, Zhang et al. described the assembly and disassembly of vesicles formed by a UV-responsive PEG (2000) terminated malachite green derivative.[89] Due to the similarity of this amphiphile to a diblock copolymer, it is also reviewed here. Malachite green (a triphenylmethane dye) is a photochromic molecule and very attractive for its physical chemistry both from a fundamental and applied point of view. This UV-responsive amphiphile self-assembled into vesicles in water (107 nm, CAC $= 7.7 \times 10^{-5}$ mol/L) in part due to hydrophobic

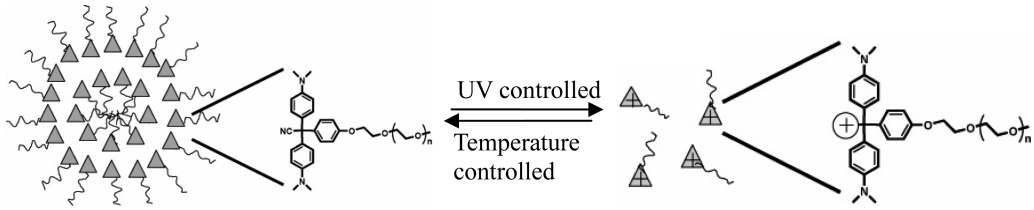

Figure 13. Vesicle formation and effective photoinduced disassembly.[89]

interactions. Upon UV irradiation, the hydrophobic malachite green was very quickly ionized to its corresponding triphenylmethyl cation and a cyanide anion, which was released to the aqueous phase, leading to disassembly of the vesicles. The cation could be thermally converted into the starting compound, resulting in reformation of the vesicles (Figure 13).

An interesting example of the combination of photochemistry and host−guest chemistry for constructing a stimuli-responsive vesicle was lately demonstrated by Zhang et al.[90] They showed the photo controlled inclusion and exclusion reaction of an azobenzene-containing surfactant (AzoC10) with α-cyclodextrin (α-CD) for fabricating a supramolecular system that can undergo reversible assembly and disassembly. Driven by hydrophobic and van der Waals interactions, the addition of α-CD to AzoC10 vesicles resulted in the inclusion of *trans*-azobenzene by α-CD, leading to the disappearance of vesicles. Upon UV irradiation for 250 s, *trans*-azobenzene was transformed to *cis*-azobenzene that could not be included by α-CD because of the mismatch between the host and guest, bringing about the reformation of the vesicles. This host−guest assembly and disassembly between azobenzene and α-CD by photo stimuli can act as a driving force to build up molecular shuttles, motors, and machines.

2.5. Hydrogen Bonding and Electrostatic Force as Stimuli. Specific recognition via hydrogen bonding can also serve as a driving force or "stimulus" to direct the formation of supramolecular structures. For example, a biszwitterion compound with a lipophilic alkyl linker in between the two charged binding sites was found to self-assemble into large vesicles in a polar medium (DMSO),[91] mainly driven by hydrogen bonding between the amide groups in the middle of the alkyl chains together with van der Waals interactions between neighboring chains. Giant recognition-induced polymersomes (RIPs, membrane thickness was ca. 43 nm) were harvested in chloroform from copolymers randomly substituted with DNA-binding motifs. Upon mixing in chloroform, the covalently attached diamidopyridine (DAP) and thymine (Thy) recognition units on the random polystyrene spontaneously formed three-point hydrogen bonding recognition dyads, leading to the formation of RIPs. The driving force was ascribed to the "pseudo-blocky" structure of the polymers, which have regions with high concentration of polar recognition elements. These regions would self-assemble into vesicle wall to minimize contact with the nonpolar bulk medium (chloroform).[92] Interestingly, by adding Thy-functionalized polymer the microspheres formed by DAP-functionalized polystyrene in nonpolar media could be transformed into vesicles, which could be transformed back to microspheres by the addition of DAP-functionalized polymer.[93] However, these recognition sensitive structures can not be used in biomedical applications, due to the complex synthesis and the use of nonpolar media.

Similar to hydrogen bonding, electrostatic forces can also trigger polymersome formation. By mixing of a pair of oppositely charged PEG-*b*-poly(amino acid)s in aqueous solutions, polymersomes were formed.[94] These polymersomes with diameters up to 10 μm and a unique three-layered membrane structure were stable in protein containing solutions. Thus, they are promising as carriers of therapeutic compounds and compartments for diagnostic enzymes.

2.6. Other External Stimuli, for Example, Magnetic Field and Ultrasound. Magnetic-sensitive polymersomes can be obtained by encapsulating magnetic nanoparticles within the membrane or aqueous interior of the polymersome. The encapsulation of magnetic nanoparticles within the interior of the vesicles brings about a great enhancement of magnetic response. These magnetic sensitive polymersomes may find applications in biomedicine and biotechnology, where the magnetic response (e.g., mobility, contrast, magnetothermal response) is to be coupled to a triggered release, offering a new pathway for diagnosis and for targeted and triggered release of drugs. For instance, Lecommandoux et al. reported that magnetic polymersomes were formed by entrapping hydrophobically modified γ-Fe_2O_3 nanoparticles into the polymersome membrane during the self-assembly process of PBD-PGA in water.[95] The deformation of these vesicles under an applied magnetic field (at the length scale of the membrane thickness) was evidenced. The magnetic polymersomes were able to respond to a magnetic field of an intensity as low as field intensity $B = 290$ G. Recently, a hybrid giant liposome system with encapsulated hydrophilic magnetic nanoparticles in the interior and hydrophobic fluorescent quantum dots in the membrane has been developed, showing the possibility of combining MRI and optical microscopy as complementary imaging techniques for in vitro and in vivo diagnosis.[18] Very recently, hydrophobic Fe_3O_4 nanoparticles (8.6 and 14.1 nm) were incorporated into polymersomes based on polyisoprene-*b*-poly(ethylene oxide) (PI_{53}-PEG_{28}) and $P2VP_{66}$-PEG_{44}.[96] The incorporation of nanoparticles into the membrane caused bridging of adjacent membranes leading to the formation of oligolamellar vesicles, which in turn resulted in a higher loading of magnetic nanoparticles, thus enhancing the magnetophoretic mobility ($v = 6-11$ μm/s) in external magnetic fields. These vesicles with a biocompatible PEG-shell are very promising as delivery vehicles with a combination of functionalities suitable for in vivo magnetic localization by external magnetic fields, for providing contrast for magnetic resonance imaging (MRI), and for magnetothermal treatment of cancer by inductive heating of the nanoparticles coupled to a thermally triggered release of encapsulated hydrophilic or hydrophobic drugs. However, so far no in vivo data were reported.

Ultrasound is a noninvasive modality of biomedical imaging. Recently, it was investigated as a sensitizer to enhance chemotherapy and to overcome drug resistance,[97] via increased intracellular drug accumulation. Ultrasound has served as a stimulus in targeted chemotherapy, triggering the release of anticancer drugs from liposomes[98] directly and efficiently into the lesions. Air encapsulated biodegradable polymersomes based on PEG-PLA were prepared via a lyophilization/rehydration procedure in the presence of mannitol and evaluated as ultrasound contrast agent.[99] It was shown that by using a medical ultrasound frequency of 7.5 MHz, polymersomes were visual-

ized as bright spots, which were backscattering signals from air inside the polymersomes. Thus these polymersomes were acoustically active, and very interesting candidates for targeted polymersome bubbles with encapsulated anticancer drugs for tumor imaging and triggered drug release for tumor killing.

3. Conclusions and Perspectives

Since their discovery, polymersomes have received tremendous attention and have been explored for various biomedical applications including drug and gene delivery, noninvasive biomedical imaging, and as membrane models. Research on functional polymersomes, especially stimuli-sensitive polymersomes, has become one of the fastest growing fields. However, despite the fact that much progress has been made in the past decade, most polymersome systems reported so far suffer from drawbacks of being not biocompatible, nonbiodegradable, slow responsiveness to stimuli, or a lack of target ability.

In the future, it will be highly desirable to develop new biocompatible and biodegradable stimuli-sensitive polymersomes that respond quickly, in a time scale of sec to h, to internal stimuli such as pH, redox potential, temperature, and glucose level. In particular, glutathione and pH-sensitive polymersomes are extremely attractive for cellular targeting and cytoplasmic delivery of drugs and genes due to a comparably low pH in the endosome and a high reducing potential in the cytoplasm and the cell nucleus. It also has to be born in mind that the size of stimuli-sensitive polymersomes should be smaller than 300 nm to be applied for tumor-targeted delivery via either the EPR effect (the pores in tumor blood vessels are 400−700 nm) or active targeting via surface decoration with targeting ligands.

It will also be of particular interest to develop polymersomes loaded with magnetic nanoparticles, MRI contrast agents, such as Gd complexes, gold nanoparticles, and quantum dots, or near-infrared fluorophores. These may provide noninvasive, functionally relevant images of organs and tissues in animals and humans. Moreover, the in vivo distribution and processing of the polymersomes in time can be determined with these polymersomes. Among these, polymersomes with near-infrared fluorophores, quantum dots, and gold nanorods are extremely interesting because they can yield images at centimeter tissue depths with very high sensitivity without disturbing cellular function. Meanwhile, this enables an optimal use of the external stimuli (magnetic or near-infrared) to trigger the release of drugs when sufficient polymersomes have been accumulated in the targeted region. This may then in turn result in a high concentration of free drug at the site of action and in an optimal pharmacotherapeutic effect.

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