Review article

Polymersome carriers: From self-assembly to siRNA and protein therapeutics

David A. Christian, Shenshen Cai, Diana M. Bowen, Younghoon Kim, J. David Pajerowski, Dennis E. Discher *

Biophysical Engineering and NanoBio-Polymers Lab, University of Pennsylvania, Philadelphia, PA, USA

ABSTRACT

Polymersomes are polymer-based vesicular shells that form upon hydration of amphiphilic block copolymers. These high molecular weight amphiphiles impart physicochemical properties that allow polymersomes to stably encapsulate or integrate a broad range of active molecules. This robustness together with recently described mechanisms for controlled breakdown of degradable polymersomes as well as escape from endolysosomes suggests that polymersomes might be usefully viewed as having structure/property/function relationships somewhere between lipid vesicles and viral capsids. Here we summarize the assembly and development of controlled release polymersomes to encapsulate therapeutics ranging from small molecule anti-cancer drugs to siRNA and therapeutic proteins.

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1. Introduction

Nature has evolved many solutions to many problems of transport at the cellular and sub-cellular scale. Small lipid vesicles or liposomes bud from cell membranes and help traffic a wide range of biomolecules both within cells and outside cells. Fluidity, flexibility, and dynamics of these carriers largely arise from the fact that lipids are low molecular weight amphiphiles. In contrast, viral capsids self-assemble from virus-encoded polypeptides that are typically 1–2 orders of magnitude larger in molecular weight than lipids. Robust, solid-like capsid structures are genetically tailored to encapsulate, protect, and deliver the viral genome, often integrating mechanisms for targeting as well as controlled intracellular release. Viral vectors are indeed capable of high infection efficiency and sustained expression of foreign genes, but they are limited to delivery of nucleic acids and their polypeptides tend to be immunogenic.

Liposomes have been pursued as non-viral gene and drug delivery vehicles for several decades, but pure lipid vesicles are generally cleared within hours from the circulation. Addition of biocompatible poly(ethylene glycol) (PEG) to a small fraction of the lipids addresses this shortcoming. PEGylation is sometimes described as emulating the glycocalyx of cell membranes and is found commercially on chemotherapeutic liposomes such as DOX-IL®. However, vesicles composed of natural lipids also generally

lack mechanisms for controlled release, and so additional synthetic schemes continue to be developed for lipids [38].

Polymersomes are formed from high molecular weight (MW) amphiphilic block copolymers composed of distinct hydrophilic and hydrophobic blocks – somewhat like a scaled up lipid. Choices of polymer MW and chemistry impart polymersomes with a broad and tunable range of carrier properties. As reviewed here, polymersomes are capable of encapsulating a large range of therapeutically active molecules and biomolecules, with considerable work being done to engineer the release of those encapsulants at the desired place and time. The accelerated use of engineered polymer systems to create polymeric micellar structures for application in the field of drug delivery motivates this current review of the role of polymersomes in non-viral delivery.

2. Polymersome structure and properties

2.1. Self-directed assembly of polymer vesicles

The principles that govern the self-assembly of natural amphiphiles such as lipids can be generalized to simple energetic and geometric arguments [44]. At solution concentrations above a critical micelle concentration (CMC) – where CMC decreases exponentially with molecular weight M_h of the hydrophobic block (i.e. CMC $\sim exp$(-MW)) – amphiphiles self-assemble to form super molecular weight aggregates. Aggregate geometry is dictated by the proportions of the hydrophilic and hydrophobic segments of the amphiphilic molecule. This idea is described by the molecular packing parameter $p = v/al_c$, in which v is the volume of the densely packed

* Corresponding author. Biophysical Engineering and NanoBio-Polymers Lab, University of Pennsylvania, 129 Towne Bldg., 220 S 33rd St., Philadelphia, PA 19104, USA. Tel.: +1 215 8984809.

E-mail address: discher@seas.upenn.edu (D.E. Discher).

hydrophobic segment ($v \sim M_h$), l_c is the chain length of the hydrophobic block normal to the interface ($l_c \sim M_h^c$; c is a scaling exponent), and a is the effective cross-sectional area of the hydrophilic chain of molecular weight M_p ($a \sim M_p^a$), group. This simple idea of a packing parameter can then be used to predict ($p \sim M_p^{1-c}/M_p^a$; with a • c • 0.5 implying $p \sim (M_h/M_p)^{1/2}$) whether the resultant morphology of these amphiphilic aggregates is spherical ($p < 1/3$), cylindrical ($1/3 < p < 1/2$), or a vesicle bilayer ($1/2 < p < 1$). It is this generality that has facilitated understanding of self-assembly to move beyond lipids and small molecular weight surfactants and on to high molecular weight amphiphiles such as amphiphilic block copolymers, polypeptides, rod-coil polymers, and dendrimers [28,5].

Block copolymers can be designed with the same amphiphilic character as lipids but consist of polymer chains covalently linked as a series of two or more "blocks". In the bulk polymer phase without organic solvent, block copolymers are known to display a wide range of ordered morphologies, including lamellar phases (with the same symmetry as a stack of paper). Hydration of these dry lamellae by aqueous solution results in a stable dispersion of amphiphilic block copolymer aggregates. Using the fully synthetic diblock copolymer poly(ethylene oxide)-polybutadiene (PEG-PBD, noting that PEO and PEG are the same), the role of the molecular packing factor in block copolymer aggregate morphology has been fully elucidated [94,45,46]. By changing the packing factor of PEG-PBD through systematic changes in the hydrophilic block fraction f (• $M_p/(M_p + M_h)$;$f \sim 0.5/(1 + p^2)$ from experiments), the morphology of the resultant aggregates in aqueous solution can be tuned to form spherical micelles ($f > 50\%$), worm-like micelles ($40\% < f < 50\%$) [95], or unilamellar polymer vesicles ($25\% < f < 40\%$) referred to as "polymersomes" [27]. The sensitivity of these morphological phase boundaries on f has not been systematically explored as a function of polymer chemistry, though they are consistent for the hydrogenated homolog of PEG-PBD, PEG-polyethylethylene (PEG–PEE).

Initial work with copolymers having values of f that consistently yield polymersomes and number-average molecular weights ranging from ~2000 to 20,000 Da has shown by cryogenic transmission electron microscopy (cryo-TEM) that the membrane core (d) increases from $d \sim 8$ to 21 nm with the scaling $d \sim M_h^b$ ($b \approx 0.55$) [6,12]. The scaling of membrane thickness as well as the morphological dependence of PEG–PEE on f has been confirmed by novel coarse-grained molecular dynamics simulations (Fig. 1a) [79,89,91]. Perhaps most revealing from simulation has been that *only* for low MW systems, the polymer bilayers exhibit a clear mid-plane of low density reminiscent of the "methyl trough" present in lipid bilayers. For high MW copolymers, the two leaflets of the bilayer interdigitate or "melt" together into a single thick and integrated shell of homogeneous density. Polymersome membranes thus present a novel opportunity to study membrane properties and performance in application as a function of membrane thickness.

2.2. Physicochemical characterization of polymersomes

The formation of giant, micron-sized vesicles allows for detailed characterization of membrane properties by single-vesicle
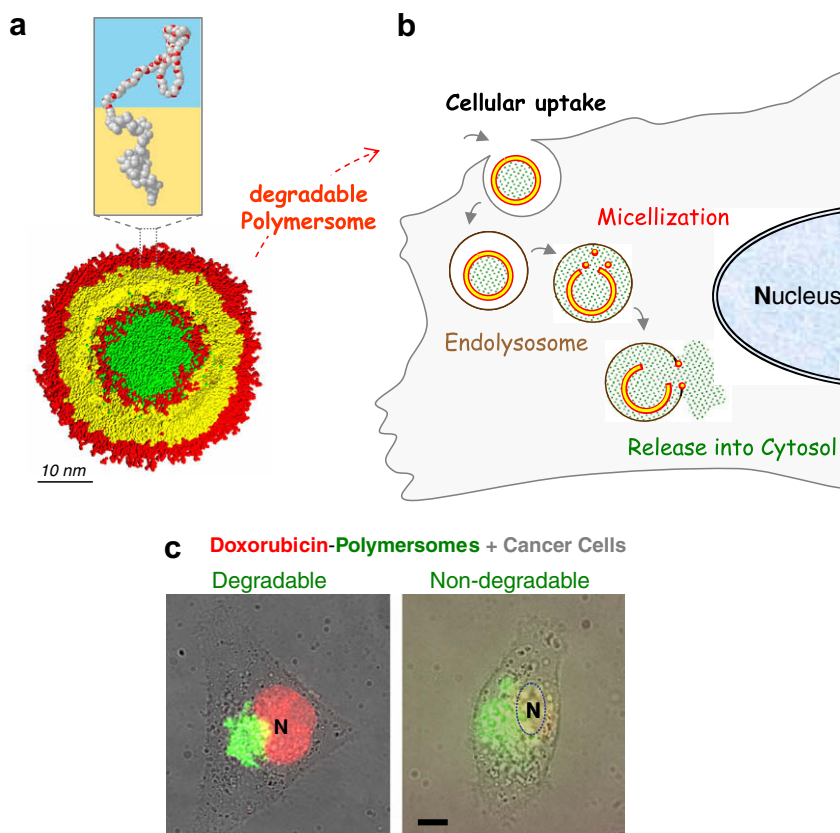
Fig. 1. Polymersome assemblies, and cellular uptake and release. (a) Coarse-grained molecular dynamics simulation of polymersomes of amphiphilic diblock copolymers in water demonstrates assembly of the vesicle bilayer. (b) Schematic of cystolic drug delivery with degradable polymersomes illustrates the uptake and degradation of polymersomes within the endolysosomal pathway. Hydrolytic degradation of polymersomes results in a high concentration of spherical micelles that mimic detergents and lyse the endolysosomal membrane to allow the release of the therapeutic into the cytosol. (c) Uptake of polymersomes labeled with a green fluorescent membrane probe and loaded with the red fluorescent anti-cancer drug Doxorubicin is demonstrated *in vitro* using a human cancer cell line. Non-degradable polymersomes show colocalization of the polymersome label and Doxorubicin near the nucleus as expected for endolysosomal cargo. With degradable polymersomes the green membrane probe remains outside the nucleus while the red Doxorubicin localizes to the nucleus.

micromanipulation methods [27]. Measurements of lateral diffusivity [60] as well as apparent membrane viscosity [26] have shown that membrane fluidity generally decreases with increasing MW, and the fluidity decreases most drastically when the chains are sufficiently long to entangle. Area elasticity measurements for PEG–PBD membranes provide an indirect measure of the monomer's effective interaction energy (ε_h) as interfacial tension ($\gamma \sim 30$ mN/m), which appears independent of MW. Opposition to interface dilation is expected to be dictated by polymer chemistry and solvent. Electromechanical stability also increases with membrane thickness up to a limit set by γ [6]. Phospholipid membranes rupture well below such limits as their thin membrane core increases susceptibility to catastrophic defects. Even when intact, permeation of water through the thick polymersome membranes is considerably reduced in comparison to lipid membranes [10]. The trends with MW generalize early measurements on liposomes [9].

In parallel with experimental studies, coarse grain molecular dynamics have also been used to study the effects of block length on the area elastic modulus and lateral chain mobility of the bilayer [89]. Simulation has indicated that the area elastic modulus is indeed independent of block length. Experimental diffusivities of the diblocks compared with simulation data for the extent of overlap and entanglement between the two leaflets of the bilayer suggest a crossover in diffusivity from the linear Rouse regime at low MW to a more viscoelastic regime dominated by entanglements at high MW. What appears most suggestive from the physicochemical studies to date is that lipid membranes have evolved in nature to be optimized more for fluidity than for stability. In contrast, robustness and solidity are hallmarks of viral capsids.

2.3. Evolution of polymersome design

2.3.1. Polyelectrolyte-based polymersomes

Although *positively* charged liposomes tend to be cleared quickly from the circulation [66], addition of charge to polymer amphiphiles presents additional opportunities for controlled release in response to external stimuli, such as pH (e.g. poly (acrylic acid) (PAA)) mediated endolysosomal rupture [48]. The shape and structure of charged diblock copolymer aggregates in solution are governed by a surprisingly delicate balance of forces. Interfacial tension between the core and bulk solvent orients, confines, and stretches the chains, but the interaction between solvated corona blocks can be repulsive through sterics and electrostatics as well as attractive if multivalent ions are involved [44].

The first morphological phase diagram of charged diblock copolymers in dilute solution was mapped out by Eisenberg and co-workers for "crew-cut" PAA–PS glassy copolymers [97]. The rich phase behavior and dynamics of a relatively symmetric and fluid diblock of PAA–PBD have now also been mapped out in water [33]. Depending on the concentration of added salt as well as pH, these diblocks are seen to self-assemble in water into stable vesicles, worm-like micelles ('worms'), and spherical micelles ('spheres'). Furthermore, using fluorescence microscopy, transitions of vesicles into worms and spheres within minutes were visualized with a sudden jump in pH or chelation of salt. Liu and Eisenberg have likewise shown rapid pH-triggered inversion of biamphiphilic triblock copolymer vesicles of PAA-PS-poly(4-vinyl-pyridine) (P4VP) in organic/aqueous mixtures [67]. With increasing bulk pH, the aggregate morphology changes from vesicles (with P4VP outside) to solid aggregates, and then inverts back into a vesicle assembly (with PAA on the outside). Some of these design principles seem likely to make their way into biocompatible polymersomes, and certainly inform us about the dramatic effects of charge in polymer systems.

2.3.2. Peptide-based vesicles

A rapidly emerging approach to polymer-based vesicles involves a return to biology with peptide-based assemblies. In contrast to viral capsids that exploit precise lock-and-key interactions within remarkable geodesic assemblies, the peptide-based vesicle assemblies are based more simply again on amphiphilicity. Most polypeptide-based amphiphilic copolymers have been composed of a hydrophilic polypeptide segment covalently coupled to a synthetic hydrophobic block [19,57,86,30,8]. The first examples of this approach used PBD as the synthetic hydrophobic block and a form of poly(L-glutamic acid) as the peptide-based hydrophilic block [19,57] to form self-assembled vesicles or "peptosomes" [57] where the polypeptide forms a well-defined α-helical structure. In aqueous solution, these copolymers form either spherical micelles or larger vesicular aggregates and exhibit pH and ionic strength dependent changes in the hydrodynamic radius and in coil-helix transitions.

More recently, Koide and co-workers have synthesized polypeptide-based copolymers that have replaced the synthetic hydrophobic block with a hydrophilic block of PEG, and coupled it to a block of charged polypeptide that is either cationic or anionic [53]. These block copolymers do not rely on amphiphilicity for assembly, but rather simple mixing of a pair of opposite and equally charged block copolymers results in a stable polyion complex (PIC) membrane that forms vesicles or "PICsomes". Both anionic and cationic copolymers are synthesized from the same precursor, PEG–poly(β-benzyl-L-aspartate) (PEG–PBLA), where the anionic PEG–poly(α,β-aspartic acid) (PEG–P(Asp)) is obtained from alkali hydrolysis and the cationic PEG–poly([5-aminopentyl]-α,β-aspartamide) (PEG–P(Asp-AP)) is achieved by aminolysis with the diamine 1,5-diaminopentane. Because these PICsome membranes do not have a hydrophobic core, they have an increased permeability to hydrophilic compounds with MWs as high as ~450 Da and can be made without the use of organic solvent, making them amenable carriers for sensitive hydrophilic compounds such as nucleic acids or proteins.

The formation of purely polypeptide polymer vesicles was first demonstrated with the amphiphilic diblock co-polypeptide [poly(N_ε-2-(2-(2-methoxyethoxy)ethoxy)acetyl-L-lysine]$_x$-(poly-L-leucine)$_y$ (K_xL_y) [11], where the size and structure of these vesicles are dictated primarily by the ordered conformation of the peptide segments. Further work with K_xL_y has demonstrated the tunability of the aggregates, where the fluidity of the vesicle membrane allows for control of vesicle size and the morphology of aggregates can be tuned with changes in the chain lengths of poly-lysine and poly-leucine [42]. By replacing the poly-lysine block of the K_xL_y co-polypeptide with poly-arginine (R_xL_y), it is possible to impart the enhanced cellular delivery properties of other arginine-rich protein-transduction domains (i.e. HIV-1 Tat peptide) to co-polypeptide vesicles [43]. Studies with $R_{60}L_{20}$ vesicles demonstrate the ability of these arginine-based vesicles to encapsulate hydrophilic compounds and successfully deliver them to cells *in vitro* with high efficacy.

In order to mimic pH-responsive synthetic block copolymer vesicles developed by Liu and Eisenberg [67], Lecommandoux and co-workers have expanded work with co-polypeptide vesicles. By synthesizing a zwitterionic block co-polypeptide composed poly(L-glutamic acid)-*b*-poly(L-lysine) (PGA-*b*-PLys), the structure of the vesicle in aqueous solution could be tuned via pH [85]. In acidic conditions (pH < 4), the PGA is neutralized and forms a hydrophobic α-helical structure resulting in a vesicle with a positively charged PLys coil brush and PGA membrane core. As pH is increased to basic conditions (pH > 10), a similar vesicle structure is formed but with an α-helical PLys membrane core and a negatively charged PGA coil brush.

In addition to forming amphiphilic co-polypeptides for vesicle assembly, polypeptides can be used as a backbone to form syn-

thetic block copolymers. The synthesis of amino acid (AA) chains composed of poly-L-lysine of poly-L-ornithine provides a linear building block that allows for the covalent attachment of different polymers through the primary amines of lysine or ornithine. By this method, Brown and co-workers successfully created an amphiphilic block copolymer chain via the addition of the hydrophilic methoxy-poly(ethylene glycol) (mPEG) and the hydrophobic palmitic acid [16]. These recent advances in polypeptide-containing vesicles from enhanced cellular delivery to pH-sensitive assembly suggest potential for therapeutic application, although immunogenicity of peptides is more likely than with synthetic polymers – which is why virus based gene therapy is problematic and limiting to gene therapy.

3. Controlled release from polymersomes

Viruses have evolved to deliver cargos effectively to a target cell, and to achieve this, viruses disassemble only when triggered by the appropriate cellular cues. Polymersomes can circulate *in vivo* for extended periods, probably because of the 100% PEGylation which scales the circulation half-life with PEG MW (M_P) as $\tau_{1/2} \sim M_P^a$ ($a \approx 0.43$) [82], but release mechanisms – triggered externally or by intracellular cues – have just begun to be engineered into polymersomes for controlled delivery. External cues include temperature changes in the tissue resulting from the application of heating or cooling devices to specific regions of the body. Since some polymersomes have been found to enter cellular endolysosomes via 'pinocytosis' pathways as sketched in Fig. 1b [4], intracellular stimuli can be environmental changes that occur in the endolysosomes, particularly a pH decrease or a change in redox condition. While some work in this area has been done with liposomes and spherical micelles, we focus here on the incorporation of release mechanisms in polymersomes.

The most common mechanism for release of encapsulated therapeutics from polymersomes is via hydrolytic degradation of hydrophobic polyester blocks such as polylactic acid (PLA) or polycaprolactone (PCL). This release mechanism takes advantage of the molecular shape-dictated morphology of block copolymer aggregates. As the chain-end hydrolyzes [34], the hydrophobic block systematically changes the molecular shape by increasing *f*. PEG–polyester copolymer molecules that begin cylindrically shaped ($f \sim 35\% \pm 10\%$) and form bilayer vesicles transition to a wedge and finally to a cone-shaped molecule. This degradation is accelerated at acidic pH as found within the endolysosomal pathway of cells [1], and at the very high copolymer concentrations (~100 mg/ml) achieved by taking up a single polymersome within an endolysosome the micelles can porate endolysosomal membranes and provide a means for drug escape into cytoplasm [3]. Eventually – upon complete hydrolysis – single PEG chains can be secreted from cells and excreted through the kidney.

Polyester-based degradable polymersomes have thus far been formed from PEG–PLA, PEG–PCL, and PEG–PMCL [2,1,71, 36,98,32]. The blending of an inert diblock such as PEG–PBD (but slowly degrading polymers could also be used) with degradable copolymers has been shown to result in homogeneously mixed polymersome membranes as verified by fluorescence microscopy [2], and this blending exploited to control polymersome degradation kinetics as the release rates of encapsulated molecules vary linearly with the mole ratio of PEG–PLA [1]. The release of these encapsulants as well as other physical measurements (fluorescence and electron microscopy images [3]) makes evident that the degradation-induced molecular shape transitions of the PEG–PLA copolymer play a significant role in the morphology taken by the blended micellar structure. While there has been initial work on these systems, the crystallinity or glassiness of pure polyester blocks at temperatures below 50 °C can complicate the morpholog-

ical phase diagram, membrane integrity at physiological temperatures, and the ability to make nano-sized polymersomes suitable for drug delivery purposes without otherwise fluidizing the aggregates [36]. Over the last 5 years or so years, various laboratories have described both vesicles and worm-like micelles made from PEO–polyester diblocks, and recent work has begun to elaborate the morphological phase diagram of PEO–PCL as a function of *f* [32,34].

In contrast to the gradual mechanism of release incorporated by hydrolytic degradation of the hydrophobic block of the copolymer, other release mechanisms relying on the systematic change in hydrophobic block polarity in response to physiological or external cues have also been incorporated into the polymersome membrane [84]. Two examples of external cues that change copolymer polarity include temperature and UV light. UV-dependent release from polymersomes has been explored in two different systems: PEG–poly(methylphenylsilane) [56] and PEG-(Malachite Green-CN) [47]. Studies with PEG–poly(methylphenylsilane) have shown that polymersomes can be formed to encapsulate hydrophilic molecules. The release of these encapsulants is achieved as UV irradiation results in the cleavage of the Si-Si bonds in the poly(methylphenylsilane) that makes up the hydrophobic core of the polymersome bilayer. Upon Si–Si bond cleavage, the polarity of the bilayer core increases, resulting in a loss of membrane integrity, polymersome disintegration, and a release of hydrophilic encapsulants. Similarly, PEG-(Malachite Green-CN) (PEG-MG-CN) has been shown by electron microscopy and dynamic light scattering to form nano-sized polymersomes in aqueous solution [47]. Exposure of these polymersomes to UV irradiation induces the loss of the cyanide anion from MG, resulting in an ionized MG molecule. This ionization significantly increases the polarity of the MG that makes up the hydrophobic core of the polymersome membrane which results in PEG-MG polymersome disassembly and release of any hydrophilic encapsulants. UV-sensitive release mechanisms are more likely to be used for *in vitro* delivery of encapsulants, as UV exposure *in vivo* has many technical challenges.

In contrast to UV-dependent release, temperature-dependent mechanisms are more feasible for *in vivo* delivery and are starting to be explored in polymer systems by making use of the polymer poly(*N*-isopropylacrylamide) (PNIPAm) [62,83]. The polarity of PNIPAm is dependent on temperature: for $T \geqslant 40$ °C, the block is hydrophobic. Therefore, by covalently attaching PNIPAm to a hydrophilic block such as PEG or poly(*N*-(3-aminopropyl)methylacrylamide hydrochloride) (PAMPA), the resulting diblock copolymer is amphiphilic at high temperatures and self-assembles to form micellar structures like polymersomes. At these higher temperatures it has been shown by fluorescence microscopy [83] and electron microscopy [62] that PNIPAm-based diblocks can indeed self-assemble to form polymersomes capable of encapsulating hydrophilic therapeutics such as doxorubicin. As predicted, decreasing the temperature of the polymersome solution results in the disassembly of polymersome structures and the transition to single copolymer molecules suspended in aqueous solution [62,83]. Initial studies have shown that this disassembly allows for the release of encapsulated therapeutics [83], inviting the application of such a carrier in conjunction with localized hypothermic therapy for targeted drug delivery.

Polymersomes with release mechanisms that respond to external cues including temperature and UV light are limited by the accessibility of the target tissue to these cues. In contrast, the effective delivery from polymersomes with release mechanisms dependent on intracellular cues is significantly less limited. Such intracellular cues include changes in pH and redox conditions in the endolysosomal pathway. In the endolysosomal pathway, pH drops from 7.4 (as present in blood plasma) to ~5. Using

polymersomes whose structure is sensitive in this pH range will allow for the intracellular release of polymersome encapsulants. While the rate of polyester hydrolysis increases with decreasing pH [1,3], such degrading polymersomes non-specifically release encapsulant prior to pH changes. Alternatively, polymers which change polarity in response to pH will maintain structural integrity and result in localized burst release. To this point, work with two different diblock copolymers – poly(2-vinylpyridine)-PEO (P2VP-PEO) [14] and poly(2-(methacryloyloxy)ethyl phosphorylcholine)-b-poly(2-(diisopropylamino)ethyl methacrylate) (PMPC-b-PDPA) [31] – has demonstrated the pH-sensitive formation of polymersomes by light or electron microscopy and encapsulation experiments. In both systems, one block of the copolymer is composed of a polybase – P2VP or PDPA – that is hydrophobic at pH values above their respective pKa values. If these polymersomes are endocytosed, the pH in the endolysosomes would progressively decrease to pH ~4.5 – below the pKa for both P2VP and PDPA. Below the pKa, each polybase deprotonates to become a cationic polyelectrolyte block which results in the disassembly of the polymersome membrane. Time lapse microscopy captures the process of polymersome membrane failure as well as the release of hydrophilic encapsulants upon exposure to an acidic environment [14].

Instead of relying on changes in pH to shift the polarity of the hydrophobic block, Hubbell and co-workers have developed release mechanisms in polymersome systems that are either oxidation [74,75] or reduction-responsive [18]. To incorporate an oxidation-responsive mechanism that can be triggered in the oxidizing environment at sites of inflammation or in the endolysosomal pathway, they used an amphiphilic triblock copolymer to form stable polymersomes composed of PEO–poly(propylene-sulfide)-PEO. Upon exposure to an oxidizing agent such as hydrogen peroxide (H_2O_2), the hydrophobic poly(propylene-sulfide)(PPS) transitions to more polar poly(sulfoxides) and poly(sulfones). This increase in polarity in the hydrophobic core thus destabilizes the polymersome membrane to release encapsulated materials. By incorporating a disulfide linkage between the hydrophilic PEO and hydrophobic PPS blocks of the diblock copolymer (PEO-SS-PPS), polymersomes can be made to remain intact in oxidizing environments (i.e. blood plasma) and to disassemble in reducing environments such as the cytosol where concentrations of reducing agents such as glutathione increase by an order of magnitude. This reducing environment results in a bursting rupture of polymersomes due to the cleavage of the disulfide bond between the PEO and PPS blocks. Studies with these reduction-responsive polymersomes have demonstrated the release of hydrophilic encapsulants such as calcein in solution as well as within cells in vitro [18]. Both oxidation and reduction-responsive polymersomes help to broaden the possibilities for selective release of encapsulated therapeutics using natural cues in vivo.

4. Encapsulation of small molecule therapeutics

For polymersomes to achieve their potential as effective delivery vehicles, they must efficiently encapsulate therapeutic agents. The ability of polymersomes to encapsulate small molecules into either the aqueous lumen of the vesicle (e.g. doxorubicin) or the hydrophobic core of the membrane (e.g. taxol) has been thoroughly studied. Early studies used fluorescent or light-absorbing molecules both to provide evidence of encapsulation in both the lumen and membrane core and to allow for the tracking of polymersome fate in vivo [27,82]. Polymersome encapsulation of small molecules has advanced to include anti-cancer therapeutics [20,3,4,61] as well as near-infrared fluorophores that are designed to be used for deep tissue imaging [65] or cell-tracking in vivo [21].

Efficient loading of the anti-cancer drug doxorubicin into the lumen of preformed polymersomes can be achieved by the pH-gradient entrapment method developed originally for liposomes [1,20]. Polymersomes are also capable of stably loading significant quantities of the hydrophobic anti-cancer drug Taxol into the hydrophobic core of the membrane – a distinct advantage over lipid-based carriers [3,4,61]. By loading both doxorubicin and taxol simultaneously into degradable polymersomes, an effective, dual drug anti-cancer therapeutic device has been developed [4]. In studying the uptake of these anti-cancer degradable polymersomes by cultured cells in vitro, delivery of doxorubicin (which is naturally fluorescent) to the nucleus indicated that these degradable polymersomes were capable of efficiently escaping the endolysosomal pathway. As mentioned above, degradable polymersomes break down with the pH decrease in the endolysosomal pathway: they transition to spherical micelles which are membrane-lytic at the very high polymer to lipid ratios of endolysosomes [4]. In fact, the circulating concentrations of copolymer are very low (mg/mL) and sub-lytic, but lytic concentrations of ~100 mg/mL are achieved in the small endolysosomes after uptake, and the accelerated degradation only promotes the release of drug into the cytosol (e.g. Fig. 1b).

The long-circulating properties of these 100% PEG-ylated degradable polymersomes allow passive accumulation in tumor sites by the Enhanced Permeability and Retention (EPR) effect [68]. By combining passive tumor site accumulation, endolytic escape and the capacity to increase tolerable doses of two anti-cancer drugs in the same carrier, anti-cancer polymersomes are able to effectively shrink tumors in vivo [3,4]. These in vivo studies tracked the size of human-derived tumors in nude mice over time. Mice were treated with saline, empty polymersomes, free drug, or anti-cancer polymersomes. Treatments with saline and empty polymersomes showed substantial tumor growth while free drug prevented further tumor growth. In contrast, treatments with anti-cancer polymersomes resulted in tumor shrinkage over time. Analysis of drug accumulation in the tumor and tumor cell death versus the different treatment methods all corroborate the results. Successful treatment of tumors in vivo with these anti-cancer polymersomes suggests a significant opportunity for the use of polymersomes in the effective delivery of other therapeutic molecules.

5. Polymersomes as non-viral gene/oligo vectors

With the effectiveness of gene therapy proven in many different disease models, the development of vectors to safely and effectively administer these materials in vivo has been an area of intense study for 10–20 years. The optimal vectors should be capable of delivering genetic material to a wide variety of cells and tissues, and considerable work has gone into re-engineering viruses. Viral vectors are certainly capable of high transfection efficiency and sustained expression of a foreign gene, but they can face problems with production scale-up (including avoidance of empty capsids) and safety concerns with an immune response. The substantial challenges facing viral vectors have led to increased efforts to synthesize effective non-viral gene delivery vectors. While such carriers do not pose the same risks as viral vectors, they are not without their disadvantages. Two of the major challenges facing work with non-viral vectors are (1) low transfection efficiency caused by the many barriers between administering the vector and delivering viable genes to the nucleus and (2) transient expression of the foreign gene. Non-viral vectors are frequently composed of the negatively charged, genetic material (DNA, RNA, etc.) complexed with a cationic lipid carrier system referred to as lipoplexes. Lipoplexes such as Lipofectamine have commonly been used to effectively deliver genetic material in vitro. The composition of these complexes has been expanded to include cationic polymers (polyplex), cationic polymers encapsulated by cationic liposomes (lipopolyplex), and now both charged and neutral polymersomes.

Mixtures of cationic and anionic surfactants at different ratios were shown by Kaler and co-workers to yield different aggregate morphologies from spheres to cylinders to planar bilayers that result in catanionic surfactant vesicles [49]. Though surfactant vesicles are more reminiscent of low MW lipid structures than polymersomes, the initial work with DNA encapsulation in non-lipid vesicle structures merits discussion. Drawing from work with lipoplexes, these vesicles were investigated for use as gene therapy vectors, and initial studies of DNA interaction with different catanionic vesicle systems showed DNA compaction to the vesicle surface that could be released upon the addition of anionic amphiphiles [70,13]. Investigation of the ability of these vesicles to encapsulate DNA for gene delivery revealed that the complexation of anionic DNA with catanionic vesicles resulted in multibilayer lamellar stacks intercalated with DNA [24,25]. Despite the ability of these vesicles to encapsulate DNA by complexation, they were not a viable vector due to the cytotoxic nature of the positively charged surfactant cetyltrimethylammonium bromide (CTAB). Therefore, Lindman and co-workers replaced CTAB with arginine-*N*-lauroyl amide dihydrochloride (ALA) as a cationic surfactant [87,88]. Catanionic vesicles composed of ALA and the anionic surfactant sodium octylsulfate (SOS) shows similar DNA intercalation as with CTAB/SOS systems, and the release of DNA from DNA/ALA/SOS complexes was shown to result in DNA/ALA complexes. While these catanionic vesicles demonstrate the ability to encapsulate DNA, complexation and subsequent intercalation between bilayers makes controlled release difficult to achieve. In addition, any exposed cation is problematic for sustained circulation *in vivo*, in part because cell surfaces are negatively charged.

To create an effective gene therapy vector based on a vesicle architecture, controlled release must be incorporated for the encapsulated gene. By encapsulating DNA in the aqueous lumen of vesicles in addition to complexation with a cationic polymer, Korobko and co-workers were able to effectively release and deliver genes *in vitro* [55,54]. The cationic amphiphilic diblock copolymer poly-(butadiene-*b*-*N*-methyl-4-vinyl pyridinium) (PBD-P4VPQI) was dissolved in toluene and added to an aqueous solution containing DNA to form cationic polymersomes encapsulating DNA in the vesicle lumen as well as intercalated into the P4VPQI brush as visualized by fluorescence microscopy. DNA intercalation provides the stability necessary for these polymersomes to be purified in air and redissolved in an aqueous PEG solution. To demonstrate that these polymersomes are not limited to small DNA molecules as are viral vectors, the DNA plasmid pUC18 was successfully encapsulated in the lumen and P4VPQI brush as shown by fluorescence microscopy [54]. Because DNA is suspended in the lumen of these vesicles, release is simply achieved by lysing the polymersomes with changes in osmotic pressure or ionic strength. The cationic polymersomes were shown effective in *in vitro* transfection: the DNA-containing vesicles stuck to a glass surface, and HeLa cells cultured on the glass took up the vesicles, resulting in successful transfections after 2 h [54]. While these cationic polymersomes allow for the controlled release of DNA by encapsulation in the lumen of the vesicle, the cationic surface charge results in a non-viable *in vivo* delivery system due to non-specific uptake and challenges to vesicle and DNA stability.

Encapsulation and delivery of DNA has also been investigated with poly(amino acid) (poly(AA))-based polymer vesicles. Brown and co-workers have created an amphiphilic triblock copolymer by attaching methoxy-poly(ethylene glycol) (mPEG) and the hydrophobic chain palmitic acid in block segments along a poly-L-lysine (PLL) or poly-L-ornithine (PO) backbone, leaving a block segment of bare, cationic PLL or PO [16]. This triblock self-assembles to form vesicle structures capable of intercalating with DNA, where the surface charge of the vesicles can be tuned by the polymer:DNA ratio. Incubation of these DNA-containing vesicles with two different cell lines shows improved DNA transfection versus poly(AA) + DNA or DNA alone. Injection of these DNA-encapsulating poly(AA) vesicles *in vivo*, where the surface charge is slightly anionic, has shown that gene transfer can be achieved in the lungs and liver [15]. However, measurements of cytotoxicity in cell culture show cell death at vesicle concentrations as low as 0.1 mg/mL of polymer, demonstrating the need for a more biocompatible polymer gene delivery vector.

As demonstrated by studies with anti-cancer polymersomes, the *in vivo* advantages of a charge-neutral, 100% PEG-ylated surface – i.e. long circulation *in vivo*, biocompatibility – make it desirable to create a PEG-based polymersome vector that is capable of effectively encapsulating and delivering genes or oligonucleotides. Initial work with degradable PEG-PLA (OLA) polymersomes has shown that the encapsulation and delivery of siRNA *in vitro* are comparable to levels achieved with the commonly used lipoplex of siRNA and Lipofectamine 2000 (LF2K) [51]. Encapsulation of an siRNA that works to silence the gene responsible for the nuclear proteins lamin A and lamin C (lamin A/C) was achieved by an emulsion technique, wherein OLA dissolved in dimethyl sulfoxide (DMSO) was added to an aqueous solution containing fluorescently labeled siRNA. Nano-sized polymersomes with a hydrodynamic radius (R_h) of 93 ± 7 nm were subsequently formed, encapsulating siRNA in the aqueous lumen, where R_h was measured by dynamic light scattering (DLS). Organic solvent and unencapsulated siRNA were removed by dialysis, and the colocalization in fluorescence micrographs of the hydrophobic membrane labeling dye (PKH26) and FITC-labeled siRNA indicate the successful loading of siRNA into polymersomes (Fig. 2a).

Size, loading efficiency, and surface charge of these OLA polymersomes (OLA-Psomes) with siRNA was similar to LF2K + siRNA lipoplexes. The ability of siRNA-containing OLA polymersomes to knockdown lamin A/C in the A549 cell line *in vitro* was then compared to LF2K + siRNA. After incubating with OLA-Psomes, LF2K, siRNA, LF2K + siRNA, or OLA-Psomes + siRNA, cells were examined at 72 h to determine protein knockdown. Lamin A/C knockdown was measured by comparing immunostain fluorescence intensity to that of control cells that were not exposed to any carrier or siRNA. Representative fluorescent micrographs of lamin A/C knockdown at 72 h display similar decreases in fluorescence intensity in cultures exposed to LF2K + siRNA and OLA + siRNA compared to controls (Fig. 2b). Analysis of lamin A/C knockdown (Fig. 2c) indicates that bare siRNA had no knockdown effect while both LF2K + siRNA and OLA-Psome + siRNA show about one-third decreases in lamin A/C expression. Viral-based methods show greater knockdown (about 60–70%; [51]), but such virus strategies are not especially viable *in vivo* as discussed above. The advantage of the polymersome system compared to LF2K is that its neutral 100% PEG-ylated surface is already known to be effective for *in vivo* delivery of small molecules.

6. Polymersome encapsulation of proteins

Therapeutic proteins are generally potent but short-lived, which has motivated recent work on direct modification of proteins with poly(ethylene glycol) (PEG) [81,40,80]. Encapsulation is another route and has been pursued with several different carrier systems, including liposomes and (micro/nano) particles. While both approaches have been shown to increase the protein plasma half-life, both have disadvantages. Liposomes have been shown to be leaky and unstable over time, and – without modification – are cleared quickly ($t_{1/2}$ < 5 h) by the body [58,82]. The process of forming (micro/nano) particles to encapsulate therapeutics can cause denaturation and thus inactivation of proteins. During particle formation, proteins are exposed to hydrophobic interfaces (i.e. organic solvent, air) and subsequently denature to form inac-
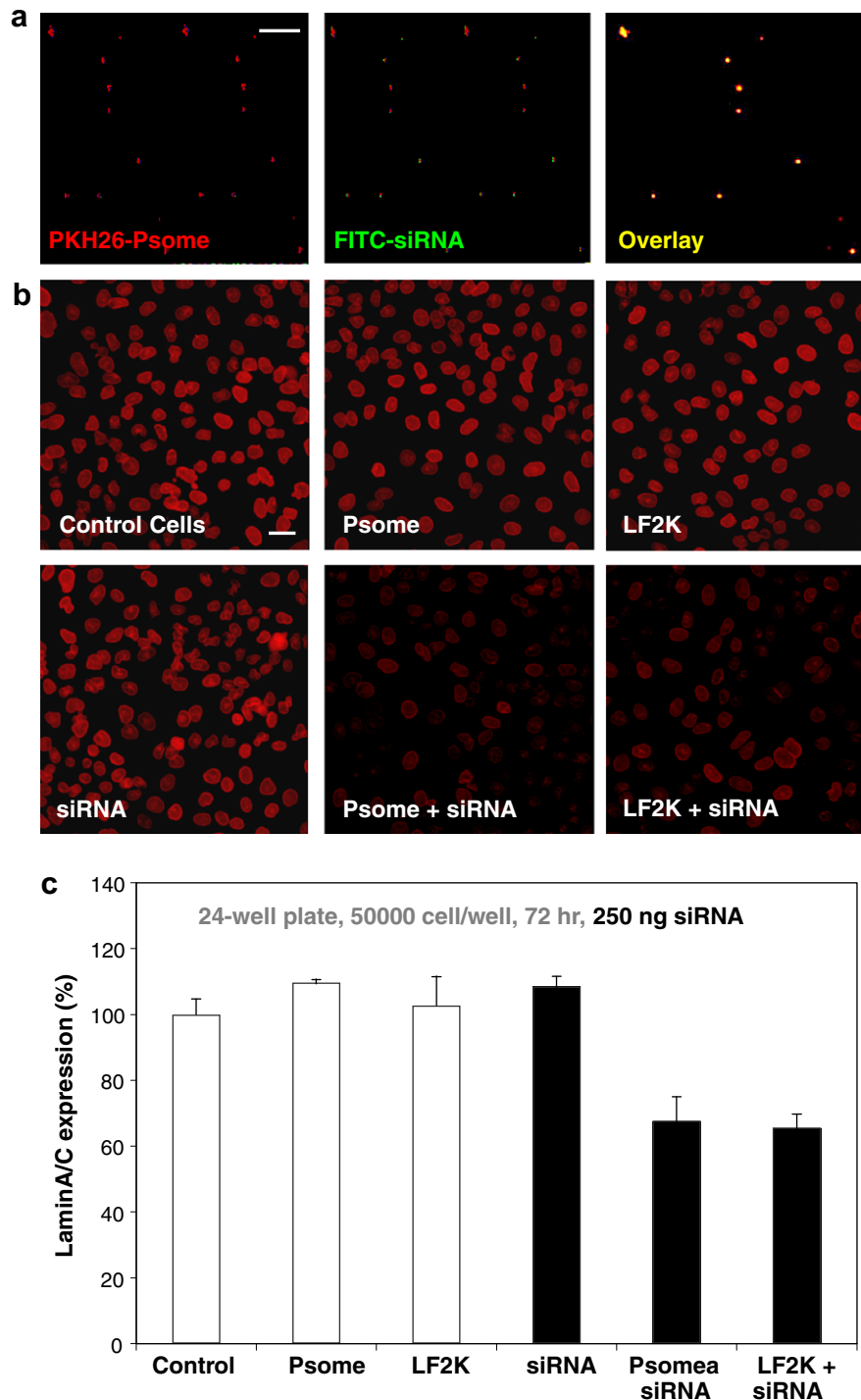
Fig. 2. Delivery of siRNA by polymersomes. (a) Fluorescent micrographs of fluorescent PKH26 labeling the membrane of nano-sized OLA polymersomes (Psome) and FITC-labeled siRNA for lamin A/C. The precise overlay of these two images indicates the encapsulation of siRNA in the aqueous lumen of OLA polymersomes. Scale bar = 10 μm. (b) Representative images of A549 cells fixed and stained for lamin A/C at 72 h display the knockdown of lamin A/C in cultures incubated with LF2K-encapsulated siRNA and OLA-Psome encapsulated siRNA when compared to controls. Scale bar = 40 μm. (c) Quantification of lamin A/C immunofluorescence in A549 cells indicates a 33–35% knockdown of lamin A/C by LF2K-encapsulated siRNA and OLA-Psome encapsulated siRNA.

tive protein conformations [81]. The covalent attachment of PEG molecules to proteins (PEGylation) has been shown to be effective in increasing the effectiveness of protein therapies. PEGylation increases the half-life of the protein in plasma as well as decreases the immune response from the body [40,80]. The drawback of PEGylation is that the non-specific covalent attachment of a large PEG molecule to the protein can lead to changes in the protein structure and thus activity [40]. In contrast to these encapsulation methods, polymersomes formed by film rehydration have been shown to incorporate transmembrane proteins in the bilayer as well as encapsulate proteins in the aqueous lumen without loss of their functional conformation.

Incorporation of proteins into polymer bilayers began by integrating membrane proteins into black block copolymer membranes – two-dimensional bilayer membranes stretched across an interface between two aqueous solutions [69]. This work proved

proteins could be incorporated into hyperthick triblock copolymer membranes while maintaining their functionality as measured by membrane conductance. Incorporation of proteins in "black films" of block copolymer has been expanded for applications in sensors [96] and protein-driven energy transduction [41] across polymeric biomembranes. By expanding their work to integrate selective protein channels into polymersome membranes, Meier and co-workers were able to form polymersome nano-reactors [76,77] as well as load DNA into polymersomes by (a very impressive) viral injection [37]. These polymersomes were formed from an ABA triblock copolymer composed of a flexible poly(dimethylsiloxane) (PDMS) hydrophobic block and two water soluble poly(2-methyloxazoline) (PMOXA) side blocks. Creation of nano-reactors is achieved by the incorporation of the channel protein OmpF which selectively allows the passage of the antibiotic ampicillin into the lumen of the vesicle where it can be hydrolyzed by the encapsulated enzyme β-lactamase [77,78]. Similarly, by incorporating the channel protein LamB – a bacterial channel protein that serves as a receptor for the λ-phage virus – into the polymersome membrane, the λ-phage virus recognizes its receptor, binds to the polymersome and injects its DNA as visualized by incredible electron microscopy micrographs [37]. To better understand how membrane proteins can be incorporated into the hyperthick polymersome membrane and maintain functionality, the insertion of pore forming membrane into a polymer bilayer was simulated using coarse-grained molecular dynamics [90]. Simulations suggest that polymer chain flexibility permits the integration of proteins with small membrane-spanning domains, but the flexibility of the hydrophilic chains can partially block the functional pore of the membrane protein, resulting in decreased functionality compared to when the proteins are incorporated in more natural lipid membranes.

Encapsulation of proteins within the aqueous lumen of polymersomes can in principle take advantage of the extended circulation kinetics and controlled release properties of polymersomes. To test the ability of neutral diblock or charged triblock polymersomes to encapsulate larger, globular proteins, initial studies were performed with FITC-labeled bovine serum albumin (FITC-BSA) as well as myoglobin and hemoglobin [59,93]. Fluorescent micrographs of either giant or fluorescently labeled, nano-sized polymersomes provide visual proof that FITC-BSA is indeed encapsulated in the aqueous lumen of the vesicles. By encapsulating different enzymes and metalloproteins in polymersomes, different groups have proven that proteins remain functional after encapsulation [77,78,74,7,52]. As previously discussed, Meier and co-workers were able to create polymersome nano-reactors by incorporating functional proteins into the membrane, while also encapsulating the enzyme β-lactamase in the aqueous lumen of the vesicle [77,78]. The activity of β-lactamase was demonstrated through the hydrolysis of ampicillin to form ampicillinoic acid. Also discussed previously was the incorporation of an oxidation-responsive release mechanism from PEG-PPS-PEG polymersomes [75]. While this mechanism can be cued in vivo (i.e. sites of inflammation, endolysosomal pathway), release can also be triggered via the encapsulation of oxidizing enzyme. Glucose oxidase (GOx) works to break down glucose to form gluconolactone by oxidation and in the process reduces oxygen to form hydrogen peroxide. Because GOx that was encapsulated in PEG-PPS-PEG polymersomes remained functional, the addition of glucose to the surrounding medium resulted in glucose crossing the polymersome membrane where it was subsequently oxidized, resulting in the formation of hydrogen peroxide [74]. Hydrogen peroxide – a strong oxidizing compound – then worked to break down the PEG-PPS-PEG polymersomes (as measured by optical density at 600 nm) by oxidizing PPS to its more polar byproducts poly(sulfoxide) and poly(sulfone). In these studies it was shown that GOx not only maintained its functionality after encapsulation, but its functionality was extended compared to GOx in solution.

The synthetic mimicry of cellular structures was the initial inspiration for the study of micellar polymer structures such as polymersomes and worm-like micelles [22]. Red blood cells (RBCs) are perhaps the easiest functional cells to mimic because they lack intracellular structures – nucleus, organelles – that make all other cells so very complex. By encapsulating the metalloproteins hemoglobin [7] and myoglobin [52] in polymersomes, an RBC 'mimic' has been created. Because oxygen is capable of diffusing across the polymersome membrane at rates similar to lipid bilayers, polymersomes encapsulating hemoglobin have been shown to have oxygen affinities similar to those of human RBCs [7]. Similarly, Kishimura and co-workers successfully encapsulated myoglobin (Mb) in PICsomes as shown by fluorescent micrographs of fluorescently labeled Mb [52]. Functionality of encapsulated Mb was determined by oxygen binding which could be monitored by absorbance at 434 nm. Though these studies have shown that encapsulation of functional proteins is possible with polymersomes, little work has been done to encapsulate therapeutic proteins.

The encapsulation of recombinant insulin in polymersomes provides a good model for the encapsulation of therapeutic proteins. Enhanced circulation kinetics and controllable release of insulin in vivo are desirable to increase patient compliance and to decrease the need for intravenous injections. While insulin is a low molecular weight protein, it has been shown to aggregate to dimers, hexamers, and eventually fibrils when the structure is disrupted by exposure to agitation or hydrophobic interfaces. Therefore, insulin was a challenging first test of therapeutic protein encapsulation in PEG-based polymersomes. Briefly, by a film rehydration method, thin films of PEG-PBD (OB2, 1.2–2.4 kDa) blended with the hydrophobic membrane label PKH26 were hydrated at 60 °C with phosphate buffered aqueous solutions (pH = 7.4) containing either fluorescein-labeled bovine insulin or recombinant human zinc-insulin at 1.5 mg/mL. As expected, film rehydration resulted in giant, membrane-labeled OB2 polymersomes encapsulating insulin in their aqueous lumen. The encapsulation of fluorescein-insulin provided visual proof of insulin encapsulation by fluorescence microscopy as micrographs of the red fluorescent membrane label overlayed perfectly with micrographs of fluorescein-insulin in the aqueous lumen (Fig. 3a).

In previous work with growth factors similar to insulin, the non-ionic detergent Tween80 was used to prevent protein denaturation and aggregation [50]. Similarly, the addition of Tween80 (0.05% v/v) to solutions of zinc-insulin was shown by circular dichroism to enhance the stability of insulin's secondary structure during hydration (Fig. 3b). Solutions of zinc-insulin alone were incubated either at 4 °C or 60 °C for 8 h with or without Tween80. Circular dichroism was performed at constant temperatures and the final protein concentrations were measured to calculate the mean residue ellipticity (θ_{MRE}). Promising circular dichroism results also indicated that the film rehydration technique allowed for the encapsulation of insulin in OB2 polymersomes without significant loss of secondary structure.

For polymersomes to be used as viable delivery vehicles, they must be sized down to a radius of approximately 100 nm. To prevent denaturation of insulin, polymersomes were sized down by repeated extrusion through sequentially smaller pore sizes without prior sonication or freeze-thaw cycles. The R_h of these nano-polymersomes was then measured to be 97 ± 6 nm using dynamic light scattering (data not shown). Fluorescein-insulin loaded, nano-polymersomes were then separated from unencapsulated fluorescein-insulin by size exclusion chromatography through a Sephacryl S-500 column, collecting fractions every 500 μl. Separation of the polymersomes from unencapsulated fluorescein-insulin was confirmed by fluorescence absorbance measurements of each elution fraction using a Fluoroskan microplate fluorimeter. After
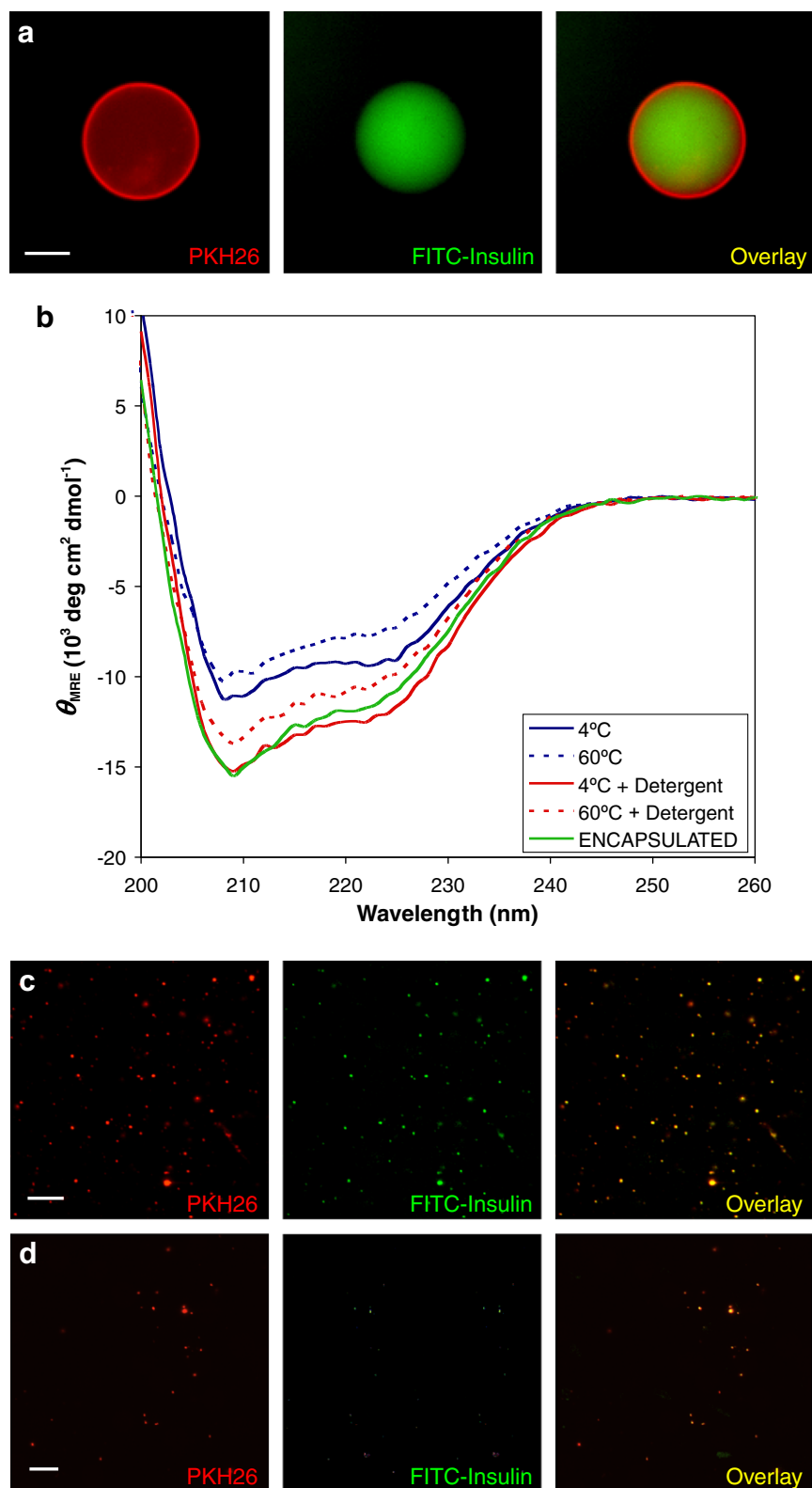
Fig. 3. Therapeutic protein encapsulation in polymersomes (a). Fluorescent micrographs of fluorescent PKH26 labeling the membrane of a giant OB2 polymersome and fluorescein-labeled bovine insulin (diluted 100×). Overlay of these images display the encapsulation of fluorescein-insulin in OB2 polymersomes. Scale bar = 10 μm. (b) Circular dichroism (CD) results for recombinant human Zn-insulin after incubation for 8 h at 4 and 60 °C indicates that the film rehydration technique does not result in the loss of insulin secondary structure. The addition of the non-ionic detergent Tween80 further stabilizes the secondary structure at both 4 and 60 °C. Most importantly, CD of insulin encapsulated in OB2 polymersomes in the presence of Tween80 does not indicate any loss of protein secondary structure. (c) Fluorescent micrographs of PKH26 labeling the membrane of nano-sized OB2 polymersomes and fluorescein-insulin (diluted 0×). The precise overlay of these two images shows that polymersomes can stably encapsulate insulin through sizing down and separation from unencapsulated insulin. Scale bar = 10 μm. d) Fluorescent micrographs of PKH26 labeling the membrane of nano-sized OB2 polymersomes encapsulating fluorescein-insulin after 10× dilution in whole blood. Fluorescence overlay indicates stable encapsulation in a biologically relevant medium up to 8 h. Scale bar = 10 μm.

sizing down and separation, these polymersomes were shown to maintain their encapsulants for months (at 4 °C) by fluorescence microscopy (Fig. 3c). While these structures were too small to visualize the presence of fluorescein-insulin in the aqueous lumen as done with giant polymersomes, the precise colocalization of fluorescent signals from the fluorescent membrane label and fluorescein-insulin confirms encapsulation. To prove these polymersomes provide a stable carrier system for therapeutic proteins, fluorescein-insulin loaded nano-polymersomes were incubated for hours in whole blood in vitro at 37 °C and shown to stably maintain their encapsulant (Fig. 3d). Encapsulation of insulin in neutral PEG-PBD polymersomes provides a promising method to increase therapeutic efficiency by maintaining protein structure – and in a carrier system that has been shown to be stable in biological environments.

7. Active targeting of polymersomes

Although few if any drug carriers in clinical use today are targeted, viruses often show preferential – although not exclusive – interactions with particular cell types and cell entry pathways. Given that polymersomes exhibit minimal non-specific adhesion to cells (initially), that they can circulate for many tens of hours, and that they can integrate controlled release mechanisms, targeted polymersomes should add an additional level of functionality – although there is an added cost for the complexity. With PEG-based assemblies, much work has been focused on attaching ligands or antibodies to the hydroxyl end-group [92,29,21,72]. Biotinylated non-degradable block copolymer assemblies have been shown to attach to surfaces coated with the biotin receptor avidin [23,64,72,73] as well as to cells where they successfully delivered the hydrophobic drug Taxol® [23]. Similar chemistry has been used to attach either an antihuman IgG, or antihuman serum to PEG-carbonate- or PEG-polyester-assembled polymer vesicles [71,63]. The attachment of HIV-derived Tat peptide – a cationic peptide shown to enhance cellular delivery of nanoparticles [39] – to PEG-PBD polymersomes has been shown to increase dendritic cell uptake in vitro [21]. Broz and co-workers [17] have modified their triblock copolymer polymersomes with polyguancylic acid to target a macrophage scavenger receptor SRA1. This scavenger receptor is a pattern-recognition antigen upregulated only in activated tissue macrophages, not in monocytes or monocyte precursor cells. These in vitro cell targeting studies have not yet been translated to in vivo conditions, where complications may arise from opsonization by serum components and competitive interactions with other cells [29]. Though promising, chemically attached targeting moieties do not act as a 'homing beacon' to the desired in vivo target. Instead, targeted carriers must contact the desired target either by convective or diffusive collisions; only then can targeted adhesion increase the likelihood of cellular uptake or localized release. Therefore, future decisions to increase carrier complexity with targeting ligands should be carefully weighed against the advantages of active targeting versus passive targeting via the EPR effect.

8. Conclusions

The tunability of polymersome structure and properties has expanded considerably with the recent advances in block copolymer chemistries. The ability to specifically tailor polymersome formulation methods, physicochemical properties, release mechanisms, and even targeting chemistries make polymersomes an ideal platform for the encapsulation of a broad range of therapeutic molecules. As demonstrated by their effective treatment of tumor models in vivo and stable encapsulation of other actives (i.e. dyes,

nucleic acids, proteins), polymersomes show great promise in moving from design and onto therapeutic application.

Lastly, one might conclude from our initial descriptions of copolymer assemblies that a library of any given block copolymer (e.g. 10% < f < 90%) could be extremely useful to develop and study, even if the initial focus was on polymersomes. Indeed, the anti-cancer performance of polymersomes loaded with hydrophobic drugs such as taxol can be readily compared to that of worm-like micelles and spherical micelles of very similar composition. This has already proven an interesting direction of study with the demonstration that very long worm-like micelles appear to circulate longer than any synthetic carrier described to date and also that worm-like 'filomicelles' more effectively shrink tumors than the smaller carriers [35].

References

[1] F. Ahmed, D.E. Discher, Self-porating polymersomes of PEG-PLA, PEG-PCL: hydrolysis-triggered controlled release vesicles, Journal of Controlled Release 96 (1) (2004) 37–53.

[2] F. Ahmed, A. Hategan, D.E. Discher, B.M. Discher, "Block copolymer assemblies with cross-link stabilization: from single-component monolayers to bilayer blends with PEO-PLA, Langmuir 19 (16) (2003) 6505–6511.

[3] F. Ahmed, R.I. Pakunlu, A. Brannan, F. Bates, T. Minko, D.E. Discher, Biodegradable polymersomes loaded with both paclitaxel and doxorubicin permeate and shrink tumors, inducing apoptosis in proportion to accumulated drug, Journal of Controlled Release 116 (2) (2006) 150–158.

[4] F. Ahmed, R.I. Pakunlu, G. Srinivas, A. Brannan, F. Bates, M.L. Klein, T. Minko, D.E. Discher, Shrinkage of a rapidly growing tumor by drug-loaded polymersomes: pH-triggered release through copolymer degradation, Molecular Pharmaceutics 3 (3) (2006) 340–350.

[5] M. Antonietti, S. Forster, Vesicles and liposomes: a self-assembly principle beyond lipids, Advanced Materials 15 (16) (2003) 1323–1333.

[6] H. Aranda-Espinoza, H. Bermudez, F.S. Bates, D.E. Discher, Electromechanical limits of polymersomes, Physical Review Letters 8720 (2001) 20.

[7] D.R. Arifin, A.F. Palmer, Polymersome encapsulated hemoglobin: a novel type of oxygen carrier, Biomacromolecules 6 (4) (2005) 2172–2181.

[8] J. Babin, J. Rodriguez-Hernandez, S. Lecommandoux, H.A. Klok, M.F. Achard, Self-assembled nanostructures from peptide-synthetic hybrid block copolymers: complex, stimuli-responsive rod-coil architectures, Faraday Discussions 128 (2005) 179–192.

[9] A.D. Bangham, Liposomes - the Babraham connection, Chemistry and Physics of Lipids 64 (1-3) (1993) 275–285.

[10] G. Battaglia, A.J. Ryan, S. Tomas, Polymeric vesicle permeability: a facile chemical assay, Langmuir 22 (11) (2006) 4910–4913.

[11] E.G. Bellomo, M.D. Wyrsta, L. Pakstis, D.J. Pochan, T.J. Deming, Stimuli-responsive polypeptide vesicles by conformation-specific assembly, Nature Materials 3 (4) (2004) 244–248.

[12] H. Bermudez, A.K. Brannan, D.A. Hammer, F.S. Bates, D.E. Discher, Molecular weight dependence of polymersome membrane structure, elasticity, and stability, Macromolecules 35 (21) (2002) 8203–8208.

[13] A. Bonincontro, C. LaMesa, C. Proietti, G. Risuleo, A biophysical investigation on the binding and controlled DNA release in a cetyltrimethylammonium bromide–sodium octyl sulfate cat-anionic vesicle system, Biomacromolecules 8 (6) (2007) 1824–1829.

[14] U. Borchert, U. Lipprandt, M. Bilang, A. Kimpfler, A. Rank, R. Peschka-Suss, R. Schubert, P. Lindner, S. Forster, PH-induced release from P2VP-PEO block copolymer vesicles, Langmuir 22 (13) (2006) 5843–5847.

[15] M.D. Brown, A.I. Gray, L. Tetley, A. Santovena, J. Rene, A.G. Schatzlein, I.F. Uchegbu, In vitro and in vivo gene transfer with poly(amino acid) vesicles, Journal of Controlled Release 93 (2) (2003) 193–211.

[16] M.D. Brown, A. Schatzlein, A. Brownlie, V. Jack, W. Wang, L. Tetley, A.I. Gray, I.F. Uchegbu, Preliminary characterization of novel amino acid based polymeric vesicles as gene and drug delivery agents, Bioconjugate Chemistry 11 (6) (2000) 880–891.

[17] P. Broz, S.M. Benito, C. Saw, P. Burger, H. Heider, M. Pfisterer, S. Marsch, W. Meier, P. Hunziker, Cell targeting by a generic receptor-targeted polymer nanocontainer platform, Journal of Controlled Release 102 (2) (2005) 475–488.

[18] S. Cerritelli, D. Velluto, J.A. Hubbell, PEG-SS-PPS: reduction-sensitive disulfide block copolymer vesicles for intracellular drug delivery, Biomacromolecules 8 (6) (2007) 1966–1972.

[19] F. Checot, S. Lecommandoux, Y. Gnanou, H.A. Klok, Water-soluble stimuli-responsive vesicles from peptide-based diblock copolymers, Angewandte Chemie-International Edition 41 (8) (2002) 1339–1343.

[20] A. Choucair, P.L. Soo, A. Eisenberg, Active loading and tunable release of doxorubicin from block copolymer vesicles, Langmuir 21 (20) (2005) 9308–9313.

[21] N.A. Christian, M.C. Milone, S.S. Ranka, G.Z. Li, P.R. Frail, K.P. Davis, F.S. Bates, M.J. Therien, P.P. Ghoroghchian, C.H. June, D.A. Hammer, Tat-functionalized

near-infrared emissive polymersomes for dendritic cell labeling, Bioconjugate Chemistry 18 (1) (2007) 31–40.

[22] P. Dalhaimer, H. Bermudez, D.E. Discher, Biopolymer mimicry with polymeric wormlike micelles: molecular weight scaled flexibility, locked-in curvatureand coexisting microphases, Journal of Polymer Science Part B-Polymer Physics. 42 (1) (2004) 168–176.

[23] P. Dalhaimer, A.J. Engler, R. Parthasarathy, D.E. Discher, Targeted worm micelles, Biomacromolecules 5 (5) (2004) 1714–1719.

[24] R.S. Dias, B. Lindman, M.G. Miguel, Compaction and decompaction of DNA in the presence of catanionic amphiphile mixtures, Journal of Physical Chemistry B 106 (48) (2002) 12608–12612.

[25] R.S. Dias, B. Lindman, M.G. Miguel, DNA interaction with catanionic vesicles, Journal of Physical Chemistry B 106 (48) (2002) 12600–12607.

[26] R. Dimova, U. Seifert, B. Pouligny, S. Forster, H.G. Dobereiner, Hyperviscous diblock copolymer vesicles, European Physical Journal E 7 (3) (2002) 241–250.

[27] B.M. Discher, Y.Y. Won, D.S. Ege, J.C.M. Lee, F.S. Bates, D.E. Discher, D.A. Hammer, Polymersomes: tough vesicles made from diblock copolymers, Science 284 (5417) (1999) 1143–1146.

[28] D.E. Discher, A. Eisenberg, Polymer vesicles, Science 297 (5583) (2002) 967–973.

[29] D.E. Discher, P.J. Photos, F. Ahmed, R. Parthasarathy, F.S. Bates, Polymersomes: a new platform for drug targeting, in: V.R. Muzykantov, V.P. Torchilin (Eds.), Biomedical Aspects of Drug Targeting, Kluwer Academic Pub., Boston, 2002, pp. 459–471.

[30] C.M. Dong, X.L. Sun, K.M. Faucher, R.P. Apkarian, E.L. Chaikof, Synthesis and characterization of glycopolymer–polypeptide triblock copolymers, Biomacromolecules 5 (1) (2004) 224–231.

[31] J.Z. Du, Y.P. Tang, A.L. Lewis, S.P. Armes, pH-sensitive vesicles based on a biocompatible zwitterionic diblock copolymer, Journal of the American Chemical Society 127 (51) (2005) 17982–17983.

[32] Z.X. Du, J.T. Xu, Z.Q. Fan, Micellar morphologies of poly(epsilon-caprolactone)-b-poly(ethylene oxide) block copolymers in water with a crystalline core, Macromolecules 40 (21) (2007) 7633–7637.

[33] Y. Geng, F. Ahmed, N. Bhasin, D.E. Discher, Visualizing worm micelle dynamics and phase transitions of a charged diblock copolymer in water, Journal of Physical Chemistry B 109 (9) (2005) 3772–3779.

[34] Y. Geng, D.E. Discher, Hydrolytic degradation of poly(ethylene oxide)-block-polycaprolactone worm micelles, Journal of the American Chemical Society 127 (37) (2005) 12780–12781.

[35] Y. Geng, P. Dalhaimer, S.S. Cai, R. Tsai, M. Tewari, T. Minko, D.E. Discher, Shape effects of filaments versus spherical particles in flow and drug delivery, Nature Nanotechnology 2 (4) (2007) 249–255.

[36] P.P. Ghoroghchian, G.Z. Li, D.H. Levine, K.P. Davis, F.S. Bates, D.A. Hammer, M.J. Therien, Bioresorbable vesicles formed through spontaneous self-assembly of amphiphilic poly(ethylene oxide)-block-polycaprolactone, Macromolecules 39 (5) (2006) 1673–1675.

[37] A. Graff, M. Sauer, P. Van Gelder, W. Meier, Virus-assisted loading of polymer nanocontainer, Proceedings of the National Academy of Sciences of the United States of America 99 (8) (2002) 5064–5068.

[38] X. Guo, F.C. Szoka, Chemical approaches to triggerable lipid vesicles for drug and gene delivery, Accounts of Chemical Research 36 (5) (2003) 335–341.

[39] B. Gupta, T.S. Levchenko, V.P. Torchilin, Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides, Advanced Drug Delivery Reviews 57 (4) (2005) 637–651.

[40] J.M. Harris, R.B. Chess, Effect of pegylation on pharmaceuticals, Nature Reviews Drug Discovery 2 (3) (2003) 214–221.

[41] D. Ho, B. Chu, H. Lee, C.D. Montemagno, Protein-driven energy transduction across polymeric biomembranes, Nanotechnology 15 (8) (2004) 1084–1094.

[42] E.P. Holowka, D.J. Pochan, T.J. Deming, Charged polypeptide vesicles with controllable diameter, Journal of the American Chemical Society 127 (35) (2005) 12423–12428.

[43] E.P. Holowka, V.Z. Sun, D.T. Kamei, T.J. Deming, Polyarginine segments in block copolypeptides drive both vesicular assembly and intracellular delivery, Nature Materials 6 (1) (2007) 52–57.

[44] J.N. Israelachvili, Intermolecular and Surface Forces, Academic Press, London; San Diego, 1991.

[45] S. Jain, F.S. Bates, On the origins of morphological complexity in block copolymer surfactants, Science 300 (5618) (2003) 460–464.

[46] S. Jain, F.S. Bates, Consequences of nonergodicity in aqueous binary PEO-PB micellar dispersions, Macromolecules 37 (4) (2004) 1511–1523.

[47] Y.G. Jiang, Y.P. Wang, N. Ma, Z.Q. Wang, M. Smet, X. Zhang, Reversible self-organization of a UV-responsive PEG-terminated malachite green derivative: vesicle formation and photoinduced disassembly, Langmuir 23 (7) (2007) 4029–4034.

[48] R.A. Jones, C.Y. Cheung, F.E. Black, J.K. Zia, P.S. Stayton, A.S. Hoffman, M.R. Wilson, Poly(2-alkylacrylic acid) polymers deliver molecules to the cytosol by pH-sensitive disruption of endosomal vesicles, Biochemical Journal 372 (2003) 65–75.

[49] E.W. Kaler, A.K. Murthy, B.E. Rodriguez, J.A.N. Zasadzinski, Spontaneous vesicle formation in aqueous mixtures of single-tailed surfactants, Science 245 (4924) (1989) 1371–1374.

[50] M. Katakam, L.N. Bell, A.K. Banga, Effect of surfactants on the physical stability of recombinant human growth-hormone, Journal of Pharmaceutical Sciences 84 (6) (1995) 713–716.

[51] Y. Kim, M. Tewari, J.D. Pajerowski, S.S. Cai, S. Sen, J. Williams, S. Sirsi, G. Lutz, D.E. Discher, Polymersome delivery of RNAi and antisense oligonucleotides, Journal of Controlled Release (2008), accepted for publication.

[52] A. Kishimura, A. Koide, K. Osada, Y. Yamasaki, K. Kataoka, Encapsulation of myoglobin in PEGylated polyion complex vesicles made from a pair of oppositely charged block lonomers: a physiologically available oxygen carrier, Angewandte Chemie-International Edition 46 (32) (2007) 6085–6088.

[53] A. Koide, A. Kishimura, K. Osada, W.D. Jang, Y. Yamasaki, K. Kataoka, Semipermeable polymer vesicle (PICsome) self-assembled in aqueous medium from a pair of oppositely charged block copolymers: physiologically stable micro-/nanocontainers of water-soluble macromolecules, Journal of the American Chemical Society 128 (18) (2006) 5988–5989.

[54] A.V. Korobko, C. Backendorf, J.R.C. van der Maarel, Plasmid DNA encapsulation within cationic diblock copolymer vesicles for gene delivery, Journal of Physical Chemistry B 110 (30) (2006) 14550–14556.

[55] A.V. Korobko, W. Jesse, J.R.C. van der Maarel, Encapsulation of DNA by cationic diblock copolymer vesicles, Langmuir 21 (1) (2005) 34–42.

[56] A. Kros, J.A. Jansen, S.J. Holder, R.J.M. Nolte, N. Sommerdijk, Silane-based hybrids for biomedical applications, Journal of Adhesion Science and Technology 16 (2) (2002) 143–155.

[57] H. Kukula, H. Schlaad, M. Antonietti, S. Forster, 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 (8) (2002) 1658–1663.

[58] D. Lasic, D. Papahadjopoulos, Medical Applications of Liposomes, Elsevier, Amsterdam; New York, 1998.

[59] J.C.M. Lee, H. Bermudez, B.M. Discher, M.A. Sheehan, Y.Y. Won, F.S. Bates, D.E. Discher, Preparation, stability, and in vitro performance of vesicles made with diblock copolymers, Biotechnology and Bioengineering 73 (2) (2001) 135–145.

[60] J.C.M. Lee, M. Santore, F.S. Bates, D.E. Discher, From membranes to melts, rouse to reptation: diffusion in polymersome versus lipid bilayers, Macromolecules 35 (2) (2002) 323–326.

[61] S.L. Li, B. Byrne, J. Welsh, A.F. Palmer, Self-assembled poly(butadiene)-b-poly(ethylene oxide) polymersomes as paclitaxel carriers, Biotechnology Progress 23 (1) (2007) 278–285.

[62] Y. Li, B.S. Lokitz, C.L. McCormick, Thermally responsive vesicles, their structural "locking" through polyelectrolyte complex formation, Angewandte Chemie-International Edition 45 (35) (2006) 5792–5795.

[63] J.J. Lin, P. Ghoroghchian, Y. Zhang, D.A. Hammer, Adhesion of antibody-functionalized polymersomes, Langmuir 22 (9) (2006) 3975–3979.

[64] J.J. Lin, J.A. Silas, H. Bermudez, V.T. Milam, F.S. Bates, D.A. Hammer, The effect of polymer chain length and surface density on the adhesiveness of functionalized polymersomes, Langmuir 20 (13) (2004) 5493–5500.

[65] V.S.Y. Lin, S.G. Dimagno, M.J. Therien, Highly conjugated, acetilenyl bridged porphyrins – new models for light-harvesting antenna systems, Science 264 (5162) (1994) 1105–1111.

[66] D.X. Liu, F. Liu, Y.K. Song, Recognition and clearance of liposomes containing phosphatidylserine are mediated by serum opsonin, Biochimica Et Biophysica Acta-Biomembranes 1235 (1) (1995) 140–146.

[67] F.T. Liu, A. Eisenberg, Preparation and pH triggered inversion of vesicles from poly(acrylic acid)-block-polystyrene-block-poly(4-vinyl pyridine), Journal of the American Chemical Society 125 (49) (2003) 15059–15064.

[68] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review, Journal of Controlled Release. 65 (1-2) (2000) 271–284.

[69] W. Meier, C. Nardin, M. Winterhalter, Reconstitution of channel proteins in (polymerized) ABA triblock copolymer membranes, Angewandte Chemie-International Edition 39 (24) (2000) 4599.

[70] S.M. Mel'nikov, R. Dias, Y.S. Mel'nikova, E.F. Marques, M.G. Miguel, B. Lindman, DNA conformational dynamics in the presence of catanionic mixtures, FEBS Letters 453 (1–2) (1999) 113–118.

[71] F.H. Meng, G.H.M. Engbers, J. Feijen, Biodegradable polymersomes as a basis for artificial cells: encapsulation, release and targeting, Journal of Controlled Release 101 (1–3) (2005) 187–198.

[72] J. Nam, M.M. Santore, Adhesion plaque formation dynamics between polymer vesicles in the limit of highly concentrated binding sites, Langmuir 23 (13) (2007) 7216–7224.

[73] J. Nam, M.M. Santore, The adhesion kinetics of sticky vesicles in tension: the distinction between spreading and receptor binding, Langmuir 23 (21) (2007) 10650–10660.

[74] A. Napoli, M.J. Boerakker, N. Tirelli, R.J.M. Nolte, N. Sommerdijk, J.A. Hubbell, Glucose-oxidase based self-destructing polymeric vesicles, Langmuir 20 (9) (2004) 3487–3491.

[75] A. Napoli, M. Valentini, N. Tirelli, M. Muller, J.A. Hubbell, Oxidation-responsive polymeric vesicles, Nature Materials 3 (3) (2004) 183–189.

[76] C. Nardin, T. Hirt, J. Leukel, W. Meier, Polymerized ABA triblock copolymer vesicles, Langmuir 16 (3) (2000) 1035–1041.

[77] C. Nardin, S. Thoeni, J. Widmer, M. Winterhalter, W. Meier, Nanoreactors based on (polymerized) ABA-triblock copolymer vesicles, Chemical Communications 15 (2000) 1433–1434.

[78] C. Nardin, J. Widmer, M. Winterhalter, W. Meier, Amphiphilic block copolymer nanocontainers as bioreactors, European Physical Journal E 4 (4) (2001) 403–410.

[79] V. Ortiz, S.O. Nielsen, D.E. Discher, M.L. Klein, R. Lipowsky, J. Shillcock, Dissipative particle dynamics simulations of polymersomes, Journal of Physical Chemistry B 109 (37) (2005) 17708–17714.

[80] S. Parveen, S.K. Sahoo, Nanomedicine – clinical applications of polyethylene glycol conjugated proteins and drugs, Clinical Pharmacokinetics 45 (10) (2006) 965–988.

[81] C. Perez, I.J. Castellanos, H.R. Constantino, W. Al-Azzam, K. Griebenow, Recent trends in stabilizing protein structure upon encapsulation and release from bioerodible polymers, Journal of Pharmacy and Pharmacology 54 (3) (2002) 301–313.

[82] P.J. Photos, L. Bacakova, B. Discher, F.S. Bates, D.E. Discher, Polymer vesicles in vivo: correlations with PEG molecular weight, Journal of Controlled Release 90 (3) (2003) 323–334.

[83] S.H. Qin, Y. Geng, D.E. Discher, S. Yang, Temperature-controlled assembly and release from polymer vesicles of poly(ethylene oxide)-block-poly(N-isopropylacrylamide), Advanced Materials 18 (21) (2006) 2905.

[84] C.J.F. Rijcken, O. Soga, W.E. Hennink, C.F. van Nostrum, Triggered destabilisation of polymeric micelles and vesicles by changing polymers polarity: an attractive tool for drug delivery, Journal of Controlled Release 120 (3) (2007) 131–148.

[85] J. Rodriguez-Hernandez, S. Lecommandoux, Reversible inside-out micellization of pH-responsive and water-soluble vesicles based on polypeptide diblock copolymers, Journal of the American Chemical Society 127 (7) (2005) 2026–2027.

[86] G.Z. Rong, M.X. Deng, C. Deng, Z.H. Tang, L.H. Piao, X.S. Chen, X.B. Jing, Synthesis of poly(epsilon-caprolactone)-b-poly(gamma-benzyl-L-glutamic acid) block copolymer using amino organic calcium catalyst, Biomacromolecules 4 (6) (2003) 1800–1804.

[87] M. Rosa, M.D. Miguel, B. Lindman, DNA encapsulation by biocompatible catanionic vesicles, Journal of Colloid and Interface Science 312 (1) (2007) 87–97.

[88] M. Rosa, M.D. Moran, M.D. Miguel, B. Lindman, The association of DNA and stable catanionic amino acid-based vesicles, Colloids and Surfaces a-Physicochemical and Engineering Aspects 301 (1-3) (2007) 361–375.

[89] G. Srinivas, D.E. Discher, M.L. Klein, Self-assembly and properties of diblock copolymers by coarse-grain molecular dynamics, Nature Materials 3 (9) (2004) 638–644.

[90] G. Srinivas, D.E. Discher, M.L. Klein, Key roles for chain flexibility in block copolymer membranes that contain pores or make tubes, Nano Letters 5 (12) (2005) 2343–2349.

[91] G. Srinivas, J.C. Shelley, S.O. Nielsen, D.E. Discher, M.L. Klein, Simulation of diblock copolymer self-assembly, using a coarse-grain model, Journal of Physical Chemistry B 108 (24) (2004) 8153–8160.

[92] V.P. Torchilin, T.S. Levchenko, A.N. Lukyanov, B.A. Khaw, A.L. Klibanov, R. Rammohan, G.P. Samokhin, K.R. Whiteman, p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups, Biochimica Et Biophysica Acta-Biomembranes 1511 (2) (2001) 397–411.

[93] A. Wittemann, T. Azzam, A. Eisenberg, Biocompatible polymer vesicles from biamphiphilic triblock copolymers and their interaction with bovine serum albumin, Langmuir 23 (4) (2007) 2224–2230.

[94] Y.Y. Won, A.K. Brannan, H.T. Davis, F.S. Bates, Cryogenic transmission electron microscopy (cryo-TEM) of micelles and vesicles formed in water by polyethylene oxide)-based block copolymers, Journal of Physical Chemistry B 106 (13) (2002) 3354–3364.

[95] Y.Y. Won, H.T. Davis, F.S. Bates, Giant wormlike rubber micelles, Science 283 (5404) (1999) 960–963.

[96] D. Wong, T.J. Jeon, J. Schmidt, Single molecule measurements of channel proteins incorporated into biomimetic polymer membranes, Nanotechnology 17 (15) (2006) 3710–3717.

[97] L.F. Zhang, K. Yu, A. Eisenberg, Ion-induced morphological changes in "crew-cut" aggregates of amphiphilic block copolymers, Science 272 (5269) (1996) 1777–1779.

[98] J.A. Zupancich, F.S. Bates, M.A. Hillmyer, Aqueous dispersions of poly(ethylene oxide)-b-poly(gamma-methyl-epsilon-caprolactone) block copolymers, Macromolecules 39 (13) (2006) 4286–4288.