Biohybrid Polymer Capsules

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1. Introduction

Compartmentalization is one of the most powerful tools in nature's arsenal to structure metabolic and signaling pathways. Many of the catalytic reactions taking place in cells comprise multiple reaction steps where a compound is processed to a final product via a series of enzymes acting in a predetermined order.[1] It is compartmentalization that helps a cell to enable a high level of control over enzyme reaction order.[2] In addition, compartmentalization may help protect the cell against its harmful contents, as is the case with lysosomes or, on a smaller scale, proteasomes.[3] Compartments can also serve as scaffolds, e.g. for the precise decoration with biomolecules, which can act as recognition elements on the surface, as catalysts in the interior of the compartment, and as selective channels in the compartment's membrane, as found for the complex protein translocation machinery in the endoplasmatic reticulum.[4] The coupling of reactions in space and time as observed in nature is of high interest to chemists, as it may help develop catalytic systems that display increased efficiencies in chemical conversions on scales that range from the laboratory bench to those of large industrial plants.[5] Currently, entire cells are being used in industry as microfactories to produce a variety of products on a large scale, underlining their ongoing use as the most advanced functional capsules to date.

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Stijn F. M. van Dongen (group, center) was born in Goirle, The Netherlands, and studied chemistry at the Radboud University Nijmegen. He received his master's degree in 2006 after traineeships in the physical organic chemistry group of Prof. R. J. M. Nolte and the synthetic biology group of Prof. D. M. Hilvert at the ETH in Zürich, Switzerland. He is currently a Ph.D. student in the group of Profs.' R. J. M. Nolte and J. C. M. van Hest, working on the exploration of polymersomes as nanoreactors in a biological setting.

Hans-Peter M. de Hoog (group, second from left) was born in Arnhem, The Netherlands, and graduated in chemistry at Utrecht University in 1998, specializing in the analysis of complex biomolecules. After a short stay at The Netherlands Organisation for Applied Scientific Research (TNO), he moved to the Radboud University Nijmegen in 2005 to pursue a Ph.D. in supramolecular and physical organic chemistry in the group of Prof. R. J. M. Nolte and J. J. L. M. Cornelissen. His research involves a collaborative project with Delft University of Technology (Prof. I. W. C. E. Arends) on the application-driven encapsulation of enzymes in polymersomes.

Ruud J. R. W. Peters (group, right) obtained his bachelor's degree in molecular life sciences at the Radboud University Nijmegen in 2008. He is currently performing his master's research on the interactions between polymersomes and biological systems in the groups of Prof. J. C. M. van Hest and Prof. R. J. M. Nolte.

Roeland J. M. Nolte (group, second from right) received his Ph.D. in physical organic chemistry from the University of Utrecht (1973), where he stayed and became assistant professor and then associate professor. In 1981, he was a visiting scientist at UCLA in the group of Prof. Donald J. Cram. In 1987, he moved to the Radboud University Nijmegen and became a full professor of organic chemistry, and since 1994 he has also been a part-time professor of supramolecular chemistry at the Eindhoven University of Technology. In 2003, he was awarded the first Royal Netherlands academy of arts and science chair in chemistry. He is currently the director of the Institute for Molecules and Materials at the Radboud University. His research interests span a broad range of topics at the interfaces of supramolecular chemistry, macromolecular chemistry, and biomimetic chemistry. In his work, he focuses on the design of catalysts and (macro)molecular materials. He and his group have published ca. 600 scientific papers.

Jan C. M. van Hest (group, left) conducted his doctoral research on molecular architectures based on dendrimers at the Eindhoven University of Technology under the supervision of Prof. Bert Meijer, for which the Ph.D. title was granted in 1996. As a postdoctoral researcher he investigated the possibilities of protein engineering for the preparation of materials under the supervision of Prof. D. A. Tirrell, at the University of Massachusetts at Amherst. In 1997 he then joined the chemical company DSM, where he worked as a research scientist and later on as group leader on the development of innovative material concepts. In 2000 he was appointed as a full professor at the Radboud University Nijmegen to set up a new group in bio-organic chemistry. His current research efforts are aimed at developing bioinspired materials and processes in order to combine the functionality of biological systems with the flexibility and robustness of synthetic structures, using a variety of synthetic techniques, such as protein engineering, peptide synthesis, and controlled polymerization methods.

Artificial nano- and microcapsules that seek to mimic their natural counterparts can be constructed in different ways, leading to a variety of properties, as will be discussed in this review.[6−8] Enzymatic conversions can take place in the lumen of such capsules, and their membranes can be used to confine and tune reaction pathways. Synthetic capsules are also attracting a lot of attention because of their promising applications in the controlled release of pharmaceuticals. Capsules that bear recognition elements have been targeted to specific tissues or organs, providing a desirable vehicle for the aforementioned release of drugs.[9] For a chemist, the successful exploitation of capsules begins with their tailor-made design and synthesis, for which cells and their organelles are the primary source of inspiration. In order to be able to do so, one needs insight into the design principles of nature to endow function to a molecule and to direct its self-assembly to a preset architecture. Although spectacular progress has been made in the field of bioinspired self-assembly,[10−13] unfortunately, the construction of an artificial cell is still not much more than a fantasy. Fortunately, more simple systems such as micelles, vesicles, and other assemblies of molecules may already partly solve the problem by providing a capsule that can be geared toward a desired application, e.g. the controlled release of drugs, as was demonstrated in the literature already quite a long time ago.[14−16] The view of life as being the result of a nanoscale phenomenon[17] is of more recent date and should rouse the interest in capsules for any chemist.

Madhavan Nallani obtained his bachelor in chemical engineering at the University of Madras, India. After one year of employment at Berger Paints Ltd., he pursued higher education, obtaining a masters degree in process engineering at the Technical University of Hamburg, Germany. His Ph.D. on employing membrane proteins and self-assembled polymer vesicles as nanoreactors was supervised by Prof. Ulrich Schwaneberg at the Jacobs University in Bremen, Germany, in collaboration with Prof. Wolfgang Meier, University of Basel, Switzerland. After a postdoctoral stay in the groups of Profs. R. J. M. Nolte and J. C. M. van Hest at the Radboud University Nijmegen to gain in-depth knowledge of block copolymer assemblies, he moved to the Institute of Materials Research and Engineering (IMRE) in Singapore to work on self-assembled biomembranes.

The aim of this review is to give an overview of the wide range of polymer-based capsules that have been constructed from synthetic and biological building blocks or from biological building blocks that are taken out of their natural environment, i.e. biohybrid capsules, using both hyperbranched and self-assembly approaches. We have focused in our review on developments in the past decade, covering those fields of organic and macromolecular chemistry that have been influenced by biology. That is, all research discussed in this review concerns either biohybrid polymer building blocks or applications in a biomedical setting. This can vary from the use of amino acids or carbohydrates in the synthesis of polymer building blocks for capsules to the in vivo targeting of tissue using an otherwise nonbiohybrid capsule. In this connection, all relevant articles are highlighted, including the papers from earlier work that have led to the foundation of this field. The capsules that are discussed can be considered as the simplest mimics of an organelle or cell and contain a cavity in which chemical reactions can take place or cargo can be stored. Our primary focus is on the various architectures of these polymer capsules and their functional exploitation. Not included is the vast area of liposomes,[18−22] i.e. hollow vesicles of phospholipids containing a bilayer membrane. Neither covered are capsules solely based on proteins,[23] such as the emerging field of viral capsids and their applications,[24,25] or the microspheres that are formed by precipitation polymerization or polymerization-induced phase separation.[26−28] The review is divided into several sections which cover the development of tailor-made capsules, starting from unimolecular dendrimer-based containers, and further expanding into large compartment-containing objects. The different architectures and applications of the dendrimer capsules are presented in section 2. Section 3 covers the construction of polymer micelles from amphiphilic block copolymers, whereas section 4 deals with the assembly of polyelectrolytes to give layer-by-layer capsules. Finally, in section 5 a survey of the developments in the field of polymersomes, i.e. hollow polymer-based vesicles formed through self-assembly, will be given.

2. Dendrimers

Dendrimers are hyperbranched molecules, emanating from a single multivalent core moiety and extending radially outward in a highly symmetric fashion. Vögtle and coworkers reported the synthesis of what might be called a first dendrimer some thirty years ago,[29] although Tomalia and Newkome were the first to identify this class of compounds.[30,31] Since then, the field has grown exponentially.[32−35] Due to their versatility, dendrimers have drawn increasing attention for their applicability as delivery vessels,[36,37] scaffolds for catalytic compounds,[38] and carriers of imaging agents[39] and therapeutically active compounds,[40,41] among others. In the past, the broad range of dendrimer applications has already been extensively reviewed.[42−58] Because dendrimers develop a distinct core−shell architecture, leading to the formation of internal cavities, they can be regarded as cross-linked micellar architectures or monomolecular capsules, and as such they will be discussed in this chapter. This section will therefore highlight the developments in the field of dendrimers, explicitly related to biohybrid dendrimers and their in vivo applications.

2.1. Synthesis of (Biohybrid) Dendrimers

Dendritic molecules are characterized by a layer by layer architecture, which provides a unique structural control over their properties. Synthesis proceeds by an iterative procedure of repeating reaction steps in which every completed cycle adds a new generation to the dendrimer. Two common paths can lead to the construction of a dendrimer, namely the **divergent** and the **convergent** approach (Figure 1).

The divergent approach was pioneered by Newkome,[30] Tomalia,[31,59] and, later on, Meijer.[36] This method features a layer by layer, polymerization-like synthesis starting from the core, continuing outward to form a characteristic treelike structure. The ease of synthesis via this divergent approach allows for the large scale production of dendrimers, of which the most commonly used ones, such as poly(amido amine) (PAMAM) and poly(propylene imine)(PPI) dendrimers (Figure 2), are even commercially available. Later, the group of Fréchet introduced a more synthetically controllable method, the convergent approach, in which branches are synthesized separately, starting from the peripheral moieties, working inward toward a focal point, after which the wedges are connected to a core molecule.[60,61] Other convergently acquired structures can be obtained by self-assembly of separately synthesized dendrons.[62,63] A major advantage of convergently synthesized dendrimers compared to divergent ones is the possibility of purification prior to dendron linkage to the core. Divergently synthesized dendrimers are more susceptible to inconsistencies due to incomplete reaction sequences, since early mistakes in the structure are amplified throughout the synthetic procedure.

For both methods, their synthesis provides a unique degree of control over the eventual structure, providing very low polydispersities and low rates of defects in the structure.[32] This feature gives dendrimers an advantage above other types of polymers used in biological systems, since polydispersity is often a problem in applying linear polymers in vivo.

There is a wide range of monomers available, since nearly any coupling reaction is applicable, and therefore many compounds can be used. The only requirement is that an AB_x type monomer is used, with $x \geq 2$, to allow for branching of the structure. Yet PAMAM and PPI dendrimers,

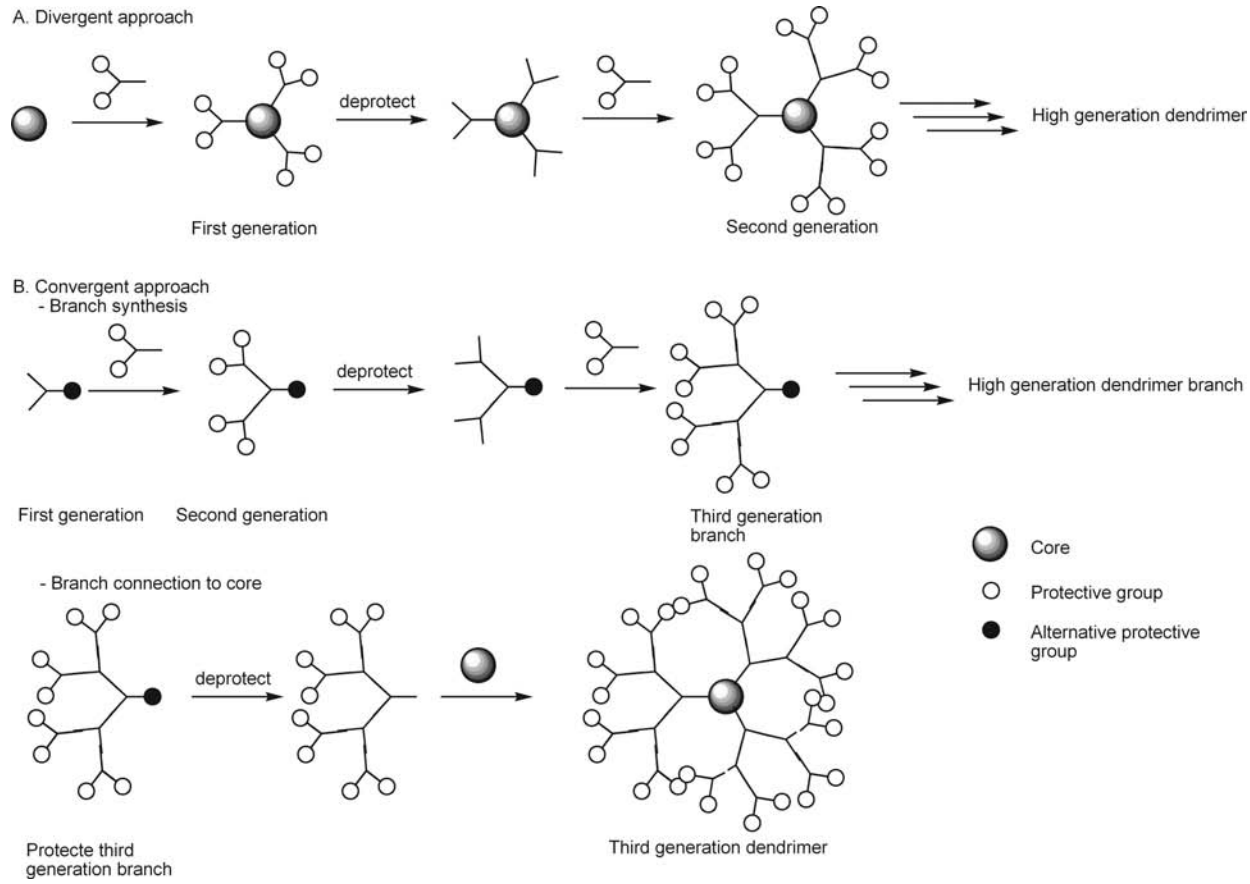


Figure 1. (A) Divergent approach to dendrimer synthesis; the dendrimer grows outward from the core molecule. (B) Convergent approach; separately synthesized dendrimer branches are coupled to a core.

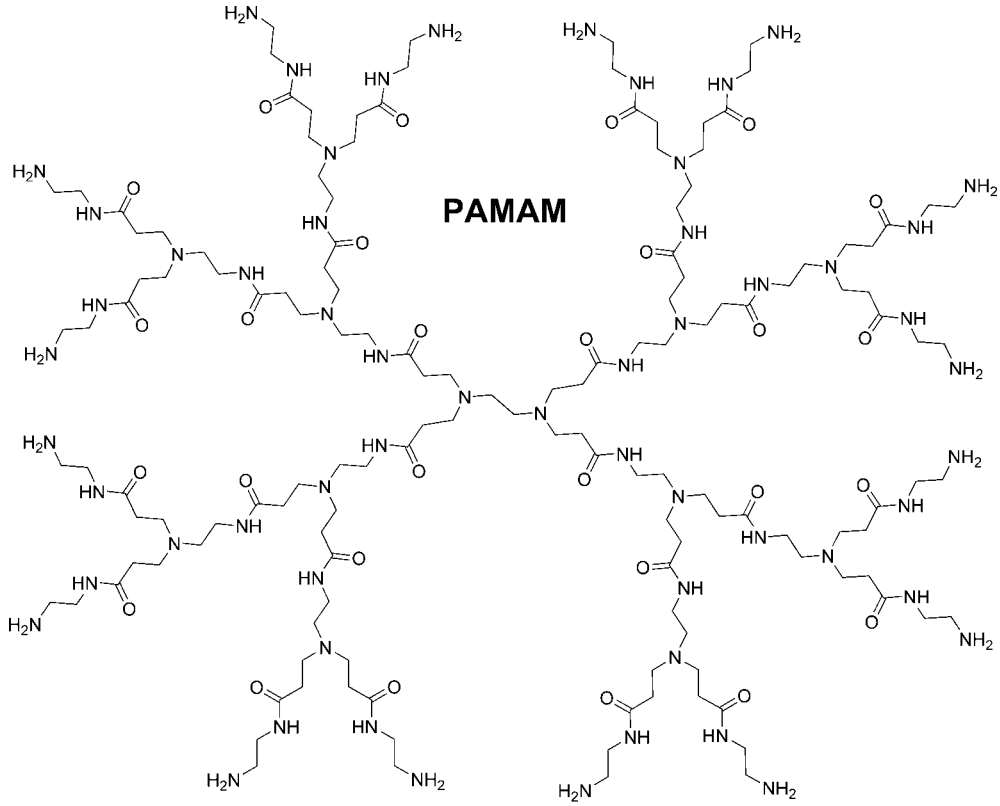
often only peripherally altered, constitute the bulk of dendrimer species found in the field, since they are commercially available and easily functionalized. A simple amide coupling can be used to functionalize the terminal groups of both PAMAM and PPI dendrimers. Virtually any functionality can be added, leading to various different applications, of which many examples will be discussed in the coming sections. Peptides are also quite common constituents of dendrimers, yet only an AB_2 amino acid, like lysine or glutamic acid, can be used to achieve the branched dendritic structure. The poly(L-lysine) dendrimers, pioneered by Denkewalter,[64] are one of the most commonly used peptide dendrimers. Applying such natural building blocks as monomers can promote biocompatibility and degradability of the dendrimer.

Another, more specific class of biohybrid dendrimers which have been extensively studied are dendrimers composed of, complexed to, or functionalized with DNA. These materials have found interest as tools in biosensing, because the branched structure offers increased sensitivity and signal amplification due to the availability of multivalent interactions. This field was recently reviewed by Caminade et al.[65] DNA dendrimers are particularly useful, since they can contain customizable terminal oligonucleotide sequences. Because DNA dendrimers can be applied to hybridize with a cDNA strand in a high affinity manner which can be easily detected, they can be conveniently applied in the diagnosis of pathogenic or genetic diseases. In an early example by Wang et al.,[66] DNA dendrimers were immobilized on a quartz crystal microbalance, increasing their hybridization capacity, and yielded a 10-fold increase in detection sensitivity of single stranded DNA. This setup enabled direct monitoring of the hybridization kinetics by means of mass-sensitive piezoelectric transducers.[66]

Li et al.[67] reported the formation of Y-shaped dendrimers, by assembly of three partly cDNA strands. By lengthening the DNA strands with other complementary sequences, a dendritic shape with multiple generations was accomplished (Figure 3). Also other dendrimer-like DNA complexes were derived by similar self-assembly procedures.[68] An even more intriguing method to obtain DNA dendrimers was reported by Yin et al.,[69] who describe the triggered self-assembly of DNA strands into higher generation dendrimer patterns (up to 5 generations) and other interesting architectures. The applied method allows for the design of molecularly preprogrammed assembly pathways of DNA-hairpin motifs. An initiator strand was used to trigger the disassembly of multiple "inert" DNA hairpin monomers. This caused their reassembly into dendrimers of predetermined generations by allowing the strands to anneal to complementary parts of other strands (Figure 4). Such structures could find use in DNA immobilization and hybridization techniques.

2.2. (Bio)degradation of Dendrimers

Dendrimers show many promising applications in vivo, yet immune responses or cytotoxic effects of dendrimer accumulation, among others, are still issues that need to be addressed. Both the dendrimer itself and its degradation products should not give rise to cytotoxic effects or immunogenic responses.[47−49] An advantage of the dendrimer structure above other polymeric structures is their highly controlled and generally lower molecular weight and uniform architecture.[50] Small compounds, such as low-generation



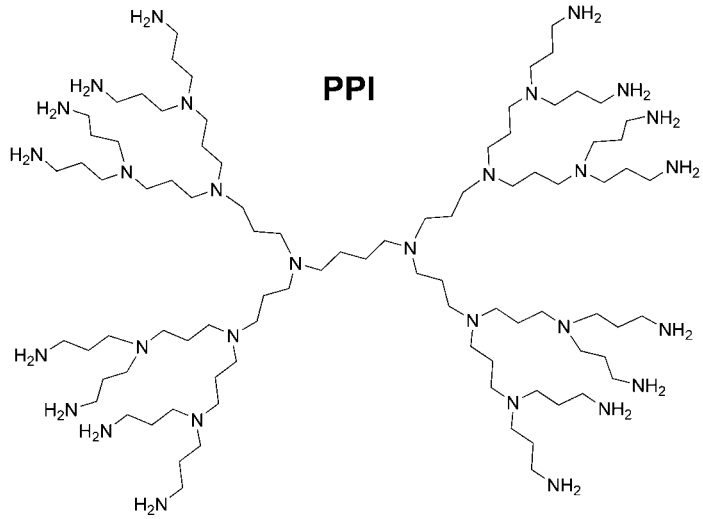


Figure 2. Two of the most commonly used dendrimers, poly(amido amine) (PAMAM, top) and poly(propylene imine) (PPI, bottom).

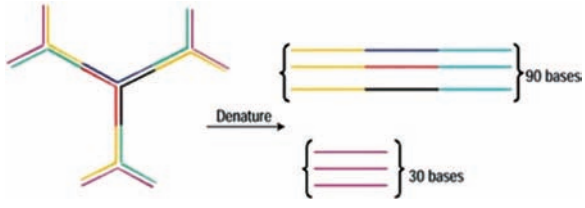


Figure 3. Composition of a self-assembled DNA dendrimer comprised of oligonucleotide strands partially complementary to one another and capped by a short oligonucleotide sequence at the periphery. (Reprinted with permission from Macmillan Publishers Ltd.: *Nature Materials* (ref 191). Copyright 2004.)

dendrimers, generally cause fewer cytotoxic effects, since they are easily removed through renal clearing. Larger compounds, such as polymers and higher generation dendrimers, are not this easily degraded and cleared by this process and can therefore cause unwanted side effects by their prolonged presence.

In order to remain biocompatible, higher-generation dendrimers should therefore be designed to be easily degradable. When accumulation does occur, polymers will be degraded if possible through prolonged exposure to enzymes. These processes can be exploited in the design of dendrimers that are to be subjected to degradation in an organism. The earliest example of biological dendrimer degradation was the enzymatic breakdown of (*R*)-3-hydroxybutanoic acid and trimesic acid dendrimers by Seebach et al. (1996).[70]

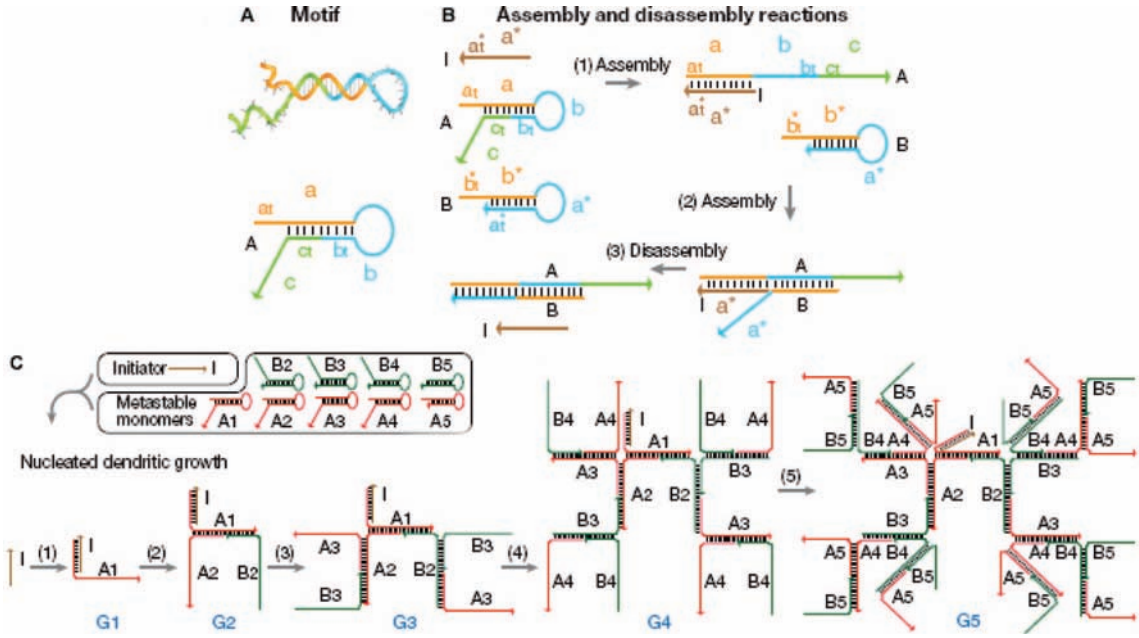


Figure 4. Programmed triggered self-assembly of DNA dendrimers: (A) structure of the short hairpin motif; (B) duplex formation by assembly and disassembly reactions of hairpin sequences; (C) formation of the dendritic secondary structure through a cascade assembly and disassembly reaction, triggered by a single initiator strand. (Reprinted with permission from Macmillan Publishers Ltd.: *Nature* (ref 69). Copyright 2008.)

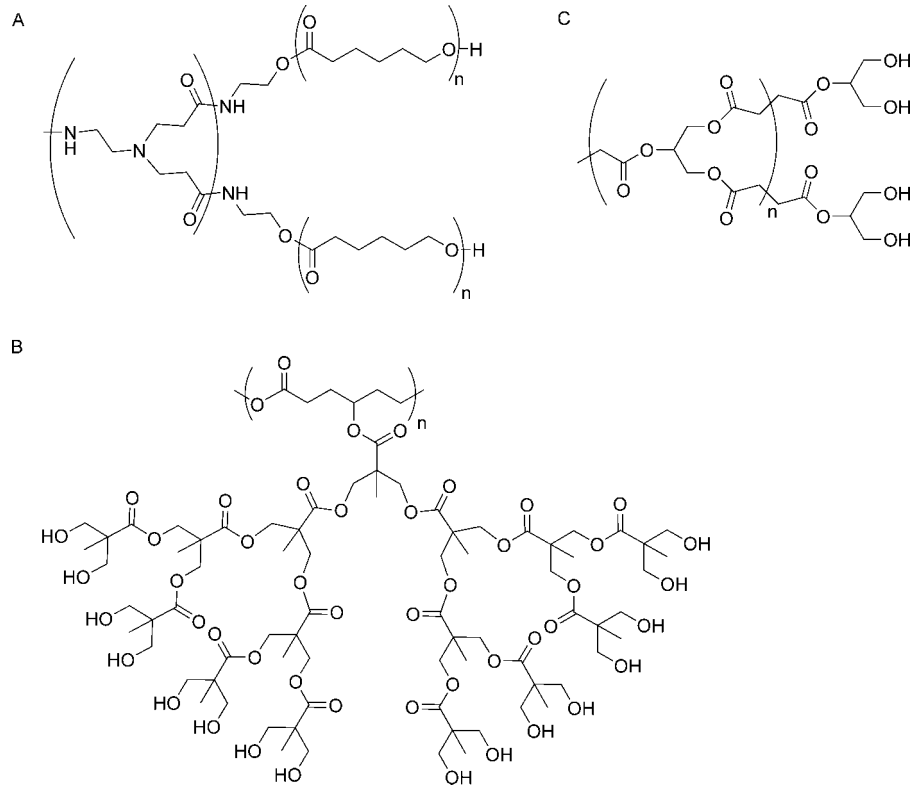


Figure 5. Biodegradable dendrimers: (A) PAMAM dendrimer core functionalized with polycaprolactone tails; (B) polycaprolactone functionalized with polyester dendrons; (C) poly(glycerol-succinic acid) (PGLSA) dendrimer.[71−73]

Enzymes can readily cleave various peptide sequences, making polypeptides suitable candidates to be used in biodegradable dendrimer synthesis, especially amino acid combinations which resemble proteolytic enzyme substrates. Besides peptide targets, enzymes can also degrade other dendrimer building blocks. Caprolactone chains fitted to a PAMAM dendrimer core (Figure 5) were degraded by *Pseudomonas cepacia* lipase and exposed to hydrolytic degradation, to yield the free PAMAM core and degraded caprolactone monomers.[71]

In a similar vein, Lee et al. synthesized polycaprolactone with polyester dendrons (Figure 5) attached and reported their susceptibility to hydrolytic cleavage, showing that these dendritic structures can be degraded at both physiological and basic pH values.[72] Grinstaff and co-workers designed dendrimers which yielded nontoxic degradation products by using natural metabolites or other biocompatible compounds as monomers for dendrimer synthesis, such as glycerol and succininc acid (Figure 5).[73]

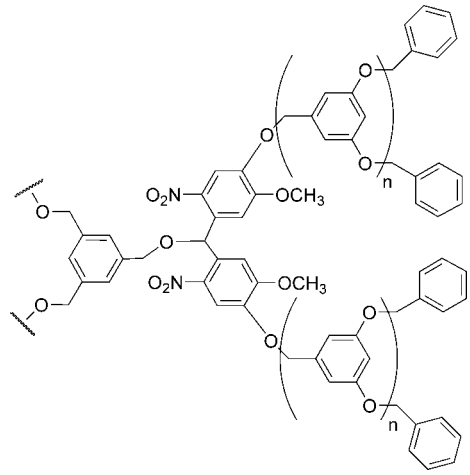


Figure 6. Photolabile, ether-bound *o*-nitrobenzoyl dendrimer core.[75]

Other dendrimers are less susceptible to such forms of degradation, like the commonly used PAMAM dendrimer, the amide backbone of which is quite resistant to hydrolysis in vivo.[74] It was found that these dendrimers only degrade in solution due to solvolysis under reflux conditions, which are considerably harsher compared to the cellular environment.

Photolytic cleavage is another possibility to break down the compound architecture, although this method is currently not easily applicable in living organisms. Irradiation by UV-light can be used to cleave bonds, and it can be applied as a triggering action for dendrimer decomposition. Cleavage of third generation benzyl aryl ether dendrimers was achieved by the incorporation of *o*-nitrobenzyl moieties (Figure 6) as linkages between the dendrons and the core molecule.[75] Irradiation by UV-light resulted in the disconnection of the dendrons from the core.

Instead of breaking every single bond, other examples of dendrimers have been reported, i.e. ones in which one reaction can trigger the complete decomposition of the molecule into its small constituent molecules. These self-immolative dendrimers can be degraded both via a peripheral reaction as well as via a reaction at the core. The group of McGrath produced two different dendrimers which could disassemble by a single triggering event. The geometric disassembly was achieved first by a linear benzyl ether depolymerization reaction, triggered by allyl deprotection,[76] and second, an amplified complete disassembly of the dendrimer, also triggered by allyl deprotection conditions, was accomplished (Figure 7).[77] Such cascade decomposition reactions can be used to liberate therapeutically active substances from dendrimer structures, a field to which both Shabat and co-workers[78−81] and De Groot et al.[82] have contributed, which will be discussed further in section 2.4.1.

2.3. Dendrimer Toxicity and Pharmacokinetics

Dendrimer cytotoxicity depends mainly on the nature of its peripheral groups. This property has been comprehensively reviewed in the past.[48] Charge is often a key factor, since neutral or negatively charged dendrimers often show little toxicity or hemolysis. Yet for dendrimers with a cationic periphery, such as PPI, poly(ethyleneimine) (PEI), and PAMAM dendrimers, a concentration-dependent and sometimes even generation-dependent effect on toxicity and hemolysis is observed.[83,84] Decreasing the positive charge of the terminal groups can often lead to a decreased toxic effect in vitro, and similar effects have been observed in vivo.

Extensive in vitro toxicity studies were performed by Chen et al.,[85] who synthesized a small library of melamine-based dendrimers with varying terminal groups of a cationic, anionic, or neutral nature, along with a PEGylated one (Figure 8). The dendrimer scaffold consisted of second generation monochlorotriazine dendrons coupled to a piperazine displaying core. They reported increasing hemolytic activity and low cell viability for dendrimers with increasing amounts of positive charges in their periphery, with toxicity also increasing with concentration. Anionic dendrimers displayed only little hemolytic activity, while the PEGylated version displayed none at all. Furthermore, cell viability remained high for both of these types of dendrimers. In vivo testing of the PEGylated dendrimer even showed no toxicity in mice at concentrations up to 2.56 g kg^{-1}.

Surface charge also seems to affect which uptake mechanism is used by the cell to internalize certain types of dendrimers and by which cells they are endocytosed. Though PAMAM dendrimers were endocytosed by fluid-phase endocytosis, irrespective of their surface functionality, other dendrimers showed varying uptake results for different cell lines. It was also shown that neutral and anionic dendrimers localize to the lysosome, while PAMAM dendrimers remained in peripheral vesicles.[86]

Similarly, Fréchet and co-workers synthesized several polyester dendrimers (Figure 9) and incubated these with cells at concentrations up to 5 mg/mL, showing no influence on cell viability. Furthermore, conjugates of these dendrimers with the anticancer drug doxorubicin also showed only little unwanted accumulation in vital organs during biodistribution studies.[87,88]

Extensive studies on the biodegradation and pharmacokinetics of ^{3}H labeled poly(L-lysine) dendrimers (Figure 10) showed a high degree of bioresorption of ^{3}H labeled L-lysine degradation products. No specific localization of the labeled products was found in rats; they were distributed throughout the major organs.[89] Capping the terminal groups of poly(L-lysine) dendrimers with aryl sulfonate groups (Figure 10) was shown to greatly influence the distribution and plasma clearance of these dendrimers. Labeled dendrimers were quickly cleared from the blood, and especially dendrimers capped with larger groups were readily phagocytosed.[90]

Despite their potentially toxic properties in vivo, cationic dendrimers possess very useful properties related to the charge effects of their peripheral moieties, such as the ability to bind DNA or to cross cellular membranes. By masking some of these charged peripheral groups, cationic dendrimers can still be used for biological applications, while the toxic effects are minimized. For example, the surface acetylation of generation two and four PAMAM dendrimers caused a significant 10-fold decrease in cytotoxicity, leading to high cell viability while maintaining the cell membrane permeability of regular PAMAM dendrimers.[91] Similar studies were performed on fifth generation PPI dendrimers, whose peripheral amines were masked by functionalization with glycine, phenylalanine, mannose, or lactose moieties.[92] Also poly(L-lysine) dendrimers functionalized with D-galactose showed a decreased toxic response, compared to the unfunctionalized cationic ones.[93] These studies investigated hemolytic, cytotoxic, and immunogenic properties and again showed decreased but still concentration-related toxicity values for protected forms of the cationic dendrimer.

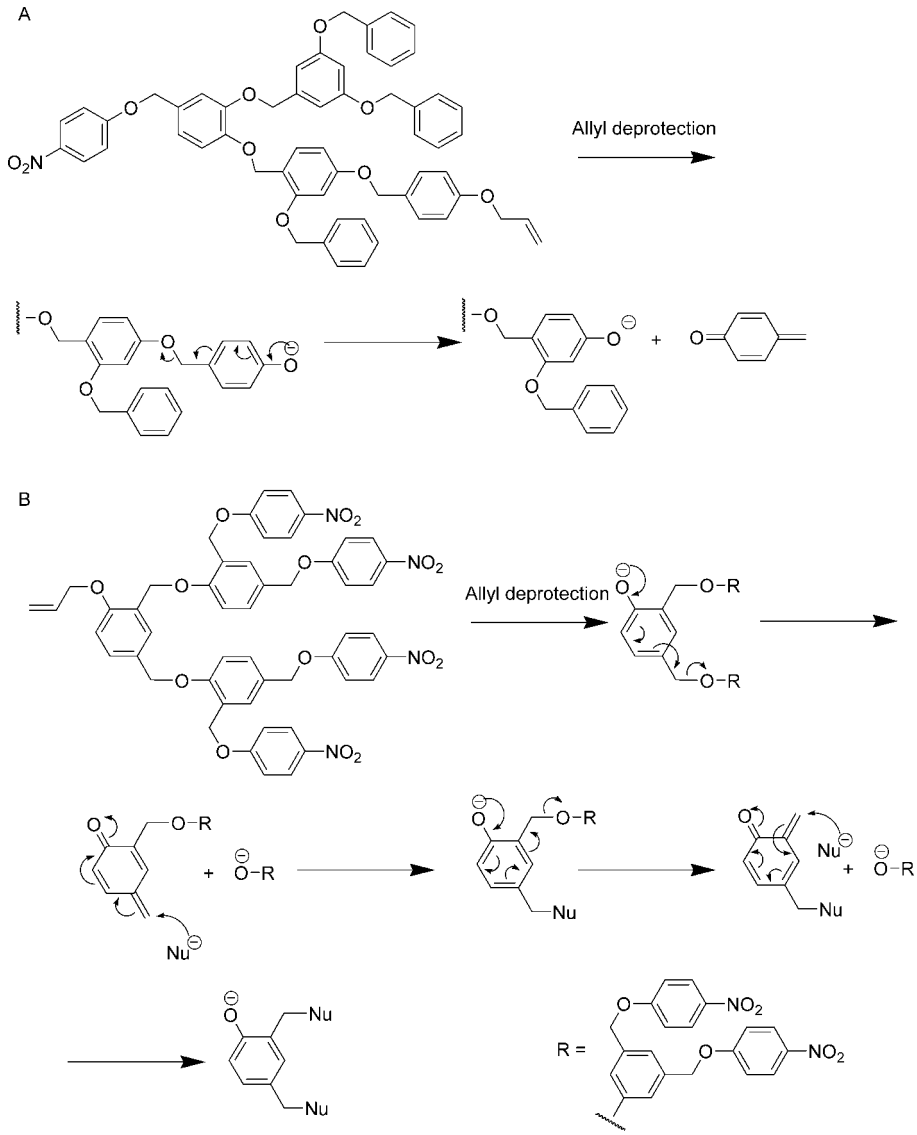


Figure 7. Triggered dendrimer disassembly: (A) linear benzyl ether depolymerization, triggered by an allyl deprotection step; (B) complete dendrimer disassembly, triggered also by allyl deprotection, yet amplified through the ortho and para positioning of the substituents compared to the initially protected oxygen. The presence of a nucleophile during the allyl deprotection enables the amplification reaction.[76,77]

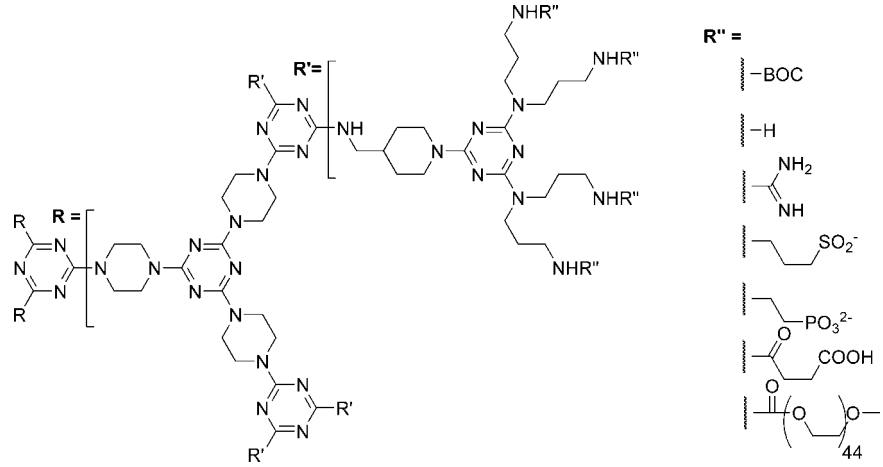


Figure 8. Library of melamine based dendrimers, listed with varying surface functionalities and charges.[85]

PEGylation is a very common concept used to decrease cytotoxic effects of surface accessible groups.[94] The effect of PEGylation on cationic poly(L-lysine) dendrimers was tested in rats by Kaminskas et al.[95] They found prolonged plasma circulation and retention times for higher molecular weight (PEG 2000 MW) chains attached to the dendrimer, compared to the unprotected cationic polymer. Eventually, the larger dendrimers showed distribution to the spleen and liver instead of the lower molecular weight versions, which were still cleared via the kidneys. None of the PEGylated

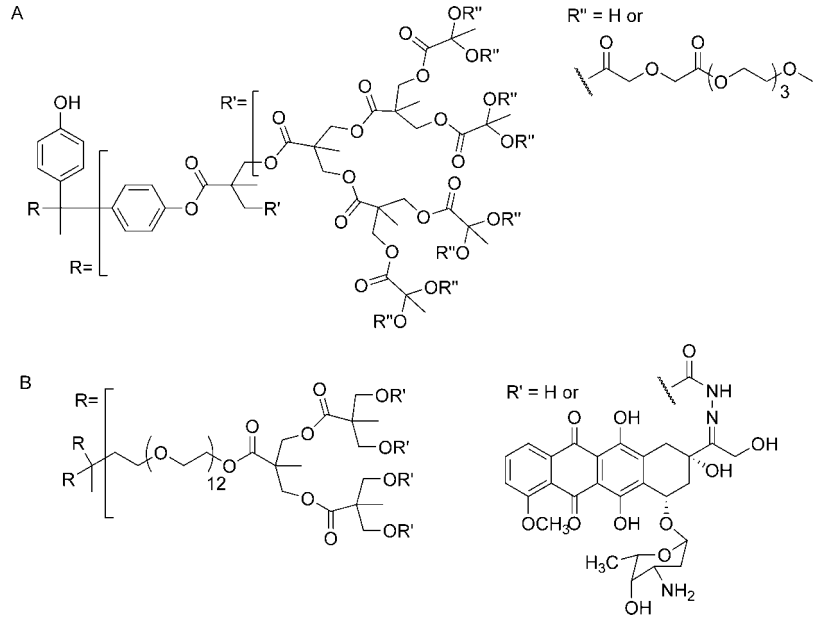


Figure 9. Polyester dendrimers: (A) polyester dendrimer with a trisphenolic core, with or without triethylene glycol tails attached; (B) three-armed polyethylene oxide star conjugated with second generation polyester groups. The terminal alcohol moieties are either free or functionalized with doxorubicin.[87,88]

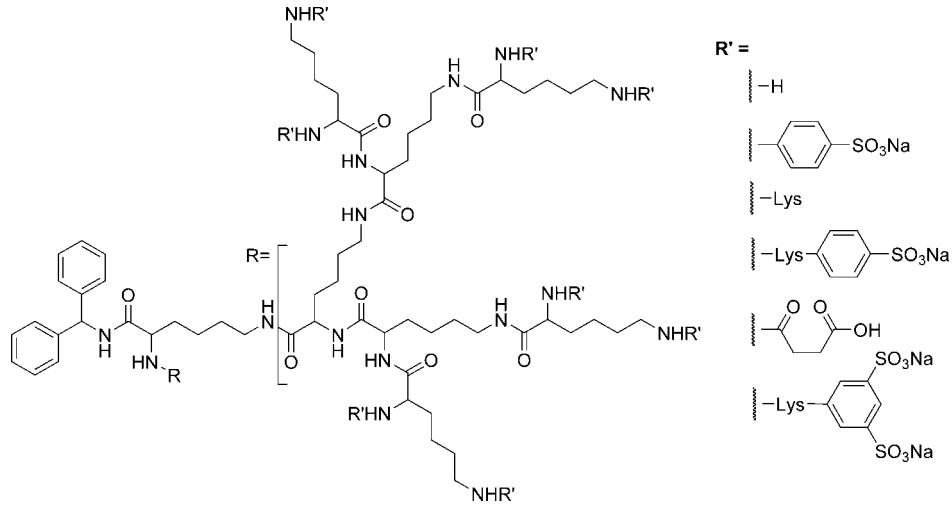


Figure 10. Polylysine dendrimer, based on a bifunctional lysine benzhydrylamide core, with varying surface functionalities.[89,90]

dendrimers gave evidence of hydrolytic degradation of the macromolecule, which might suggest that PEG tails protect the core from enzymatic breakdown through their steric bulk.

PPI dendrimers were also found to show decreased cytotoxicity after PEGylation,[96] and results from studies on PAMAM dendrimers again showed decreased toxicity for both partially PEGylated dendrimers, dendrimers decorated with varying PEG chain lengths, and fully PEGylated ones. Yet these reports also described a decreased cell viability at higher concentrations when PEG 2000 MW tails were attached to all peripheral groups.[97] The sudden increase in toxicity might be caused by intramolecular aggregation of PEGylated dendrimers, breaking with the rule of thumb that more PEG results in less toxicity.

2.4. Dendrimers as Delivery Vessels

Changing terminal moieties, monomer composition, and merely the size of the dendrimer can lead to interesting new properties and applications. Since their discovery, there has been an increasing number of biological applications for which dendrimers have been utilized. Many of these applications have already been reviewed in the past.[47,50,51,98,99]

Enclosed cavities formed by the membrane-like dendrimer periphery can be conveniently used for storage of various payloads of compounds of biological interest, turning dendrimers into containers or delivery vessels. This peripheral barrier is formed in higher generation dendrimers, through the tight packing of terminal groups at the surface of the dendritic sphere. These cavities are thus created in the interior of the dendrimer and shield the payload from the outer environment. Guest molecules can be included into the structure during buildup of the dendrimer, either covalently or by passive enclosure. When these macromolecules are designed to penetrate cellular membranes,[100] they can deliver their cargo to the intracellular environment. Using a dendritic carrier has various advantages: it can increase cargo solubility, it provides the ability to target and penetrate specific cell types, and it prevents biodegradation before the target is reached, thus increasing the overall lifespan of the encapsulated molecules.[50]

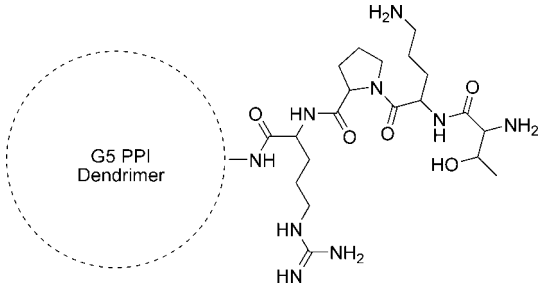


Figure 11. Fifth generation PPI dendrimer functionalized with the macrophage activator peptide tuftsin, capable of loading efavirenz.[102]

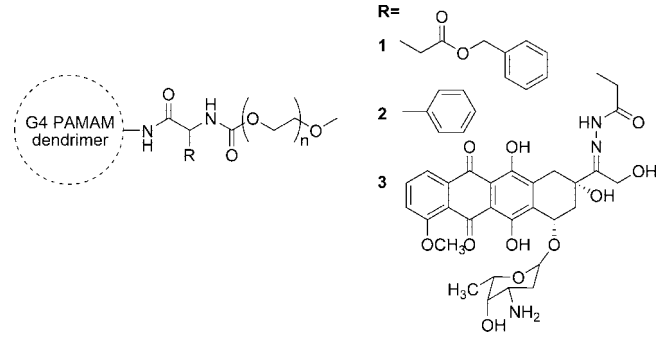


Figure 12. Fourth generation PAMAM dendrimer, conjugated with either γ-benzyl-L-glutamate (1), phenylalanine (2), or γ-adriamycin-L-glutamate (3), all with an N-terminally attached PEG tail.[104,105]

2.4.1. Drug Loading and Release

One of the first examples of molecular enclosure in a dendrimer is of a poly(propylene imine) dendrimer with 64 terminal amino groups,[36] to which a fluorescent dye was added. It was subsequently locked into the "dendritic box" by reacting the terminal amines with a Boc-protected phenylalanine derivative, to form a rigid shell. Further investigation brought forward that size selective liberation of these guest molecules was possible from the dendrimer cavities.[101]

More recently, fifth generation PPI dendrimers were functionalized with tuftsin by Dutta et al.,[102] to mask the cytotoxic positive charges and enhance uptake by HIV-infected macrophages. Tuftsin is a tetrapeptide which serves as a macrophage activator, increasing their natural killer activity. In AIDS patients, macrophages act as reservoirs for spreading the HIV virus through the rest of the body. Tuftsin functionalized PPI dendrimers (Figure 11) were loaded with efavirenz, a nucleoside analogue reverse transcriptase inhibitor, used in highly active antiretroviral therapy. These dendrimers showed a prolonged drug circulation, with the drug protected inside tuftsin-conjugated dendrimers, and an increased cellular uptake compared to the free drug. The dendrimer-drug conjugate was found to cause a 99% decrease in viral load in vitro.

PEGylation of this PPI dendrimer was used by the same group for the delivery of an anti-inflammatory drug, aceclofenac. Drug loading was increased through the conjugation of PEG tails to the dendrimer, while simultaneously decreasing drug leakage and hemolytic acitivity as compared to the non-PEGylated version.[103]

PAMAM dendrimers were functionalized by Kono et al.[104] with a shell of hydrophobic amino acids, either phenylalanine or γ-benzyl-L-glutamate, to improve encapsulation of guest molecules (Figure 12). PEG chains were conjugated to the amino acid layer, to improve solubility. The shell of hydrophobic amino acids was found to reduce unwanted release of guest molecules by sterically compressing the size of the dendrimer.[104] Functionalization with glutamic acid instead of hydrophobic protected residues, connected to PEG chains, led to an efficient delivery vesicle for adriamycin. This anticancer drug was covalently bound to the carboxylic acid side chain of the glutamate, by amide or hydrazone bonds (Figure 12) and displayed pH dependent release from the dendrimer.[105]

Many more surface groups can accomplish enclosure of guest molecules, as long as they form a tightly packed shell. For example, Klajnert et al.[106] improved the incorporation of fluorescent 1-anilinonaphthalene-8-sulfonic acid molecules into maltose functionalized PPI dendrimer cavities, using the tightly packed maltose surface moieties to form a dense shell, shielding the fluorescent probe from the hydrophilic exterior.

Besides encapsulation of guest molecules such as drugs, dendrimers can also easily be surface functionalized with therapeutical compounds. The first actual use of dendrimers as drug delivery agents is as an antitumor drug, with cis-platinum conjugated to carboxylate terminated PAMAM dendrimers. This method greatly improved solubility of the drug and caused a 5-fold increase in drug concentration in targeted tissue compared to the free drug at the same dose.[107]

Fréchet et al.[108] described a "bow tie" dendrimer, in which PEG and polyester dendrons, functionalized either with a therapeutical compound or with a solubility enhancing agent, were covalently connected (Figure 13). Biological studies were performed, and the amount of PEG arms attached to the dendron was varied, showing longer circulation half-lives for increasing amounts of PEG arms, with retention of biodegradability.[109]

A more advanced delivery system in which drugs can be released utilizes the earlier described "self-immolative" dendrimer.[46,110] These molecules can degrade through a single activation step, causing a cascade reaction to break down the entire structure into small (in)active molecules. This single activation step could also release therapeutical compounds and break down the rest of the molecule into inactive metabolites. The drug can thus be delivered site specifically, while the carrier is biologically degraded upon release of its payload. An example by de Groot et al.[82] showed a specifier being used to target a drug-carrying dendrimer at specific cells (Figure 14) and deliver paclitaxel, an antitumor drug. The drug was conjugated as a terminal moiety to a second generation dendrimer structure and subsequently released through a cascade elimination, triggered by a single activation step. Activation occurred by reduction of the nitro group at the core to an amino group, effectively eliminating all end groups.

The group of Shabat has also developed various self-immolative dendrimers, which are triggered by enzymatic activity. A diethylenetriamine trigger linker was used as a building block for completely biodegradable dendrimers, whose disassembly occurs by an intracyclization reaction. Changing the surface substrate moieties allowed for different enzymes to be used to initiate the decomposition (Figure 15A).[78]

More recently the same group reported an example of multidrug delivery, which can be used to increase the therapeutic effect by using drugs that act on different sites in the targeted process. By means of a retroaldol reaction, instigated by catalytic antibody 38C2, camptothecin, doxo-

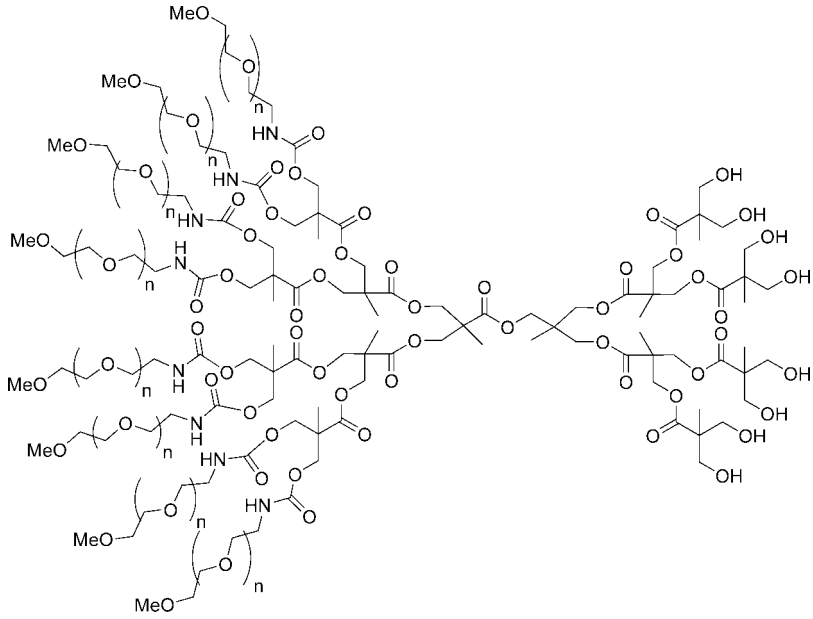


Figure 13. Bow-tie dendrimer, consisting of a second generation polyester core, functionalized with PEG tails on one side.[108]

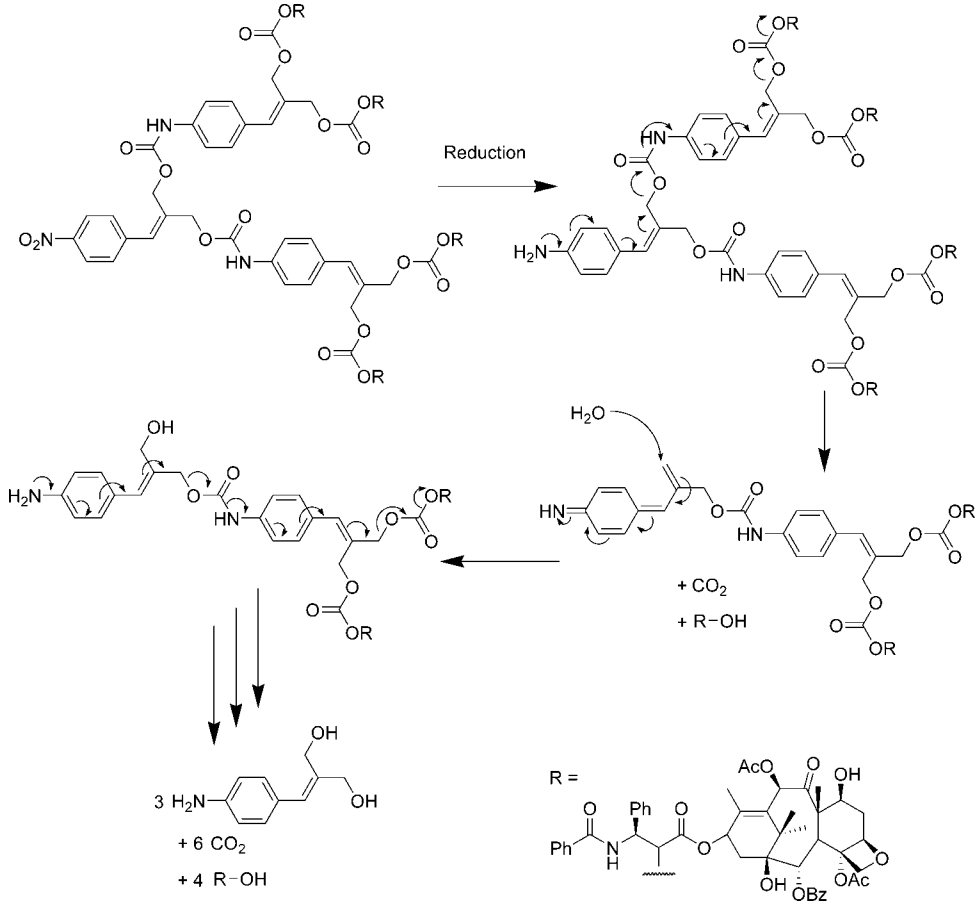


Figure 14. Second generation cascade release dendrimer. Reduction of the nitro group at the focal point leads to elimination of all paclitaxel end groups (R), releasing them into the environment.[82]

rubicin, and etoposide were released from a single dendritic structure.[81] The same group also reported release of camptothecin from a second generation self-immolative dendrimer, conjugated with two PEG5000 tails, by enzymatic activation through penicillin-G-amidase (Figure 15B).[80] PEG-tails were attached by a copper catalyzed cyclization reaction between a PEG-azide and the alkyne functionalized dendrimer. Conjugation with PEG-tails prevented aggregation of the hydrophobic prodrug moieties attached to the dendrimer and thereby enabled the enzyme to reach the triggering substrate.

In Alzheimer's disease, formation of β-amyloid peptide plaques in the cerebral cortex is believed to be a major cause for neurodegeneration. These peptides show a relatively high binding affinity to sialic acid moieties on the cell surface. Removal of these moieties also attenuates binding of the β-amyloid peptides. In this light, sialic acid residues were

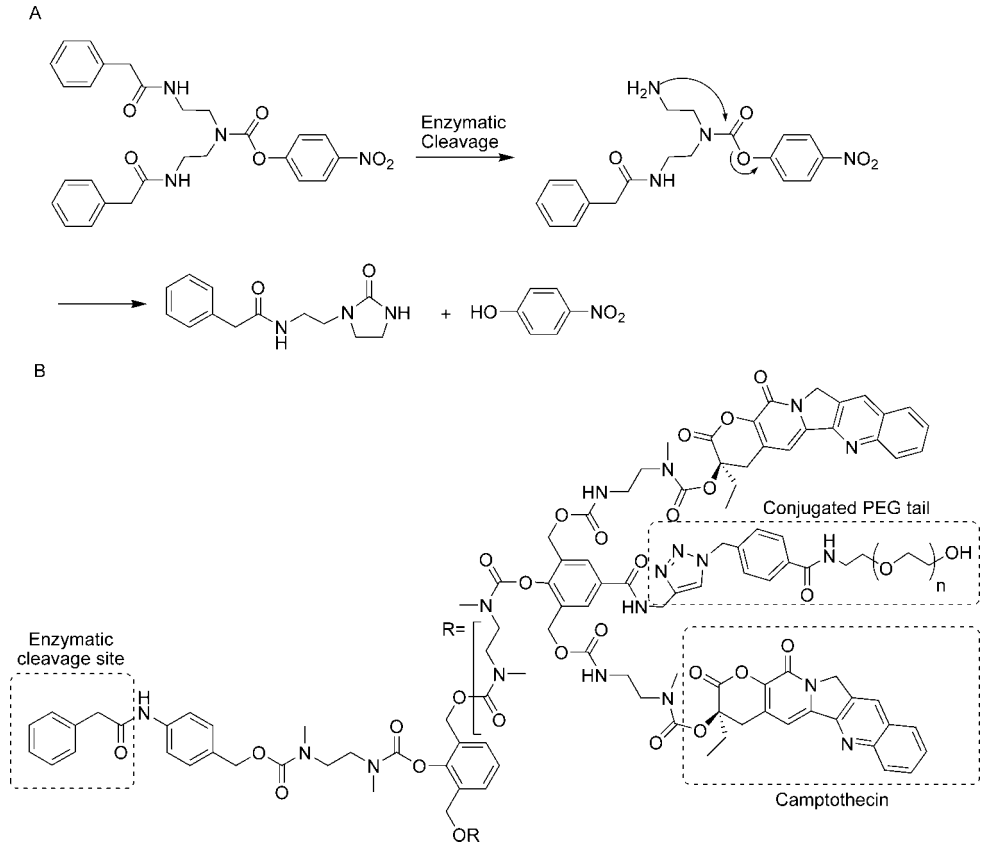


Figure 15. (A) The use of diethylenetriamine as a branching agent. (B) Self-immolative dendrimer with PEG5000 tails attached for increased solubility. Its disassembly is triggered by cleavage of the enzymatic substrate by penicillin-G-amidase. The subsequent cascade of elimination reactions releases the camptothecin prodrugs.[78,80]

conjugated to generation two through four PAMAM dendrimers. Micromolar concentrations of the dendrimer were used to successfully inhibit β-amyloid binding in a size dependent manner, showing the highest inhibition for the fourth generation dendrimer.[111,112]

Boron neutron capture therapy (BNCT) is used for treatment of cancer. Irradiation of a boron compound with thermal or epithermal neutrons produces high energy α-particles and $^{7}Li^{3+}$ ions, the former of which can effectively kill cells. Localization of these boron compounds to tumor sites can direct this radiation but poses a problem in itself. Tumor-specific antibodies have therefore been conjugated to boron compounds to provide the necessary targeting specificity. A sufficient amount of boron atoms ($>10^{9}$) has to accumulate in the targeted tissue for irradiation in order to be able to yield a sufficient amount of high energy particles, which can damage the tumor cell's ability to enter mitosis.[113,114] Dendrimers suitable for BNCT were prepared by incorporation of boron into the dendrimer structure, either through synthesis with ^{10}B containing monomers or by binding boron moieties to the peripheral groups of the dendrimer. The multivalent dendritic structure provided a method of easily concentrating high amounts of ^{10}B at tumor cells. Generation two and four PAMAM dendrimers, functionalized with a water-soluble isocyanatoborane (Figure 16), were synthesized with a tumor selective monoclonal antibody attached, to give boron-containing immunoconjugates[40] with high concentrations of ^{10}B. Attaching the small polypeptide epidermal growth factor (EGF) yielded even more specificity in tumor targeting to the brain.[115,116] Recently another report from the group of Barth showed a generation five PAMAM dendrimer, which contained nearly 1100 ^{10}B atoms in

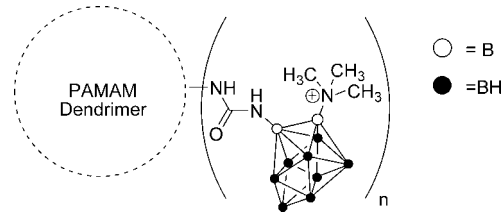


Figure 16. PAMAM dendrimer, functionalized with cationic decaborane-derived ($Na(CH_3)_3NB_{10}H_8NCO$) moieties.[40]

decaborate moieties.[117] The dendrimer was functionalized with a vascular endothelial growth factor (VEGF), used to target the VEGF receptor, which is overexpressed in tumor neovasculature. Cetuximab (IMC-C225) and L8A4, both monoclonal antibodies, were also attached to these boronated dendrimers for specific targeting to a mutated EGF receptor, expressed in brain tumors.[118,119]

2.4.2. Gene Delivery

Besides drug delivery, gene delivery can also be accomplished by methods involving dendrimers. The positive charges of for example high generation (5 or higher) PAMAM dendrimer terminal amino groups can interact with the negative charges of phosphate groups in the DNA-backbone. An excess of positive charge is crucial to achieve cell penetration and subsequent transfection. When these PAMAM dendrimers were combined with DEAE-dextran, a commercially available transfection agent which promotes cell-adhesion, the dendrimer−DNA conjugate was 20,000- to 40,000-fold more effective when compared to DEAE-dextran on its own.[120] DeLong et al.[121] have shown that also lower generation PAMAM dendrimers are capable of com-

plexation with phosphorothioate-linked oligonucleotides. Increased cellular uptake was observed for both 1:1 and 1:20 ratios of oligonucleotide−dendrimer complex (the ratio's based on the amounts of the protonated amines in the dendrimer to the anionic phosphate linkers in the DNA backbone), yielding a 3- to 4-fold and a 50-fold increase, respectively.

Dissociation of DNA from the DNA−dendrimer complex is important for the transfected gene to actually be transcribed. The dendrimer complex generally inhibits binding of transcriptional initiator proteins, by destabilizing the secondary and tertiary structure of DNA. Studies by Bielinska et al.[122] revealed that, at a certain DNA−dendrimer charge ratio, transcription proceeded, while the complex still protected the DNA against nuclease activity.

Delivery of plasmid DNA encoding the region of the hepatitis B surface antigen by fifth generation PPI dendrimers was used as a genetic immunization method.[123] The optimum ratio for transfection was found to be 1:50 (DNA−phosphate to dendrimer−amine ratio). This method provides higher transfection efficiency and increased circulation half-life for the plasmid DNA compared to traditional hepatitis B vaccination based on the injection of naked plasmid DNA. Recent reports on poly(L-lysine) dendrimers[124,125] and arginine functionalized PPI dendrimers,[126] showing similar positively charged surface groups, demonstrated that these dendrimers were also capable of more efficient DNA complexation and transfection.

It was furthermore shown that modifying positively charged surface groups does not undo the transfection capabilities of the dendritic carrier. Wada et al.[127] reported on PAMAM dendrimers functionalized with galactosylated alfa cyclodextrins, which could act as efficient gene transfection agents, showing no cytotoxicity. Plasmid DNA coding for short hairpin RNA was later transfected effectively via this system, which enabled the efficient use of dendrimers in this powerful siRNA-like technique.[128]

Delivery of siRNA is becoming increasingly important for the inhibition of gene expression, yet there are still many obstacles to be overcome. These short RNA sequences show only limited transfer across the cellular membrane, offer only little resistance against enzymatic degradation, and are easily cleared from the body. Mounting siRNA on a dendrimer carrier, such as a cationic dendrimer, could help increase both membrane permeability and the stability of the siRNA. Patil et al.[129] used acetylated and hydroxyl-terminated generation four PAMAM dendrimers to achieve improved delivery of siRNA into the cell. Formation of the siRNA−dendrimer complex resulted in improved cellular uptake, and only low toxicity was observed. Poly(L-lysine) dendrimers were also successfully used as siRNA delivery agents for the inhibition of several enzyme functionalities, including GAPDH activity.[130] Similar attempts were undertaken with tat functionalized PAMAM dendrimers,[131] though with moderate effect on uptake, despite the cell penetrating capabilities of the tat peptide.

2.4.3. Dendrimers as Imaging Agents

Imaging has proven itself a vital tool for diagnosis in medical practice. Many of the imaging techniques rely on a radioactive isotope or contrast agent of some kind, which is attached to a tag to target specific tissues. Magnetic resonance imaging (MRI) often uses contrast reagents containing a metal complex to enhance the relaxation rates of water. A drawback of small molecule chelates is their rapid clearance from the bloodstream. Dendrimers can be functionalized to enable metal complexation, either on the inside or at the periphery of the molecule, to serve as larger carriers, which are less quickly eliminated from circulation. Gadolinium is a metal commonly used for these purposes. This field was recently reviewed by Meijer and co-workers.[132]

Wiener et al.[39] used PAMAM dendrimers, functionalized with 2-(4-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid (DTPA), which contained chelated gadolinium. This gave improved proton relaxation, providing better image contrast, in comparison to other poly- or monomeric chelators for MRI. Later they were able to target this dendrimer to the folate receptor, expressed by many cancers, by functionalizing the dendrimer with folic acid.[133] Recent in vivo studies in mice for both PAMAM[134] and poly-L-lysine octasilsesquioxane[135] dendrimers, targeting the folate receptor, support these findings. Landmark and co-workers[136] used folic acid-functionalized PAMAM dendrimers for the coating of superparamagnetic iron oxide nanoparticles and to target these particles to cancer cells.

Boswell et al.[137] applied a PAMAM dendrimer, functionalized with an RGD cyclic peptide and either a fluorescent or Gd(III) chelating ligand to target the α-v-beta-3 integrin receptor for tumor angiogenesis imaging. Gadolinium was also used for tumor imaging by Brasch and co-workers, who synthesized a poly(L-lysine) dendrimer, based on a large PEG core, functionalized with Gd −1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) terminal groups, to determine endothelial leakage in order to distinguish breast cancer tissue from normal soft tissue.[138,139]

A three-step targeting approach for tumor imaging was designed by Zhu et al.[140] to reduce background noise and enhance the selectivity of the imaging process. They prepared a Gd(III) chelating DTPA functionalized PAMAM dendrimer which was biotinylated and a biotinylated tumor-specific antibody. Avidin was added to link both biotinylated compounds. Unfortunately, no increase in specificity was observed for this approach, likely due to the presence of the biotinylated antibodies preventing binding of the contrast agent to avidin.[140] This was improved when another Gd(III)-DTPA functionalized PAMAM dendrimer contrast agent was built from a disulfide core by Xu and co-workers.[141] The disulfide bond was reduced and biotin moieties were attached to each dendron, after which the dendrons were bound to avidin. This complex was capable of efficiently targeting ovarian cancer cells and could carry both MRI contrast agents and fluorescent probes.

Structurally related is the dendrimer-based imaging agent presented by Koyama et al.,[142] who also designed a PAMAM dendrimer, with both near-infrared fluorophores and Gd(III) chelating groups attached, which was used to monitor lymphatic drainage and to localize sentinel lymph nodes in mice.

The use of higher generation dendrimers increases the circulation half-life of the contrast agents and also increases the contrast of the images.[143,144] The biocompatibility of these systems does not improve, though, since toxicological effects can be caused by the release of the heavy metal ions from the imaging complex through uncontrolled degradation of the dendrimer and its conjugated ligands. To solve this problem, an easily biodegradable disulfide linker was introduced between the dendrimer and the Gd-chelating complex, which was gradually broken down and which led to improved

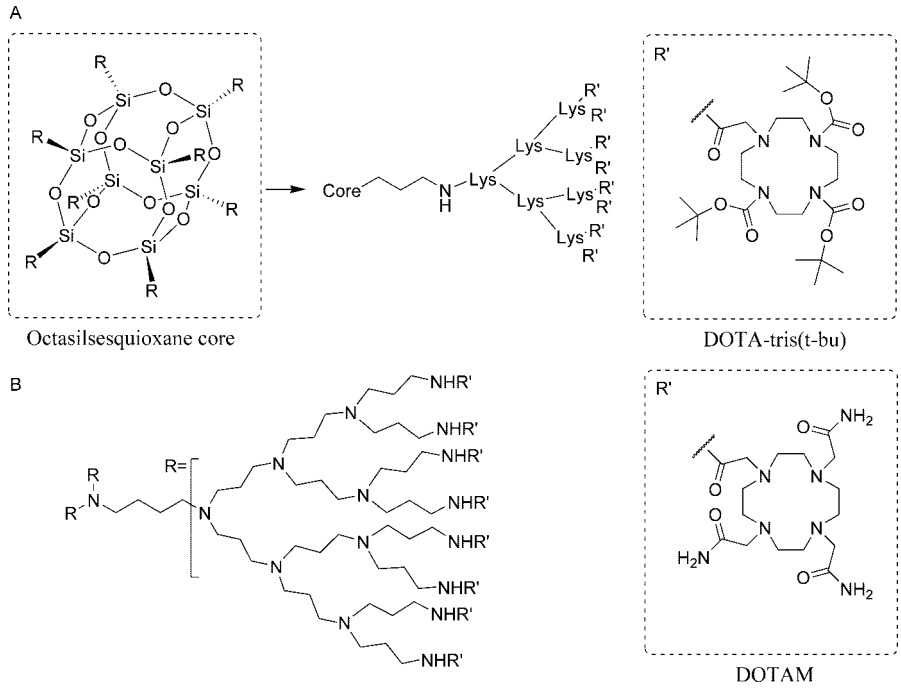


Figure 17. (A) Poly(L-lysine) dendrimer grown from an octasilsesquioxane core, terminated with DOTA-tris(*t*-Bu) groups for gadolinium coordination. (B) Third generation PPI dendrimer, terminated with DOTAM groups for ytterbium coordination.[135,148]

renal clearance of the degradation products, since heavy metals that are still chelated by DOTA, for example, are more easily cleared from circulation and hence cause less stress.[145,146]

Another strategy to counter the decreased biocompatibility of larger dendrimers lies in enhancing their efficacy: when lower concentrations of the compounds are needed, less toxicity is induced. In this vein, Kaneshiro et al.[135] synthesized a vascular contrast agent, namely a (Gd-DOTA-monoamide)poly-L-lysine octasilsesquioxane dendrimer (Figure 17), which has a well-defined globular structure. The dendrimer showed longer circulation times and higher renal clearing when compared to more conventional imaging agents, which allows for the use of a low dosage of contrast agent to be used.[135]

Gd(III)-DOTA conjugated poly(amino dipropanoyl) dendrimers were used by Amirkhanov and co-workers[147] as genetic contrast agents, capable of mRNA targeting due to their functionalization with peptide nucleic acid hybridization probes. An insuline-like growth factor analogue facilitated receptor-mediated cellular uptake of the complex, allowing the probes to localize and subsequently bind the desired mRNA sequences, allowing the spatial mapping of genetic expression through MRI.

Pikkemaat and co-workers reported the use of Yb(III)DOTA conjugated PPI dendrimers as pH mapping agents for MRI, in which higher generation dendrimers provided increased sensitivity due to their amine-rich scaffold (Figure 17).[148] Similar work by Ali et al.[149] features a PAMAM dendrimer, with Gd(III) chelating and pH responsive ligands. Relaxation behavior and responsiveness of the contrast agent was found to be pH dependent and could therefore be used to diagnose cancer or kidney failure through the reduced extracellular pH observed in these tissues.

Another dendrimer imaging application was found in photonic oxygen sensing. This analysis technique is used to diagnose whether a tumor will respond to treatment, since the oxygen concentration present in the tissue is an indication for treatment possibilities. Therefore, accurate determination of these oxygen levels is of high importance. In the group of Vinogradov a method was devised for using metalloporphyrins as guest molecules inside poly(glutamic acid), poly(aryl ether), and poly(ether amide) dendrimers as water-soluble oxygen sensors. Inside the targeted tissue, phosphorescence was induced by visible or near-infrared light. This phosphorescence was quenched when dissolved oxygen was encountered.[150,151] Currently this technique is applied both in vitro and in vivo, yet the limitation lies in the penetration depth of light into human tissue, which probably gives the nod to near-infrared based versions because of their ability to more deeply penetrate this tissue.

2.4.4. The Multivalent Effect

Besides being able to serve as delivery vessels for various therapeutically active payloads, certain dendrimers themselves can portray druglike behavior. Supattapone et al.[152] have shown that dendrimers terminated with positively charged moieties were able to remove PrP^{Sc} proteins, a protease resistant isoform of the prion protein, from scrapie infected neuroblastoma cells. It was suggested that these dendrimers stimulate the cellular pathways for removal of these abnormal protein accumulations and that this principle could be extended to many other degenerative diseases. Low noncytotoxic concentrations of PAMAM, PEI, and PPI dendrimers were incubated with these neuroblastoma cells, and a nearly complete reduction of the accumulated PrP^{Sc} was observed in all cases. Maltose functionalized PPI dendrimers were also found capable of interacting with prion peptides and could very well be used as antiprion agents.[106] Instead of using charge interactions, these dendrimers interacted by means of the hydrogen-bonding properties of the maltose units. Yet also, electrostatic interactions are deemed important for dendrimer−peptide interactions. Positively charged PPI dendrimers were capable of destabilizing and precipitating insulin in a generation dependent manner.[153]

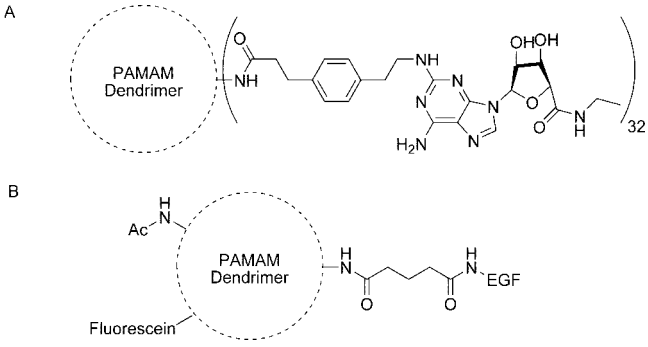


Figure 18. Dendrimers as agonists: (A) PAMAM dendrimer terminally functionalized with the CGS21680 nucleoside. (B) PAMAM dendrimer partially acetylated and functionalized with a fluorescein isothiocyanate label and epidermal growth factor (EGF).[159,160]

Surface modification with protein denaturing groups adds to the electrostatic effects of the dendrimers.

Polyvalent interactions are defined by the binding of multiple ligands of a single object to multiple receptors of another object, a quite common phenomenon in biology.[154] Due to the higher number of interactions that occur at the same time, a much stronger effect and higher levels of specificity are observed compared to monovalent interactions. In most cases multivalency is found in protein−protein interactions and interactions between carbohydrates and proteins. Natural carbohydrate binding proteins for example often aggregate to achieve a higher affinity for the target receptor, since multiple ligands can be concentrated and bound at the same time. This is known as the "cluster (glycoside) effect".[155] These higher valency molecules will be able to force an effect, whereas the monovalent version cannot.

Besides the fact that polyvalent interactions are of vital importance to many functions of the human body, they can also be misused by malignant viruses and bacteria. Inhibition of these undesired interactions provides possibilities for interfering in processes, such as host−pathogen recognition. Also, in this case monovalent binding of carbohydrate ligands to the pathogen receptors only provides low binding affinity, insufficient to achieve a significant effect.

In order to mimic polyvalency, dendrimers are useful scaffolds for presenting the necessary ligands and for controlling such interactions, being naturally equipped with a large number of spatially well-ordered functional groups. Similar to nature, glycodendrimers functionalized with suitable carbohydrates at their periphery provide a much larger binding affinity, as also described for sugar-coated polymersomes in section 5.1.2. Various examples exist of high valent carbohydrate dendrimers designed to use the cluster effect. The group of Meijer[156] synthesized a PPI-carbohydrate dendrimer, in which the 64 terminal saccharides were separated from the dendrimer by a methylene spacer. Roy and co-workers synthesized a lactose functionalized PAMAM dendrimer, with up to 128 terminal carbohydrates.[157] Later they reported another PAMAM dendrimer, terminally functionalized with α-thiosialosyl moieties, which was capable of inhibiting the binding of human α_1 acid glycoprotein to *Limax flavus* (slug lectin) 210 fold better than monovalent α-thiosialosyl.[158]

Kim et al.[159] produced a PAMAM dendrimer conjugated with the CGS21680 nucleoside, a multivalent G-protein-coupled receptor agonist (Figure 18). The dendrimer was capable of inhibiting human platelet aggregation induced by adenosine 5′-diphosphate (ADP). A partially acetylated PAMAM dendrimer functionalized with EGF moieties and a fluorescein isothiocyanate label (Figure 18) was found to increase stimulation of the EGF receptor on various cell types. In this case, the EGF-dendrimer complex acted as a multivalent superagonist, causing increased cell growth when compared to free EGF.[160]

A particular application of the multivalent effect is as a tool for synthetic vaccines and antibody production. This application was first explored by Tam in 1988, who used dendrimers as multiple antigenic peptide (MAP) carriers.[41] This specific type of dendrimer was not synthesized from a separate core moiety but was built up from a simple trifunctional amino acid, such as lysine, to which additional amino acids were linked; in the final step the dendrimer was functionalized with antigenic peptides on the outer surface. This design was based upon the lysine dendrimers of Denkewalter et al.[64] Up to four layers of lysines were connected to the core, and when functionalized with antigenic peptides, up to sixteen could be attached. Higher generation lysine dendrimers were difficult to synthesize in this manner.[161] Due to their immunogenic effect, easy synthesis, and high customizability, they are ideal carriers for antigenic peptides in vaccination.

Tam et al.[162] also synthesized lysine based dendrimers, functionalized with tetra- and octapeptides portraying antimicrobial properties. These antimicrobial dendrimers provided higher solubility of the peptides and increased resistance against proteolysis over conventional antimicrobial peptides. Furthermore, their results suggest that clustering of charges, hydrophobic residues, and peptides improves the antimicrobial properties. Their findings were supported by more recent reports of Klajnert et al.,[163,164] who performed extensive studies on the biological properties of small peptide dendrimers. The degree of dendrimer branching and steric distribution and the types of aromatic and cationic moieties proved important for the antimicrobial functionality and their toxicity.

Anionic amphiphilic dendrimers (Figure 19) have been found to portray antimicrobial properties against Gram-positive bacteria, and they only showed little cytotoxic effect on eukaryotic cells. Their antimicrobial activity was higher compared to conventional commercial antibacterial amphiphiles, as a result of the multivalent effect.[165]

Besides inhibition or agonization of receptors, the multivalent effect can also enhance catalysis. As shown by Zaupa et al.,[166] RNA cleavage was catalyzed by multivalent zinc-functionalized dendrimers, the rate of which was increased due to multiple catalytic sites acting upon the substrate simultaneously.

2.5. Dendrimers as Scaffolds

Besides the use of dendrimers as capsules for delivery purposes, they have also served as scaffolds for various purposes. For example, aldehyde terminated phosphorus dendrimers were used to form a reactive, covalently bound layer on top of a glass surface on which in a next step DNA was immobilized. This dendrimer layer was used for hybridization with, and detection of, targeted cDNA sequences.[167,168] Another way of preparing these so-called DNA microarrays is by microcontact printing. Reports from the group of Reinhoudt described the construction of a positively charged stamp surface by deposition of generation

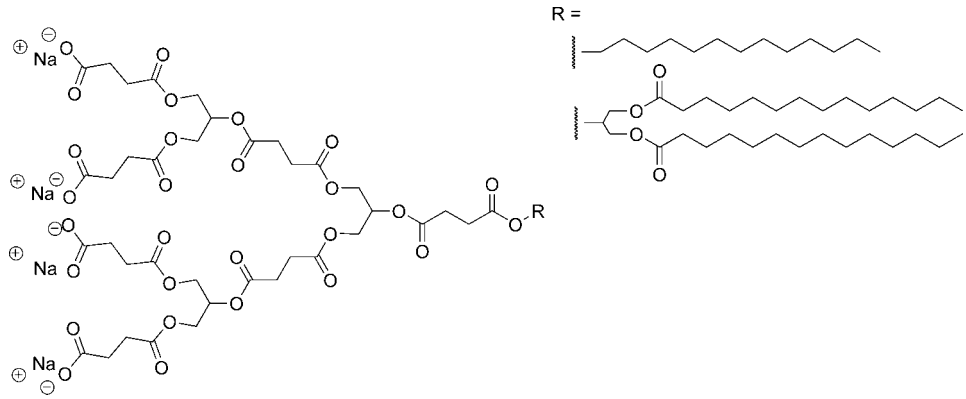


Figure 19. Anionic dendritic amphiphiles with antimicrobial properties, built of glycerol, succinic acid, and myristic acid.[165]

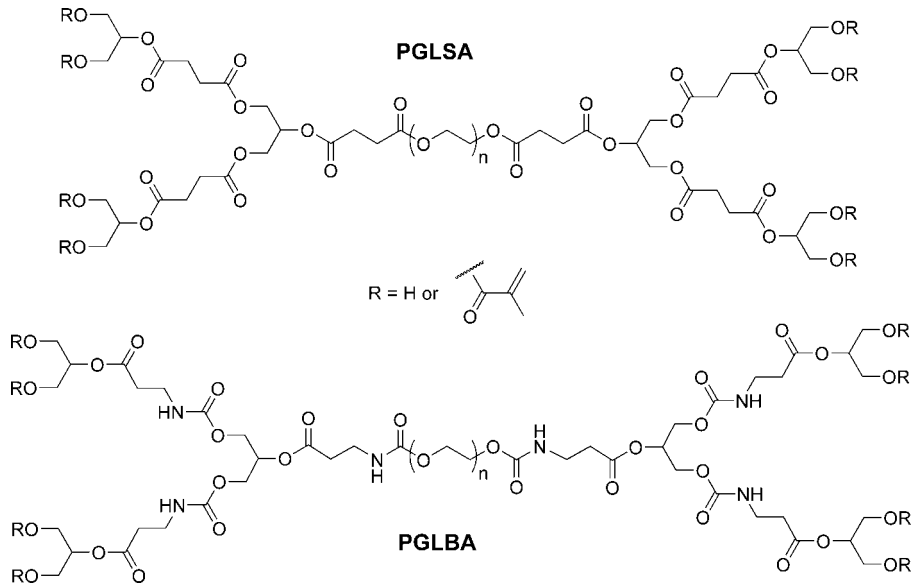


Figure 20. PGLSA and PGLBA dendrimers with PEG chain cores and cross-linkable by acrylate moieties at the terminal positions.[172,173]

five PPI dendrimers. This layer was capable of attracting negative charges in a "layer-by-layer" fashion (see section 4 of this review) and was used to transfer print DNA or RNA to the microarray surface. Afterward the dendrimer was washed off, leaving behind polynucleotide strands.[169,170] Dendrimers with cross-linkable peripheral groups were used to form networks, or scaffolds, on which new tissue could grow, offering possibilities for wound sealing and tissue regeneration. Furthermore, single dendrimers have served as a scaffold on which enzymes, or biohybrid mimics thereof, were immobilized and locally concentrated to increase their catalytic efficiency. Many of these dendritic scaffolds have been used for interesting biological applications, which will be discussed in this section.

2.5.1. Scaffolds for Tissue Repair

Water-insoluble dendrimers functionalized with cross-linkable groups were used as a scaffolding microstructure to seal corneal wounds[73,171] or to produce a scaffold for cartilage tissue engineering[172] by forming an insoluble hydrogel. The hydrogel was formed by UV-photoinitiation of cross-linkable peripherally positioned acrylate groups on a poly(glycerol succinic acid) (PGLSA) dendrimer. Furthermore, a linear PEG chain was used to connect two separate dendrons, effectively incorporating PEG as the core moiety for the PGLSA dendrimer (Figure 20). The first generation PGLSA-PEG-PGLSA molecules were able to accelerate the natural sealing of corneal wounds by up to five times. The dendritic gel could maintain pressure in the tissue at similar levels compared to the conventional method of suturing. This demonstrates that dendrimers can provide a biodegradable scaffold for tissue self-repair.

Other work by the same group features a photo-cross-linkable poly(glycerol beta-alanine) (PGLBA) dendrimer (Figure 20) as a scaffold for osteochondral tissue repair, for which good mechanical properties and decent attachment were observed in vivo. Production of collagen II and glycosaminoglycans was discovered in tissue treated by this method, as an indication of tissue repair.[173]

Second generation PPI dendrimers were used by Duan and co-workers[174] to cross-link corneal collagen in order to form gels that might also be used as corneal tissue repair scaffolds. The dendrimers served as amino terminated bridging molecules between the collagen residues' carboxylic acid side chains in an EDC coupling. The gels formed by this reaction showed improved mechanical and optical properties compared to existing methods for corneal tissue repair. Although these results are promising, the authors state that it still is a challenge to equal natural cornea due to the lack of control over the formation and alignment of the collagen fibrils formed.[174]

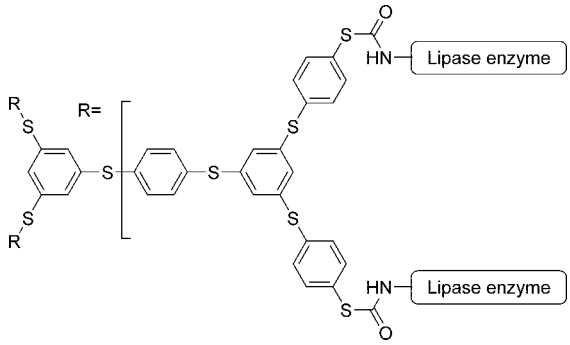


Figure 21. Catalytic poly(phenyl sulfide) dendrimer, with terminal lipase enzyme functionalities.[177]

2.5.2. Scaffolds for Catalysis

With their branched architecture, dendrimers can be used as enzyme mimics or as scaffolds for catalysis, since they can hold many enzymes or other catalysts together at the surface or inside their structure.[175,176] This close proximity of active sites holds advantages for both multistage reactions, involving different enzymes, or catalysts working in a cascade reaction, taking advantage of the precise spatial positioning that dendrimer structures may offer. It can also promote cooperativity effects between multiple copies of an active catalyst, the so-called multivalent effect, in which both the number of occupied catalytic sites and the amount of cooperating catalytic moieties determine the efficiency of multivalent catalysts.[166]

Yemul et al.[177] used second and third generation poly(phenylene sulfide) dendrimers as scaffolds to covalently bind *Burkholderia cepacia* lipase (Figure 21). Hydrolysis of olive oil to fatty acid was monitored, and activity was found to remain high. The advantage of scaffolding in this case was found in the fact that the optimum temperature and pH ranges widened for immobilized enzyme compared to free enzyme. The enzyme−dendrimer conjugate could also be recycled, with high retention of hydrolysis activity, even after 20 cycles.[177]

Biomimetic peptide dendrimers, containing histidine and serine/threonine moieties have been proven to show catalytic activity in the hydrolysis of 8-acyloxypyrene 1,3,6-trisulfonates. Substrate binding of the dendrimer was correlated to the amount of histidines present in the dendrimer, with three histidines binding each substrate and with specificity for the sulfonate groups, since the ester derivatives were not bound and hydrolyzed by the dendritic catalysts (Figure 22).[178] A peptide dendrimer with a single histidine at the core and two arginines located at the first generation branch points showed increased binding of the trisulfonate substrates through assistance of the arginine residues. Catalysis was further enhanced by aromatic residues positioned at the outer generations,[179] mimicking active site regions of natural enzymes (Figure 22). Multiple other catalytic peptide combinations have been found lately by screening a library of peptide combinations for function and placing them on a dendrimer scaffold to assess their activity.[180]

A recent report from the group of Fréchet showed multiple PAMAM dendrimers, each having a different enclosed catalyst, performing a multistep reaction.[181] This mimics the approach taken by nature, in having multiple processes function without influencing each other, via compartmentalization. In the experiment, three incompatible catalysts were encapsulated inside the dendrimer, allowing organocatalysis by iminium, enamine, and hydrogen bonding moieties to perform a cascade reaction that could otherwise not be performed within the same reaction vessel (Figure 23). This strategy makes use of the fact that dendrimers have a sufficiently dense outer layer to rule out interpenetration, effectively preventing the enclosed catalysts from touching base.

2.6. Outlook

In general, dendrimers are incredibly versatile molecules with broad applications in the field of biomedicine. Their unique structure provides a monodisperse and customizable platform for drug and gene delivery, imaging, tissue repair, and various other applications with considerable advantages over other carriers. For example, dendrimers can provide improved solubility,[104,106] controlled cellular targeting and uptake,[147] tuned circulation half-lives,[135] and improved sensitivity and receptor binding by way of multivalent interactions,[159,160] merely by attaching different functional terminal groups.

Besides surface modification of existing dendrimer species, the introduction of nucleotides, amino acids, and sugars as actual building blocks instead of mere peripheral groups has added a wide range of new possibilities for dendrimers. Dendrimers made of amino acids have been designed as enzyme mimics,[178−180] to closer mimic actual active sites. Preprogrammed self-assembling structures have been constructed using DNA-dendrimers,[67−69] and polyvalent receptor-binding dendrimers can be found in the form of glyco-dendrimers.[158−160]

The cytotoxicity of dendrimers has been a major problem for in vivo applications,[85] especially for cationic dendrimers. Fortunately, recent discoveries have offered solutions to increase biocompatibility and biodegradability through conjugation of various moieties to the periphery of the dendrimer. Examples are the induction of "stealth" behavior using PEGylation,[94−96] or offsetting the effect of positive charges by acetylation.[91] Moreover, the use of self-immolative dendrimers as completely degradable delivery vessels[81,82] has opened up many opportunities for new in vivo applications.

3. Polymer Micelles

Of the capsule-like structures that are the focus of this review, polymeric micelles are probably the most intensively studied. Although not strictly capsules, polymer micelles can be viewed as having an inner core and outer shell (or corona) which differ in polarity. These architectures are mainly assembled from amphiphilic block copolymers, which phase separate in selective solvents as a result of the solubility difference between the blocks. The distinct polarity difference between the bulk solution and the hydrophobic interior allows hydrophobic molecules to partition preferentially in the hydrophobic core. Indeed, the potential of polymeric micelles as drug delivery vehicles has resulted in a considerable amount of literature that will not be the focus of this section.[182−187] Rather, the main focus will be on the different biohybrid polymer architectures that were found to assemble into micelles, their stabilization by cross-linking, and several approaches to impart function to the carriers, such as the use of temperature-sensitive and pH-sensitive polymers or immobilization of proteins.[188] The first and foremost part will cover polymeric micelles that contain (poly-)peptide blocks. The second and the third part cover polymeric micelles based

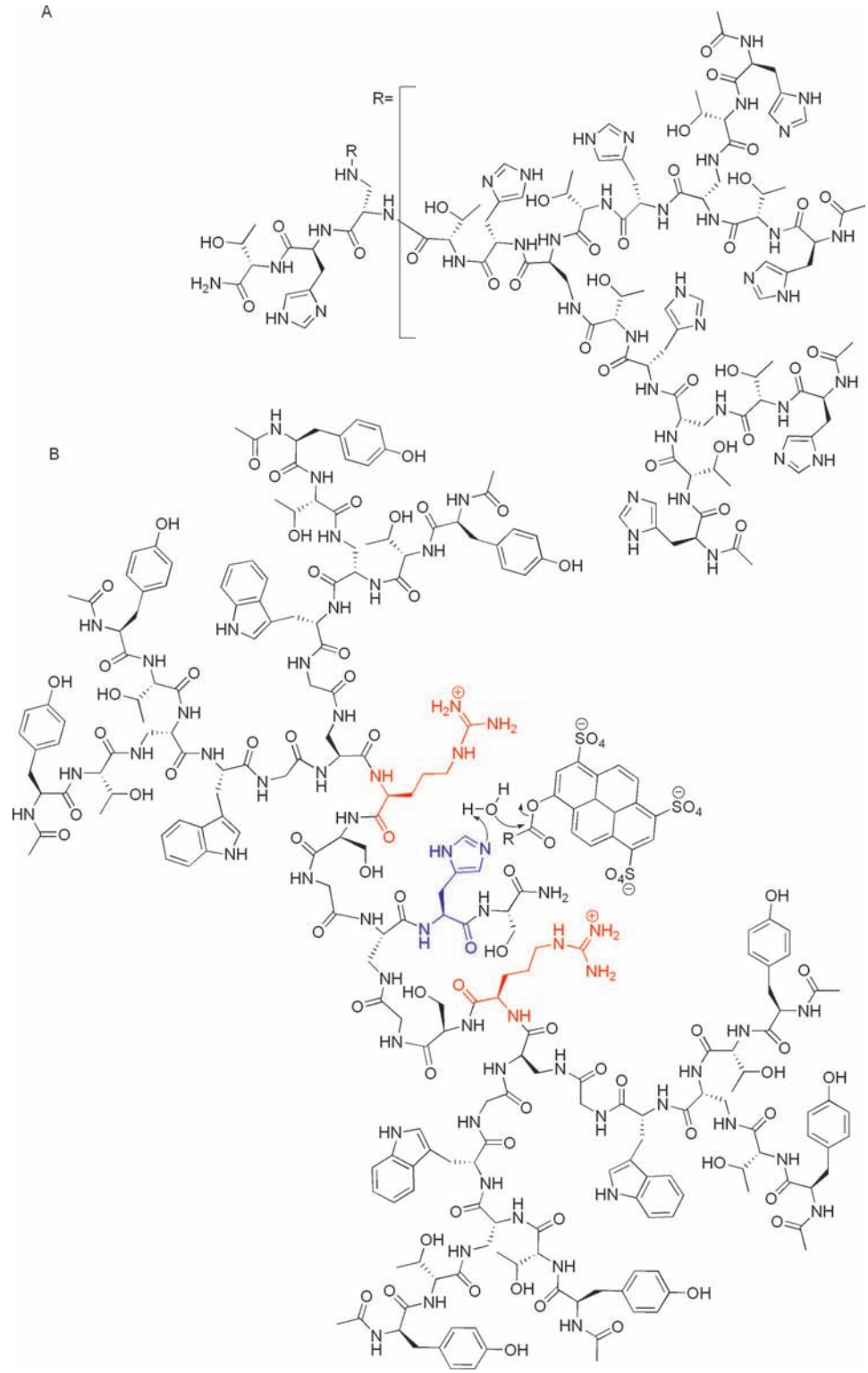


Figure 22. Catalytic peptide dendrimers: (A) consisting of Thr-His-diamino propionic acid monomers or (B) containing a single His core (blue), with two adjacent Arg residues (red) to bind the charges of the substrate, and hydrophobic Tyr and Trp residues at the surface to provide a hydrophobic pocket for the substrate to reside in.[178,179]

on glycopolymers and nucleic acids, respectively. An interesting step forward in complexity is that of multicompartment micelles,[189] which were introduced by Ringsdorf[190] but which only have received major attention relatively recently after seminal work by Lodge et al.[191] The concept bears resemblance to natural cells with their many divided compartments. Potential steps toward biocompatibility have been taken, replacing perfluorated polymer blocks by polyesters.[192] To the best of our knowledge, however, no biohybrid polymers constituting these systems or investigations on biological applications have been reported yet.

3.1. Micelles Based on Poly(amino acids)

Most literature on micelles based on poly(amino acids) concerns block copolymers with a polypeptide block that may form the corona or the inner core, and a block of a "classical" polymer such as PS, PEG, or stimuli-responsive

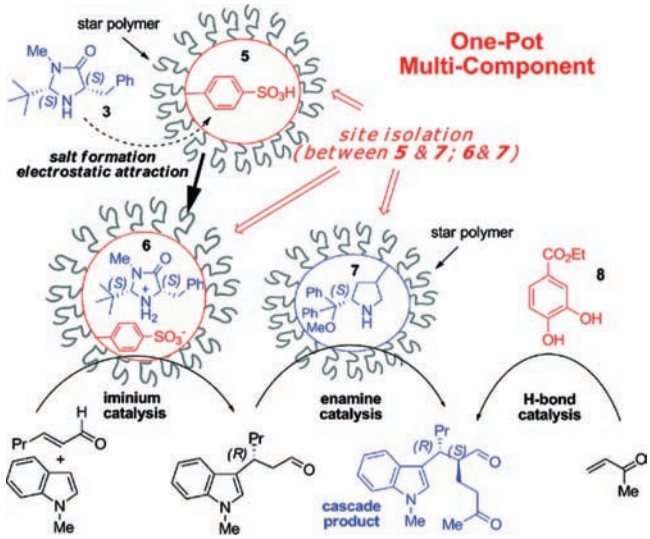


Figure 23. One-pot multicomponent cascade reaction with dendrimer encapsulated catalysts that are otherwise incompatible. (Reproduced with permission from ref 181. Copyright 2008 American Chemical Society.)

polymers such as PNIPAAm (Chart 1).[193,194] In earlier reports, the peptide block was synthesized from the protected *N*-carboxyanhydrides (NCA) of benzylglutamate, benzylaspartate, and ε-benzyloxycarbonyl lysine, as their polymerization is well controlled.[195] However, in the past five years the preparation of polypeptides by solid phase peptide synthesis and protein-engineering has gained importance, as have, even more recently, noncovalent approaches to construct block copolymers.[196] Along with this development, novel techniques to couple biomolecules to synthetic polymers have emerged.[197−200]

3.1.1. Micelles Based on Poly(aspartic acid)

Much pioneering work on polypeptide containing block copolymers which form micelles in aqueous solution has been performed on the system poly(ethylene glycol)-*block*-poly(aspartic acid) (PEG-p(Asp)) and derivatives thereof, by Kataoka and co-workers.[201] The interaction of PEG-p(Asp) with the block copolymer PEG-*block*-poly(L-lysine) (PEG-PLL) led to the concept of polyion complex (PIC) micelles with exceptional monodispersity.[202−204] Not only synthetic polyions were used to form PIC micelles with PEG-p(Asp), but also biological polyions such as DNA or enzymes such as lysozyme and trypsin were trapped.[205,206]

Fréchet et al. reported the derivatization of the aspartate moiety of PEG-p(Asp) with benzylidene acetals. At physiological pH (pH = 7.4) the block copolymer formed micelles, which at pH = 5 (the pH of lysosomal compartments in cells) rapidly disassembled. The disassembly resulted from hydrolysis of the acetal groups to diols, which rendered the polymer more hydrophilic.[207] Recently, a strategy was reported involving the derivatization of the aspartate-moiety with citraconic anhydride using ethylene diamine as a linker. The resulting citraconic amide was stable at neutral and basic pH but degraded at acidic pH. This PEG-poly[(*N*′-citraconyl-2-aminoethyl)aspartamide] formed PIC micelles that could contain lysozyme in its core, and degraded rapidly when brought into an acidic environment (∼2 h at pH = 5), releasing the enzyme (Figure 24).[208]

3.1.2. Micelles Based on Polylysine

PLL-based block copolymers with PEG have been investigated for drug delivery purposes, mainly in PIC micelle systems as described above, because the positively charged backbone interacts strongly with DNA or oligonucleotides.[209,210] Interestingly, the PEG-block had a profound influence on the secondary structure of the PLL-block; where the homopolymer of PLL assumed a random coil conformation, PLL of the same block length but conjugated to PEG was found to be in an α-helical conformation.[211] In a related approach, linear dendritic block copolymers of PEG-PLL were synthesized in order to convey a better control over the polymer topology and subsequent drug delivery properties.[193,212,213] The release of a model drug (doxorubicin) from the micelles showed, however, that the structure and the degradation of the aggregates was influenced by whether or not any drug was present.[214]

The self-assembly of a series of polystyrene-*block*-PLL (PS-PLL) was investigated by Lübbert et al.,[215] who found that its critical micelle concentration (cmc) showed a parabolic dependence on the polypeptide block length. For the shorter blocks, this behavior was attributed to an increase in ionizable groups, which for longer blocks was offset by the decrease in aqueous solubility of the peptide. Similar behavior was also found for PS-*block*-poly(acrylic acid) (PS-PAA).[216]

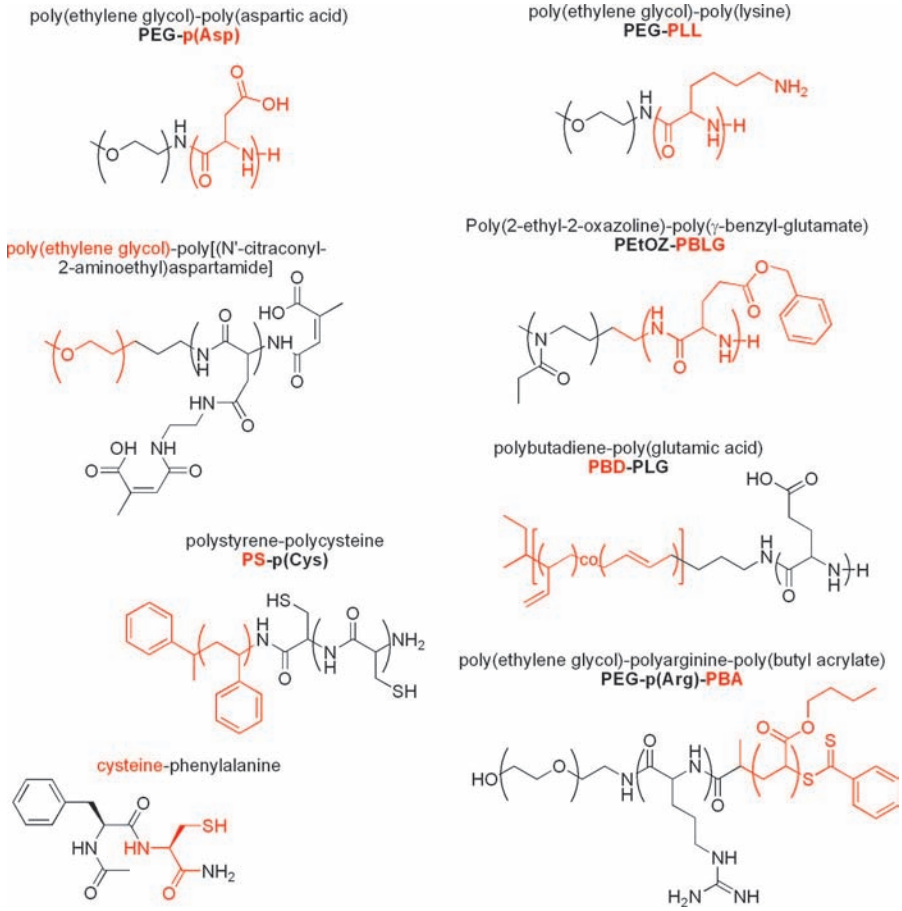
Gebhardt et al.[217] investigated the pH-dependent behavior of a polybutadiene-*block*-PLL (PBD-PLL) and found that for the block lengths PBD_{107}-PLL_{200} an increase in hydrodynamic radius (R_H) from 62 nm at pH = 10 to 82 nm at pH = 2 could be observed. This was ascribed to charge repulsion and osmotic swelling of the deprotonated amino acid residues. The samples with lower lysine contents showed more rodlike aggregates.[217] It should be noted that for block copolymers of PB_{165}−PLL_{88} the observed pH-responsiveness of the aggregate size could be explained merely in terms of colloid stabilization, as opposed to secondary structure effects.[218]

3.1.3. Micelles Based on Poly(glutamic acid)

Besides polyaspartic acid and polylysine, poly(L-glutamic acid) (PLG) and ester derivatives thereof have been extensively exploited in micelle forming block copolymers. In particular, environmental effects on micelle formation and morphology, such as pH and type of solvent, have been studied in depth for this class of block copolymers. Cho et al. prepared block copolymers of a hydrophobic poly(γ-benzyl-L-glutamate) (PBLG) block and a hydrophilic poly(*N*-isopropylacrylamide (PNIPAAm) block, which yielded micelles in an aqueous environment with PBLG forming the core. A series of this block copolymer in which part of the benzyl glutamate moieties were derivatized to N^5-hydroxyalkylglutamate was prepared as well.[219,220] Poly(2-ethyl-2-oxazoline)-*block*-PBLG (PEtOZ-PBLG) assembled into spherical micelles in water[221] but displayed a diverse array of aggregate morphologies in solvents in which the PBLG-block assumed a rodlike shape.[222]

PBLG grafted with PEG[223] and PBD-PLG copolymers were exploited to show pH-dependent swelling behavior that was correlated to a change in polypeptide conformation. At the critical pH (pH = 6), the size of the PBD-PLG micelles changed. This pH value corresponded to the value where the peptide undergoes a transition from an α-helix (at acidic

Chart 1. Structure Formulas of Various Polymeric Micelle-Forming or Related Compounds



pH) to a random coil conformation (at basic pH).[224] Light scattering showed that, for PBD-PLG block copolymers with relatively long PLG blocks, at pH = 12 micelles were observed with core-sizes of 7 nm and a total radius, including the solvated corona, of 38 nm. At pH values below 7, the radius of the aggregates dropped to 28 nm. Even at salt concentrations higher than 0.5 M NaCl a change in radius was observed upon pH variation, albeit with a lower amplitude.[225]

In analogy with studies initiated by Wooley et al. (vide infra), the shells of micellar aggregates formed from the PBD-PLG were cross-linked by reacting the carboxylic acid groups with 2,2-(ethylenedioxy)bis-ethylamine.[226] The resulting aggregates did not show a difference in micellar dimensions. But swelling studies with THF, which is a selective solvent for the core, showed that where the non-cross-linked micelles gave an 8 times increase in radius, swelling was absent for the cross-linked aggregates. Micellar aggregates of polyisoprene-*block*-PLL (PI-PLL) showed the same behavior upon cross-linking.[226,227] A reversible cross-linking procedure with dithiothreitol was reported for micelles of poly-L-cysteine-*b*-poly(lactic acid).

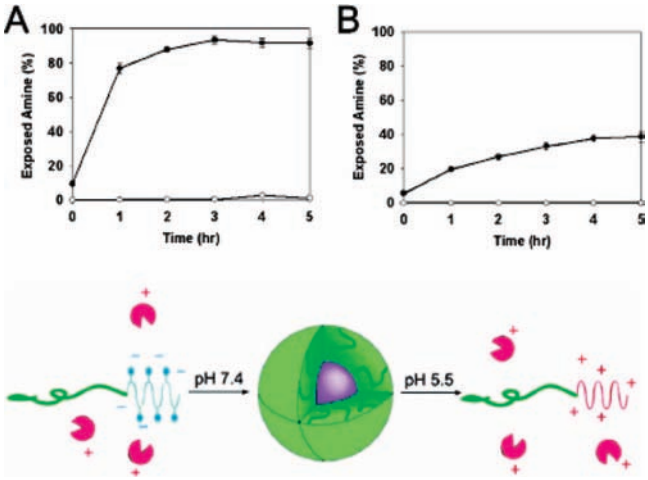


Figure 24. (Top) Degradation of the polymer at pH = 5.5 (A) and pH = 7.4 (B). (Bottom) Schematic representation of the micellar assembly of the polymer with lysozyme and its degradation with time at acidic pH. (Adapted with permission from Figures 1 and 2 from ref 208. Copyright 2007 American Chemical Society.)

Tian and Hammond[228] reported the assembly into micelles of a comb-dendritic block copolymer based on poly(γ-*n*-dodecylglutamate) as a comb-block and a hydrophilic polyester dendron, modified at the end-groups with an ω-carboxylic acid PEG (Figure 25). Upon changing the pH, a decrease in micellar size was observed at basic pH, in contrast to the swelling behavior observed for the PLG-based block copolymers reported above. This was explained by the persistent shape of the dendritic block and the high density of ionizable carboxylic acid groups at the PEG chain ends, resulting in a significant increase in the headgroup area and thereby lowering the aggregation number and the size of the micelles.[229] Biodegradable and hyperbranched PEG-polyethyleneimine-PBLG (PEG-PEI-PBLG, with the PEI being hyperbranched) and PEI-PBLG were also shown to form micelles in solution.[228] In aqueous solution, the latter showed micelles that increased in diameter with increasing PBLG length. Unimolecular micelles were formed in chloroform that could efficiently capture ionic dyes such as methyl-orange between pH 2.3 and 10.3.[230]

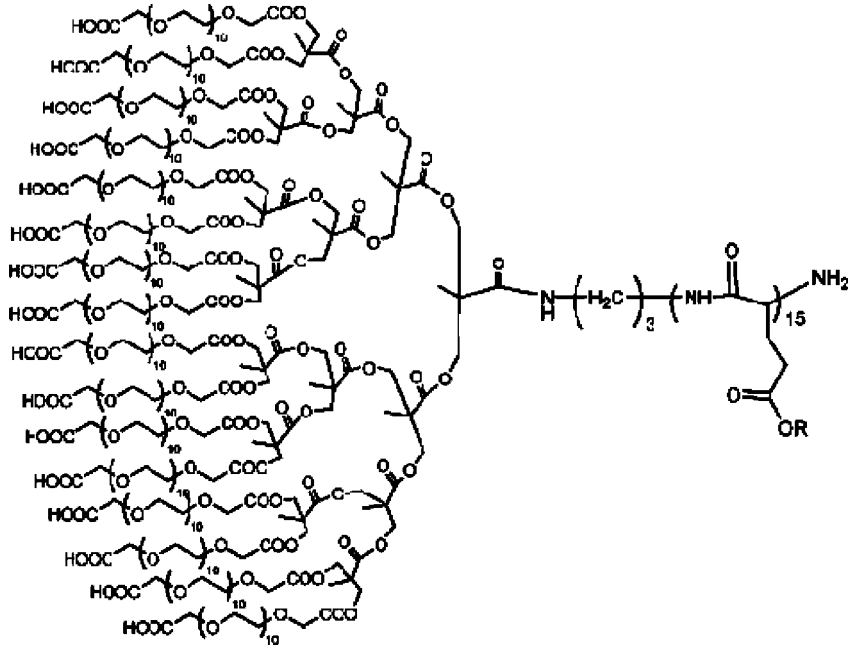


Figure 25. Chemical structure of the micelle-forming comb-dendritic block copolymer based on a poly(γ-*N*-dodecylglutamate) comb block and a hydrophilic polyester dendron modified with PEG. The high persistence length of the rodlike helical polypeptide backbone within the comb block resulted in a "treelike" architecture for this comb-dendritic block copolymer. In aqueous solution, the macromolecules self-assemble into spherical micelles with the hydrophobic comb blocks forming the inner core and the hydrophilic dendritic blocks forming the exterior shell. (Adapted with permission from Scheme 1 from ref 229. Copyright 2005 American Chemical Society.)

The synthesis of multiblock copolymers based on polypeptides has also been reported.[231−233] A pentablock copolymer of two PEO-PBLG blocks linked by a perfluoroether block, prepared by Thünemann et al.,[234] showed a mixture of spherical and cylindrical micelles in aqueous solution, with a diameter of 22−27 nm and a length of 100−200 nm. The core was formed by the fluoroether, which was surrounded by a thin shell (~2 nm) of glutamates in a β-sheet conformation and a corona of PEG-chains, giving core−shell−corona assemblies (Figure 26).[234]

3.1.4. Micelles Based on Double Hydrophilic Block Copolymers

Apart from amphiphilic block copolymers as a basis for micelles, some recent advances have been made in the field of micelles based on double-hydrophilic block copolymers (DHBCs).[235−241] Polymer aggregates formed from DHBCs are in equilibrium with their unimers rather than being kinetically trapped structures, which makes the aggregation properties of this class of block copolymers especially sensitive to changes in physicochemical parameters such as pH, temperature, and ionic strength.[242,243] At basic pH and room temperature, PLG_{110}-$PNIPAAm_{65}$ was molecularly dissolved but aggregated into micelles with a PNIPAAm core and a PLG corona when heated above the lower critical solution temperature (LCST) of PNIPAAm (32 °C). When the solution was changed to acidic conditions (pH = 2) at temperatures below the LCST, the PLG block transformed from a random coil conformation to a helix, resulting in micelles with a PLG core.[238] Remarkably, the same block copolymer, but with different block lengths ($PNIPAAm_{55}$-*b*-$PLGA_{35}$), showed similar "schizophrenic" behavior.[244] But instead of spherical micelles, now rodlike micelles were observed at high pH and temperatures above the LSCT.

Diblock and AB_2 star-polypeptide DHBCs of PLL and PLG were prepared by conjugating one or two PBLG-N_3 blocks to the (protected) monoalkynyl or dialkynyl PLL blocks using the Huisgen [3 + 2] dipolar cycloaddition. The product, a star-shaped polypeptide, was investigated for its pH responsiveness. The PLG blocks showed phase separation in water below pH = 4.2, and the PLL blocks separated above pH = 9.6, resulting in micelles with a PLG core at pH = 2 and micelles with a PLL core at pH = 12. At their isoelectric point (pH values between 4.6 and 6.2), the polymers precipitated (Figure 27).[245,246]

3.1.5. Micelles Based on Other Polypeptides

While the majority of biohybrid micelles described in the literature contain aspartic acid, glutamic acid, or lysine, incidental studies have reported on micelle formation of polypeptides such as polyhistidines,[247−249] polycysteines,[250,251] polyvalines,[252,253] and polyalanines.[254] For block copolymers containing these polypeptides, the "classical" block has been varied from biodegradable polymers such as poly(ε-caprolactone) (PCL) and poly(lactic acid) (PLA)[219,241,255−258] to organometallic poly(ferrocenyldimethylsilane)s.[259,260] For instance, poly(histidine)-PEG block polymers have been described that showed more or less stable aggregates at pH = 8 but disassembled at acidic pH as a result of protonation of the histidine imidazole ring, rendering the block copolymers soluble.[247−249] PS_{100}-*block*-$polycysteine_{20}$ assembled into micelles (of diameter 150−300 nm) with PS as the core and polycysteine as the corona. Interestingly, when the block copolymer was added to a solution of gold nanoparticles (AuNPs), they were readily encapsulated by the polymer, with the cysteine blocks interacting with the AuNPs and the PS forming a hydrophobic outer shell. The aggregates were stable without any additional modification.[251] By means of a radical addition reaction a dipeptide (cysteine-phenylalanine) was grafted on the PBD block of PBD-PEG, which caused a change in aggregate morphology from micellar for the original polymer to wormlike micellar and vesicular for the grafted copolymer. The transition was explained by a reduction in weight fraction of the PEG block by conjugation

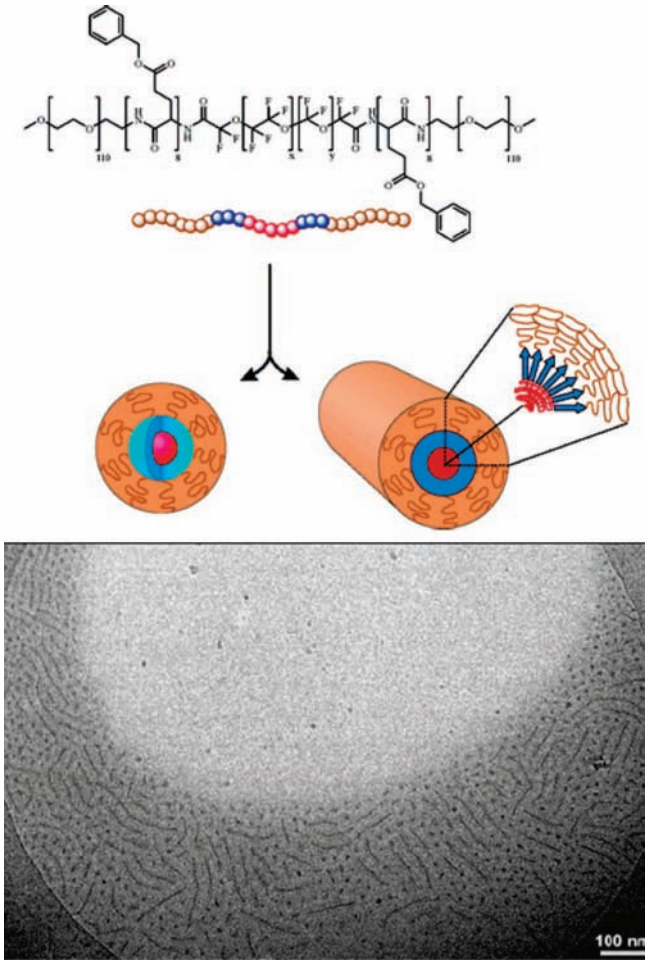


Figure 26. (Top) Chemical structure of a pentablock copolymer with two PEG-PLG diblocks connected by a perfluoroether, and the micellar and wormlike architectures it forms in water. (Bottom) Cryo-SEM picture showing the dense perfluoroether cores of the aggregates. (Adapted with permission from Figures 1 and 7 from ref 234. Copyright 2006 American Chemical Society.)

to the hydrophobic peptide, which shifted the aggregate structure to a state of lower interfacial curvature.[250]

Instead of the ring-opening polymerization of amino acids to construct block copolymers, more recently also ring-opening metathesis polymerization (ROMP)[261] and controlled radical polymerizations (CRPs),[262] such as reversible addition−fragmentation chain transfer (RAFT),[263−266] have been used. Temperature- and pH-responsive micelles were formed by block copolymers containing a poly(*N*,*N*-dimethylacrylamide) (PDMA) block and a statistical block of *N*-isopropylacrylamide and *N*-acrylolylvaline. Between pH = 2 and pH = 5, micelles were formed which could be reversibly "cross-linked" above the critical micelle temperature by complexing the poly(*N*-acryloylvaline) with a cationic polymer.[267] Triblock copolymers of PDMA-*block*-(*N*-acryloylalanine)-*block*-PDMA and analogues containing *N*-acrylolylvaline were shown to have similar properties.[268]

3.1.6. Micelles Based on Nonpolymerized Polypeptides

Besides the use of polymerization techniques to incorporate amino acids into the block copolymer architecture, a more recent alternative approach has been the modification of preformed polymers or micelles and their subsequent conjugation with peptides prepared by solid phase methods. An example of this strategy is the work of Liu et al.[269] Shell cross-linked nanoparticles (SCKs—this abbreviation actually expands to *shell cross-linked knedel*.[270−272] The word "knedel" is Polish and describes a food of meat surrounded by a dough layer.), obtained by the micellization of PCL-PAA followed by cross-linking of the shell by amidation, were covalently attached to the tat peptide, an oligomeric cell penetrating peptide[273] sequence from the HIV tat protein. This tat peptide was prepared by a solid phase protocol.[269] The particles showed cell surface binding and uptake.[274] A variety of supramolecular structures, among which micelles, were formed by connecting similar tat peptides to a hydrophobic lipid dendrimer.[275] In a different approach, tritrpticin (an antimicrobial peptide) was prepared on a solid phase and functionalized with an atom transfer radical polymerization (ATRP) initiator. Subsequently, while still on the resin, a block copolymer was polymerized from the peptide. The resulting block-copolymers self-assembled in aqueous solution to form micelles. Importantly, the peptide maintained its antimicrobial activity (Figure 28).[276]

Oligo-arginines are also known to be potent cell-penetrating peptides.[273,277] In the group of Börner,[278] a solid phase supported peptide synthesis technique was applied to prepare an ABC block copolymer. In this block copolymer, a central Arg_{10} oligopeptide block, a hydrophilic outer PEG block, and a hydrophobic poly(butyl acrylate) (PBA) block were combined. This PEG_{60}-Arg_{10}-PBA_{115} triblock copolymer assembled into spherical micelles. The functional Arg_{10}-domain was suggested to be positioned at the interface of the core−shell aggregates.[278] Another biologically active moiety, the tripeptide arginine-glycine-aspartic acid (RGD), is an extensively studied cellular binding domain found in the extracellular matrix.[279] It has also been the focus of studies in which the peptide motif was either grafted to micelles[280] or introduced directly into the polymer chain before self-assembly.[281]

A noncovalently connected multiblock copolymer was constructed from a set of polymer−peptide conjugates, in which the polymers were either PS or PEG and the peptide blocks were two complementary random coil peptide blocks.[282] In solution, these complementary peptide blocks associated to form an α-helical heterodimer. Interestingly, these structures initially formed rodlike micelles. The rodlike micelles were suggested to consist of a rodlike PS block which was shielded from the aqueous buffer by clusters of coiled-coil peptides around which PEG was closely folded. After the reversible dissociation of the heterodimer, by heating to 96 °C and cooling again, the system did not return to its initial state but instead formed spherical micelles. These consisted of a PS-core with a PEG-polypeptide corona (Figure 29).[282]

Instead of specific peptide sequences, also entire proteins were successfully attached to polymeric micelles.[283,284] Micelles that had furan groups anchored to the hydrophilic PEG shell were conjugated by Diels−Alder chemistry to a maleimide-functionalized anti-HER2 antibody (a therapeutic antibody used to treat breast cancer). The functionalized micelles specifically bound to cells overexpressing the HER2-receptor.[285,286] SCKs bearing aldehyde groups on the outer surface were conjugated to lysozyme via Schiff base formation. However, the residual activity of the enzyme was not assessed.

Instead of using preformed micelles to attach proteins, the group of Nolte used proteins as the hydrophilic headgroup to form giant amphiphiles,[287] which assembled into a range

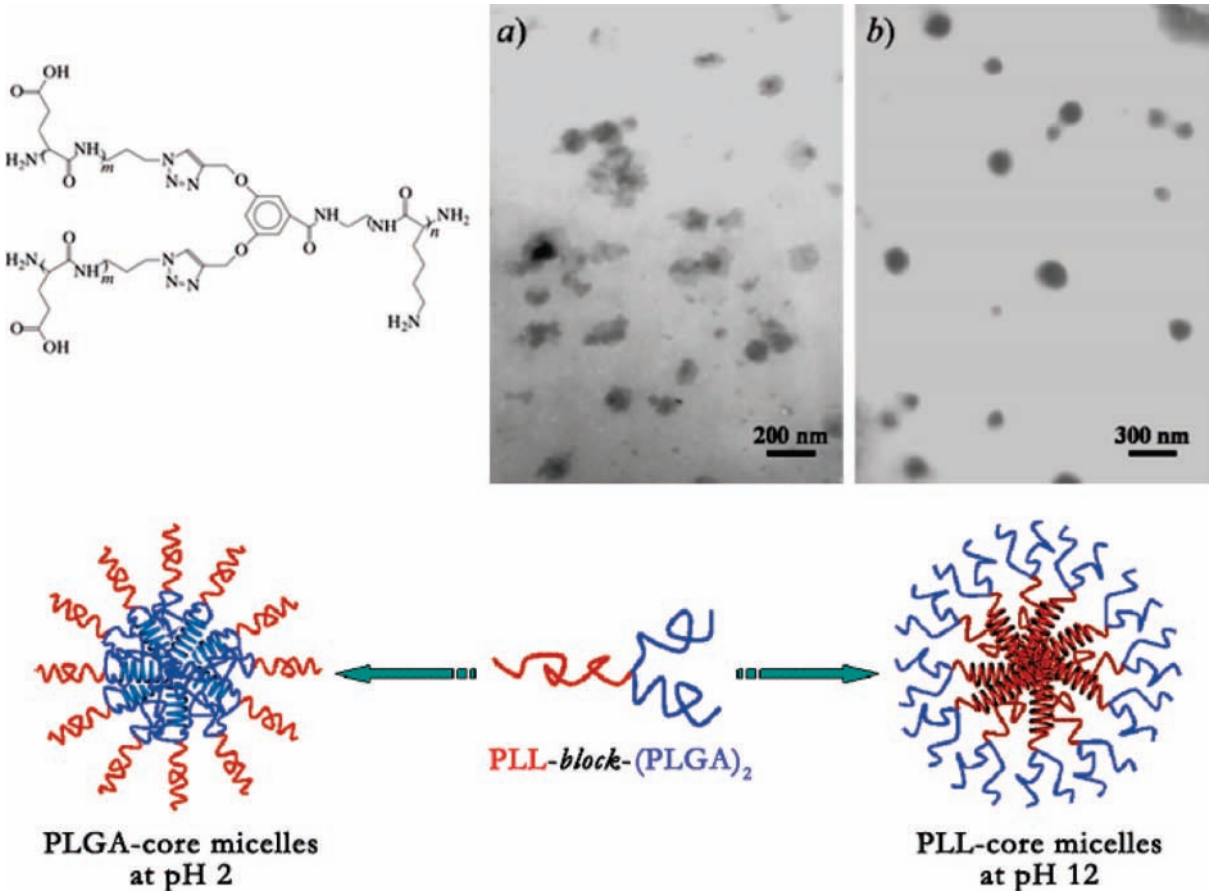


Figure 27. (Top left) chemical structure of a Y-shaped star-polypeptide block copolymer; (top right) the micellar aggregates it forms in water at (a) pH = 2 and (b) pH = 12; (bottom) schematic representation of the "schizophrenic" micellization behavior that results from the pH-dependent solubility of the respective polypeptide blocks. (Adapted with permission from Figure 8 and Schemes 1 and 2 from ref 245. Copyright 2008 American Chemical Society.)

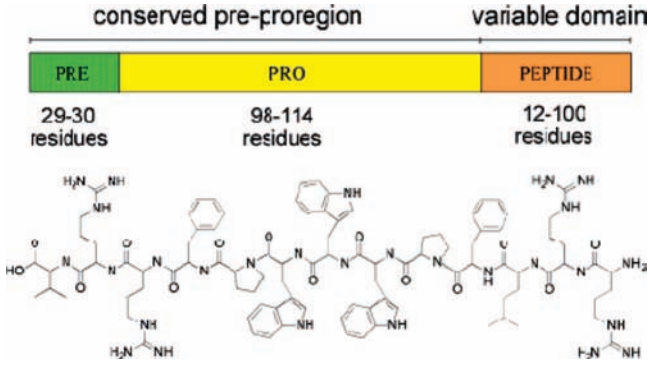


Figure 28. Chemical structure of tritrpticin (VRRFPWWWP-FLRR), a variable domain at the end of the larger prepro domain, which is largely conserved within the Cathelicidin family of antimicrobial peptides. The distinct fold in the 3D structure of this peptide and the separation of the cationic residues from the hydrophobic residues result in an amphiphilic structure, which is believed to be the structural feature that leads to antimicrobial activity. (Reproduced with permission from ref 276. Copyright 2005 American Chemical Society.)

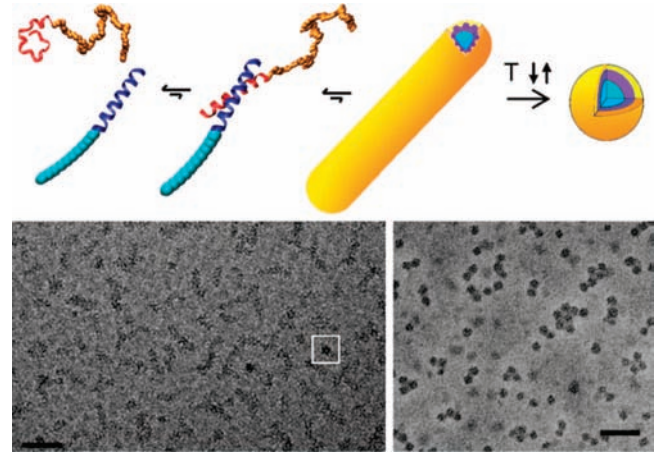
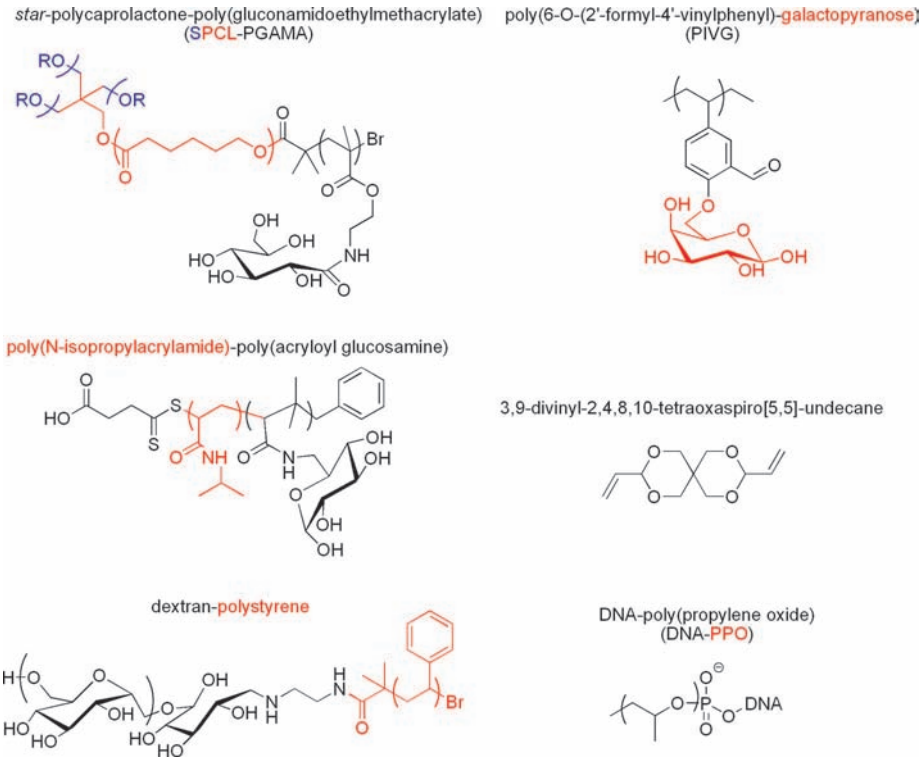


Figure 29. (Top) schematic representation of peptide−polymer conjugates of PS (light-blue) and PEG (orange). The peptides form a heterodimeric α helix, thus forming a noncovalent triblock copolymer; (below) cryo-SEM micrographs of the assemblies formed in water before (left) and after (right) annealing of the helix dimers. (Adapted with permission from Figures 4 and 6 and the table of contents image from ref .) Copyright 2008 American Chemical Society.)

of supramolecular structures, depending on the enzyme and polymer used.[288] For example, an initial study in which *Candida antarctica* lipase B (CalB) was connected to a maleimide appended PS-block via a reduced disulfide bridge on its surface yielded micellar tubes that were still catalytically active.[289] This strategy was extended to the conjugation of either a coumarin-labeled tripeptide or bovine serum albumin (BSA). In these cases the Cu catalyzed Huisgen cylcoaddition reaction was used between azides and alkynes, employing alkyne functional peptides or BSA, and azide functional polystyrene. In aqueous solution the polymer−BSA hybrid showed micellar aggregates.[287]

Because of its ability to combine amino acid sequence control with the ability to produce high molecular weight polypeptides, protein engineering has become a popular tool

Chart 2. Structure Formulas of Various Polymeric Micelle-Forming or Related Compounds



in materials science. Polymers containing genetically encoded poly(amino acids) can nowadays be created with well-defined physical properties, such as stimuli-responsive self-assembly.[290] For example, elastin-like polypeptides (ELPs) of the general structure Val-Pro-Gly-Xaa-Gly, where Xaa can be any amino acid except proline, show LCST behavior. The LCST can be varied by varying, among others, the Xaa-residue. Using this approach, a 165 kDA triblock copolymer with an elastin−mimetic polypeptide sequence was expressed. The triblock copolymer had a hydrophobic (LCST $\sim$ 20 °C) middle block and a hydrophilic end-block. At T = 5 °C, even below the LCST of the middle block, micelles could be observed. At temperatures above T = 25 °C the micelles decreased in size and formed denser structures, which was ascribed to an α-helix to β-sheet arrangement transition in the micellar core.[291]

Dreher and co-workers have prepared a series of ELP block-copolymers with different ratios of hydrophobic blocks (low LCST) and hydrophilic blocks (high LCST) by protein engineering.[292] It was found that monodisperse spherical micelles were formed at temperatures between the LCST of the separate blocks when the hydrophobic−hydrophilic ratio was between 1:2 and 2:1. Eventually, a set of ELP block copolymers could be selected that showed self-assembly at the desired temperature of T = 42 °C, with the block copolymers still molecularly dissolved at T = 37 °C.[292] These target temperatures took advantage of the fact that certain types of tumors have an elevated temperature, thermally triggering self-assembly of the micelles. Conjugation of the ELP block copolymers to RGD ligands thus enabled thermal triggering of multivalent RGD presentation, using polypeptide micelles as a scaffold.

3.2. Micelles Based on Glycopolymers

With the advent of CRP techniques, some progress has been made in the synthesis of new (amphiphilic) polymer architectures containing carbohydrates (see Chart 2).[293−295] A main strategy to access such polymers has been the polymerization of monomers containing pendant carbohydrate groups, the so-called glycopolymers. Nowadays this terminology is used in a more general sense, meaning any type of synthetic polymer containing carbohydrate moieties. The majority of studies reporting the synthesis of amphiphilic glycopolymers does not focus on their self-assembly, but rather on the synthesis and characterization of the polymers. Conceivably, this is a result of the complex synthesis methods involved. However, the introduction of RAFT polymerization, with its wide tolerance for functional groups, has made these architectures more accessible.

3.2.1. Micelles Based on Glycopolymers Prepared by CRP Techniques

Before the arrival of RAFT, a few groups reported the synthesis of amphiphilic glycopolymers, mainly by nitroxide-mediated radical polymerization, that assembled into micelles.[296−303] Star-shaped block copolymers of PCL-*block*-poly(gluconamidoethylmethacrylate) (PGAMA) were prepared by ATRP of the unprotected glycomonomer from a star-shaped PCL macroinitiator. The crystallinity of the PCL_{15}-block decreased significantly with the weight fraction of the PGAMA-blocks (n = 7, 11, or 18). The aggregate morphology changed from spherical micelle to wormlike micelle and then to vesicle, upon the decrease in weight-fraction of the PGAMA-block. The interaction of a sugar-binding protein, Concanavalin A (Con A), with the aggregates led to a distinct change in size of the aggregates, but this was not further investigated.[304] The same authors also reported a micelle-forming ABA triblock copolymer of PGAMA-PCL-PGAMA, in which the PCL-block was threaded with α-cyclodextrin (αCD).[305] Micelle-forming amphiphilic poly(macromonomers)[306] containing different sugars (galac-

tose, mannose, ribonic-γ-lactone, and xylose) were prepared by ROMP.[307]

3.2.2. Micelles Based on Glycopolymers Prepared by RAFT

RAFT has been successfully applied to the synthesis of glycopolymers, using either protected or unprotected monomers.[308−311] The monomer 1,2,3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)galactopyranose was polymerized by RAFT.[312] The resulting polymer had hydrophilic side-chains and a hydrophobic backbone, which led it to form micelles in aqueous solution. The presence of an aldehyde group allowed for the immobilization of BSA on the micellar surface.[312] A RAFT-synthesized thermosensitive poly(acryloyl glucosamine)-*block*-PNIPAAm block copolymer assembled into micelles above the LCST of the PNIPAAm block, and its core (PNIPAAm) was cross-linked by reaction with the acid sensitive cross-linker 3,9-divinyl-2,4,8,10-tetraoxaspiro[5,5]undecane. At temperatures below the LCST and at pH = 8, the aggregates were stabilized but rapidly degraded (t = 30 min) to unimers at pH = 2. The micelles (at t = 0) showed a remarkable variation in size at the pH studied (pH = 2, 310 nm; pH = 8, 85 nm).[313]

3.2.3. Micelles Based on Grafted Glycopolymers

The introduction of carbohydrates into block copolymers has also been accomplished by employing sugar-derived initiators ("grafting from")[314−316] or by the introduction of the carbohydrate moiety after polymerization ('"grafting-to").[317,318] Micellar aggregates formed by these polymers were studied for their ability to bind lectins (carbohydrate binding proteins) in a qualitative manner.[314,315,317] Wooley et al. reported the preparation of mannosylated SCKs prepared by cross-linking the shell of PAA-PMA by varying degrees of mannosylation (0, 1, 2, 10, and 100%). The 1% and 2% mannose-bearing particles were found to interact with Con A (a lectin that has also been used in studies described above), as evident from an increase in diameter of the aggregates. The ability of the nanoparticles to inhibit the agglutination (clustering) of red blood cells due to Con A was also examined. It was found that when a higher mannose density was present on the particles, the minimum concentration of micelles needed to inhibit agglutination decreased. However, the studies were somewhat obscured by the observation that even the sample that contained 0% mannose showed significant inhibition.[316] Using isothermal titration calorimetry (ITC), Rieger and coworkers studied the interaction of mannosylated PEG-PCL micelles with BclA lectin, which binds more strongly to mannose than Con A does. The thermodynamic parameters calculated from ITC showed that mannose binding was similar in all cases, irrespective of whether the mannose was immobilized on the surface of micelles or not.[318]

The introduction of carbohydrate monomers into polymers and their self-assembly has also been studied intensively by Lim et al.[319] A short PEG block (n = 23) with a hydrophobic tetra(*p*-phenylene) was synthesized together with two other amphiphiles that either had a smaller PEG block (n = 12) or a different hydrophobic block, e.g. a di[tetra(*p*-phenylene)]. These rod−coils were terminated by a mannose moiety via glycosylation of the free hydroxyl group on the hydrophilic ethylene glycol terminus. The first compound was found to assemble into small spherical micelles, while the others assembled into vesicles and cylindrical micelles, respectively. Agglutination tests (see above) showed an 1800-fold decrease in the minimum inhibitory concentration for the micellar aggregates. For the vesicles and micellar rods, an 800-fold and 1000-fold increase was found. It was suggested that the spherical micellar objects, with their higher curvature, are more efficient inhibitors than the larger vesicular and cylindrical objects.[320]

Glycopolymers have also been prepared by polymerization from macroinitiators that were derived from bioavailable oligo- and polysaccharides.[321] End-functionalized PS with βCD was prepared with this approach and showed aggregates with a cyclodextrin core in benzene.[322] Comparable polymers with a longer PS block were prepared by Felici et al.[323] and were found able to form polymersomes, as further discussed in a later section. Likewise, a series of polystyrenes were prepared that were end-functionalized with malto-oligosaccharides, i.e. oligomers of glucose, with one to six oligomerized glucose units. The deprotected glycoconjugates showed reverse micellar assemblies,[324] as probed by SLS, which increased in aggregation number with increasing MW of the polymers. ATRP of styrene from a bromo-isobutyramide-terminated dextran macroinitiator yielded dextran-PS block copolymers which adopted various structures in aqueous solution, including micelles.[325] Instead of creating a polysaccharide functional block copolymer by initiating the polymerization from the carbohydrate block, the reverse was also demonstrated. For instance, the synthesis of PS-amylose by modification of amino-terminated PS with maltoheptaose moieties was reported. The maltoheptaose moieties served as initiators for the enzymatic polymerization of glucose-1-phosphate using potato phosphorylase.[326,327] Using a modular approach, a double hydrophilic block copolymer, dextran-PEG, was prepared by the conjugation of amino terminated PEG with end-lactonized dextran. At acidic pH, tight micellar aggregates were formed, which converted to more loosely aggregated structures at higher pH.[328]

3.2.4. Micelles Based on Noncovalently Connected Glycopolymers

Noncovalent approaches to glycopolymer aggregates have recently been described. A DHBC was constructed based on the host−guest inclusion complex between βCD and the adamantyl-group. The βCD and the adamantly moiety were first converted to their trithiocarbonate analogues and applied in the RAFT-polymerization of 4-vinylpyridine and PNIPAAm, respectively. After removal of the trithiocarbonate group, the polymers formed inclusion complexes when dissolved in equimolar ratios in aqueous solution. In the narrow pH-range of pH = 4.2−5, these inclusion complexes formed micelles as a result of partial deprotonation of the poly(4-vinylpyridine) chains. At temperatures above the LCST of the PNIPAAm-block, the inclusion complex formed vesicles, as discussed in section 5.1.2 (Figure 30).[329] Using a different approach, supramolecular micelles were prepared from the partial threading of a maleic anhydride appended αCD onto PCL. The threading of the cyclodextrin rendered the polymer partially hydrophilic. The resulting amphiphile assembled into micelles in water. This approach was suggested to be especially interesting for drug delivery applications, as both PCL and CDs are FDA approved materials.[330]

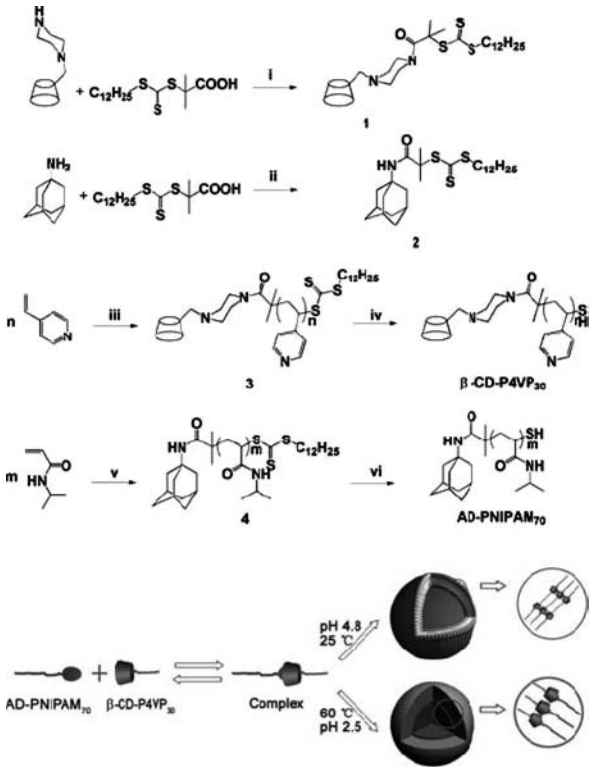


Figure 30. Synthesis procedure for the preparation of adamantyl-P4 VP and cyclodextrin-PNIPAAm, and formation of the inclusion complexes of the polymers. The double hydrophilic polymers formed distinctly different aggregates in solution in response to pH and temperature, leading to either micelles or polymersomes. (From ref 329. Reproduced by permission of The Royal Society of Chemistry.)

3.3. Micelles Based on Nucleic Acids

The rationale behind the use of polymers with nucleobase functionalities or oligonucleotide sequences is to harness the superior self-organizing properties of nucleic acids for the supramolecular organization of macromolecules, either in bulk or in solution.[331−333] CRP has been used by several research groups to synthesize polymers containing nucleobase motifs with the aim of introducing H-bonding as an intermolecular driving force for aggregation.[334−336] As is the case for glycopolymers, these studies have not directly led to thorough investigations into the self-assembly of these polymers in solution, although some studies report the formation of vesicles, tubes, and large compound micelles.[335,337−339]

The formation of polymeric micelles from the combination of oligonucleotides and synthetic polymers has mainly been restricted by the low availability of large quantities of synthetic oligonucleotides, exacerbated by their liability to degradation.[340,341] The field has gained impetus, however, by publications from the group of Mirkin[342] and Jeong and Park.[343] The former used a solid phase synthesis method similar to conventional oligonucleotide synthesis to prepare oligonucleotides that were coupled by their 5′-hydroxyl end to a phosphoramidite-polymer derivative.[344] This technique has been adopted and improved by the group of Alemdaroglu and Herrmann, and a recent review has appeared from their hand, which gives a concise overview of the research on DNA block copolymer micelles, to which the reader is referred.[345] Here, we will only cover the very recent contributions. The basis for the work of Herrmann et al. is a DNA-*block*-poly(propylene oxide) (DNA-PPO) micelle with the hydrophobic PPO having a low glass-transition

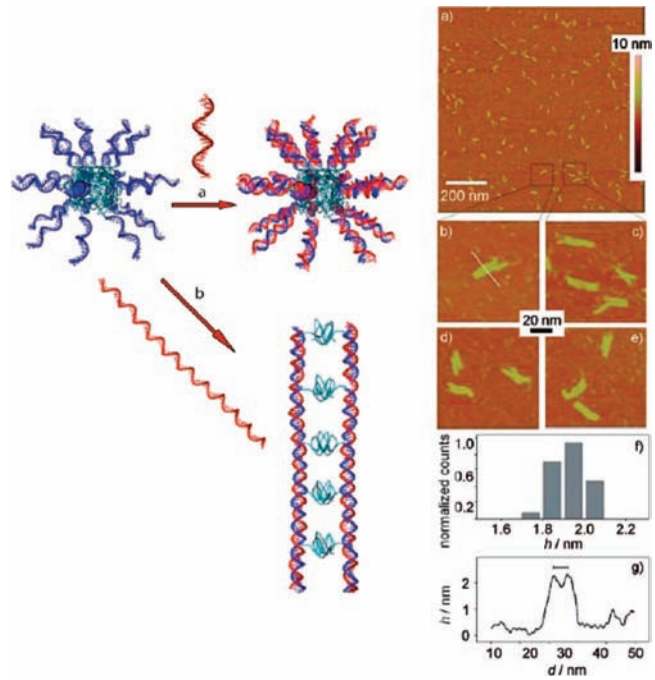


Figure 31. (Left) micellar aggregates formed by DNA-PPO block copolymers and the supramolecular structures they adopt upon the addition of antisense oligonucleotides: (a) 1 repeat unit or (b) 5 repeat units; (right) SFM topography image of the rodlike aggregates formed. (Adapted with permission from Figures 1 and 3 from ref 349. Copyright 2007 Wiley.)

temperature (−70°).[346] These micelles were used for drug delivery, for the assembly of thermoreversible organic/inorganic networks, and as programmable nanoreactors.[345] Recently, it was demonstrated that the single-stranded DNA on the corona of the micelles could be extended by the template-independent DNA polymerase Terminal deoxynucleotidyl Transferase (TdT). Scanning force microscopy (SFM) on the particles showed that upon the addition of TdT, and in the presence of Co^{2+} and mononucleotides, the height of the particles increased from 4.9 to 11.2 nm after 16 h. This corresponded to an increase in length of the DNA block with 62 nucleotides.[347] The growth of the micelles was also followed in situ, by immobilizing the aggregates on mica and following the growth by STM. It turned out that the immobilization of the micelles caused a saturation of the increase in height of the micelles after 1 h.[348] Furthermore, the influence of hybridizing the DNA-block with different lengths of antisense oligonucleotides was investigated. Whereas incubation with short complementary sequences did not affect the micellar architecture, long DNA-sequences with multiple repeats of the antisense DNA led to rodlike aggregates with two parallel aligned double helices (Figure 31).[349] The cellular uptake of the latter rodlike aggregates was 12 times more efficient than that of spherical architectures.[350] In the view of drug delivery, the DNA-block was hybridized with a folate-conjugated antisense-oligonucleotide. The resulting hybrid was found to be efficiently taken up by human cancer cells, which have a high density of folate receptors on their membranes. The cancer cells could be efficiently killed by loading of doxorubicin in the hydrophobic core of the aggregate. The straightforward functionalization of the block copolymers by hybridization, as compared to the functionalization of block copolymers by synthetic methods, seems to be an advantageous property of these nanoparticles.[351]

3.4. Outlook

In summary, the field of micelles based on polypeptides has since its introduction expanded past the study of polyaspartates, polyglutamates, and polylysines; it now encompasses the study of other poly(amino acids) and more complex micelle-forming star block copolymers. In addition, the opportunity of designing specific amino acid sequences or motifs found in natural peptide polymers, and constructing them by genetic engineering, has been added to the toolbox chemists can use for the design of responsive polypeptide-based micelles.

Compared to their peptide counterparts, glycopolymer micelles lag far behind, regarding the possible structures that have been found to assemble into micelles. The focus here lies mainly on the design of new glycopolymers, with the study of their self-assembly being only incidental. In nature, sugars play a pivotal role in recognition and multivalent binding processes,[155] offering researchers a huge reservoir to tap from for the design of vehicles for targeted drug delivery. In this respect, the grafting of (oligo-)sugars to preformed micelles seems to be a valid approach for applications where recognition and binding processes are involved, such as drug delivery.

The way in which nucleic acids can be designed to assemble into higher order structures and made to respond to outside stimuli is nigh unrivaled. Their incorporation into block copolymers for the assembly of micelles has only recently been explored, mainly by the contribution of the group of Herrmann and Alemdaroglu (see section 3.3), which shows the exciting opportunities made possible by this high level of structural control.

To conclude, the field of biohybrid polymer micelles is tilted to the development and application of polymers incorporating peptide sequences. The basic explorations of their physical properties, in particular their response to pH and temperature changes, have led to the design of polymer structures that show a controlled temperature and pH responsiveness. The development of the field has kept pace with the advent of new polymerization techniques, such as RAFT polymerization, which may especially make the development of new glycopolymers more accessible; this may cause the current focus on their synthesis to shift to the study of their self-assembly behavior and biological properties. The ability to produce high molecular weight and structurally well-defined polypeptides by genetic engineering is expected to lead to an even higher level of structural and functional diversity, with the same holding true for the field of micelles based on nucleic acids.

4. Layer-by-Layer Capsules

Layer-by-layer (LbL) capsules are composed of thin polymer shells built up from multiple polymer layers. Consecutive layers of polymer interact with one another, keeping them in place. These interactions are almost exclusively based on electrostatic attractions between oppositely charged polyelectrolytes. The relative ease of layer deposition makes this an efficient method for the construction of capsules. However, as opposed to the variety of biohybrid polymers reported in the construction of dendrimers, polymeric micelles, or polymersomes, reports on LbL capsules based on this class of polymers are scarce. Therefore, in this section, first the basics of the technique will be explained including examples in which biomolecules, and more specifically proteins, are used. Next, an overview of the encapsulation of biomolecules inside hollow LbL vesicles, as well as their incorporation into LbL membranes, will be given. Finally, different biomedical applications of functional LbL capsules will be discussed.

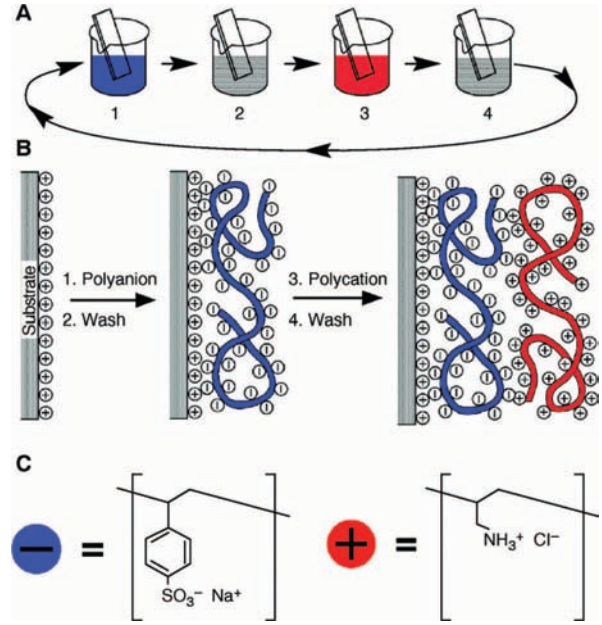


Figure 32. Schematic representation of layer by layer deposition. (A) Immersing slides in beakers containing polyanion and polycation, respectively, in a cyclic fashion. (B) Simplified molecular picture of the first two adsorption steps, depicting film deposition starting with a positively charged substrate. The polyion conformation and layer interpenetration are an idealization of the surface charge reversal with each adsorption step. (C) Chemical structure of polyions used. (From ref 356. Reprinted with permission from AAAS.)

4.1. Introduction to Layer-by-Layer Architecture

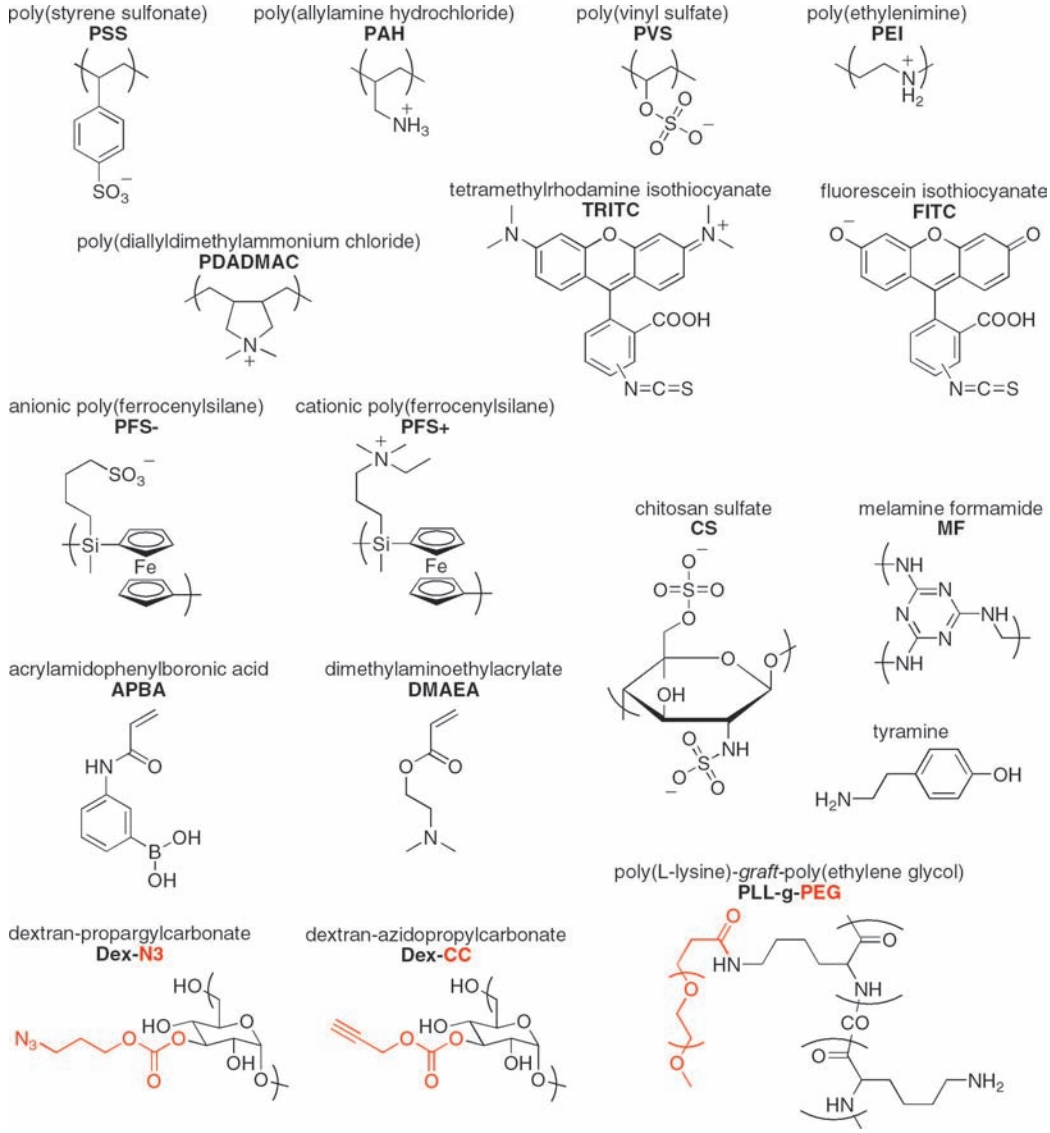
4.1.1. Layer-by-Layer Deposition on Thin Solid Films

In the early 1990s Decher and co-workers developed a new technique for constructing ultrathin organic films, creating multilayer assemblies by consecutive, layer-by-layer (LbL) adsorption of anionic and cationic polyelectrolytes.[352−355] The principle of the multilayer assembly is shown in Figure 32. Film deposition on a glass slide can be carried out in ordinary beakers. A glass slide with a positively charged surface is immersed in a solution containing an anionic polyelectrolyte, and a layer of polyanion is adsorbed. After rinsing in pure water, the substrate is immersed in a solution containing the cationic polyelectrolyte. By repeating these steps in a cyclic fashion, alternating multilayer assemblies are obtained.[356] Importantly, this technique can be applied to flat surfaces as well as to spherical particles.

For these initial studies, two typical polyelectrolytes were used for multilayer formation, namely sodium poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH). However, in principle, it is possible to incorporate many different polyelectrolytes into the multilayer assemblies, including biological polyelectrolytes such as DNA and proteins (see Chart 3).[357]

This concept was expanded by creating a multilayer of proteins of opposite charges.[358] At pH 6.5, lysozyme is positively and glucose oxidase (GOx) negatively charged. On the template particle, first a precursor film was deposited

Chart 3. Structure Formulas of Various Polyelectrolytes and Related Compounds



which consisted of two layers of both PEI and PSS (denoted as $(PEI/PSS)_2$). This precursor was then coated with an outermost layer of the positively charged lysozyme. Glucose oxidase molecules were added to adsorb onto the positively charged lysozyme layer. However, an additional polyelectrolyte layer of (PSS/PEI) on top of the lysozyme layer had to be introduced to enable attachment of negatively charged glucose oxidase molecules. It was argued that these difficulties for adsorbing oppositely charged proteins might be due to the crucial role of the density and location of charge on the protein surface.[358] Using this successful strategy of coating with polyelectrolyte in between enzyme layers, a multienzyme film containing GOx and horseradish peroxidase (HRP) layers was prepared. The enzyme activities of these films on the substrates glucose and H_2O_2 were investigated by the coloration reaction of 10-(carboxymethylaminocarbonyl)-3,7-bis(dimethylamino)phenothiazine sodium salt (DA67), which demonstrated that the enzymes retained their catalytic activities.[359] The multilayer formations were characterized using physical techniques, such as quartz crystal microbalance (QCM) and small-angle X-ray reflectivity, confirming their formation.[357,358]

4.1.2. Layer-by-Layer Deposition on Spherical Particles

Formation of multilayers has been transferred from thin solid films to spheres by Sukhorukov et al.[354,360] To this end, polystyrene latex particles modified with sulfate groups were chosen for the adsorption of multilayers. The multilayers were assembled by one of two methods: either by adsorbing at supersaturating bulk concentrations, with three repeated intermediate centrifugation cycles for washing, or by adsorption without centrifugation, at polyelectrolyte concentrations that were just beyond the onset of ζ-potential saturation for polystyrene particles smaller than 100 nm in diameter. The formation of polyelectrolyte multilayers was monitored by the electrophoretic mobility of the particles.[360]

Multilayers of fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA), alternating with poly(diallyldimethylammonium chloride) (PDADMAC), and immunoglobulin (IgG), alternating with PSS, were fabricated (Figure 33).[361] The assembly of these protein multilayers was followed by electrophoretic mobility measurements. To obtain quantitative evidence of stepwise protein multilayer growth, the technique of single particle light scattering (SPLS) was employed. SPLS is a sensitive optical technique

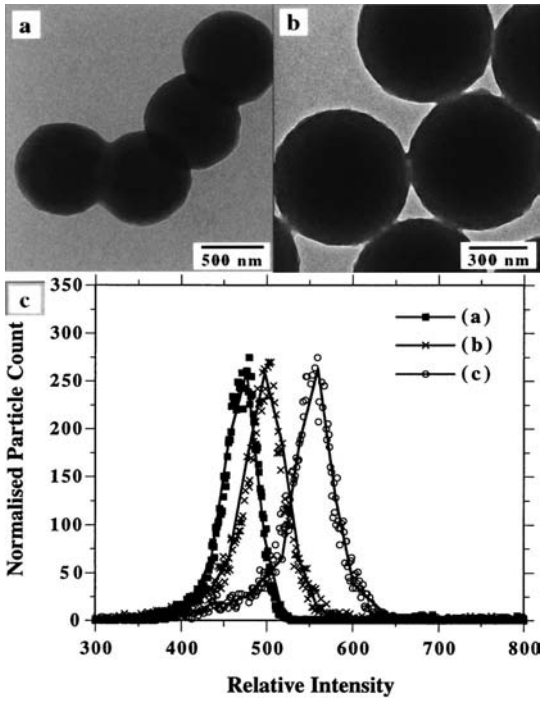


Figure 33. TEM micrographs of (a) IgG multilayers assembled onto $(PAH/PSS)_2$-coated PS latex particles and (b) $(FITC\text{-}BSA/PDADMAC)_2$/FITC-BSA coated on PDADMAC/PSS/PDADMAC-coated PS latex particles. (c) Single particle light scattering intensity distributions of (a) PDADMAC/PSS/PDADMAC)-modified PS latex particles, additionally coated with (b) one or (c) three layers of FITC-BSA/PDADMAC. (Adapted with permission from Figures 4, 7, and 8 from ref 361. Copyright 1999 American Chemical Society.)

that enables determination of the thickness of layers assembled onto colloids, as well as the state and degree of the coated colloids with respect to aggregation. Figure 33c shows the normalized SPLS intensity distributions for (PDADMAC/PSS/PDADMAC)-modified PS latex particles (curve a), and the same particles coated with one (curve b) and three (curve c) FITC-BSA/PDADMAC multilayers. When additional layers of electrolyte were added, the corresponding increase in particle size was clearly observed with this technique. Enzymes were also assembled onto the particles, and their catalytic activity was studied.[362−364]

4.1.3. Hollow Layer-by-Layer Capsules

LbL deposition onto charged colloidal particles in solution was explored to construct hollow polyelectrolyte shells through the stepwise adsorption of polyelectrolytes onto a decomposable colloidal template. This template was subsequently removed after formation of the multilayer shells was realized (Figure 34).[365]

In this case, instead of polystyrene (PS) latex particles, weakly cross-linked melamine formaldehyde (MF) colloidal particles were used. These particles decompose in aqueous media at pH values below 1.6.[366] The PSS/PAH polyelectrolyte multilayer film was built up beginning with adsorption of the negatively charged polyelectrolyte onto the positively charged MF particles. When these coated MF particles were exposed to low pH, the core decomposed (Figure 34), and the residual MF oligomers were expelled from the core, since they could permeate through the polyelectrolyte layers that form the shell. These MF oligomers were separated from the hollow shells by centrifugation. SEM images of capsules

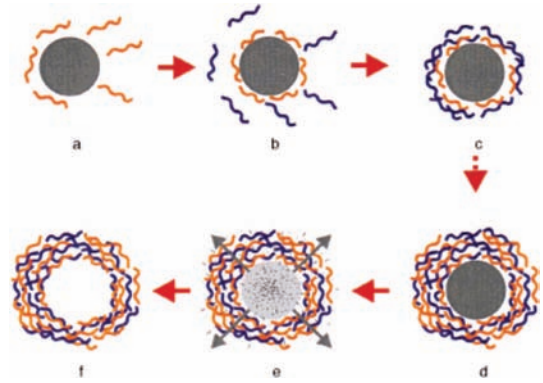


Figure 34. Shematic representation of the polyelectrolyte deposition process and of subsequent melamine formaldehyde core decomposition. The initial steps (a−d) involve stepwise film formation by repeated exposure of the colloids to polyelectrolytes of alternating charge. (e) After the desired number of polyelectrolyte layers was deposited, the coated particles were exposed to 100 mM HCl. The core immediately decomposed, as evidenced by the fact that the initially turbid solution became essentially transparent within a few seconds. Three additional washings with 100 mM HCl ensured removal of the dissolved MF oligomers. (f) Finally, a suspension of free polyelectrolyte hollow shells was obtained. (Reproduced with permission from ref 365. Copyright 1998 Wiley.)

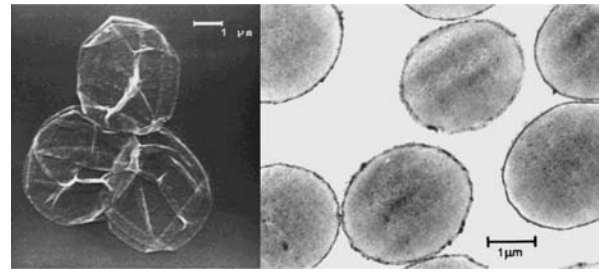


Figure 35. Nine-layer $[(PSS/PAH)_4/PSS]$ polyelectrolyte shells. (Left) SEM images of the polyelectrolyte shells after solubilization of the MF core. (Right) TEM images of the stained samples of polyelectrolyte shells. (Adapted with permission from Figures 2 and 3 from ref 365. Copyright 1998 Wiley.)

consisting of nine layers $[(PSS/PAH)_4/PSS]$ are shown in the left panel of Figure 35. Numerous folds and creases were observed, indicating the shells had low bending resistance. The diameter of these particles was approximately 4.0 μm, which was larger than that of the template MF particles (3.3 μm), indicating that layer deposition had successfully taken place on the MF core. TEM images of the same hollow polyelectrolyte shells are displayed in the right panel of Figure 35. By embedding the polyelectrolyte capsules in a resin, the homogeneous curvature of the shells was preserved.[365]

MF particles are widely used as core templates and have been very well characterized. They are favored above PS-particles because of their decomposable character but have several disadvantages, such as their nonbiocompatibility. Furthermore, the oligomers formed after decomposition can partially remain inside during the extraction process and there is an increased resistance to decomposition with time. In order to overcome these disadvantages, other biocompatible and decomposable templates for LbL techniques have been investigated.[367] The two most extensively studied template materials are poly-DL-lactic acid (PLA) and poly(DL-lactic-*co*-glycolic acid) (PLGA). Both are biodegradable and biocompatible and also used for the construction of polymersomes, as described in section 5.1.4.

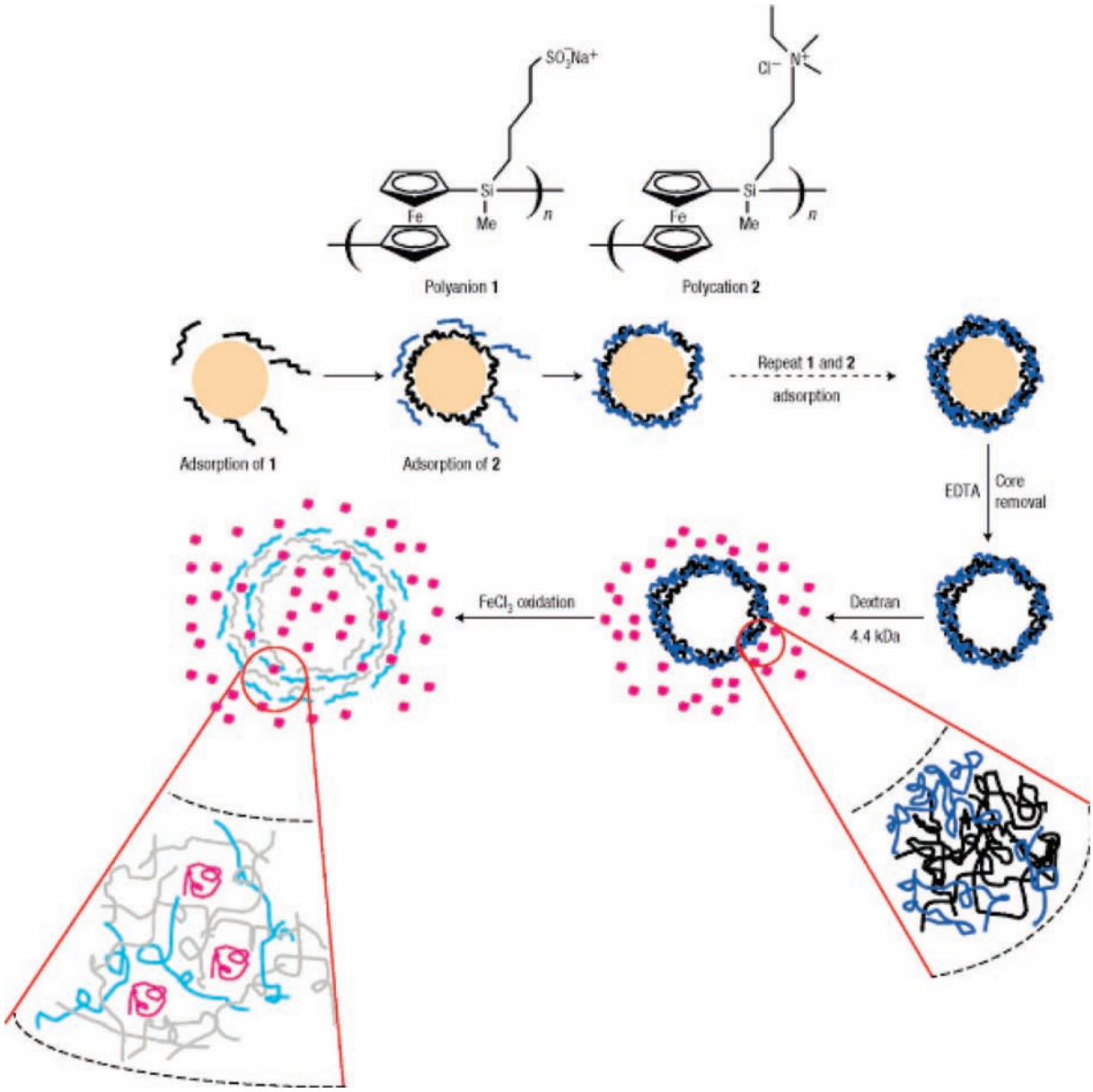


Figure 36. Schematic representation of organometallic capsule formation and of oxidative permeability control of the resulting capsule. (Reprinted with permission from Macmillan Publishers Ltd.: *Nature Mater.*, ref 368. Copyright 2006.)

Degradable microparticles based on these biopolymers were prepared using the oil/water emulsion−solvent evaporation technique. Briefly, a solution of PLGA in dichloromethane was introduced into an aqueous phase containing a polymeric emulsion stabilizer, under constant mechanical agitation. High-speed emulsification was followed by slow magnetic stirring to evaporate organic solvent from the bulk. The resulting particles were collected by centrifugation and washed with ultrapure water. The microparticles produced by this technique ranged from 50 nm to 50 μm and could be further processed to obtain a more homogeneous size distribution. PSS and PAH were chosen as polyelectrolytes to coat onto the biodegradable templates. The next step was the removal of the core by dissolution, which was achieved by dissolving the polymers in a mixture of NMP/acetone in a 1:1 volume ratio. The efficiency of coating these core templates was characterized by ζ potential measurements, and the morphology of the resulting architectures was characterized by SEM and SFM (scanning force microscopy), revealing the successful formation of spherical LbL particles.[367]

Ma et al. used organometallic polyions based on poly(ferrocenylsilane) (PFS) to form polyelectrolyte shells.[368] Instead of an MF core, metal carbonate crystals were used.[369] These cores can be removed easily by ethylenediaminetetraacetic acid (EDTA) solution. The advantage of using these organometallic polymers for incorporation into the capsule walls is that they allow changing the permeability of these walls. Their unique chemical composition ensures a redox-responsive permeability: when ferric chloride ($FeCl_3$), chosen as the oxidant due to its effectiveness in oxidizing PFS, was added, the LbL membrane expanded, randomly opening up pores. Tetramethylrhodamine isothiocyanate (TRITC)-labeled dextran with a molecular weight of 4.4 KDa was used as fluorescent probe to monitor the resulting permeability, which was sufficient to let this macromolecule diffuse past the membrane. The scheme of formation of the organometallic multilayer capsule formation and permeability control is shown in Figure 36.[368] As will be described in the next section, the $CaCo_3$ template particle has found good use for encapsulation of biological compounds, since it can be dissolved under benign conditions.

4.2. Encapsulation in Layer-by-Layer Microcapsules

4.2.1. Assembly of Compounds as Part of Layer-by-Layer Shells

Caruso et al.[370] combined the organic protocols described above with inorganic silica nanoparticles (SiO_2). SiO_2 particles with a diameter of 25 nm were self-assembled on polyionic shells, which were fabricated on a template of PS latex particles with a size of 640 nm. The polyionic shell was made of PDADMAC, which is excellent for inducing the self-assembly of SiO_2 nanoparticles by electrostatically attracting them. Subsequent removal of the template core, by exposure of the assemblies to tetrahydrofuran, resulted in hollow inorganic silica spheres and inorganic-hybrid spheres.[371] Other inorganic/organic nanocomposite hollow microcapsules were developed with high mechanical stability and controllable encapsulation and release of molecules.[372,373]

With a small modification in the preparation procedure of conventional LbL assemblies, low molecular weight fluorophores were incorporated into polyelectrolyte shells. The deposition of water-insoluble fluorophores was carried out in a nonaqueous solution, and the macromolecules were deposited in aqueous solution, temporarily keeping the fluorophores in place before they were kinetically trapped by the layer of polyelectrolyte. In this case, chitosansulfate (CS), PSS, and PAH were used. Adsorption of fluorophores such as *N*,*N*, *N*′,*N*′-tetrakis[*p*-di(*n*-butyl)aminophenyl]-*p*-benzoquinonebis(imoniumhexafluoroantimonate) (IR), indocyanine green (ICG), 2,9,16,23-tetra-*tert*-butyl-29H,31H-phthalocyanine zinc complex (PC), and 1,1′-di(*n*-butyl)-3,3,3′,3′-tetramethyl indodicarbocyanine perchlorate (IDC) was all proven by confocal laser scanning microscopy.[374]

Fang and co-workers[375] prepared LbL capsules with latex cores using PSS and PEI. These multilayered systems also contained layers of GOx, along with layers of magnetite nanoparticles.[375] The capsules could stir themselves when placed on a rotating magnetic field, which increased the relative activity of the encapsulated GOx. (At appropriate places, this review discusses more examples where enzymes are assembled as part of an LbL film.)[362−364,376]

4.2.2. Encapsulation of Compounds inside Hollow Layer-by-Layer Capsules

Since this review focuses on the biological aspects of capsules, not much attention will be paid to the many studies that have been performed on the encapsulation and membrane permeability of ions in LbL capsules. This work has been reviewed by Sukhorukov et al.[377]

Enzyme crystals were used as a template to form capsules by Caruso et al.[378,379] Despite their delicacy compared to polystyrene latex particles, suitable conditions could be developed that facilitated polymer multilayer deposition on the crystal surfaces but did not destroy the enzyme crystal morphologies. When the enzymes catalase[378] or chymotrypsin[380] were used as a template, they even kept their biological activity. To verify a successful coating of the enzyme crystals with polyelectrolyte multilayers, a fluorescently labeled polyelectrolyte (FITC-PAH) was used for each alternate layer in the build-up process. During the coating of polyelectrolytes, no notable changes to the shape of crystal were observed. However, when the pH was reduced to 2, the morphology of the polymer capsules changed from the shape of the crystal to a spherical one. The morphology change of the polymer capsules at pH 2 is due to the dissolution of the enzyme crystal, turning the enzymes into molecularly dissolved encapsulated species. This dissolution can easily occur because the polyelectrolyte multilayers are highly permeable to small molecules such as ions and water. The stability of the capsules was indicated by their survival at low pH, as well as their sustained survival when the pH was subsequently increased to 11.[378]

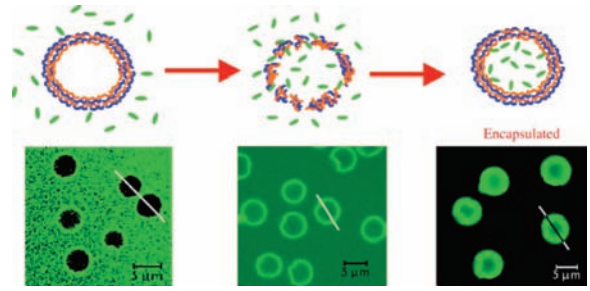


Figure 37. Encapsulation of macromolecules into hollow polyelectrolyte capsules. Bottom: confocal images of MF-derived capsules in FITC−dextran (MW 75000) solution at pH > 8 (left), at pH < 6 (middle), and returning to pH > 8 after removing the bulk FITC−dextran by centrifugation (right). (Reproduced with permission from ref 398. Copyright 2001 Wiley.)

Proteins can also be encapsulated without the necessity to crystallize them first. Instead of MF or polystyrene, inorganic calcium carbonate microparticles were used as core forming template by Antipov et al.[381] As described above for hollow PFS capsules, these $CaCO_3$ templates could be dissolved at neutral pH using EDTA, which allowed proteins to pass through during the encapsulation process without forcing them to be exposed to biologically undesirable levels of pH.[382−384]

Another approach to encapsulate (bio)macromolecules is by using the electrochemical properties of the polyelectrolytes that constitute the capsules. The presence of these polyelectrolytes makes them sensitive to physicochemical parameters such as ionic strength, solvent condition, temperature, and particularly pH. Much research has been performed to investigate this stimulus-responsive behavior of LbL capsules.[381,385−396] A pH change can induce electrostatic repulsion between the two types of polyelectrolyte, while osmotic pressure can build up when ions diffuse to the interior of the vesicles to compensate the charges. The resulting forces form holes in the polyion shells.

At pH 4, the bulk of adsorbed PSS and PAH polymers was not capable of blocking the formation of these types of holes, thus allowing macromolecules to enter into the capsule. After the capsules were loaded with macromolecules in this way, they were washed at pH 8 to remove the nonencapsulated macromolecules or proteins.[397] At this pH, the pores were "sealed" closed, trapping the compounds that had diffused inside. A schematic representation of loading these "reversible" capsules and an example of FITC-dextran macromolecule encapsulation is shown in Figure 37.[398] To control the permeability, the capsule shell was modified with inorganic nanoparticles, proteins, and lipids.[399−401]

As described above, in the formation process of hollow polyelectrolyte capsules using the MF core template, the MF core dissolves into small particles which penetrate through the capsule wall to move out and can be washed away. While this process occurs, the first layer (PSS, for example) partially dissociates from the wall and is released into the interior of

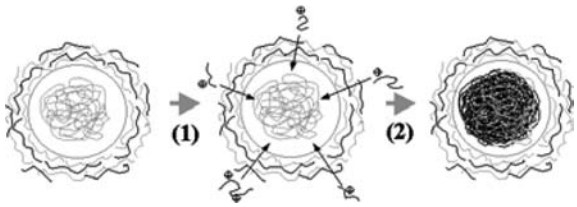


Figure 38. Preparation of spontaneously deposited microcapsules: (1) a PSS/PDADMAC microcapsule after MF particle decomposition. The inner PSS layer was released from the multilayers and formed a negatively charged complex with MF monomers during the core removal process. The PSS/MF complex is confined inside the intact capsule due to its large size. (2) Compounds with a positive charge at low pH deposit onto the PSS/MF complex. (From ref 403. Reproduced by permission of The Royal Society of Chemistry.)

the capsule, where it aggregates with positively charged MF particles to form a negatively charged complex. This complex is much too large to diffuse out of the capsule. Gao and coworkers have exploited this process of complex formation, having discovered that it promotes the driving force for water-soluble molecules to penetrate through the capsule wall and deposit themselves inside, as shown in Figure 38.[402] In this way, it was possible for many water-soluble substances to be spontaneously encapsulated. Since the large PSS/MF aggregate is negatively charged, it may not come as a surprise that positively charged species displayed a more prominent tendency for this self-deposition.[402,403] A variation on this concept has been exploited to selectively accumulate dyes with a negative charge inside LbL capsules that were covered on the inside by positively charged MF remnants.[404]

To encapsulate proteins, mammalian cells have also been explored as biological templates.[405−407] Donath et al. produced hollow capsules by LbL deposition of PSS and PAH on glutaraldehyde-treated human erythrocytes as core templates. Subsequent solubilization of the cytoplasmatic constituents by NaOCl, a deproteinizing agent, dissolved the template cell. The obtained hollow films preserved both the size and shape of the cells, which opens up a route for the production of polymeric capsules with a wide range of size and shape, by using a variety of biological templates. As with most PSS/PAH-based capsules, the polymer shells were found to be permeable to small molecules and ions but not to macromolecules. However, these capsules could be made porous to proteins[405] or nucleic acids[406] by increasing the ionic strength of the solution. The thickness of the membrane was smaller than that of regular LbL capsules, as was found after small angle neutron scattering (SANS) investigations.[408] Next to mammalian cells, fungal cells[409] and yeast cells[410−412] have also been explored as templates for multilayered polymeric capsules.

A layer-by-layer technique not based on electrostatic interactions was presented by the group of Hennink.[413] Degradable polymeric microcapsules were fabricated by so-called "click" chemistry using two dextrans, modified with either azide or alkyne moieties.[413,414] The alternating layers of the two dextrans were covalently bonded by virtue of the 1,3-dipolar Huisgen cycloaddition reaction (Figure 39). The modifications that introduced the azide or alkyne groups were based on hydrolyzable carbonate esters, making these LbL capsules biodegradable.

4.3. Applications of Layer-by-Layer Capsules

As described above, accurate control over membrane thickness, combined with flexibility in the choice of building blocks, make polyelectrolyte microcapsules promising for diverse applications in materials and life science, and a variety of biological applications have been reported,[415,416] For example, they can be used as reactor vessels, delivery capsules, and even as "body armor" for cells. Two important fields of application—LbL capsules as enzyme reactors and LbL capsules in biomedical applications—will be discussed in more detail in the next sections.

4.3.1. Layer-by-Layer Hollow Capsules as Enzymatic Reactors

LbL hollow capsules were used as reactors to synthesize inorganic materials and polymers.[417−420] In some cases, synthesis of materials was carried out with enzymes inside the polyelectrolyte capsules. For example, urease-containing polyelectrolyte capsules were used for biomineralization. Urease catalyzes the decomposition of urea to form carbonate anions. These carbonate anions subsequently interact with the metal cations present, causing the precipitation of $CaCO_3$ to occur. Urease was encapsulated inside hollow PAH/PSS polyelectrolyte capsules by the so-called solvent-controlled permeability method.[376,421] In this method, PAH/PSS polyelectrolyte capsules were exposed to a urease-containing water/ethanol mixture. This solvent mixture permeabilized the capsule walls, allowing the urease molecules to enter the capsule's lumen. After removing the ethanol by centrifugation filtration, the urease-containing capsules were introduced into a mixture of 0.5 M urea and 1 M $CaCl_2$. Urea decomposition and CO_3^{2-} formation both occurred inside the polyelectrolyte capsule. In order to prevent the formation of $CaCO_3$ in solution, a high concentration of calcium ions was maintained inside the capsule by keeping the total concentration in the solution sufficiently high. At Ca^{2+} concentrations of 0.5 M and above, $CaCO_3$ synthesis was almost exclusively observed inside the capsules. The growth of calcium carbonate inside the polyelectrolyte capsules was monitored by confocal scanning microscopy. Images taken at different reaction times are shown in Figure 40.[381]

Horseradish peroxidase (HRP) was encapsulated inside the inner compartment of hollow LbL capsules.[422] To this end, the polyelectrolyte capsules were added to a solution of HRP at pH 4.0. At this pH, the shell wall of the capsules is permeable to the proteins. Once HRP diffused inside, the pH was readjusted to 8.5, closing the capsule walls and retaining the enzymes inside. The polyelectrolyte capsules containing HRP were studied for the enzyme-catalyzed polymerization of phenols. Tyramine was chosen as a monomer because the polymer formed by a successful polymerization is highly fluorescent. Both tyramine and H_2O_2 can permeate freely into the reactor capsules because of their low molecular weights. As expected, polymer formation was found to occur inside the capsules only in the presence of all the three ingredients (tyramine, hydrogen peroxide, and HRP). The fluorescent product obtained by the tyramine polymerization both inside the capsules and in bulk solution was analyzed by fluorescence spectroscopy. The deviation between the spectra suggests that polymer synthesis in submicrometer volumes gives materials with a structure different from that of polymer formed in bulk.[422]

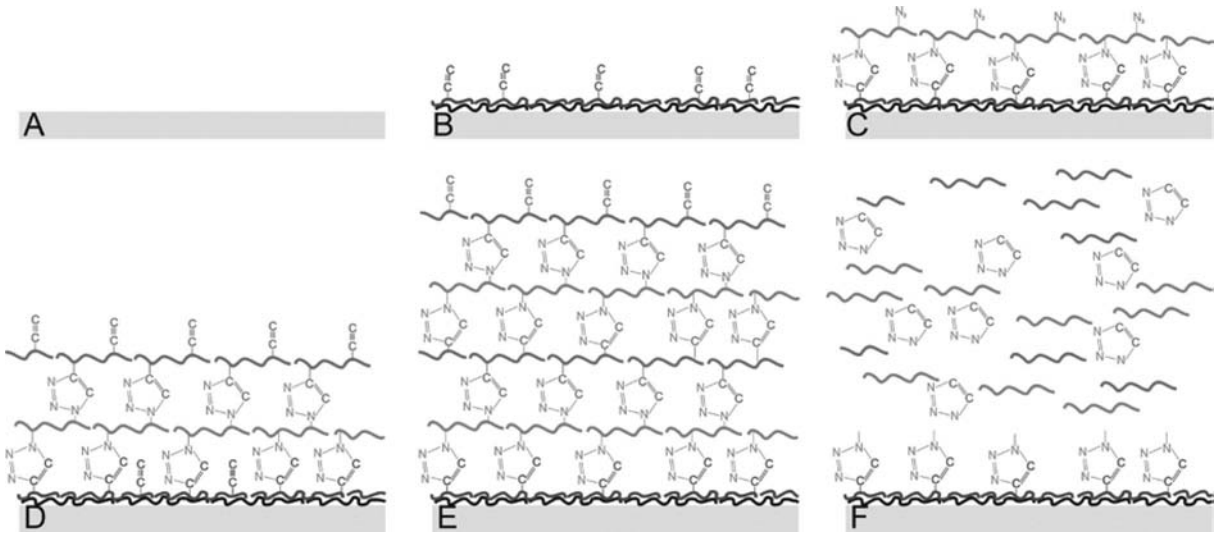


Figure 39. Schematic representation of the build up of a degradable "clicked" multilayer. A bare substrate (A) is precoated with a PEI/PAA−alkyne layer (B) to make the surface reactive toward click chemistry. A Dex-N3 layer (C) is clicked to the PAA−alkyne layer followed by the clicked deposition of consecutive layers (D and E). Hydrolysis of the carbonate esters, which link the triazole bonds to the dextran backbones, leads to the disassembly of the clicked multilayer film. (Reproduced with permission from ref 413. Copyright 2008 Wiley.)

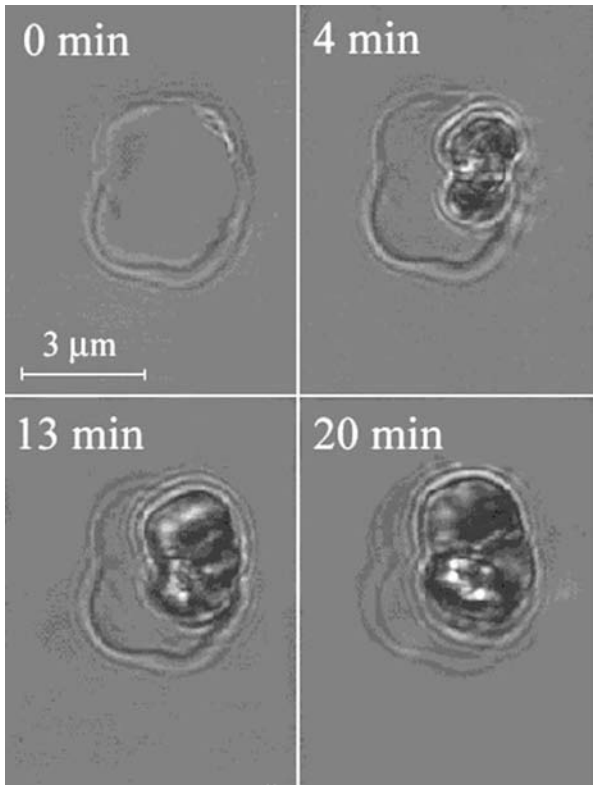


Figure 40. Optical microscopic images of $CaCO_3$ growth at different stages inside a urease-filled polyelectrolyte capsule. (Reproduced with permission from ref 421. Copyright 2003 Wiley.)

Lvov and co-workers have also reported peroxidase-catalyzed polymerization of phenol. However, in their system the peroxidase was assembled with PSS in the shell of the polyelectrolyte capsules instead of inside the hollow polyelectrolyte vesicle. 4-Hydroxyphenylacetic acid was used as a monomer, and the formation of additional polymer on the surface of the shell provided the possibility to tune its permeability.[423]

A case where HRP worked in concert with a second enzyme, GOx, was reported by Balabushevich and co-workers.[424] Their MF-templated LbL hollow capsules consisted of the polyanion dextran sulfate and protamine, an arginine-rich protein involved in DNA-binding. Spontaneous deposition (Figure 38) was used to accumulate both HRP and GOx inside the hollow capsules. The resulting bienzyme system was shown to be active, demonstrating the viability of LbL capsules as reactor vessels for two-enzyme cascade reactions.[424] The same cascade reaction was used by the group of Trau,[425] who templated their capsules on an agarose hydrogel containing the enzymes. Transfer of these microgel templates to an organic phase prior to polyelectrolyte deposition resulted in almost quantitative protein inclusion.

The same group recently reported amplification of DNA by the polymerase chain reaction inside LbL capsules.[426] This "microcapsule-PCR" involved temperature stable PSS/PAH microcapsules deposited on an agarose core containing a PCR reaction mixture of polymerase enzymes and oligonucleotide primers. Nucleotide building blocks were supplied externally during the PCR and diffused freely through the capsule wall. The high molecular weight PCR products were retained within the vesicle. A microcapsule-PCR experiment involving different capsules with different primers demonstrated that the reactors are individual compartments that do not exchange templates or primers between microcapsules during PCR cycling, as might be the case for thermally cycled recognition induced polymersomes (RIPs, see section 5.1.3)

As an extension to the LbL capsule method, a capsule was built inside another capsule, with each capsule containing human serum albumin (HSA) but with different fluorescent labels.[427] To construct this architecture, TRITC-labeled HSA and magnetite nanoparticles were immobilized within spherical calcium carbonate template microparticles by coprecipitation. On this precursor, five layers of PSS and PAH were adsorbed. The resulting core−shell particles were subjected to a second coprecipitation step with single core $CaCO_3$ and HSA labeled with Alexa 488, to create a template already containing an LbL wall. This template was then used as a scaffold to produce a new set of layers, giving a capsule-in-capsule architecture. The magnetic properties of these capsules, imparted by the magnetite used in the first core, enabled separation of the templates using a magnetic field.

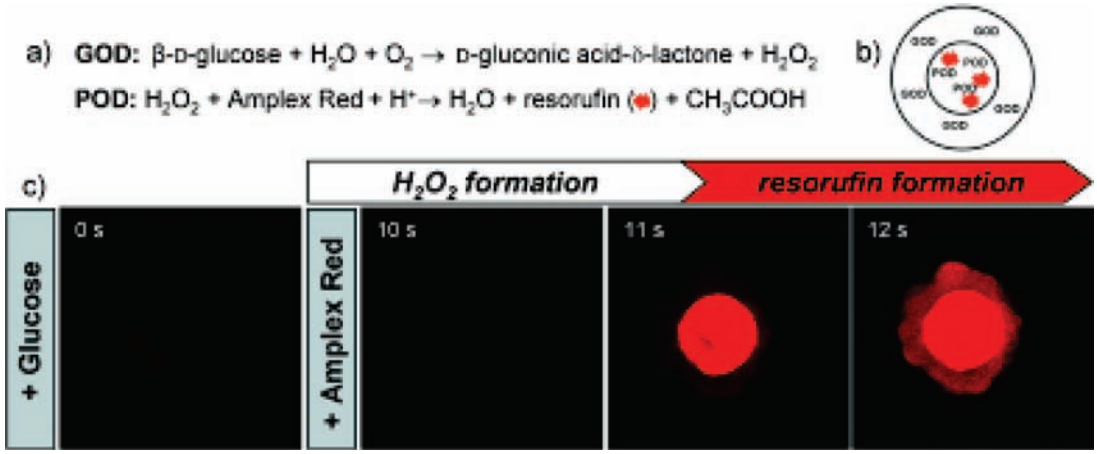


Figure 41. Coupled enzymatic assay with GOx (GOD in the image) and HRP (POD in the image) inside shell-in-shell capsules: (a) reaction scheme; (b) schematic representation of GOx (GOD) and HRP (POD) in a capsule-in-capsule setting; (c) confocal micrograph image of resorufin formation inside the capsules. (Reproduced with permission from ref 427. Copyright 2007 Wiley.)

Following the above method, GOx was encapsulated in the outer, surrounding, compartment and HRP was included in the inner capsule. The presence and catalytic activity of both enzymes was assessed by the amplex red assay, as shown in Figure 41.[427]

In a similar approach, near-infrared light absorbing gold nanoparticles were incorporated into the inner shell or into the inner capsule.[428] To trigger mixing of the contents of the shell-in-shell capsules, the assemblies were irradiated using near-infrared laser light. This irradiation heated the gold particles, which led to degradation of the inner capsule. Hence, the contents of the inner shell were released into the outer shell, providing a route for controlling reactions in these confined volumes, via the on demand intermixing of the contents of both compartments. For demonstration of this principle, TRITC-labeled dextran was encapsulated in the inner compartment and FITC-labeled dextran was confined in the outer compartment (Figure 42). Both compartments were separated by six layers of PSS and PAH. The laser induced mixing of both compartments was demonstrated using confocal scanning fluorescence microscopy. Before the irradiation trigger, all components were effectively spatially separated (Figure 42a). After irradiation (Figure 42b), the TRITC-labeled dextran left the interior compartment and it subsequently admixed with the FITC−dextran in the exterior shell. However, a homogeneous distribution of both compounds was not observed. Also, in some cases the inner capsules were released during the laser irradiation process (Figure 42c).[428]

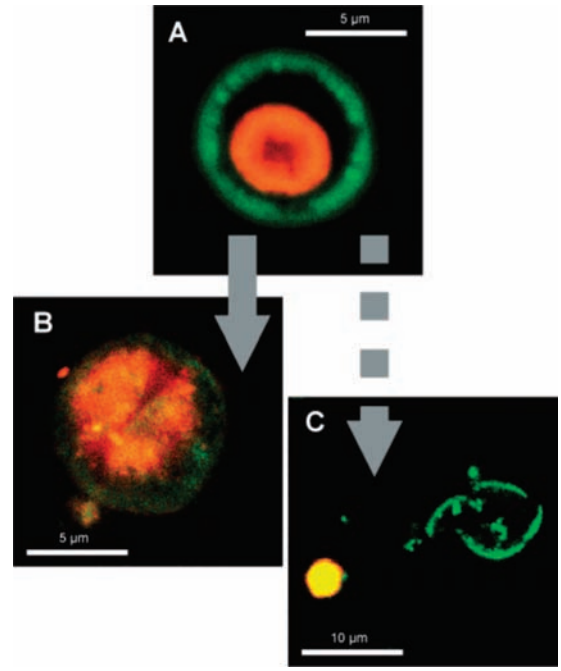


Figure 42. Confocal micrograph images of a polyelectrolyte shell-in-shell capsule loaded with TRITC−dextran in the inner compartment and FITC−dextran in the outer compartment. The inner shell is doped with gold nanoparticles. (A) Before laser irradiation. (B) After irradiation of the inner shell. (C) Some of the particles lose their inner capsule during laser irradiation. (Reproduced with permission from ref 428. Copyright 2007 Wiley.)

4.3.2. Biomedical Applications

As recently reviewed, LbL capsules can be used in a variety of biomedical settings.[126,415,429−431] For example, polyelectrolyte capsules as potential drug delivery or cargo systems have been reported by the groups of Sukhorukov and Möhwald, De Smedt, and many others.[432−438] Near-infrared fluorescent particles[439] and metal nanoparticles[440] were encapsulated inside polyelectrolyte capsules. Silver metal nanoparticles inside LbL capsules were used to activate and to release the contents by laser irradiation. This mechanism was shown to function even when the capsules were internalized in living cells.[441] As a comparable method, triggered release by ultrasound was reported by the same group.[442,443] It was applied as a release mechanism for plasmid DNA that was adsorbed as one of the polyelectrolytes in a PAH/DNA coated microparticle.[442−444] Another capsule system did not have a mechanism for triggered release but slowly degraded because biodegradable polyelectrolytes were used, namely polyarginine and dextran sulfate.[445] It was shown that the stability of the capsules after cellular uptake was determined by the number of polyelectrolyte layers.[446] Related studies using PDADMAC/PSS capsules showed that capsule stability inside cells could be further increased by incorporation of citrate-stabilized gold nanoparticles in the LbL shells.[447]

An interesting release system was described by Borodina et al.,[437] who prepared self-disintegrating LbL capsules based on polyarginine and oppositely charged poly(aspartic acid). These layers were deposited on a $CaCO_3$ core containing Pronase, a commercially available protease that can degrade polypeptides. Extraction of the $CaCO_3$ core released this

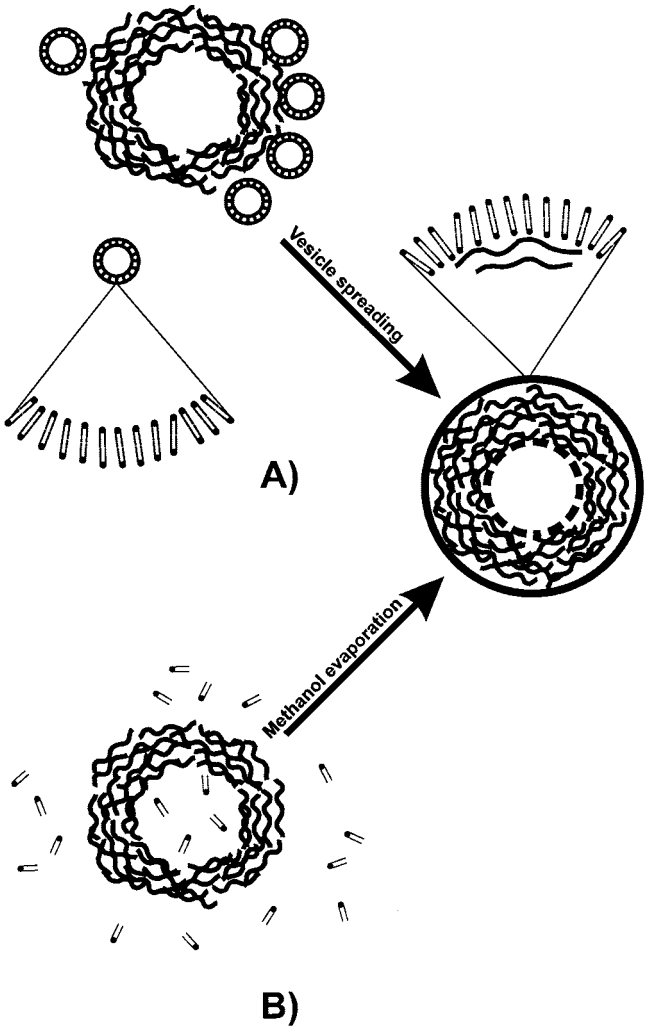


Figure 43. Scheme of lipid layer formation onto polyelectrolyte capsules: (A) via adsorption and spreading of preformed vesicles; (B) via deposition of lipids onto polyelectrolyte capsules from a lipid solution in methanol. In this case a bilayer may be formed at the inside as well. (Reproduced with permission from ref 449. Copyright 2000 American Chemical Society.)

enzyme into the capsule's interior, where it then started to degrade its encapsulating shells. Any compound that was coimmobilized in the carbonate core was subsequently released. The successful triggered release of DNA by this method indicated the possible application of this system in drug delivery. The release of nucleic acid-based therapeutics from polyelectrolyte assemblies has recently been reviewed by Jewell and Lynn.[448]

The release of encapsulated compounds from LbL particles can be slowed down by coating the LbL membrane in a further layer of phospholipids (Figure 43).[449−451] The group of Möhwald prepared PSS/PAH polyelectrolyte capsules that were covered in a variety of phospholipids and found that the permeability of the LbL membrane was greatly reduced, approaching the impermeability of liposome membranes. Compared to liposomes, these hybrid systems had a greater mechanical stability due to the supporting polymer scaffold. Also, their shape could be predetermined by tuning the shape of the LbL capsule. This platform was expanded further by incorporation of channel-forming peptides in the lipid part of the membrane,[452] allowing membrane potential studies to be conducted on these cell-mimicking assemblies.

De Rose, Zelikin, and co-workers internalized LbL hollow capsules containing a peptide vaccine in cells.[453] Their strategy for vaccine delivery was to target nanoengineered capsules to antigen presenting cells (APCs), which involved the surface coating of LbL capsules with a final layer of either PAH, PGA, or poly(L-lysine) (PLL). These polymer layers caused the capsules to be passively bound by white blood cells in blood. Subsequent internalization by these cells was followed by release of the encapsulated peptide vaccine, which in the APCs was intracellularly trafficked for presentation. This mechanism thus successfully elicited an immune response against the encapsulated peptide.[453]

LbL capsules for delivery were also created with stimuli-responsive shells. For example, glucose-sensitive capsules were prepared by assembly of a copolymer of dimethylaminoethylacrylate (DMAEA) and acrylamidophenylboronic acid (APBA) with PSS as shell material.[454] Comparable carbohydrate-sensitive capsules were built by multilayer assembly, via the formation of ester bonds between a polysaccharide and a phenylboronic acid grafted onto poly(acrylic acid).[455] In the former architecture, the presence of glucose solubilized the copolymer, destabilizing the capsule membrane. In the latter, free carbohydrates competed with the polysaccharide for ester bonds, undermining the actual interactions between the two polymers. In both cases, the destabilization led to the release of fluorescently labeled BSA, as a model therapeutic protein.[455]

De Geest and co-workers have thoroughly explored self-rupturing microcapsules.[456−458] These capsules consisted of a biodegradable dextran-based microgel template surrounded by a polyelectrolyte membrane, such as $(PSS/PAH)_3$.[456] Degradation of the microgel template led to an increase of the swelling (osmotic) pressure inside the microcapsules. Upon degradation at pH 9, the microgels were able to rupture their surrounding coating, resulting in self-rupturing microcapsules, while at pH 7 the coating did not rupture upon degradation, leading to hollow capsules. This was explained by the pH-dependent permeability of the $(PSS/PAH)_3$ coating for the degradation products of the microgels.[457] Membranes that do not naturally have a high permeability, such as lipid bilayers, are ruptured at a wider range of pH levels and are not able to produce hollow capsules.[459] Encapsulation of microgels in lipid bilayers was one of the first methods for pulsed drug delivery, though these examples needed an external trigger to initiate release.[460]

Self-rupturing LbL capsules like these may be suitable candidates for pulsed drug delivery. With the term pulsed drug release it is meant that after administration initially no drug release occurs. Only after a defined period, the therapeutics are released, which could be advantageous for a variety of reasons, such as single-shot vaccination.[461] As a step toward these possible biological applications, the use of biodegradable polyelectrolytes such as poly(aspartic acid) and polyarginine for layer-by-layer coating of these microgels has been investigated.[458]

The attractiveness of LbL capsules as delivery vehicles is considerable. This may have prompted the expansion of the self-exploding delivery platform to include the delivery of other LbL capsules. De Geest and co-workers embedded LbL capsules in a hydrogel, which was then used as the template for an even larger, self-exploding capsule.[462] It was shown that embedded LbL capsules could be successfully released from larger, self-exploding LbL species. Using FITC-labeled latex beads as a model for the small LbL cargo, this system

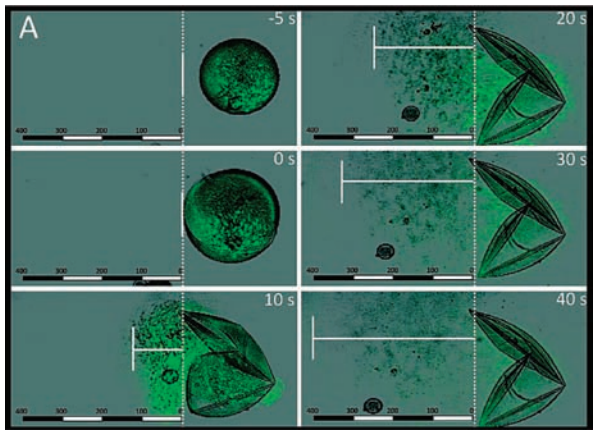


Figure 44. Confocal microscopy snapshots taken at regular time intervals of a self-exploding LbL capsule containing FITC-nanoparticles during the NaOH-triggered degradation of the microgel core. The microcapsule explodes 10 s after addition of NaOH. The edge of the propagating front of released nanoparticles is marked by the vertical white line. The unit of the scale bar is μm. (Adapted with permission from Figure 3 from ref 463. Copyright 2008 American Chemical Society.)

was further characterized.[463] After ejection from the larger microcapsules, the traveling velocity of the nanoparticles in water was 800-fold higher than that caused by Brownian motion. For most delivery devices, the released therapeutic entities have to slowly spread into the environment by diffusion. The nanoparticles released from these exploding microcapsules, though, were propelled into the environment, which allowed them to travel relatively large distances in a short time (Figure 44).

Some cargo does not need to be released. In fact, there are instances where the encapsulation of compounds and the protection granted by the polymeric shell are advantageous. Kreft and co-workers[464] constructed PAH/PSS microcapsules containing a high molecular weight dextran in its lumen which was conjugated to SNARF-1. The latter is a fluorophore which shifts its emission wavelength from 650 nm (red) under basic conditions to 580 nm (green) in acidic conditions. When these capsules were internalized by cells, their environment changed from alkaline in the cell growth medium to acidic in the endosomes, which was readily detectable through monitoring of the fluorescent emission. A pH-independent fluorophore was coencapsulated in the vesicles as an internal reference standard. The concentration of the fluorescent signal in the capsules, as opposed to its diffusion throughout the entire cell, facilitated its detection.

Another sensory application used digitally encoded particles (such as barcoded ones) as a template for PSS/PAH multilayers.[465] The multilayer shell contained ferromagnetic CrO_2 nanoparticles to align the spheres in a magnetic field, facilitating a correct readout of the digital code (Figure 45). For their application as a sensor, an outer layer of poly(acrylic acid) (PAA) was adsorbed to provide a homogeneous surface of carboxylic acid moieties, which allows the covalent coupling of antibodies.[466] These antibodies can bind their corresponding antigens and thus label them with the barcode inscribed in the bead. Reminiscent of ELISA techniques,[467] a second, fluorescently labeled antibody against the same antigen signals that a specific bead is "loaded" with its analyte. The identity of the immobilized receptor can subsequently be read out with the bar code. This setup was shown to function even in whole blood, detecting proteins such as tumor necrosis factor (TNF-α) and follicle stimulating hormone (FSH). The bar code approach allowed multiplexing immunoassays, i.e. the simultaneous detection of multiple analytes interacting with their receptors.[467]

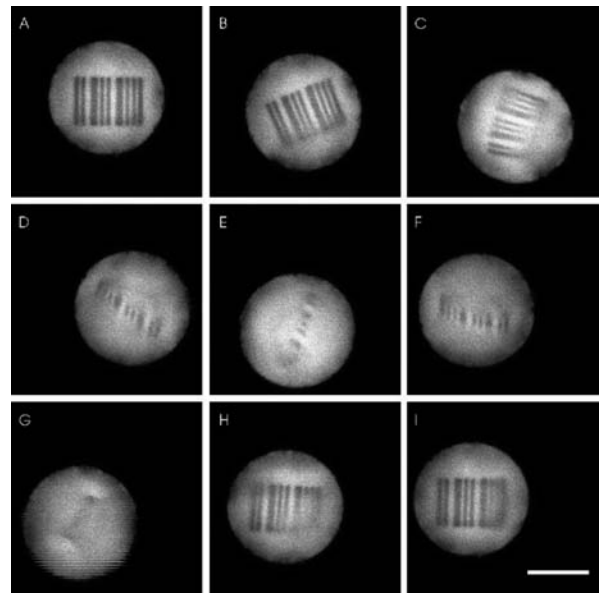


Figure 45. Magnetic LbL coating of a microsphere labeled with a bar code brings the sphere into a correct readout position after application of a magnetic field. (A) Confocal image of the central plane of a magnetic LbL-coated microsphere just after being encoded with a bar code. (B−H) Confocal images of the central plane of the microsphere while randomly moving. (I) Confocal image of the central plane of the microsphere after bringing the microsphere back into a magnetic field, upon which it returns to its original orientation (compare images A and I), permitting reading of the code. The scale bar is 20 μm. (Reproduced with permission from ref 465. Copyright 2007 American Chemical Society.)

LbL-coated bar-code beads were also used as sensors to detect proteases.[468] Protease substrates were fluorescently labeled and incorporated in the LbL membrane surrounding the labeled bead. In the presence of active proteases, any peptides that could be cleaved were degraded. This caused the cleaved products, including the fluorescent label, to detach from the labeled particle. As a result, any bar-code bead that lost its fluorescence had detected its corresponding protease. With strict fluorescent substrate and bar-code combinations, these LbL particles could be applied in a multiplex assay for protease activity.

PEGylation has been investigated to improve the stealth properties of many synthetic capsules that are brought into contact with biological materials.[469] Polyelectrolyte microcapsules have been PEGylated using a PLL polymer randomly grafted with PEG tails (PLL-*g*-PEG).[470] These PLL-*g*-PEG-coated capsules adsorbed only 0.1% of the streptavidin adsorbed by the same capsules without the protective copolymer layer, demonstrating the effective antifouling characteristics imparted by the PEG layer. When functionalized with biotinylated PLL-*g*-PEG, the capsules adsorbed 40 times more streptavidin than their unfunctionalized counterparts, as a result of the specific binding between biotin and streptavidin. These studies demonstrated the feasibility of controlled surface-immobilization of specific bioligands onto LbL particles.

The use of cells as templates for hollow LbL capsules has been discussed in a previous section. But LbL polyelectrolyte coatings have also been used to either protect or functionalize cells. The former, i.e. a protective coating, was applied by

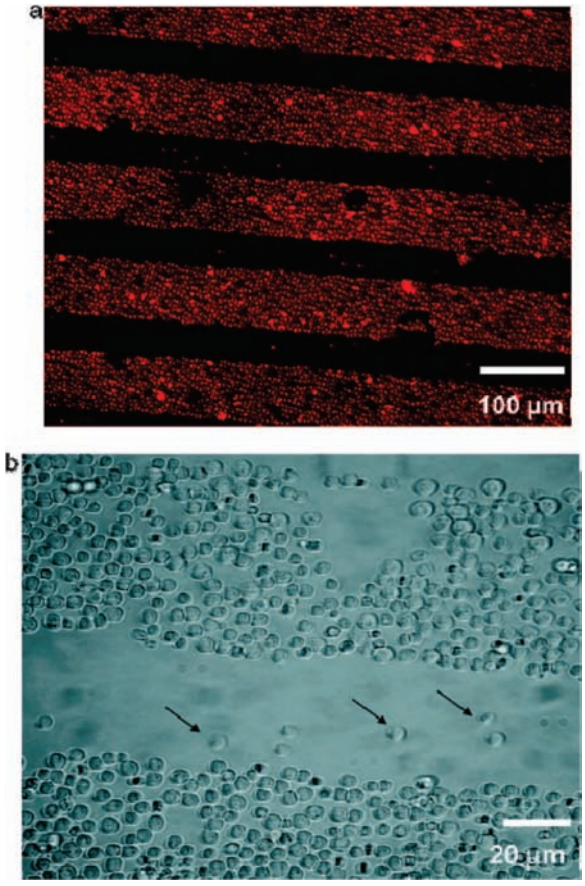


Figure 46. (a) Fluorescence micrograph showing selective coated cell adsorption on a patterned substrate. One of the PAH layers coating the cells was labeled with Alexa 555. (b) Phase contrast image of encapsulated yeast cells attached by electrostatic self-assembly to a 100 μm stripe structure. Unattached cells in the similarly charged region of the glass are blurred, due to movement (arrows). (Reproduced with permission from ref 472. Copyright 2005 American Chemical Society.)

Germain et al.,[471] who employed a mammalian cell line that produced a fluorescent protein upon contact with estradiol. This biosensor was sensitive to low levels of analyte, but it was also fragile, since it lacked a cell wall as yeast or plant cells have. Encapsulation of the cells in PSS/PAH layers revealed that cell viability was maintained while their biosensing functionality was uncompromised.

The immobilization of cells into well-defined arrays is important for the development of cell-based biosensors such as the one described above. Krol et al.[472] presented an approach in which *Saccharomyces cerevisiae* yeast cells were coated with polyelectrolytes $(PAH/PSS)_2$. As was the case for the previously described biosensor, the cells were protected by the coating. By microcontact printing of polyelectrolytes (PEI/PSS/PAH), a surface was then prepared with regions of opposite charge (as compared to the outer layer surrounding the cells) adjacent to regions of the same charge. Hereby, cell adhesion only occurred at the designated oppositely charged areas (Figure 46).

Encapsulation of cells not only protects the cells from their environment; it also ensures that the environment is not influenced by the encapsulated cells. In the group of Fussenegger, genetically modified cells were encapsulated in large CS/PDADMAC capsules.[473] Some of these cells were engineered for trigger-induced expression of a cellulase that can degrade the enclosing LbL capsule. This approach was combined with the encapsulation of different producer cell lines that expressed therapeutic proteins. The therapeutics were too large to diffuse through the CS/PDADMAC membrane, trapping them inside the vesicle. Upon the triggered expression of the cellulase, which started to degrade the capsule walls, the encapsulated producer cells and their previously expressed reserves of therapeutic proteins were released.

4.4. Outlook

Initial research on layer-by-layer capsules was focused on interesting (in)organic polymer shells, but it did not take long for biological materials to get incorporated, either into the interior of the shells or as part of the layers.[357] While the number of biohybrid materials for the construction of LbL capsules is limited, their ease of preparation makes the range of biohybrid applications numerous, varying from sensors[472] and vehicles for pulsed drug delivery[461] to protective layers for cells.[473]

Their method of preparation seems to present a technical limitation to the diameter (commonly between 5 and 10 μm, which may explain their relatively limited use for delivery purposes, i.e., where truly nanoscale small capsules are desired. However, the advantages of LbL capsules, in the sense that capsules with semipermeable properties can be prepared with relative ease and in a biocompatible manner, makes them more versatile for certain applications than "competitors" in the field, such as polymersomes and liposomes.

The use of degrading microgels as a template to obtain self-rupturing capsules is an example where traditional templates for LbL assembly were eschewed to gain more function. We expect a considerable body of future literature to explore the blessings of combining LbL assembly techniques with other structurally controlled architectures, such as microgels, liposomes, viruses, and whole cells. Indeed, there is already a sizable group of reports on cells with new functionalities endowed to them by their encapsulation in polyelectrolyte shells.[471−473] In these cases, the polyelectrolyte layers confer the new function to the cells, directing their preferred proliferation sites or toughening the cells themselves. Hence, it seems reasonable to conclude that future advances in the field of LbL capsules may be found there where just the properties of the layers are needed. The ability to take a surface and tweak its properties by polyelectrolyte deposition is a powerful one.

5. Polymersomes

Polymersomes are hollow vesicles with a polymeric membrane, generally built from amphiphilic block copolymers of the AB or ABA-type.[474−478] In almost all cases, the vesicular membrane has an insoluble middle and a soluble outer layer.[474] The driving force for their formation by self-assembly is considered to be the microphase separation of the insoluble blocks.[479−483] Some commonly observed assemblies of different types of block copolymers are shown in Figure 47. For linear amphiphiles in aqueous solution, the resulting morphology is dictated by the time-average molecular shape of the polymer chain.[477] This shape can have the form of a cylinder, a wedge, or a cone (Figure 48) Most simply put, it is a reflection of the so-called hydrophilic fraction *f*, since, in water, lyotropic behavior (the tendency to phase-separate in response to solvent conditions) mostly

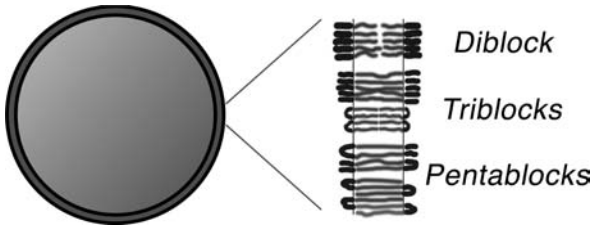


Figure 47. Some of the most common methods of block copolymer packing in polymersome bilayer membranes.

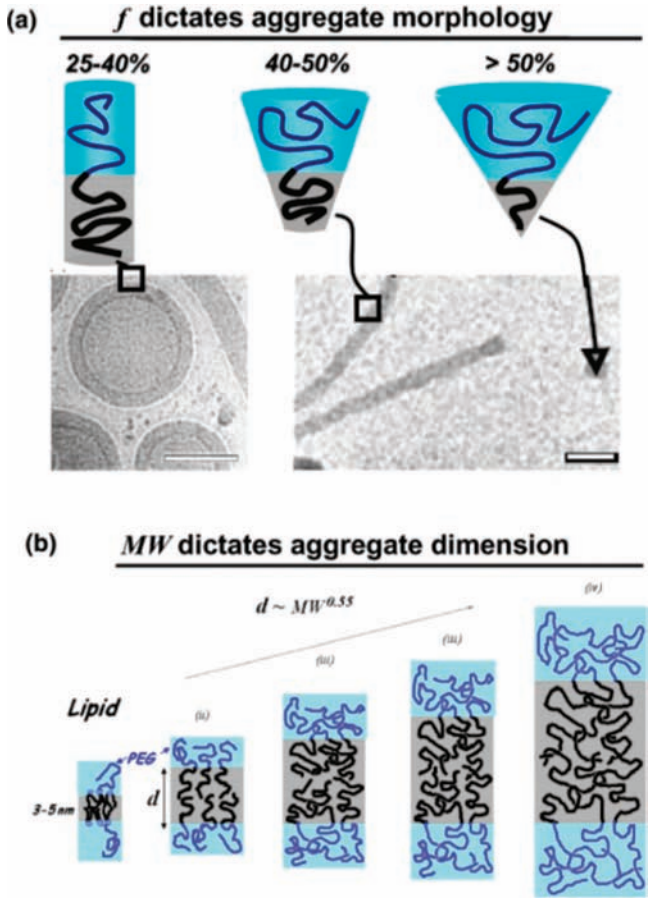


Figure 48. (a) Schematics of block copolymer hydrophilic fractions "*f*" with respective cryogenic transmission electron microscopy images showing vesicles or worm micelles and spherical micelles. (b) Schematic scaling of polymersome membrane thickness with copolymer molecular weight (MW). (Reproduced with permission from ref 606. Copyright 2006 Wiley.)

originates from hydrophobicity of one of the blocks. For ABA-type triblock copolymers, the central "B"-block is often shielded from the environment by its flanking "A"-blocks, whereas AB-type copolymers aggregate in bilayers, placing two hydrophobic blocks tail-to-tail, much to the same effect.[484,485]

This latter architecture has many similarities to the bilayer of liposomes,[486] which are basically the same, yet on a smaller scale. The use of the name "polymersomes" for block copolymer vesicles has even been inspired by their similarities in the bilayer morphology.[476] Polymersomes, however, are typically more mechanically and thermodynamically stable, which can for a large part be attributed to the lower critical aggregation concentration of their constituent amphiphilic macromolecules.[481,487−489] Furthermore, the versatility which can be applied in block copolymer synthesis[62,490−492] or solvent system composition[493] enables a greater control over the properties of polymersomes as compared to liposomes. This greater versatility has made polymersomes a subject of intensive research, with current applications ranging from imaging agents[494−497] and biological delivery vehicles[498−502] to nanoreactors.[8,503−505] Even industrial applications have already been reported.[485,506,507]

In this section we will first focus on the different types of biohybrid building blocks that have been reported. This includes block copolymers containing biomolecules, and block copolymers of which at least one block is biodegradable, such as polyesters or polyanhydrides. The subsequent sections will discuss the many ways of incorporating biological material into polymersomes, after or during their formation. These techniques can be both noncovalent, as is the case with encapsulation of proteins, or covalent, as for surface conjugation. This section is combined with a description of two main application areas of hybrid polymersomes, i.e. the use of polymersomes as nanoreactors and their application in the biomedical field for imaging and delivery.

5.1. Introduction to Building Blocks

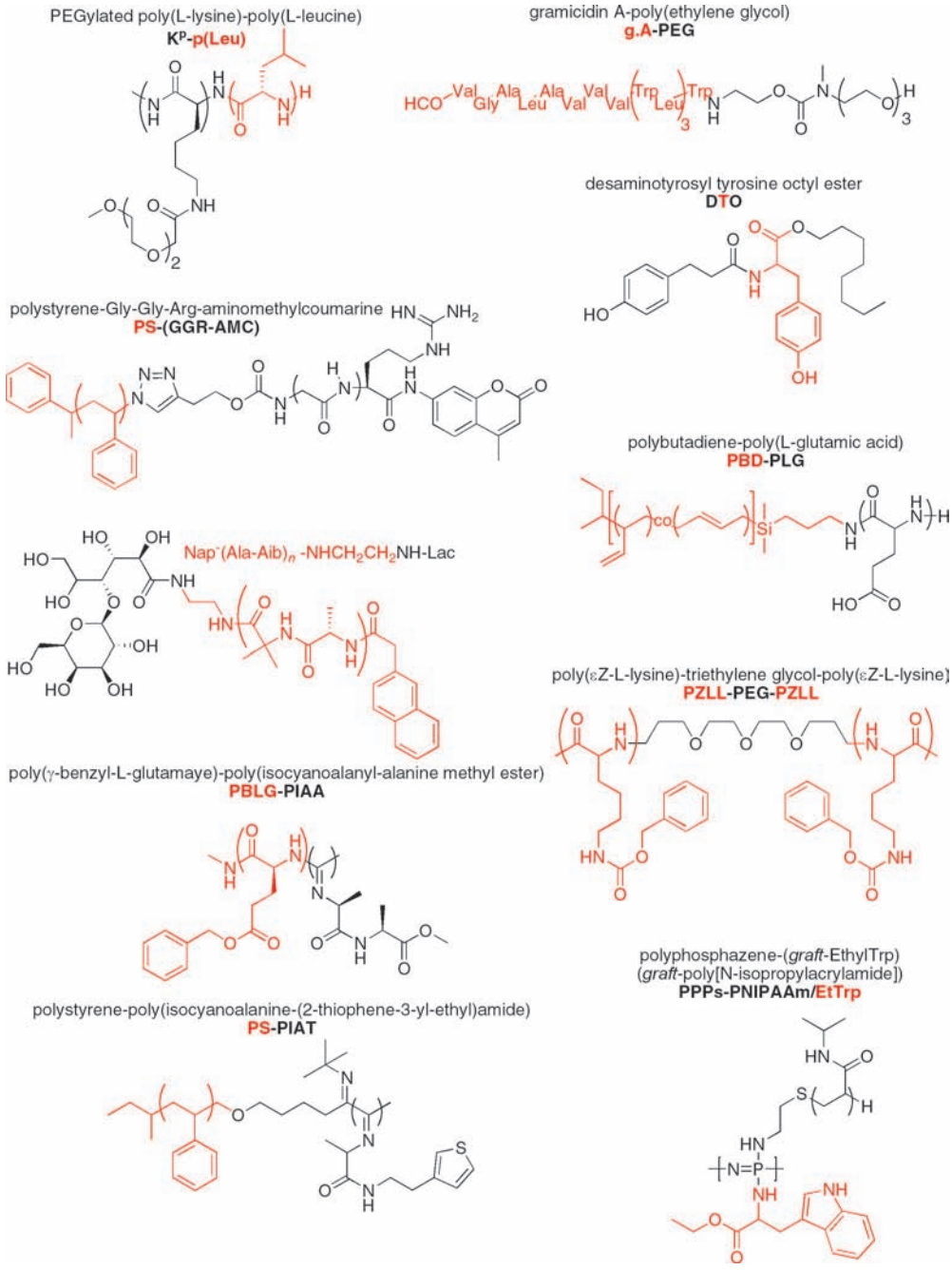
Although polymersome membranes on average are much thicker than conventional lipid bilayers, a comparison of polymersomes with naturally occurring lipid vesicles is inevitable.[508] This may have led to the multitude of bioinspired block copolymer building blocks featuring biological moieties such as peptides and saccharides (see Chart 4).[340,509−511] The use of biomolecular building blocks enables the introduction of biological functionality into or onto the polymersomes, which makes them suitable to mimic or interfere with biological processes. Furthermore, the well-defined folding properties of biomolecules can be used to create block copolymers with specific types of topologies, suitable for polymersome formation.[512] In the next sections, polymersomes based on amino acids, saccharides, and nucleobases will be discussed. Finally, biodegradable block copolymers used for polymersome formation will be described.

5.1.1. Amino Acid-Containing Building Blocks

One of the earliest examples of polymersomes based on peptide-containing block copolymers was described by Kimura et al.[513] Interestingly, the hydrophobic, naturally occurring peptide gramicidin A was used as the biocomponent; it forms rather rigid helices that act as ion channels in lipid membranes. The hydrophobic peptide was made amphiphilic by conjugation to PEG_{13}, which led to polymer−polypeptide amphiphiles that formed vesicles in aqueous solution, separating gramicidin A-helices from the aqueous environment by PEGylation (Figure 49). These vesicles have been termed "peptosomes" and were used to encapsulate fluorescently labeled PEG chains. The same group reported an alternative peptosome architecture,[514] based on the helix forming hydrophobic poly(alanine-aminoisobutyric acid), coupled to the hydrophilic lactobionic acid. These helices were shown to resist unusually high concentrations of guanidine hydrochloride (3.5 M), an agent commonly used to denature secondary structures of polypeptides. This stability presumably originates from the densely packed state of the helices in the hydrophobic membrane and is an example of the stabilizing effect that vesicle formation can have on participating amphiphiles.

An example that uses an oligopeptide sequence as a hydrophilic headgroup was provided by Dirks and co-

Chart 4. Structure Formulas of Various Amino Acid-Containing Polymersome-Forming Compounds



workers.[287] They reported the preparation of biohybrid amphiphiles via the Huisgen [3 + 2] dipolar cycloaddition between an azide-terminated polystyrene block and an acetylene-functionalized Gly-Gly-Arg-(7-amino-4-methylcoumarin) (GGR-AMC) tripeptide. The resulting amphiphiles were able to form stable polymersomes that were fluorescently labeled. These architectures might exhibit interesting biological activity, since GGR-AMC is a suitable substrate in thrombin generation tests.[287] Another point of interest is the modularity of the approach taken toward amphiphile formation, which was extended in the same report toward the formation of PS-protein amphiphiles.

The transition metal-catalyzed ring-opening polymerization of the *N*-carboxy anhydrides of amino acids, pioneered by Deming,[195] has enabled the controlled polymerization of polypeptides with increased block length.[515] This technique was used by Wyrsta and Bellomo et al.[516] to produce block copolymers that were entirely based on (modified) polypeptides, namely poly(L-lysine)$_{100-200}$-*block*-poly(L-leucine)$_{20-40}$ (PLL-p(Leu)). The high charge density normally present in polylysine, which would hamper its aggregation, was offset by the coupling of ethylene glycolic acid to the amines present in the lysine side chains. The second block of the block copolymer was comprised of enantiopure p(Leu), which forms a stable hydrophobic helix. In a variation on this makeup, the hydrophobic block consisted of a copolymer of L-leucine and up to 70% L-lysine. At sufficiently high pH, unprotonated polylysine is not water-soluble, preventing the disruption of either the helicity or hydrophobicity of the leucine domain. At lower pH values, the lysine is protonated, which vastly increases its solubility, ultimately leading to dissociation of the vesicle membrane. The ability of these asymmetric block copolymers to form vesicles was based on their helical secondary structure; accordingly, polypeptides

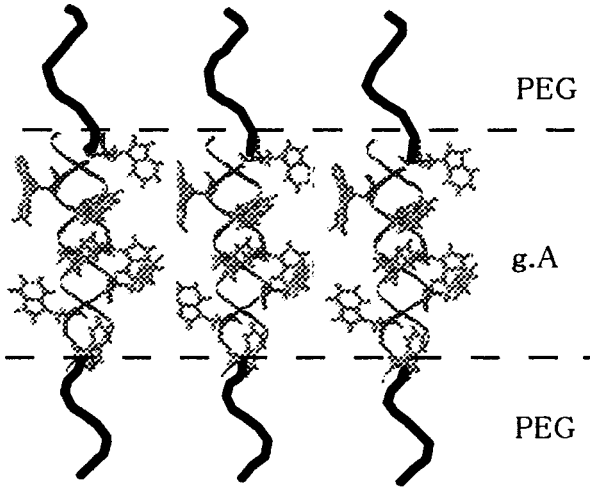


Figure 49. Illustration of the helix peptide gramicidin A separated from the aqueous environment by PEGylation, forming a "peptosome" membrane. (Reproduced with permission from ref 513. Copyright 1999 American Chemical Society.)

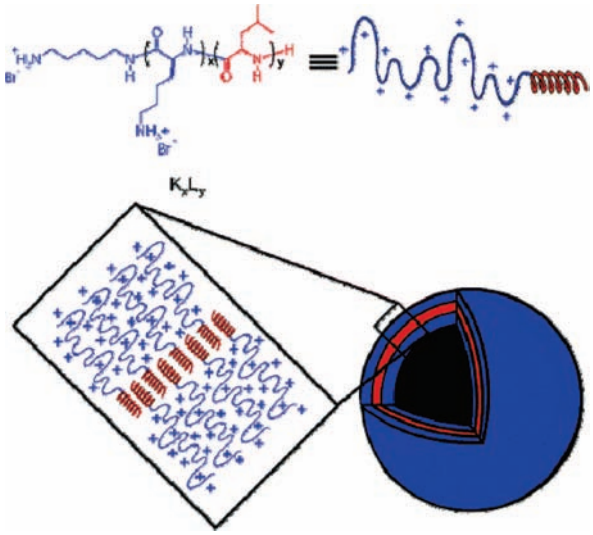


Figure 50. Schematic drawing showing the proposed self-assembly of $PLL_{60}p(Leu)_{20}$ into polymersomes. The usual sheet formation of the p(Leu) block is disrupted by the charge−charge repulsion in between the PLL blocks, allowing the formation of spherical aggregates. (Reproduced with permission from ref 517. Copyright 2005 American Chemical Society.)

based on a racemic mixture of amino acids did not engage in vesicle formation.[516]

Charged, non-PEGylated peptides have also been used to form vesicles, as demonstrated by the same group.[517] To overcome the charge−charge repulsion, smaller block lengths were applied, as demonstrated with PLL_{60}-$p(Leu)_{20}$ and, designed by analogy, poly(L-glutamate)$_{60}$-*block*-$p(Leu)_{20}$ (PLG-p(Leu)). In these polypeptides, the presence of charge was used as an advantage; that is, to disrupt the sheet-formation, poly(L-leucine) tends to be engaged in driving the aggregates toward a vesicular architecture (Figure 50). Phenylalanine can replace leucine in the hydrophobic block, as shown by Sun et al.[232]

Apart from p(Leu), γ-benzyl-protected polyglutamate is another viable choice for the hydrophobic block, as demonstrated by Iatrou and co-workers,[518] who prepared PLL-*block*-poly(γ-benzyl-d7-L-glutamate)-*block*-PLL (PLL-PBLG-PLL). This triblock copolymer was shown to form vesicles for various block length distributions. Not only did this

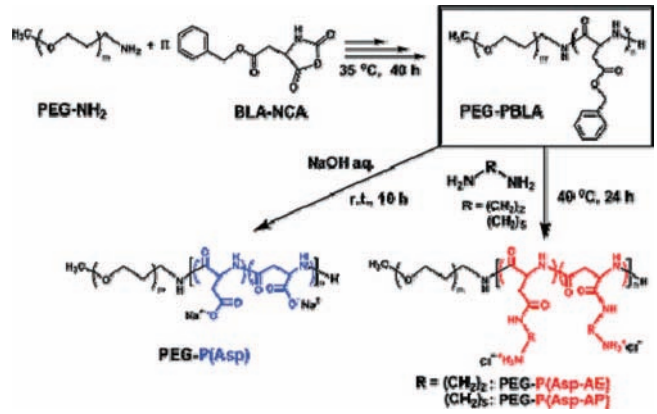


Figure 51. Synthesis of a pair of oppositely charged block copolymers. These double hydrophilic block copolymers can form polymersomes based on the electrostatic attraction between the oppositely charged blocks, forming polyion complexsomes, or PICsomes. (Reproduced with permission from ref 519. Copyright 2006 American Chemical Society.)

system profit from the secondary structure of the polypeptide, the charge on the lysine side chains was also exploited. The capture of plasmid DNA in the hydrophilic leaflet of the membrane resulted in larger yet still stable polymersomes, turning these polypeptide aggregates into potential gene delivery vehicles.

Another system where the charged side chains of amino acids were put to good use is that of the PICsome (polyion complexsome) reported by Koide et al.[519] Notably, these PICsomes were constructed from PEG-PLG derivatives (Figure 51), in which both blocks were water-soluble. Their aggregation behavior stemmed from the polyelectrolyte character of the glutamate blocks, which are either polyanions or, when coupled to a diamine, polycations. Equimolar mixing of these oppositely charged copolymers led to their close association in aqueous buffers, forming a membrane that was isolated from the environment by PEG. The lack of a tightly packed membrane made these polymersomes resistant to high osmotic pressures, allowing them to survive in a medium containing 10% fetal bovine serum at 37 °C. A later report described how a drop in pH levels to those found in cellular endosomes caused these PICsomes to become more permeable to solutes, suggesting future use as biological delivery vesicles.[520]

The combination of PEG and polypeptides can also be found in the work of Sun and co-workers,[521] who produced a hydrophobic poly(ε-benzyloxycarbonyl lysine) that was separated into two blocks by a relatively short PEG block, thus creating a triblock-copolymer. These ABA-type building blocks assembled into large vesicles, driven by hydrophobic interactions between the protected lysine monomers. A comparable system[522] was based on the more hydrophobic amino acid tyrosine, coupled to desaminotyrosine and *n*-octanol to form desaminotyrosyl tyrosine octyl ester (DTO). DTO was oligomerized using subaric acid to yield hydrophobic oligo(DTO suberate), which was flanked by two PEG blocks. The polymersomes formed from this ABA-block copolymer were shown to be both biocompatible and biodegradable.[522]

Polypeptides have also been exploited as the hydrophilic part in biohybrid amphiphilic block copolymers. A number of designs employed hydrophilic amino acids combined with poly(lactic acid)[523] or the biocompatible poly(butadiene) (PBD). An early example of the latter is PBD-PLG, almost

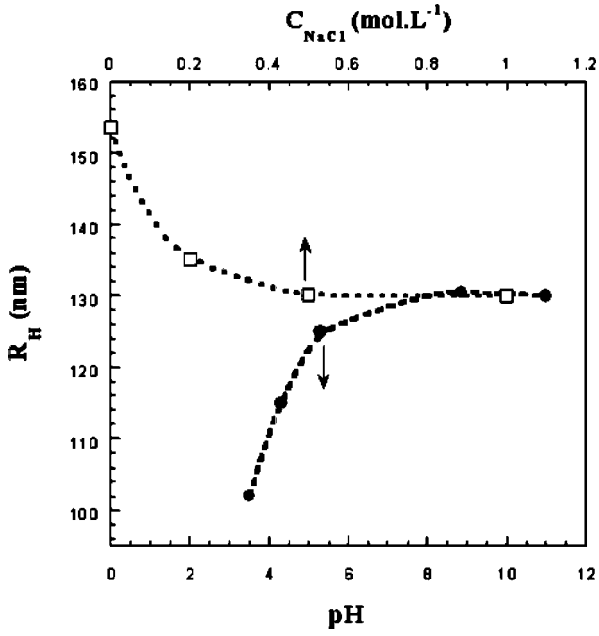


Figure 52. Hydrodynamic radius (RH) of PBD_{40}-PLG_{100} vesicles in water measured by DLS (90°) as a function of ionic strength (NaCl salt concentration) at pH = 11.5 (empty squares) and as a function of pH with constant ionic strength (1 M NaCl, solid circles). (With kind permission from Springer Science & Business Media: *Eur. Phys. J. E.*, From supramolecular polymersomes to stimuli-responsive nanocapsules based on poly(diene-*b*-peptide) diblock copolymers, *Vol. 10*, 2003, pp 25−35, Chécot et al., Figure 8.)

simultaneously reported by Kukula et al.[499] and by Chécot et al.[524] In these reports, the acidic nature of PLG was used to cause pH-induced helix-to-coil transitions in the peptide block. For shorter block lengths, this did not influence vesicle morphology, but longer PLG blocks provided vesicles that indeed responded to this external stimulus, viz. by increasing their hydrodynamic radius.[524] A later report showed that variations in ionic strength can also influence these responsive capsules, as shown in Figure 52.[224] The responsiveness and resulting secondary structure effects in polymersomes and micelles formed from PBD-PLG or PBD-PLL of different block weights have been thoroughly investigated using, among other techniques, circular dichroism, neutron scattering, and dynamic light scattering.[194,225,525] The responsiveness of polymersomes as described here is receiving increased attention, since this is an important issue in drug delivery using polymeric capsules, closely related to triggered destabilization.[498,526,527]

A departure from the more convenient block copolymer building block was provided by Lee et al.,[528] who suggested poly(2-hydroxyethylaspartamide) grafted with lactic acid oligomers (PHEA-*g*-LA) as a building block. The hydrophobic grafts, when present in a high enough percentage of repeating units, drove this amphiphilic comb toward microphase separation after dispersion in water, leading to polymersome vesicles where the hydrophilic "heads" of each grafted "tail" were, in fact, parts of the polypeptide backbone. Such grafted comblike polymer architectures are frequently encountered for another type of polymersome, namely the recognition induced polymersome (RIP), which will be extensively covered in section 5.1.3.

While amino acids can be polymerized via amide bonds, as naturally found in proteins, there is also a class of polymers that feature amino acid residues in their side chains.

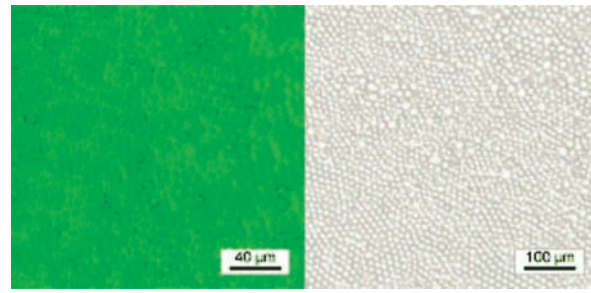


Figure 53. Confocal laser scanning (left) and optical microscope (right) images showing the typical morphologies of PBLG-PIAA. The sample was prepared by spreading drops of dilute chloroform solutions (1 mg mL^{-1}) on glass slides. (Reproduced with permission from ref 529. Copyright 2005 Wiley.)

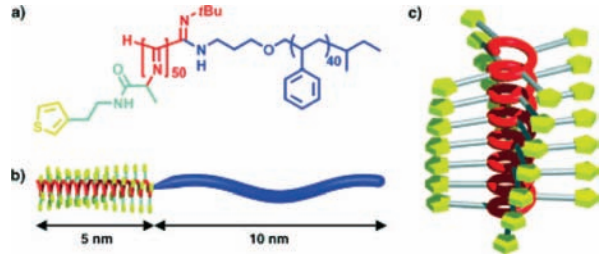
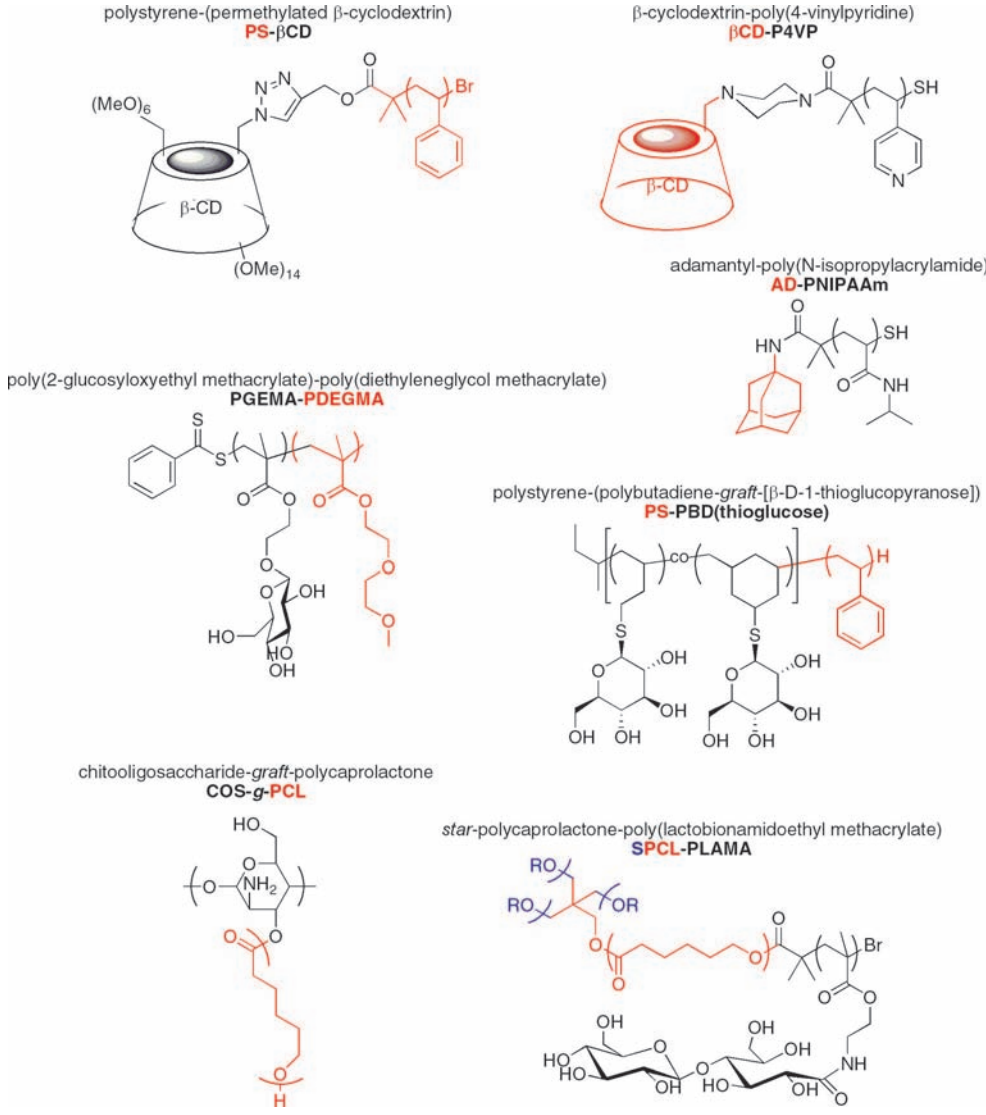


Figure 54. (a) Chemical structure of PS-PIAT. (b) Schematic representation of PS-PIAT. (c) Schematic representation of the PIAT block showing the stacks of thiophene groups. (Reproduced with permission from ref 532. Copyright 2003 Wiley.)

An example that consists of a conventional polypeptide block and a block featuring alanine in its side chain was given by Kros et al.,[529] who synthesized poly(γ-benzyl L-glutamate)-*block*-poly(L-isocyanoalanyl-L-alanine methyl ester) (PBLG-PIAA). This rod−rod block copolymer was shown to form polymersomes upon fast drying of a solution of PBLG-PIAA in chloroform (Figure 53). The polymersome formation is thought to be dependent on the helical motif of PIAA, which is reinforced by the internal hydrogen bonding arrays between the alanine moieties located parallel to the polyisocyanide backbone.[530] This feature was corroborated by the finding that comparable self-assembly characteristics could not be maintained when polyisocyanides that did not contain amino acids were used to replace PIAA.

The aforementioned PIAA block was coupled to polystyrene by Cornelissen and co-workers, who found that the resulting PS-PIAA was capable of forming bilayered vesicles,[531] among other interesting superstructures. Another interesting example of a block copolymer of PS and a polyisocyanopeptide is that of polystyrene-*block*-polyisocyanoalanine-(2-thiophene-3-yl-ethyl)amide (PS-PIAT, Figure 54), which was first reported by Vriezema et al.[532] and has been investigated in-depth by De Hoog et al.[533] The thiophene groups located in the hydrophilic leaflets of the membrane could be cross-linked to give polymerized polymersomes, allowing for regulation of aggregate rigidity and additionally enabling the construction of conducting architectures. Furthermore, these polymersomes are permeable to small molecules, which enabled a range of applications, as will be discussed in section 5.2.1. These block copolymers were also subjected to electroformation as a technique for vesicle production,[534] which resulted in giant polymersomes with diameters up to 100 μm, as opposed to the maximum diameter of 1.5 μm obtained when more conventional

Chart 5. Structure Formulas of Various (Carbohydrate-Containing) Polymersome-Forming Polymers



polymersome-formating methods were used (i.e., injection of a solution of block copolymers in THF into an aqueous phase).

Another example of an amphiphilic polymer containing an amino acid residue in one of its side chains was given by Zhang et al.[535] They reported a polyphosphazene (PPPs) grafted with poly(N-isopropylacrylamide) (PNIPAAm) and ethyl tryptophan, creating a cocontinuous microstructure with a hydrophobic tryptophan and a hydrophilic PNIPAAm chain side, interlinked by PPPs. The polymeric aggregates produced from this polymer were directly tunable through the controlled addition of indomethacin, a hydrophobic compound that can H-bond to the amide moieties in PNIPAAm, virtually turning it into a more hydrophobic block segment. Networks purely made of this PPPs-based copolymer could be transformed into various types of vesicles using the hydrophobicity induced via indomethacin.[535]

To obtain amino acids in the side chains, one can also graft these compounds to a pre-existing backbone, as demonstrated by the group of Schlaad,[536] who took advantage of the vinylic moieties that are present in PBD. Free radical addition of thiols to the double carbon−carbon bonds present in the polymer resulted in a covalently grafted random copolymer, since the reaction can either add a single thiol to a single vinyl moiety, or the double bonds can cyclize as a side reaction.[537] When cysteine, with its thiol side chain, was used to graft to PBD, an amphiphilic polyelectrolyte was formed, with a hydrophobic backbone and hydrophilic pendants. This random copolymer assembled into pH-responsive vesicles with the polymeric backbone parallel to the membrane, instead of perpendicular as observed for most previous copolymers.[536] This platform has been expanded to sulfur-containing carbohydrates, which will be covered in the next section.

5.1.2. Carbohydrate-Containing Building Blocks

Sugars have increasingly been recognized as more than just building blocks for cell walls or as an energy source for metabolism.[538,539] Through multivalent effects,[155] they play vital roles in areas such as cell−cell recognition or signal transmission,[540] which justifies the amount of research being directed toward glycopolymers.[294,295] Saccharide-functional polymersomes could be of particular interest, since they can be used to mimic or interfere with the multivalent cellular interactions that occur when the saccharides are exposed at the polymersome surface. However, as opposed to the abundance of polypeptides found in polymersome building blocks, the number of glycopolymers used for polymersome formation is still limited (see Chart 5); in the few examples

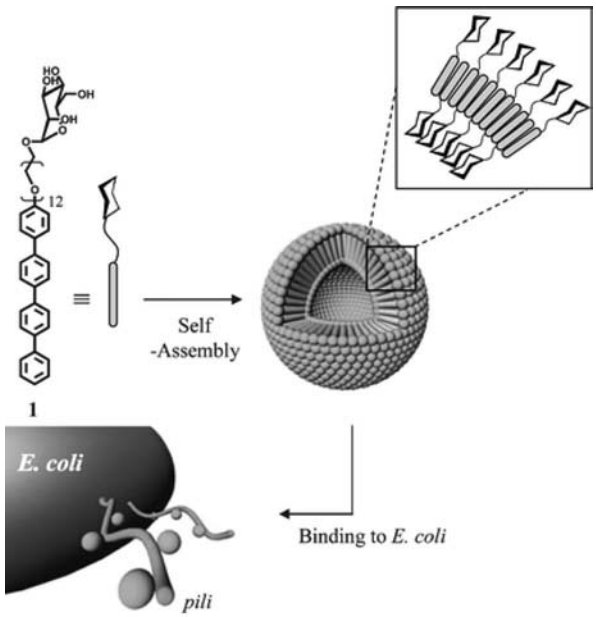


Figure 55. Schematic representation of the binding of *Escherichia coli* pili to carbohydrate-coated nanocapsules. (From ref 320. Reproduced with permission of The Royal Society of Chemistry.)

reported in the literature, carbohydrates are most often found in the side chain of the constituent block copolymers or at their termini. This may be attributable to the lack of a convenient route toward oligosaccharides with well-defined secondary structures, which in turn results from difficulties in obtaining stereospecific monomers. This is a problem that thus far has hindered progress in this discipline. Increasing input and demand from the field of glycomics[538−540] may help take this hurdle, though, in the future.

We have already mentioned a carbohydrate-terminated amphiphile in the previous section.[514] A related example of a mannose-terminated amphiphile is tetra(*p*-phenylene)-*block*-PEG_{12}-α-D-mannopyranoside,[320,541] which was already discussed in section 3.2.3 dealing with glycopolymeric micelles. This glycopolymer could also self-assemble into bilayered vesicles with an unusually small average diameter of 40 nm (Figure 55). These small mannose-covered polymersomes showed a specific binding ability to the pili of the ORN 178 *Escherichia coli (E. coli)* strain. Galactose-terminated amphiphiles also formed polymersomes but did not exhibit the same binding behavior, demonstrating the significance of the terminal carbohydrate.

Another carbohydrate-terminated amphiphile was reported by Felici et al.,[323] who showed that permethylated β-cyclodextrin (βCD) is a sufficiently polar headgroup to drive a conjugated PS_{66} toward polymersome formation. βCD is a water-soluble receptor molecule with a hydrophobic interior.[542] The layer of receptor molecules thus presented on the polymersome periphery was shown to be a viable surface for the immobilization of adamantane-appended enzymes. A similar polymer was almost simultaneously reported by Guo and co-workers,[543] who introduced a triblock variant using polyether imide (PI) capped on both termini by βCD. This building block was also shown to form stable polymersomes, and the hosting capacity of the resulting βCD-surface was assayed using adamantyl-conjugated PEG and isothermal titration calorimetry (ITC). Surprisingly, a quantitative inclusion of adamantyl moieties into the βCD cavities was found, suggesting that this polymersomal bilayer was penetrable to molecules such as PEG.[543]

A system that also uses the receptor properties of βCD is that of Jing et al.,[544] who made bilayered vesicles out of a self-assembling amphiphile featuring a bis(ethylhexyl)-modified naphthyl moiety. This docked inside the cyclodextrin to provide it with a rather small tail—but sufficiently large for bilayer formation. A similar but more truly polymer-related approach came from the group of Zhang, who used βCD to noncovalently couple βCD-poly(4-vinylpyridine) (βCD-P4 VP) to adamantyl-*N*-isopropylacrylamide (AD-PNIPAAm).[329] The resulting block copolymers assembled into a variety of architectures (see section 3.2.4), one of which was vesicular (Figure 30).

A block copolymer with saccharides in the polymer side chain was prepared by Pasparakis and Alexander,[545] who prepared the double hydrophilic poly(2-glucosyloxyethyl methacrylate)-*block*-poly(diethyleneglycol methacrylate) (pGEMA-pDEGMA). The difference in relative hydrophilicity between the blocks was sufficiently large to elicit lyotropic behavior, leading to polymersomes with block length-dependent diameters ranging from 251 nm ($pGEMA_{10}$-$pDEGMA_{50}$) to 500 nm ($pGEMA_{28}$-$pDEGMA_{36}$). Like the mannose-presenting polymersomes discussed above, these glycocalyx-endowed vesicles could interact with *E. coli* cells. The multivalent glucose even allowed for the transfer of encapsulated ethidium bromide from the vesicle interior to the bacterial cytoplasm:[545] a remarkable feat, since individual glucose moieties exhibit only weak interactions with receptors. The macromolecular assembly of the polymersome thus enabled the polyvalent presentation of this carbohydrate recognition element.

Similar architectures based on polystyrene have also been reported, combining this hydrophobic coil with glucose-appended polyacrylate, with polystyrene-*block*-poly(2-(β-D-glucopyranosyloxy)ethyl acrylate) (PS-PGEA),[546] or with thiosugar-grafted PBD, prepared analogously to the cysteine-grafted PBD described in section 5.1.1.[536,537] 1-Thioglucose-grafted PBD also formed unilamellar vesicles on its own, without the hydrophobic PS-block and assumed the same architecture as described for cysteine-grafted PBD in section 5.1.1.[536] The morphological variance in response to different organic solvent systems was investigated for all of these so-called glycosomes.

Zhou et al.[547] have reported on biodegradable, star-shaped block copolymers consisting of PCL and poly(lactobionamidoethyl methacrylate) (PLAMA), which aggregated into rather stable polymersomes with a lactose shell. The benefit of the star-shape, which effectively preorganizes four block copolymers, was demonstrated by comparison with a linear analogue, which possessed a twelve times higher critical aggregation concentration. Hence, star-shaped PCL-PLAMA are suggested to be more stable than their linear counterparts.[547] A variant with a poly(gluconamidoethyl methacrylate) block was reported by the same group.[304]

Chitooligosaccharide, a tough polymer based on naturally occurring chitin, has been used as the backbone for a grafted polymer that also formed polymersomes, as reported by the group of Li,[548,549] who investigated the properties of chitooligosaccharide-*graft*-poly(ε-caprolactone) (COS-g-PCL).[549] The hydrophilic nature of the polysaccharide contrasted sufficiently with the hydrophobic caprolactone grafts, leading to lyotropic behavior and the formation of micelles or vesicles. Interestingly, the bilayer membrane of the vesicles

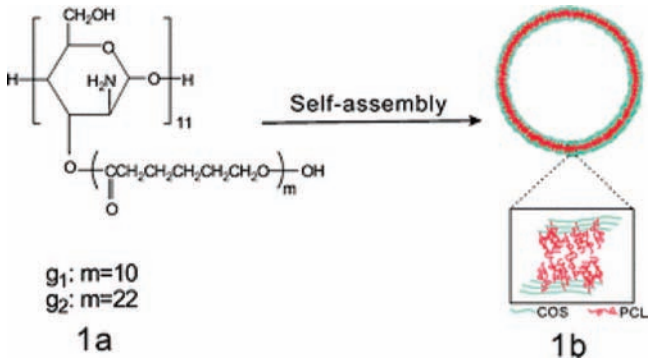


Figure 56. (a) Chemical structure of COS-*g*-PCL, where "m" represents the degree of polymerization of the PCL grafts. (b) Proposed architecture of the polymersomes formed. (From ref 550. Reproduced with permission of The Royal Society of Chemistry.)

did not resemble those formed by amphiphilic block copolymers. It turned out that the hydrophilic polymer backbone was exposed to the solvent, shielding the hydrophobic grafts (Figure 56), which could be loaded with small hydrophobic organic molecules such as pyrene. A later report discussed the exceptional size tunability of the COS-g-PCL vesicles via variations in the dioxane/water ratio used for vesicle formation, allowing vesicle sizes ranging from 0.5 to 400 μm.[550]

5.1.3. Nucleobase-Containing Building Blocks

Deoxyribonucleic acid (DNA) is one of the most fascinating macromolecules occurring in nature, due in part to its almost perfectly defined secondary structure and in part to its invaluable capability for storing information. Because of the former aspect, DNA has inspired polymer chemists to design similar self-assembled synthetic systems (see Chart 6).[551,552] The unique ability of cDNA strands to engage themselves in self-recognition events has proven to be transferable to a variety of polymeric architectures, including synthetic polymers[334,553,554] and block copolymers[555,556] exhibiting similar recognition capabilities through incorporation of nucleobases in their architectures.[333,557−559]

The group of Rotello provided some of the first reports on recognition induced vesicle formation in a noncompetitive solvent, i.e. a solvent that does not induce phase separation. An example is poly(styrene-*co*-4-chloromethylstyrene), which had several side groups modified with one of two recognition elements, leading to either a diacyldiaminopyridine-appended chain (DAP polymer) or a complementary thymine-1-acetic acid-functionalized random copolymer (Thy polymer).[560] Vesicle formation was induced by dissolution of equal volumes of the two complementary polymers in chloroform (Figure 57). These vesicles were stable for long periods of time but dissociated rapidly upon heating, which is reminiscent of the melting of DNA strands.[561] These vesicles have been dubbed recognition-induced polymersomes (RIPs) and predominantly consist of random copolymers instead of block copolymers, eschewing lyotropic behavior in aqueous solution in favor of recognition-driven assembly in organic solvents. However, a "blocky" phenomenon was proposed to occur for these random copolymers,[562] since more densely functionalized polymers or parts of polymers tended to segregate to the inner vesicle wall, whereas less densely functionalized polymers or parts of polymers tended to localize at the periphery, somewhat mimicking block copolymer-like microphase separation.

The DAP polymer did not necessarily need the Thy polymer to assemble into RIPs: low molecular weight double thymine-functionalized cross-linking agents[563] or thymine-conjugated gold nanoparticles[337] could also provide the needed amount of interchain interactions for stable polymersome formation. In turn, the use of monovalent complementary H-bonders such as flavin disrupted the membrane.[337] Using small double thymine containing cross-linkers, Thibault et al. showed that the repeated heating and cooling of RIPs between 25 and 50 °C (not unexpectedly) led to their repeated destruction and reformation. The interesting part of this experiment is the observation that this thermal cycling not only could be repeated indefinitely but also narrowed the size distribution of the polymersomes involved, creating more monodisperse particles.[563]

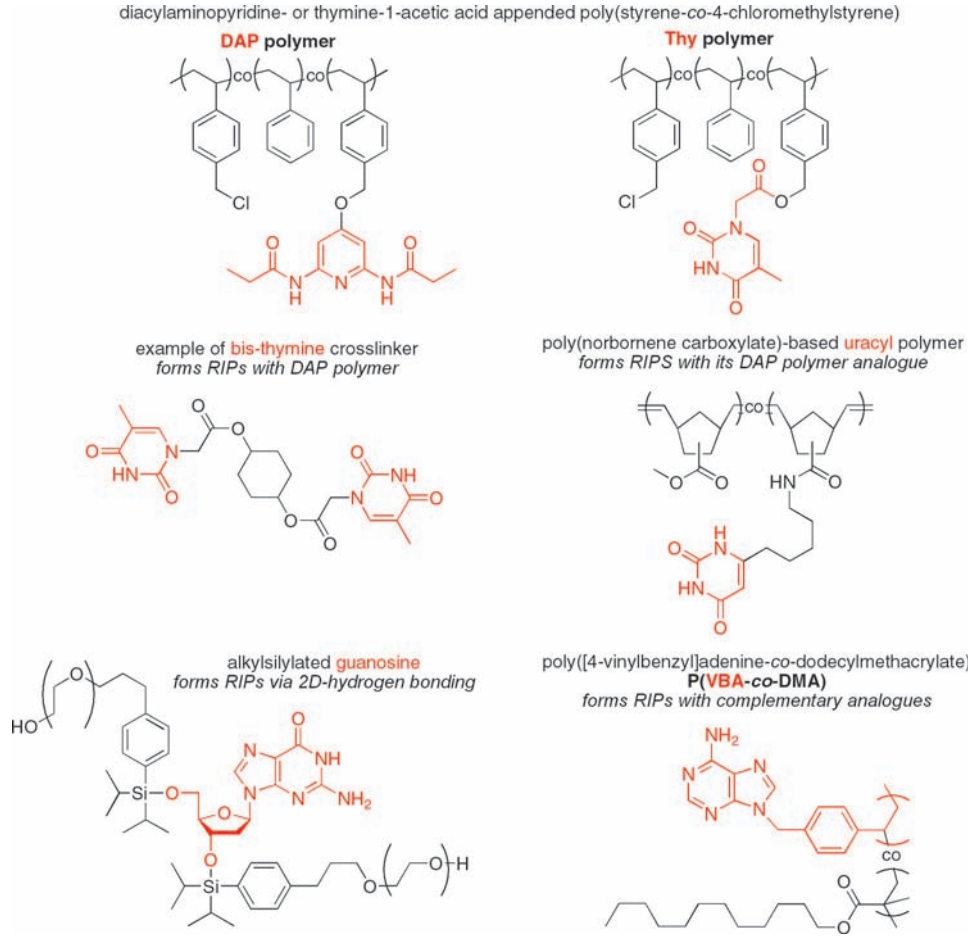
DAP/Thy polymer-based RIPs can be "frozen" by photochemical cross-linking of the thymine residues,[564] which led to vesicles with a static, stabilized membrane. Cross-linking provided excellent size control over the vesicles, since any size could be locked in at any desired moment. Additionally, the H-bonding between the DAP and thymine moieties was no longer of high importance for the structural integrity of the polymersomes, enabling the incorporation of thymine-functionalized CdSe nanoparticles into the cross-linked membrane. The highly structured spherical arrays of nanoparticles created in this way are a direct result of the recognition-mediated assembly enabled by the use of DAP and thymine.

Another system from the group of Rotello[565] used amino-derivatives of diacyldiaminopyridine and uracyl. These triple H-bonders were appended to a random copolymer of methyl norbornene-2-carboxylate and succinimidyl norbornene-2-carboxylate, employing the activated ester in the latter monomer for its facile decoration with the recognition elements.[565] The RIPs formed by these two copolymers were shown to be metastable. Initially formed polymersomes slowly fused into larger aggregates, leading, in a matter of hours, to the formation of a solvent-swollen gel. This demonstrated the influence of the polymer structure on the membrane dynamics of RIPs, since the polystyrene-based system did not exhibit this fusion behavior on the same time scale.[560]

An example of polymersome-forming copolymers that included two complementary nucleobases was given by Lutz et al.,[566] who used 1-(4-vinylbenzyl)thymine (VBT) and the complementary 1-(4-vinylbenzyl)adenine (VBA) to prepare random copolymers with dodecylmethacrylate (DMA), obtaining either P(VBA-*co*-DMA) or P(VBT-*co*-DMA). These copolymers formed polymersomes when dissolved in dilute concentrations in dioxane or chloroform, with the aliphatic tails shielding the nucleobases from the organic solvent. The driving force behind this RIP formation, H-bonding between the complementary nucleobases, was demonstrated not only by the reversible melting and annealing of the vesicles but also by the fact that the large spherical aggregates could be broken down into smaller ones by addition of free complementary monomers (Figure 58).

The membrane dynamics of the P(VBA-*co*-DMA) and P(VBT-*co*-DMA) vesicles was further investigated by labeling the two polymers with two separate fluorescent probes.[335] A "green" thymine-based vesicle dispersion was preformed and mixed with a preformed "red" adenine-based dispersion of RIPs. These single-base vesicles were found to be far less stable than those based on complementary nucleobases, as

Chart 6. Structure Formulas of Various (Nucleobase-Containing) Polymersome-Forming or Related Compounds



demonstrated by their subsequent equilibration upon mixing, which led to a spontaneous rearrangement of the polymers to interact with their complementary bases instead of with themselves. The appearance of both a "red" and a "green" signal in all RIP membranes observed after equilibration indicated the physical rearrangement of the polymers (Figure 59).[335]

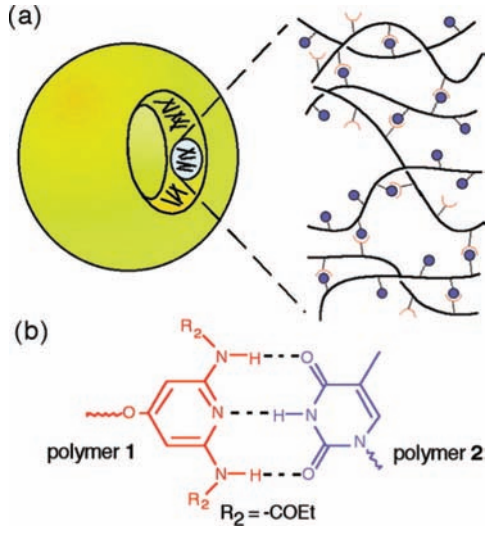


Figure 57. Schematic demonstration of vesicle formation between diaminopyridine-based polymer 1 and thymine-based polymer 2. (a) Illustration showing molecular recognition within the vesicle wall. (b) The corresponding recognition dyads. (Reproduced with permission from ref 560. Copyright 2000 American Chemical Society.)

A similar system reported by Yoshikawa et al.[567] used only "monomers" of the alkylsilylated nucleobase guanosine. It was shown to form vesicles as well, through 2D-hydrogen bonding in between the guanosine moieties. Though the constituent building blocks cannot be called polymers, their likeness to P(VBA-*co*-DMA) and similar modes of aggregation sparks interest. Furthermore, it is a rare example of RIP-formation in aqueous solution.

A final example is one in which the recognition capacity of the nucleobase was not used as the driving force of polymersome formation. Teixeira Jr. et al.[339] synthesized a

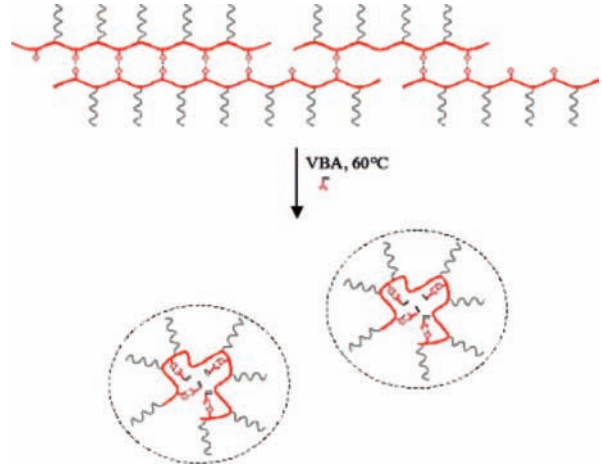


Figure 58. Schematic illustration of the destruction of P(VBT-*co*-DMA) supramolecular assemblies in the presence of the complementary monomer 1-(4-vinylbenzyl)adenine (VBA). (Reproduced with permission from ref 566. Copyright 2005 Wiley.)

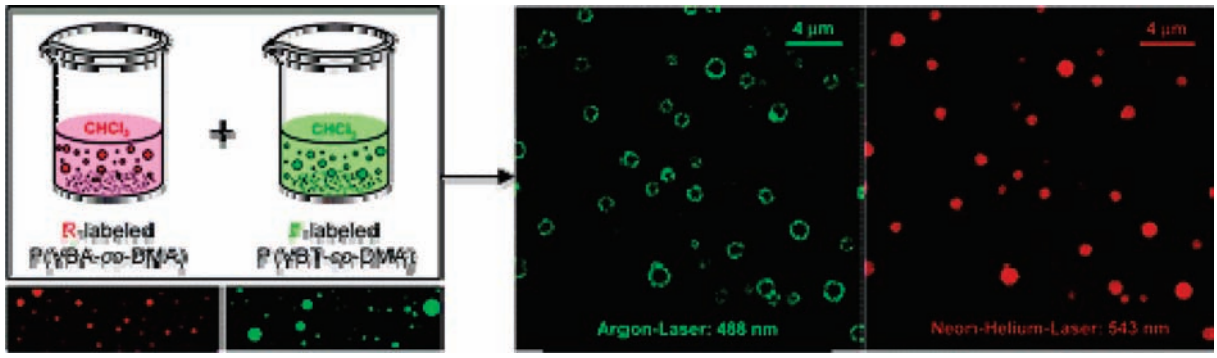
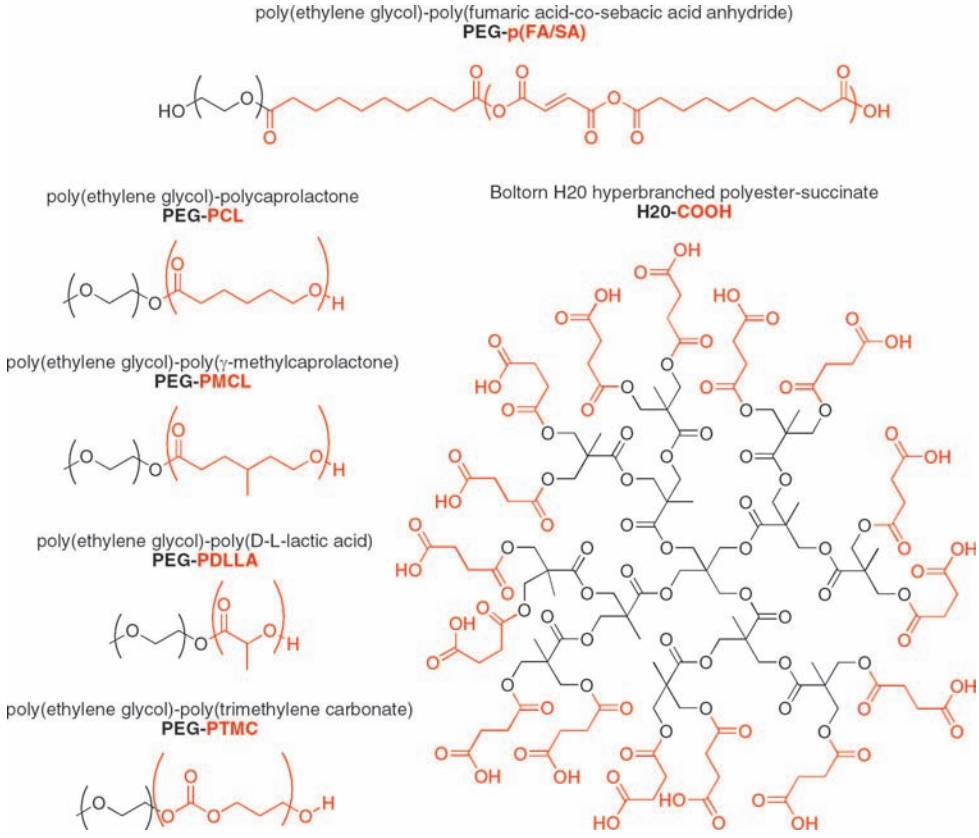


Figure 59. Example of fused vesicles, exhibiting both green and red fluorescence, observed by confocal fluorescence microscopy 10 min after mixing individual chloroform solutions of P(VBT-*co*-DMA) and P(VBA-*co*-DMA). (Reproduced with permission from ref 335. Copyright 2006 American Chemical Society.)

Chart 7. Structure Formulas of Various Biodegradable Polymersome-Forming Polymers



PBD chain that was covalently linked to an oligonucleotide sequence, which can be considered to be a water-soluble block. The resulting amphiphilic diblock copolymer formed vesicular structures in dilute aqueous solution. The vesicles were completely surrounded by a spherically closed nucleotide shell, suggesting future applications of these vesicles as biocompatible delivery capsules, which can be recognized by microorganisms.

5.1.4. Biodegradable Polymersomes

Besides the aforementioned polymersomes that contain biological building blocks, there is a large category of vesicles that do not necessarily contain these biocomponents but of which the field of application is tightly associated with that of biological systems, i.e. biodegradable polymersomes (see Chart 7). Biodegradability is a desirable aspect of polymersomes when they are to be used in vivo for delivery or imaging applications[568,569] (see section 5.2.2), since the organism itself will not be left with polymeric remnants that might accumulate in certain organs or tissue types. In addition, biodegradable vesicles have a built-in mechanism for delayed or gradual release.

Although polymersomes made from nonmodified polypeptides often are biodegradable,[523,528] the majority of the polymersomes described thus far are hybrids in which one of the blocks is a biocompatible or bioinert polymer, e.g. PEG, PBD, or short PAA, or in which a synthetic polymer (e.g., a methacrylate derivative) is used as a backbone for functionalization with biomolecular entities. These polymers are not biodegradable, purely in the sense that they do not get metabolized or broken down into smaller pieces by cells or organisms, but they are readily excreted and do not linger in the organism.[469] In this section we designate a polymersome as "biodegradable" if the vesicular morphology can be broken down and if at least one of the blocks can be metabolized and (the metabolites of) the block copolymers

can be removed from the organism without accumulation or adverse effects.

The first report on biodegradable polymersomes was by Najafi and Sarbolouki,[502] who used PEG as the hydrophilic block in di- and triblock copolymers with poly(fumaric acid-*co*-sebacic acid anhydrides)(p(FA/SA)) of the form PEG-p(FA/SA) and PEG-p(FA/SA)-PEG. As a model hydrophilic drug, calcein was encapsulated in the aqueous compartment of the resultant polymersomes. The gradual degradation of the polyanhydrides by hydrolysis in pure water was measured, along with the accompanying release of calcein. Diblock-based vesicles were fully degraded after 30 days, whereas triblock vesicles were more stable, having only degraded for approximately 80% in the same time span. The trapped calcein was completely released after 100 h for both architectures.[502]

Another early example of biodegradable polymersomes was presented by the group of Feijen, who reported a variety of hydrophobic polyesters and polycarbonates, which were combined with PEG to form amphiphilic block copolymers.[491] In order to synthesize the block copolymers, the catalyst zinc bis[bis(trimethylsilyl)amide] was used in combination with methoxy PEG to initiate the ring-opening polymerization of lactides and lactones. This catalyst has a low toxicity,[570] which is attractive for the synthesis of block copolymers that are to be used for in vivo applications. The polymersomes were studied for up to three months, and only marginal leakage of the cargo compound fluoresceinamine was detected. The impermeable vesicle membranes were expected to be completely degraded after 12 to 16 months, based on studies with pure PDLLA.[571] A later report described the gradual release of an encapsulated small molecule (carboxyfluorescein) in greater detail,[572] concluding that thicker membranes commonly slowed down the release rate of cargo molecules, as did the higher glass transition temperatures of the hydrophobic leaflets. A higher temperature increased the release rate. However, the addition of nonalbumin serum proteins to the medium slowed down the release rate, indicating that these biomacromolecules effectively thickened the vesicle membrane. This suggests that the PEGylated periphery of the polymersomes is not completely inert toward aspecific protein adsorption.[572]

The above observation is remarkable, since PEGylation of polymersomes does in general give them "stealth" properties—a term that is used to indicate that the particle is not actively expelled from circulation by a living organism and does not trigger an immune response.[469] This was demonstrated by a macrophage challenge experiment performed by the group of Discher,[488] where PBD-PEG polymersomes were brought in contact with red blood cells but did not show any adhesion or invoke any cellular response. In comparison, neither did yeast particles exhibit any of this behavior. This is contrasted by white blood cells, which within 2 min of contacting a yeast cell began to spread and actively engulfed the yeast particle, fully internalizing it in 4 min. Over the same length of time and longer, PBD-PEG polymersomes appeared to be inert to the phagocytes. The outer PEG surface of these polymersomes repelled phagocyte adhesion. This property of PEGylation will be encountered again in the section on biomedical applications of polymersomes (section 5.2.2).

Ahmed and Discher described the same type of biodegradable block-copolymers as Feijen et al. (see above)[498] and expanded the platform by mixing PCL-PEG or PLA-PEG

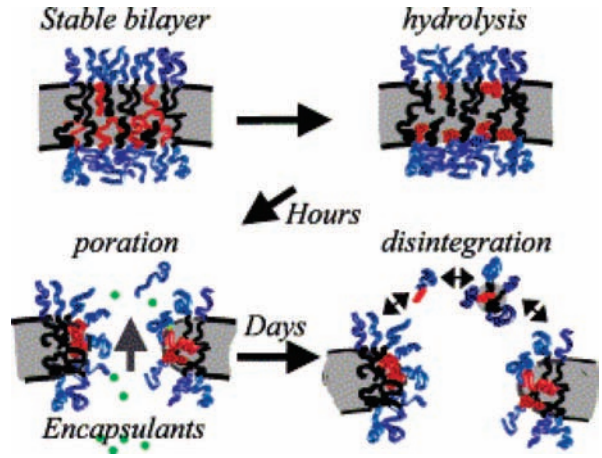


Figure 60. Polyester trigger of encapsulant release and disintegration of polymersome vesicles. Red chains are degradable polyesters, and black chains are inert. (Reprinted from *J. Controlled Release*, vol. 96 issue 1, Ahmed et al. Self-porating polymersomes of PEG-PLA and PEG-PCL: hydrolysis-triggered controlled release vesicles, p 37,[498] Copyright 2004, with permission from Elsevier.)

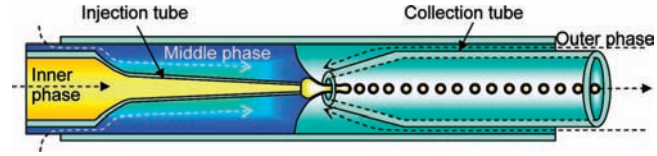


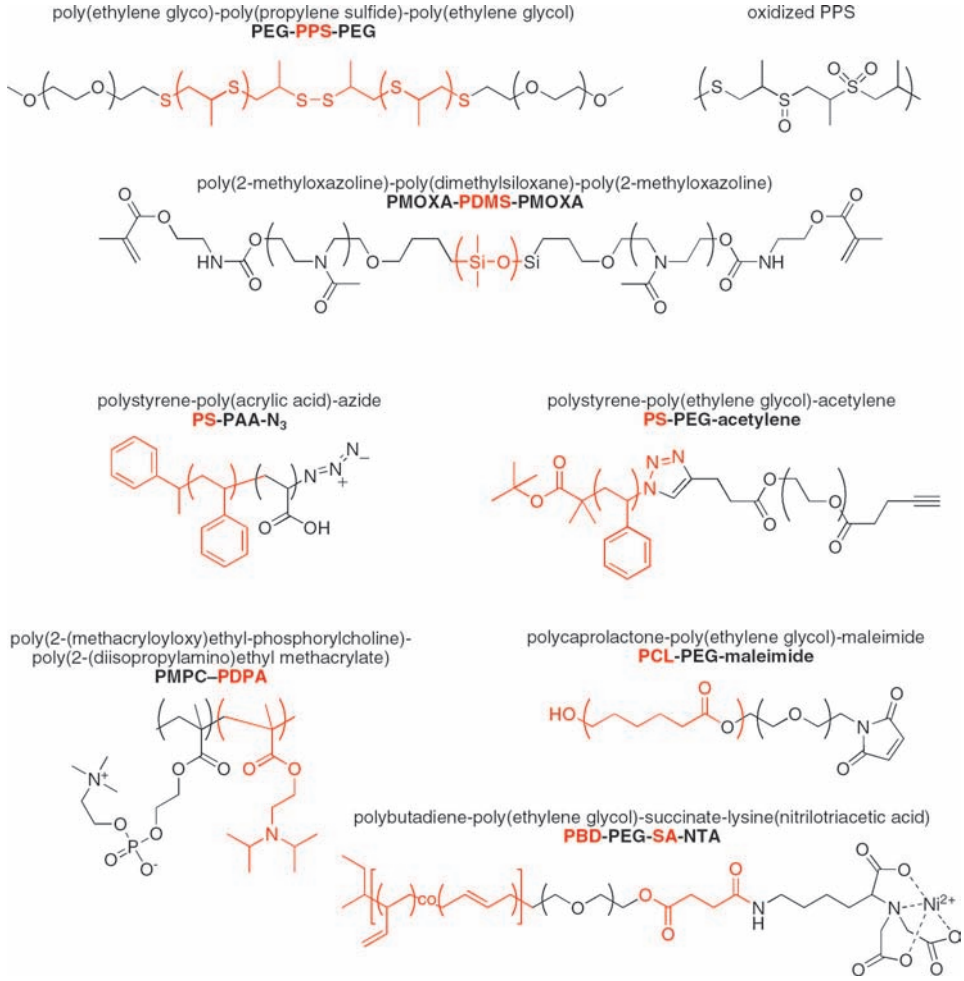
Figure 61. Schematic of the microcapillary geometry for generating double emulsions for use in polymersome formation. The geometry requires the outer phase to be immiscible with the middle phase, which in turn is immiscible with the inner phase. However, the inner phase can be miscible with the outer phase, as it is here. Both the injection tube and the collection tube are tapered from glass capillary tubes with an outer diameter of 1000 μm and an inner diameter of 580 μm. Typical inner diameters after tapering range from 10 to 50 μm for the injection tube and from 40 to 100 μm for the collection tube. (Reproduced with permission from ref 579. Copyright 2008 American Chemical Society.)

with PBD-PEG. It was found that the hydrolysis of the ester-based block copolymers destabilized the entire vesicle, leading to gradual release of its cargo (Figure 60). By decreasing the polyester content of the membrane, the leakage rate was consequently decreased, which opened the path to tunable release systems.

Due to their ease of preparation, PCL-PEG and PLA-PEG are commonly used to construct biodegradable polymersomes. Hence, the effects of, among others, variations in weight ratio between the blocks or fabrication method,[501,573−575] as well as encapsulation efficiency,[501,574−577] leaking kinetics,[500,578] and even circulation times[500,574] have been well-investigated. The work of Shum et al. was also based on PLA-PEG, but distinguished itself by the use of a microfluidic system for polymersome formation, schematically illustrated in Figure 61.[579] This produced highly monodisperse and exceptionally well-defined polymersomes that were, however, highly sensitive to osmotic pressure differences. By tuning the block ratios of the block copolymer, the technology allowed precise control of polymersome properties such as membrane thickness, permeability, thermal stability, and encapsulation efficiency.[579]

Carbohydrate containing polymersomes that are biodegradable have been discussed in the previous sections, e.g. the chitooligosaccharide-based COS-*g*-PCL reported by Wang et al.[548] and Gao et al.[549,550] or the lactobionic acid-based SPCL-PLAMA reported by Zhou et al.[304,547]

Chart 8. Structure Formulas of Various Functional Polymersome-Forming Block Copolymers



A rather simple way to obtain interesting biodegradable vesicles was shown by Shi and co-workers,[286] who used a commercially available hyperbranched polyester that after reaction with succinic anhydride became decorated with carboxylic acid groups. This caused it to exhibit pH-responsive self-assembly behavior in water. When the acid groups were protonated, the hyperbranched polymers formed vesicles with a size dependent on the generation of the dendritic molecule. This feature was explained by the fact that lower generation hyperbranched structures have a smaller dendritic core and hence a higher content of hydrophilic groups compared to their larger counterparts, and thus prefer to self-assemble into a membrane with smaller curvature. The cheap and simple synthesis of this system, combined with the facile way for preparing vesicles with a controlled size, makes this a highly accessible way to form biodegradable polymersomes.[286] Using a related strategy, the dendrimer was terminated with PEG, leading to large multicompartment aggregates instead of polymersomes.[580]

5.2. Biohybrid Applications of Polymersomes

Besides the use of premade hybrid building blocks for the construction of biohybrid polymersomes, an alternative method is to introduce biological units during or after the polymersome formation process (see Chart 8). Polymersomes have a defined architecture that can be used as a scaffold for the precise positioning of (bio)molecules. The possible applications of this property range from the simple encapsulation of proteins[488] to the incorporation of membrane proteins in the polymeric bilayer.[581] The surface of a polymersome can also be conjugated to molecules ranging from cellular signaling molecules[495,582] to complete proteins or enzymes.[583,584] All these possibilities made it possible for biohybrid polymersomes to be applied for various purposes, e.g. as reaction vessels[8,585] or as vehicles for a host of biomedical applications.[568,569]

5.2.1. Polymersomes as Enzymatic Reactors

The first encapsulation experiments with proteins were performed with myoglobin, hemoglobin, and albumin.[488] These compounds were encapsulated via the addition of the solid block copolymer PBD-PEG to an aqueous solution of the desired solute, after which the mixture was incubated for a day. The efficiency of this method varied considerably, ranging from ~5% for albumin, as determined by fluorescence spectroscopy, to more than 50% for myoglobin, as estimated by bright field microscopy.

The interest in stimuli-responsive vesicles led Napoli et al. to investigate glucose oxidase (GOx) containing polymersomes.[586] These vesicles were prepared by encapsulating GOx within polymersomes of PEG-*block*-poly(propylene sulfide)-*block*-PEG (PEG-PPS-PEG). The hydrophobic middle block contained thioethers that were converted to more hydrophilic sulfoxides and sulfones when oxidized by H_2O_2. The latter is produced when glucose is oxidized to gluconolactone by the encapsulated GOx. This oxidation thoroughly destabilized the membrane, leading to vesicle degradation

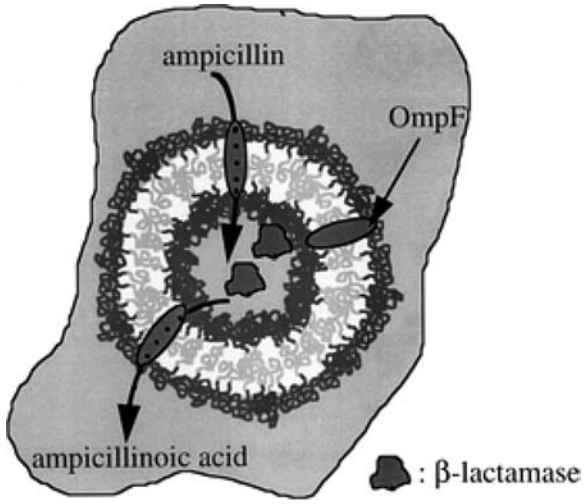


Figure 62. Schematic representation of a nanoreactor containing the enzyme β-lactamase, prepared from an amphiphilic triblock copolymer and the porin OmpF. The enzyme converts ampicillin to ampicillinoic acid. (From ref 590. Reproduced by permission of The Royal Society of Chemistry.)

and hence to aggregates that were self-immolative when glucose was added.

Membrane permeability is an important parameter when encapsulated enzymes are supposed to come into contact with solutes in the medium. Battaglia et al. have developed a method for determining the permeability of polymersome membranes by encapsulating the highly hydrophilic 3,3′,3″-phosphinidynetris-benzenesulfonic acid and subsequently monitoring its reaction with 5,5′-dithiobis-2-nitrobenzoic acid.[587] For this test system, it was found that the diffusion over polymersome membranes is in good agreement with Fick's first law. Unfortunately, a universal method to describe the diffusion of all types of solutes (charged, bulky) over a variety of membranes (high glass transition temperatures, rigid rods, triblocks) does not exist, but individual systems can most probably be investigated using similar methods.

For sufficiently small substrates, e.g. $O_2^{\bullet -}$ for the enzyme Cu,Zn superoxide dismutase, membrane permeability is not an issue.[588] The limitations of low membrane permeability for larger substrates have been circumvented by incorporating channel proteins in the polymer membrane, which can transfer substrates to their enzymes. The group of Meier has developed a nanoreactor by incorporating the so-called OmpF channel protein in the membrane of a poly(2-methyloxazoline)-*b*-poly(dimethylsiloxane)-*b*-poly(2-methyloxazoline)(PMOXA-PDMS-PMOXA)[589] polymersome (Figure 62).[581,590] Transport of small molecules over the membrane was greatly facilitated by the nonspecific OmpF channel protein embedded in the membrane, allowing passive diffusion of solutes up to a molecular weight of 400 g mol^{-1}. The thus readily accessible lumen contained β-lactamase enzymes. The activity of the encapsulated β-lactamase was determined by an iodine-starch assay, detecting the catalyzed formation of ampicillinoic acid from the substrate, ampicillin.

An interesting characteristic of the OmpF channel protein is its responsiveness to a transmembrane potential, closing itself when the potential is increased above 100 mV.[581] This property was put to good use by the addition of the sodium salt of poly(styrene sulfonate) to the dispersion. This negatively charged polymer is too large to pass through the membrane, whereas its sodium counterions can readily get into the vesicle. This establishes a so-called Donnan potential,

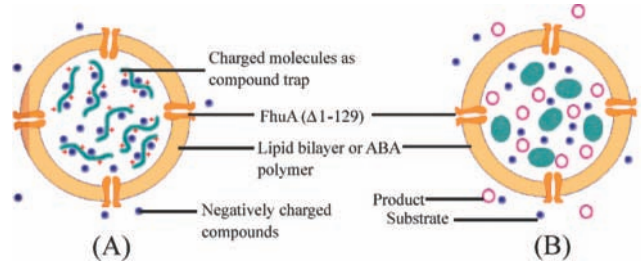


Figure 63. Schematic representation of two polymersome systems designed for (A) selective product recovery by loading the capsules with positively charged macromolecules as traps for negatively charged compounds and (B) biocatalytic conversions of substrates by enzymes encapsulated inside the polymersome. (Reprinted from *J. Biotechnol.*, vol. 123 issue 1, Nallani et al. A nanocompartment system (Synthosome) designed for biotechnological applications, p 50,[591] Copyright 2006, with permission from Elsevier.)

which closes the membrane protein. The conversion of ampicillin was effectively stopped by the creation of this potential but could be started again by dilution of the dispersion or by the addition of NaCl, both of which opened the channels again.

Another, larger channel protein that has been incorporated in PMOXA-PDMS-PMOXA polymersome membranes was a mutant of the so-called FhuA protein.[591] Polymersomes loaded with the enzyme horseradish peroxidase (HRP) and equipped with FhuA channels in their membranes were shown to be catalytically active, proving the functionality of both the enzyme and the channel (Figure 63, right). In a second application of the channel, polyelectrolytes, for example polylysine, were used as a "trap" inside the polymersome to selectively recover a product, e.g. the negatively charged sulforhodamine B, from the medium (Figure 63, left). The sequestering of product molecules inside catalytically active polymersomes was also achieved with the OmpF channel by using a soluble substrate that became insoluble upon enzymatic conversion, leading to accumulation of precipitated product molecules inside the polymersomes.[592]

Chemical modification of the FhuA channel prior to reconstitution in the polymersome membrane turned it into a reduction-responsive channel. Onaca et al.[593] modified the lysine residues on the inside of the channel with 2-[biotinamido]ethylamido-3,3′-dithiodipropionic acid *N*-hydroxysuccinimide ester or 3-(2-pyridyldithio)propionic acid *N*-hydroxysuccinimide ester. These groups sterically blocked the channel, not allowing encapsulated calcein molecules to diffuse out of the polymersome. Upon reduction of the disulfide bonds with dithiothreitol, the remaining bulk on the lysine residues was sufficiently small to allow the encapsulated calcein to pass through the channel.[593]

A variety of ion-carrying ionophores have been embedded in the PMOXA-PDMS-PMOXA triblock membrane, facilitating the transport of calcium ions.[594] To demonstrate this feature, polymersomes prepared in phosphate buffer were dialyzed to remove free, nonencapsulated phosphate ions, after which a $CaCl_2$ solution was added. Subsequently, the vesicles were incubated with one of three different ionophores. After one hour, the growth of calcium phosphate crystals on the inner membrane could be observed by electron microscopy. After 24 h, a significant part of the polymersomal lumen was filled with calcium phosphate crystals. Polymersomes not treated with ionophores did not show this crystal formation.

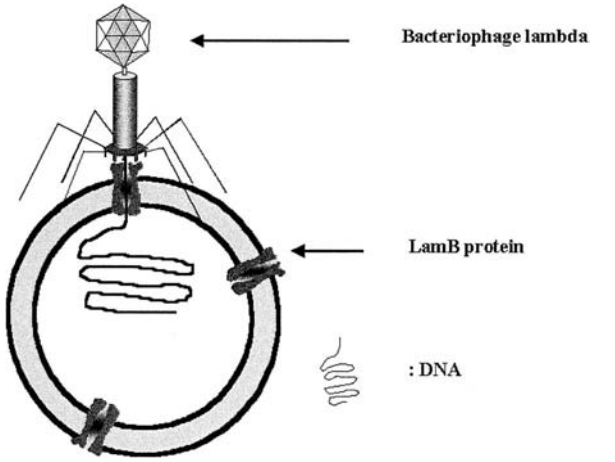


Figure 64. Schematic representation of a PMOXA-PDMS-PMOXA vesicle interacting with a λ phage. The phage binds a LamB protein that is embedded in the capsule's membrane. Subsequently, the DNA is transferred across the block copolymer membrane. (Reproduced with permission from ref 595. Copyright 2002 National Academy of Sciences, U.S.A.)

A more complex protein that was embedded in a PMOXA-PDMS-PMOXA polymersome was the bacterial channel-forming protein LamB.[595] The outer membrane protein LamB is a transport protein that is specific for maltodextrins but can also bind λ phages and trigger them to inject their DNA into the polymersome (Figure 64). The preserved functionality of LamB in an artificial environment is noteworthy, since it effectively tricks a phage into recognizing a synthetic polymer vesicle as a cell.[595]

Another system meeting the concept of artificial cells is based on a variation of the triblock copolymer used in the examples above, namely poly(2-ethyl-2-oxazoline)-*block*-PDMS-*block*-poly(2-ethyl-2-oxazoline) (PEtOz-PDMS-PEtOz). This polymer was used by Choi et al.[503] as a membrane in which some of the cellular machinery for the biosynthesis of ATP was reconstituted.[503] Bacteriorhodopsin (BR) is a light-driven transmembrane proton pump, which was embedded in the PEtOz-PDMS-PEtOz polymersome membrane to build and maintain a proton gradient over it. This gradient fueled the action of F_0F_1-ATP synthase, the rotary motor protein that catalyzes the phosphorylation of ADP to produce ATP. This biomimetic polymersome thus successfully replicated the biosynthesis of ATP, demonstrating the feasibility of performing biosynthesis in polymersomes (Figure 65).[503]

The group of Meier has taken the concept of biosynthesis in another direction, aiming to integrate a polymersome nanoreactor with a living cell.[596] This integration was achieved by labeling the outer surface of PMOXA-PDMS-PMOXA polymersomes with polyguanylic acid (polyG), a signal that is recognized by certain macrophages and triggers internalization. The polyG was immobilized on the polymersome periphery via conjugation of biotin to the PMOXA-termini, after which streptavidin was used to connect the vesicle to polyG, which also carried a biotin label.[582] In this way, trypsin-loaded, polyG-labeled polymersomes were prepared, without channel proteins in their membranes. These vesicles were internalized in macrophages. After internalization, all trypsin activity was inhibited via the addition of specific trypsin inhibitors to the cell growth medium. In spite of these inhibitors, trypsin activity was detected in macrophages containing the polymersome nanoreactors.[596]

Besides the biotin−streptavidin interaction mentioned above, also the host−guest binding between βCD and

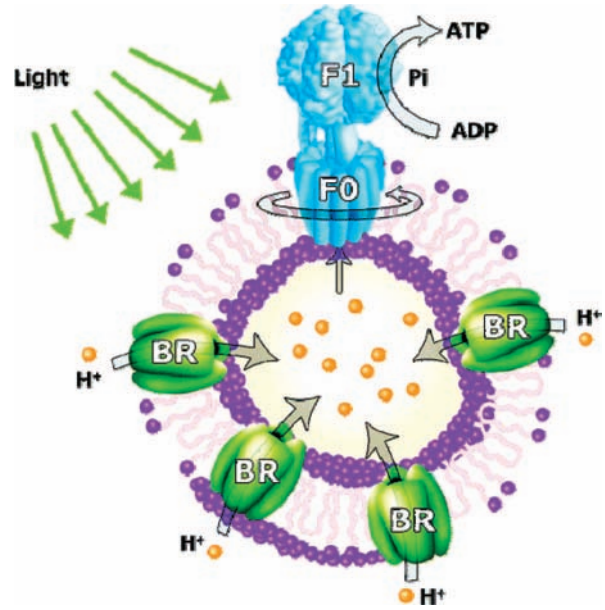


Figure 65. Schematic representation of polymersomes reconstituted with both bacteriorhodopsin (BR) and F_0F_1-ATP synthase. ATP synthase uses an electrochemical proton gradient generated by bacteriorhodopsin to synthesize ATP from ADP and inorganic phosphate (P_i). (Reproduced with permission from ref 503. Copyright 2005 American Chemical Society.)

adamantane was employed to functionalize (βCD-covered) polymersome surfaces with (adamantane-appended) enzymes, as was demonstrated by Felici et al.,[323] using the PS-appended βCD described in section 5.1.2. The enzyme in question, HRP, was equipped with a PEG_{68}-spaced adamantyl group. These surface-immobilized enzymes were incubated with hydrogen peroxide and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), of which the former is used by HRP to turn the latter into its radical cation, which strongly absorbs light at 420 nm. Indeed, this transformation was readily detectable in the polymersome dispersions. After multiple washing steps, however, the noncovalently linked HRP was present in smaller numbers, suggesting that the βCD−adamantane interaction is too weak to withstand prolonged washing.

A covalent surface immobilization procedure would solve this problem, and one such method was introduced by Opsteen et al.[583] A PS-PAA polymer was terminated with an azide moiety, which covered the polymersome surface after the assembly of the PS-PAA into polymersomes. Azides can be selectively and efficiently reacted with acetylenes using the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition, commonly referred to as a "click" reaction. To exploit the reactive polymersome surface, a variety of acetylene-bearing molecules were reacted with the "clickable" polymersomes. An acetylene-dansyl probe led to fluorescent vesicles. A functionalized biotin group enabled streptavidin recognition, which was proven by the successful conjugation of streptavidin-labeled colloidal gold particles to the biotinylated vesicles. Using pentynoic acid *N*-succinimidyl ester, green fluorescent protein was provided with acetylene groups, and this intact protein could also be conjugated to the polymersome surface, as shown by confocal fluorescence microscopy (Figure 66).

A more modular approach to surface decoration of polymersomes was given by Van Dongen and co-workers, who reported the use of a functional PS-PEG block copoly-

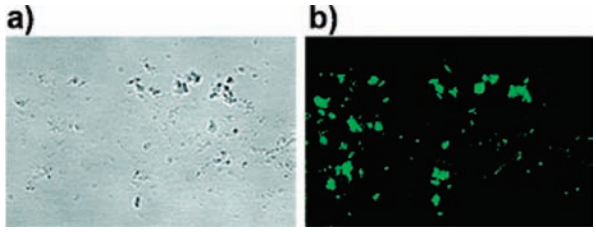


Figure 66. Confocal laser-scanning microscopy images of PS-PAA-N_3 polymersomes with acetylene-functionalized GFP conjugated to their surfaces (transmission [a] and fluorescence excited at 488 nm [b]). (From ref 583. Reproduced by permission of The Royal Society of Chemistry.)

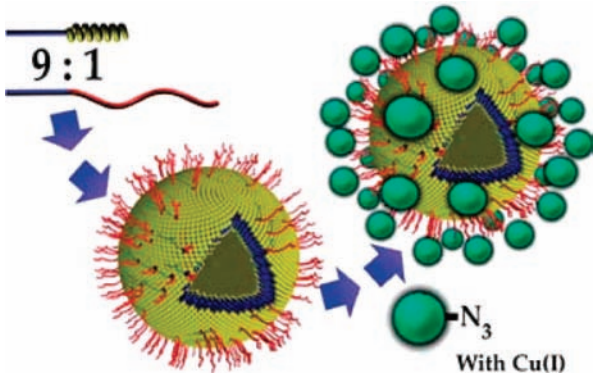


Figure 67. A percentage of acetylene-functionalized block copolymer is blended with regular vesicle forming polymers. The resulting polymersomes can then be functionalized at their surfaces using the Cu(I)-catalyzed [3 + 2] bipolar Huisgen cycloaddition. An immobilized lipase enzyme was shown to retain its activity. (Reproduced with permission from ref 584. Copyright 2008 Wiley.)

mer which could be admixed with other block copolymers (Figure 67).[584] This PS-PEG, provided with an acetylene group on its hydrophilic end, was shown to be able to expose its functionality to the surface of a variety of aggregates, as demonstrated for polymersomes built from PS-PIAT or PS-PEG. The reactivity of the PS-PEG-acetylene was proven by the successful and nearly quantitative immobilization of an azido-functionalized enzyme, *Candida antarctica* lipase B (CalB), on the polymersome, while retaining the catalytic activity of the lipase.[584,597] CalB activity was detected with the help of the substrate 6,8-difluoro-4-methylumbelliferyl octanoate (DiFMU octanoate).

A modular approach was also sought by Nehring et al.,[598] who coupled a nitrilotriacetic acid (NTA) moiety to the hydrophilic terminus of PBD-PEG, using succinic acid as a short linker. After equipping the block copolymer with the NTA chelator, it was incubated with either Ni^{2+} or Cu^{2+}, sequestering the ions. The resulting metalated block copolymers were admixed with unfunctionalized PBD-PEG in a 1 to 10 ratio to form polymersomes displaying, for example, Ni-NTA on their surfaces. In the field of proteins, the selective interaction between Ni-NTA and the terminal oligohistidine residues of a protein (a so-called His-tag) is well-known.[599] This interaction was used to noncovalently bind His-tagged model proteins to a polymersomal surface.

The enzyme CalB was also used in another block copolymer-based nanoreactor, which was constructed by encapsulating the lipase inside polymersomes of PS-PIAT.[532] The enclosed CalB enzymes retained their catalytic activity toward DiFMU octanoate, and the PS-PIAT bilayer membrane was shown to be permeable to this low molecular

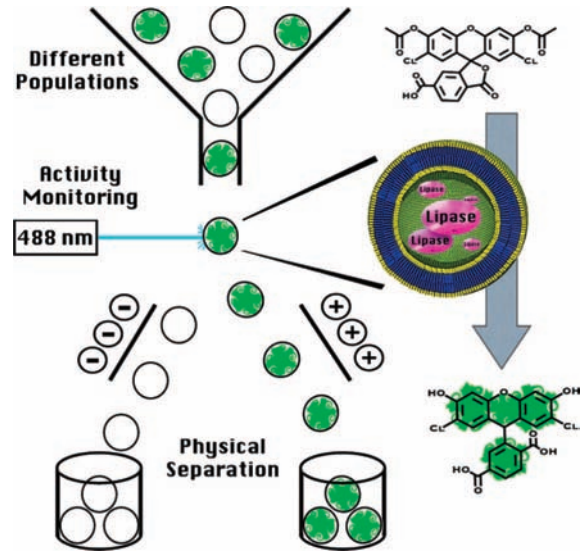


Figure 68. Enzyme-filled polymersomes that are permeable to low-molecular-weight (profluorescent) substrates can be sorted based on the activity of encapsulated enzymes using flow cytometry. Enzymatic activity is screened by the buildup of fluorescent product that is prevented from leaking out by the coencapsulation of a trapping agent. (Reproduced with permission from ref 601. Copyright 2009 Wiley.)

weight substrate. Of interest is the fact that this permeability was obtained without the use of channel proteins embedded in the membrane.

The membrane of PS-PIAT polymersomes could also be specifically loaded with enzymes,[504] by first encapsulating an enzyme in the lumen followed by lyophilization of these aggregates. After redissolving the resulting biohybrid in THF, it was redispersed in an aqueous medium, leading to immobilization of CalB inside the polymersome membrane. This methodology was used by Nallani et al.,[600] who investigated the ring-opening polymerization of several lactones by CalB when positioned in either the membrane or the water pool of a polymersome. It was found that the activity of the latter assembly was comparable to that of the free enzyme (high molecular weight esters were formed) whereas the membrane-bound CalB only produced short oligoesters. In both cases, the formed polymers were found to destabilize the vesicle membrane.

The activity of encapsulated CalB could also be probed by flow cytometry, a powerful technique that is routinely used for high-throughput fluorescence activated cell sorting (FACS).[601] In this case carboxyfluorescein diacetate (CFDA), which is converted to the fluorescent carboxyfluorescein by CalB, was used to assess the activity of CalB inside PS-PIAT polymersomes. To prevent diffusion of the fluorescent product out of the polymersomes, poly(L-lysine) was coencapsulated with CalB. This polycation trapped the negatively charged carboxyfluorescein product molecules and thus helped colocalize the fluorescent signal with the active catalysts. The thus labeled nanoreactors could be separated from other nanoreactors by flow cytometry (Figure 68), resulting in the identification of active populations of the bioreactors. The same procedure was followed for polymersomes encapsulating fluorescent markers such as GFP.

Apart from CalB, also other enzymes have been encapsulated in PS-PIAT polymersomes. Both glucose oxidase and horseradish peroxidase were trapped inside PS-PIAT-based polymersomes and were shown to be active.[602] The action

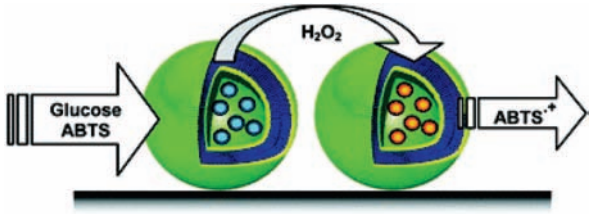


Figure 69. Schematic representation of a cascade reaction between separate polymersomes. The left polymersome contains GOx, and the right one contains HRP. (From ref 602. Reproduced by permission of The Royal Society of Chemistry.)

of GOx and HRP can be coupled in a cascade reaction where the hydrogen peroxide produced by GOx is used by HRP to convert ABTS into its radical cation. When both enzymes were separately encapsulated in polymersomes and combined in a single dispersion, the nanoreactors participated in a cascade reaction, without the need of transport mediators to facilitate transport of substrates between polymersomes (Figure 69). In addition, it was shown that the addition of a protease did not stop the reaction, as would be the case with free enzymes, indicating that the enzymes were protected by their polymersome armor.

The concept of a three-enzyme cascade reaction involving catalytically active polymersomes was presented by Vriezema et al.,[504] who made use of the possibility for positional assembly of enzymes that polymersomes offer. In this system, GOx was encapsulated into the lumen of PS-PIAT polymersomes while HRP was entrapped in the membranes, turning these polymersomes into two-enzyme nanoreactors that could perform the same cascade reaction as described above for two separate polymersomes. The cascade reaction was expanded by coupling it to the esterase activity of CalB, which was freely dissolved in the polymersome dispersion. In this case, instead of glucose, the substrate glucose tetraacetate was used; it was readily converted into free glucose via the hydrolytic action of CalB, thus producing the substrate for GOx in situ.

The most complex system incorporating the same three-enzyme cascade reaction was realized by combining lumen- and membrane-encapsulation with the surface immobilization of enzymes, in one PS-PIAT polymersome.[597] In this work, GOx was included in the lumen, and CalB was embedded in the polymersome bilayer. With the help of the PS-PEG-acetylene block copolymer described above,[584] azido-HRP was conjugated to the surface of the polymersome, selectively positioning three different enzymes at three different locations of the same polymersome (Figure 70). The resulting three-enzyme cascade was kinetically analyzed, and it was found that the activity of HRP had no influence on the overall reaction rate, suggesting that its spatial position made it not involved in or before the rate determining step of the reaction. Furthermore, the efficiencies of encapsulation of the enzymes were investigated. The surface conjugation of azido-HRP to the acetylenes presented via the PS-PEG-acetylene block copolymer was shown to be near quantitative. The incorporation efficiencies of GOx in the water pool reached 25%, while CalB was retained in the bilayer membrane with an efficiency of ca. 17%, suggesting that a mere statistical inclusion of enzymes was not taking place.

Reiner et al. created long, hollow tubes from PBD-PEG polymersome membranes by pulling them out using micropipets or optical tweezers (Figure 71).[603] This tube formation allowed the creation of networks that may enable

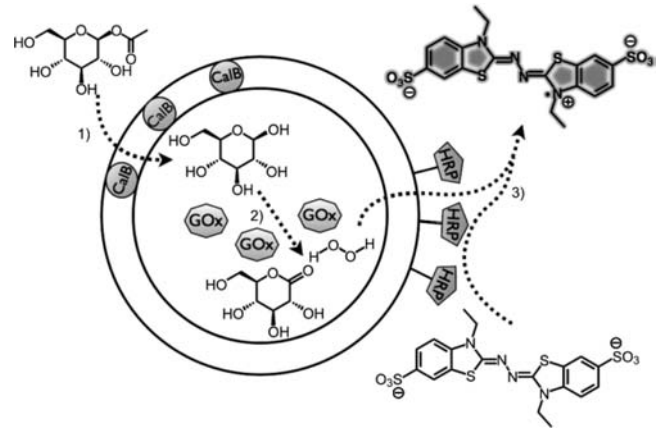


Figure 70. Schematic representation of a multistep reaction performed in PS-PIAT polymersomes. (1) Monoacetylated glucose is deprotected by CalB, which is embedded in the polymersome membrane. (2) In the inner aqueous compartment, GOx oxidizes glucose to gluconolactone, providing a molecule of hydrogen peroxide. (3) Hydrogen peroxide is used by HRP to convert ABTS to $ABTS^{\cdot+}$. HRP is tethered to the polymersome surface. (Reproduced with permission from ref 597. Copyright 2008 Wiley.)

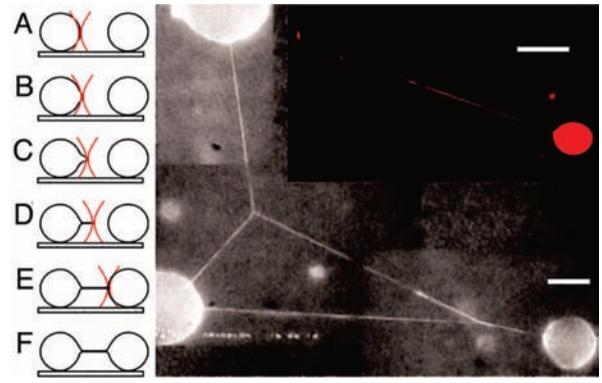


Figure 71. Images illustrating the creation of polymer nanotube−vesicle networks. (Left) Sequence of images illustrating the pulling of a nanotube from the membrane of a polymersome using optical tweezers (shown in red) and the attachment of the nanotube to another polymersome. (Right) Composite image from video fluorescence microscopy of a network of polymer nanotubes and polymersomes containing a membrane dye, assembled using optical tweezers. (Scale bar: 10 μm.) (Inset) Scanning confocal microscopy image of a nanotube pulled from a polymersome encapsulating the sulforhodamine B dye in buffer. Variations in the intensity are due to movement of the nanotube during the scan. (Scale bar: 10 μm.) (Reproduced with permission from ref 603. Copyright 2006 National Academy of Sciences, U.S.A.)

nanoscale reactor setups, since polymersomes were successfully interconnected by these channels. The PBD blocks allowed for UV-cross-linking of the hydrophobic membrane leaflet, leading to increased structural stability.[604] Also, permanent kinks or bends could be created in the channels by means of spot-curing the membrane. The ability of these polymersomal channels to transport (bio)molecules was demonstrated by electrophoresis of DNA through one of these nanotubes. Although these results, at present, do not involve catalysis of any kind, the demonstration of microfluidic facilitation may inspire the study of more complex polymersome cascade reaction setups in the future.

The opportunities that polymersomes offer as nanoreactors are manyfold, and it may not be long before practical applications will be realized. The desire to closely mimic nature has already resulted in systems of high complexity.[503] Catalysts encapsulated in polymersomes are not only pro-

tected from their environment, e.g. enzymes protected from the action of proteases,[602] but are also more easily removed, for example via filtration of the solution.[597] The precise ordering of enzymes inside polymersomes and the tuning of reactions by their spatial positioning opens the door to many applications in the future.

5.2.2. Biomedical Applications

As discussed in the previous section, PEG is one of the most widely used hydrophilic polymers in the field of polymersome science. PEGylated liposomes are widely used for stealth purposes, since they can stay in circulation for tens of hours. This makes PEG-based polymersomes even more interesting, since they have a greater PEG content and a higher stability,[605] while still evading macrophage attention, as described above.[488] The stealth properties provided by the dense PEG surface of polymersomes have been shown to increase the in vivo circulation time to up to twice that of liposomes, viz. 20−30 h in rats. Eventually, the polymersomes are cleared from the circulation via the liver and the spleen, by the process of opsonization.[605] Opsonization stands for the deposition of plasma proteins onto the membrane of the vesicle, which provides a "grip" for phagocytes to interact with the polymersome despite its layer of PEG. The combination of long circulation times with aggregate stability has led to a variety of in vitro and in vivo applications of polymersomes.[606]

Prolonged circulation increases the effectivity of imaging. For biomedical applications visible light fluorescence is not as useful as infrared and near-infrared (NIR) radiation, which has a far higher degree of tissue penetration and, thus, can be used for imaging in vivo. This property was taken advantage of by Ghoroghchian et al.,[576,607] who used a variety of porphyrin-based dyes to load the hydrophobic leaflet of PBD-PEG polymersomes. The fluorescent properties of these dyes could conveniently be varied by changing the substituents on the porphyrins involved.[496,607] Confocal NIR microscopy showed that the dye was uniformly dispersed throughout the polymersome membrane. These brightly fluorescent polymersomes were injected in a 9 L glioma-bearing rat, which was then imaged at various time points.[497] The total fluorescence emanating from the polymersomes gave rise to a localized signal of sufficient intensity to penetrate the dense tumor tissue of this living animal (Figure 72), giving a signal-to-background ratio of at least 10 to 1.

This platform was later expanded by combining these imaging agents with a cell permeable peptide (tat) which was conjugated to the surface of the PBD-PEG polymersomes using succinimidyl carbonate.[495] Dendritic cells (DCs), which are essential to the human immune system, were shown to be efficiently targeted by these emissive polymersomes. The cellular function of the DCs was not disrupted after internalization of the tat-labeled fluorescent vesicles. These studies may enable the in vivo tracking of labeled DCs in small animals by NIR fluorescence based imaging, since this type of fluorescence can still be perceived up to a centimeter tissue depth and shows low photobleaching.

Another imaging strategy is the enhancement of magnetic resonance signals with the help of a contrast agent. One effective contrast agent is chelated gadolinium, due to fast exchange rates between bound water molecules and surrounding bulk water. The need to interact with bulk water has hampered its use in liposomal carriers, since these are only sparsely permeable. This limitation was overcome by the group of Tsourkas, who, instead of liposomes, used porous polymersomes of PBD-PEG and a phospholipid, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC).[494] The block copolymer was cross-linked, after which the POPC was extracted with surfactant, generating a highly porous membrane for increased water permeability. To prevent leakage of Gd chelates, they were attached to dendrimers, which greatly increased their steric bulk. From magnetic resonance contrast studies, the vesicles were estimated to be a factor of 105 more efficient as contrast agents than nonencapsulated Gd chelates.[494]

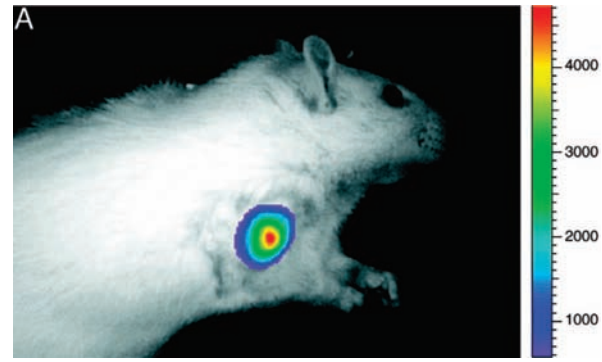


Figure 72. In vivo fluorescence image of 300-nm-sized NIR-emissive polymersomes taken 10 min after direct tumor injection of a 9 L glioma-bearing rat. The fluorescence signal intensity corresponded to only half the integrated emission from the polymersomes, and it remained constant between successive images taken during a 20-min interval postinjection. (Adapted with permission from ref 497. Copyright 2005 National Academy of Sciences, U.S.A.)

Ahmed et al.[608] demonstrated the efficacy of passive targeting combined with release using PCL-PEG vesicles. Polymersomes were systemically injected in mice and were found to accumulate in tumorous tissue, presumably as a result of passive extravasation. The accumulation, followed by the expected degradation of the polymersomes, led to the passively targeted release of an anticancer cocktail of a hydrophilic drug loaded in the lumen (doxorubicin) and a hydrophobic drug loaded in the polymeric membrane (paclitaxel). A single injection of the encapsulated cocktail showed a higher maximum tolerated dose along with a 2-fold higher cell death in the tumors when compared to the free drugs, effectively shrinking the tumors by 50%.[608] These results are very promising for the future development of biodegradable polymersomes as vehicles for passive delivery of pharmaceuticals.

Active targeting, as with the surface conjugated tat peptide described above, may seem to be more laborious than passive targeting but does also enable strategies that cannot otherwise be achieved—mainly because passive targeting cannot be tweaked. In the section on carbohydrate building blocks, the mannose-terminated amphiphile shown in Figure 55 was mentioned. The small mannose-covered polymersomes formed by this amphiphile showed a specific binding ability to the type 1 pili of the ORN 178 *E. coli* strain.[320,541] Type 1 pili are mannose-binding proteinacious fibers that protrude from the surface of many Gram-negative bacterial cells. This is an example of polymersomes where the constituent polymer provides the aggregate with active targeting behavior.

As described in detail above (section 5.2.1), polymersomes labeled with polyG through streptavidin−biotin chemistry were successfully internalized by macrophages via their SRA7 receptor protein.[582] Transgenic expression of this

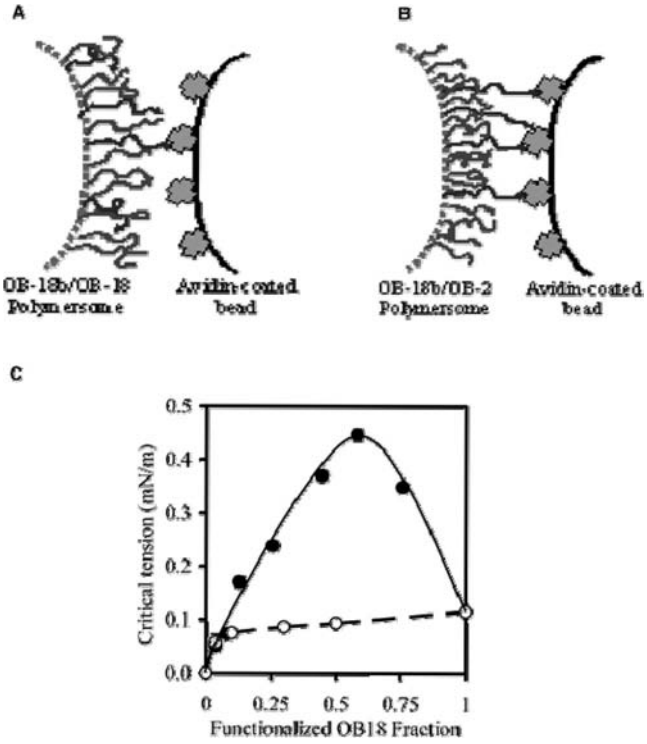


Figure 73. Binding strength measurements: (A) Illustration of PBD-PEG-biotin in a PBD-PEG polymersome with the same chain lengths. (B) Illustration of the same PBD-PEG-biotin in a polymersome with shorter PEG blocks. In the latter case, the difference in the chain length between the two polymers leads biotin to be presented only beyond the surface of the polymersome. (C) Dependence of critical tension (mN/m) vs the percentage of PBD-PEG-biotin polymers. For long biotinylated PEG chains in polymersomes with short PEG blocks, the critical tension increased as the percentage of functionalized polymer increased to a maximum, after which the adhesiveness decreased again with increasing biotin concentration. When functionalized PBD-PEG was mixed into polymersomes with equal block lengths, the critical tension increased as the percentage of functionalized polymer increased, shown as the dashed line. The adhesion strength for longer biotinylated PEG chains in a short system was above that of equal length chains at all concentrations. (Reproduced with permission from ref 609. Copyright 2004 American Chemical Society.)

SRA7 receptor in COS7 cells led to successful receptor-mediated internalization of the polymersomes in those cells as well.

The labeling of polymersomes using the avidin−biotin couple was also applied by Lin et al.,[609] who investigated the binding strength between avidin-coated polystyrene beads and biotinylated polymersomes. PBD-PEG-biotin was mixed with nonfunctional PBD-PEG at different ratios and using block copolymers of different block lengths. The polymersomes were then brought into contact with the PS beads using micropipet manipulation and subsequently drawn apart. Interestingly, it was found that the presentation of biotin was most effective when it was conjugated to a longer PEG-chain and not overabundantly present, as though it were floating freely above the bulk of the polymersome membrane, tethered by its own PEG chain (Figure 73).

This system was expanded by coating the polymersomes with an antibody, anti-ICAM-1.[610] These were "stuck to" ICAM-1-coated polystyrene microspheres. The two spheres were subsequently drawn apart via micropipet manipulation. It was found that the adhesion strength was linearly related to the surface density of the antibody (Figure 74). This result

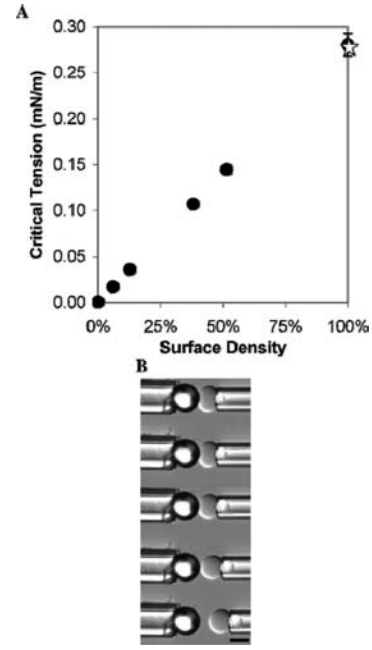


Figure 74. Critical tension measurements of an anti-ICAM-1-coated polymersome to an ICAM-1-coated polystyrene microsphere. (A) The critical tension required breaking all the anti-ICAM-1 and ICAM-1 bonds formed between a polymersome and a microsphere scales proportional to the surface density of anti-ICAM-1. The closed circles are anti-ICAM-1-coated polymersomes using 55 mol % PBD_{125}-PEG_{80}-biotin in PBD_{46}-PEG_{26} as the underlying membrane. (B) Representative sequence of the critical tension experiment. The anti-ICAM-1-coated polymersome was first allowed to adhere to an ICAM-1-coated microsphere. The micropipet on the left-hand side statically held the microsphere, while the one on the right-hand side came in to just touch the polymersome. As the suction pressure on the polymersome increased, the contact distance between the polymersome and the microsphere decreased. The scale bar is 10 μm. (Reproduced with permission from ref 610. Copyright 2006 American Chemical Society.)

contrasts with the adhesion between biotinylated polymersomes and avidin-coated microspheres described above, which had an optimal tethered biotin surface density of 55%.[609]

An increase in complexity was achieved by combining this antibody-covering of polymersomes with coconjugation of sLe^x, a selectin ligand (Figure 75).[611] sLe^x can undergo a relatively weak binding interaction with selectin, a mechanism employed by leukocytes to slowly roll over vascular walls, removing them from the free bloodstream. Bonds between selectin and its ligand continuously break and reform during this rolling. In immune systems, leukocytes are subsequently immobilized by the much stronger binding of integrin with its ligands at sites where they are required. In this synthetic system the role of integrin was mimicked by the antibody against ICAM-1. Using a flow chamber with a selectin-covered surface, it was shown that these polymersomes indeed roll at speeds typical for rolling leukocytes, i.e. 20 μm s^{-1}. When the surface was covered with both selectin and ICAM-1, the combined binding forces significantly slowed down the rolling speeds of the polymersomes, even causing long pauses in their movement. Variations in the surface-density or ratio of the ligands accordingly led to variations in relative movement speeds. The leukocyte-like rolling behavior of these biohybrid aggregates has earned them the moniker "leuko-polymersomes".[611]

The brain delivery of peptides by antibody-covered polymersomes was studied by Pang et al.,[500] who used thiols

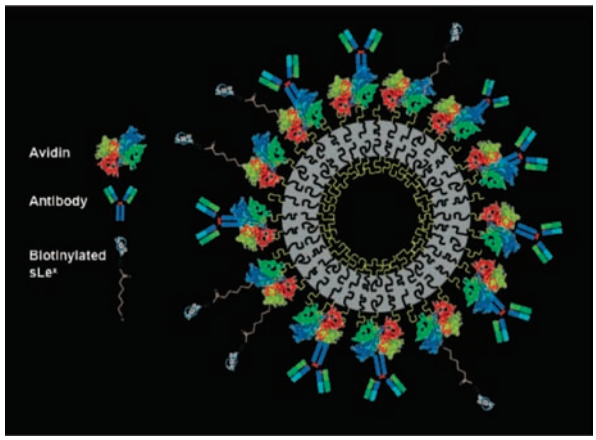


Figure 75. Schematic representation of a functionalized polymersome that mimics a leukocyte. Biotinylated sLe^x, a selectin ligand, or biotinylated antibody against ICAM-1, or both were added to the polymersome shell to mediate adhesion. For clarity, the biotin on the vesicle or the ligands is not shown. This type of vesicles is dubbed "leuko-polymersome". (From ref 611. Reproduced by permission of The Royal Society of Chemistry.)

present in the monoclonal antibody OX26 to decorate a PCL-PEG polymersome with maleimide functions on its surface. OX26 is an antibody against the transferrin receptor which can initiate the endogenous receptor-mediated transcytosis of a particle across the blood brain barrier. It was shown that coupling of OX26 to a polymersome enabled the polymersome to indeed pass the blood brain barrier and thus become a viable carrier for the delivery of pharmaceuticals to the brain. Since the constituent building blocks of the carrier are based on a polyester and PEG, they are biodegradable, so the polymersomes should degrade in time to release their contents.[500]

We have thus far encountered methods for imaging the polymersomes and for specifically targeting them. Both these techniques are most optimally exploited when the polymersome has a payload to deliver at its targeted locale, such as for instance the OX26-covered polymersomes. We have already seen a variety of biodegradable polymersomes that can deliver their cargo via passive degradation of the polymeric membrane.[498,519,548,578,612] Another much applied method makes use of stimulus-responsive block copolymers in the delivery vesicles, which has already been discussed in the section on amino acid-containing building blocks.[194,225,498,525−527] For example, this technique can take advantage of two characteristics of cancerous tissue: first, its poorly built vascular walls are susceptible to passive extravasation, allowing for accumulation of the responsive polymersomes. Second, the local pH is almost always lower inside tumors, possibly triggering the destabilization of these types of polymersomes.

pH-induced destabilization may also be desired in DNA transfection.[518,613,614] Lomas and co-workers have used the pH-sensitive poly(2-(methacryloyloxy)ethylphosphorylcholine)-*co*-poly(2-(diisopropylamino)ethyl methacrylate) (PMPC-PDPA) diblock copolymer to encapsulate DNA at neutral pH. These DNA-loaded polymersomes have stealthlike properties, since PMPC has similar biocompatibility characteristics as PEG does. This stealth character was expected to produce relatively long circulation times. When the solution pH was lowered, the formation of polymer−DNA complexes occurred, in which the nucleic acid was protected from the acidic environment by the block copolymer. When this transition was confined within a semipermeable organelle membrane, the concurrent increase in osmotic pressure was found to rupture the lipid membrane, allowing the DNA to escape into the cell cytosol. The use of PMPC-PDPA polymersomes to transfect a gene coding for GFP to various cell types has been reported to be successful.[613,614]

A second platform for intracellular delivery of cargo by polymersomes was presented by Cerritelli et al.,[615] who coupled a PEG block to poly(propylene sulfide) (PPS) via a disulfide bond. Like other polymersomes, the aggregates obtained from this PPS-PEG polymer protected their contents. When taken up by endocytosis, they burst within the early endosome, since the intracellular concentration of cysteine was sufficient to break the disulfide bridge. This caused the polymersomes to collapse and release their payload before entering the harsh conditions of the lysosome, ensuring that the cargo evades this fate. In cellular experiments, polymersome uptake, disruption, and the release of cargo upon polymersomes degradation were observed within 10 min of exposure to cells. A payload of calcein was used to demonstrate the functionality of these polymersomes.[615]

A reusable form of delivery makes use of biodegradable and biocompatible polymersomes as oxygen carriers through the encapsulation of hemoglobin.[501,574] The group of Palmer used PCL-PEG or PLA-PEG-based polymersomes to encapsulate either bovine or human hemoglobin (Hb), producing polymersome-encapsulated hemoglobin (PEH). The aggregate size distribution and Hb encapsulation efficiency were tunable by varying either the initial Hb concentration or the concentration of block copolymers. The oxygen affinity of the complex was found to be slightly lower than that of red blood cells, and PEHs were slower to saturate with O_2. This may be due to the increased membrane thickness of polymersomes compared to biological membranes. Nonetheless, these parameters were consistent with those required for efficient oxygen delivery in the systemic circulation and the polymersomes show promise as therapeutic oxygen carriers for biomedical applications.[574]

5.3. Outlook

Polymersomes may be viewed as a more stable alternative to liposomes, which have a well-established line of applications. The high stability and low fluidity of their membranes warrants analogies of polymersomes with capsids as stable as those of viruses,[606] providing ample motivation for scientists to bring together all the separate goals reached thus far: there are examples of active,[495] even antibody-mediated[500] targeting methods, and there are several different conditions that can cause the release of any type of cargo.[608,615] The in vivo behavior of PEG-covered polymersomes has been studied,[605] and the combination of antibodies with other targeting peptides was demonstrated to be effective.[611] It may be only a matter of time until a fully fledged drug delivery vehicle based on polymersomes is reported.

When used as reactor vessels, polymersomes have transcended the stage where they merely provide a shell around a given catalyst. Substrate-specific gateways, in the form of membrane proteins reconstituted in polymersome membranes, have been used to mimic biosynthetic pathways.[503] Also, the advantage of a polymersomal membrane as protection against proteases[602] or harsh environments[596] has been proven. Intricate control over enzyme positioning is possible, and this may possitively influence the rate of some

enzymes.[597] Nonfunctional polymersomes can even be weeded out of a population to guarantee a dispersion of only active nanoreactors.[601] Nowadays, all of these principles have been proven; but the wait is still on for the first synthetically or biologically relevant application of polymersome nanoreactors. Given the curiosity and determination of the average scientist, though, it will probably not take long for reports like this to seep in.

6. Conclusions

The literature shows that there are many different types of polymeric capsules for which a variety of applications can be foreseen, and a number of these have been discussed in this review. This raises the question whether there is a favorable capsule architecture for a specific task or application. How do these capsule types measure up? Every one category has its distinct advantages, not to mention its drawbacks. Regarding the availability of starting materials, the LbL approach is the one to follow; it only needs two different homopolymers and a suitable template, most of which are commercially available. Strikingly, however, the tuning of LbL capsule properties by variation of their chemistry has lagged behind, at least for biohybrid polymers. The advances made in, for instance, tuning properties such as permeability for imparting function have for the major part only been realized with the combination PSS/PAH. That the strategy of expanding the chemical diversity in polymers used for LbL capsules is a viable one was, for example, demonstrated by De Geest et al.[413] Although the polydispersity of LbL-capsules often is not an issue, the tuning of their size to that of the nanoregime is difficult, which hampers their use for in vivo applications. This may become a focus for future investigations.

The chemical tool box available for constructing polymer micelles and polymersomes is much larger, and natural motifs have been actively incorporated into their designs. Combined with the recent reports showing their facile surface functionalization, we envision an ongoing exploration of these polymeric aggregates, for example in targeted drug delivery applications. In this respect, the report of Hammer et al.[611] on "leukopolymersomes" may serve as an example of where the combination of membrane rigidity and scaffold function can be taken. It is a pity that rigidity and impermeability of polymer membranes often go hand-in-hand. This problem may be circumvented by applying specific block copolymer chemistry designs or by incorporating membrane proteins, which have the possibility to introduce substrate selectivity, making them superior to LbL-architectures, which usually only display passive diffusion properties.

The decoration of leukopolymersomes with tetrasaccharide (sLe^x) ligands is a beautiful example of the integration of biofunctional carbohydrate moieties into architectures such as polymersomes and polymeric micelles. Either by post-functionalization or by direct polymerization of glycopolymers, their combination with capsules could very well aid in the realization of full-fledged targeted drug delivery vehicles. Whereas polymersomes are only formed in a narrow window of block ratios (see Figure 48), polymer micelles are observed in most other cases, partially explaining the abundance of literature on this type of aggregates even when only biohybrid polymers are considered. While polymer micelles lack a distinct interior volume, their ease of preparation has made them to already enter the stage of clinical trials as the carriers of hydrophobic drugs. Dendrimers also lack a real interior compartment and they are the most synthetically demanding capsule-like entities, but this has only inspired chemists to come up with novel designs and synthetic pathways. The monodispersity of dendrimers, their inherent polyvalency, their availability, and their covalent nature has made these architectures well-suited for applications where durability and uniformity are of key importance, as in the case of contrast agents.

Regarding LbL, polymersome, or polymeric micelle nanoreactors, it can be foreseen that nature's own arsenal of biocatalysts will be increasingly used for encapsulation in these synthetic systems, holding promise for future nanoscale diagnostic devices. One of the main challenges in this field will be the effective stimulation of responsive polymersomes in vivo since most stimuli reported thus far cannot be applied in living organisms. The great potential of encapsulated organic and inorganic catalysts, not to mention their combination with enzymes, so far has remained virtually unexplored. Many factors are important for each separate application, and it may well be that a combination of strategies will lead to the best results, as the future may show.[616]

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8. References

(1) Jeong, H.; Tombor, B.; Albert, R.; Oltval, Z. N.; Barabásl, A. L. *Nature* **2000**, *407*, 651.
(2) Alberts, B. *Cell* **1998**, *92*, 291.
(3) Baumeister, W.; Walz, J.; Zühl, F.; Seemüller, E. *Cell* **1998**, *92*, 367.
(4) Matlack, K. E. S.; Mothes, W.; Rapoport, T. A. *Cell* **1998**, *92*, 381.
(5) Bruggink, A.; Schoevaart, R.; Kieboom, T. *Org. Process Res. Dev.* **2003**, *7*, 622.
(6) Meier, W. *Chem. Soc. Rev.* **2000**, *29*, 295.
(7) Lensen, D.; Vriezema, D. M.; van Hest, J. C. M. *Macromol. Biosci.* **2008**, *8*, 991.
(8) Vriezema, D. M.; Aragones, M. C.; Elemans, J. A. A. W.; Cornelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M. *Chem. Rev.* **2005**, *105*, 1445.
(9) Prokop, A.; Davidson, J. M. *J. Pharm. Sci.* **2008**, *97*, 3518.
(10) Douglas, T. *Science* **2003**, *299*, 1192.
(11) Elemans, J.; Rowan, A. E.; Nolte, R. J. M. *J. Mater. Chem.* **2003**, *13*, 2661.
(12) Hamley, I. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 1692.
(13) Capito, R. M.; Azevedo, H. S.; Velichko, Y. S.; Mata, A.; Stupp, S. I. *Science* **2008**, *319*, 1812.
(14) Chang, T. M. S. *Science* **1964**, *146*, 524.
(15) Chang, T. M. S.; Poznansky, M. J. *Nature* **1968**, *218*, 243.
(16) Chang, T. M. S. *Nature* **1971**, *229*, 117.
(17) Mann, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 5306.
(18) Hargreaves, W. R. *Biochemistry* **1978**, *17*, 3759.
(19) Barenholz, Y. *Curr. Opin. Colloid Interface Sci.* **2001**, *6*, 66.
(20) Walde, P.; Ichikawa, S. *Biomol. Eng.* **2001**, *18*, 143.
(21) Oberholzer, T.; Nierhaus, K. H.; Luisi, P. L. *Biochem. Biophys. Res. Commun.* **1999**, *261*, 238.
(22) Morigaki, K.; Walde, P. *Curr. Opin. Colloid Interface Sci.* **2007**, *12*, 75.
(23) Daamen, W. F.; Geutjes, P. J.; Van Moerkerk, H. T. B.; Nillesen, S. T. M.; Wismans, R. G.; Hafmans, T.; Van Den Heuvel, L. P. W. J.; Pistorius, A. M. A.; Veerkamp, J. H.; Van Hest, J. C. M.; Van Kuppevelt, T. H. *Adv. Mater.* **2007**, *19*, 673.
(24) Douglas, T.; Young, M. *Nature* **1998**, *393*, 152.
(25) Singh, P.; Gonzalez, M. J.; Manchester, M. *Drug Dev. Res.* **2006**, *67*, 23.
(26) Berg, J.; Sundberg, D.; Kronberg, B. *J. Microencapsulation* **1989**, *6*, 327.
(27) Freiberg, S.; Zhu, X. X. *Int. J. Pharm.* **2004**, *282*, 1.
(28) Basinska, T. *Macromol. Biosci.* **2005**, *5*, 1145.
(29) Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155.

(30) Newkome, G. R.; Yao, Z. Q.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985**, *50*, 2003.
(31) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117.
(32) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A. *Angew. Chem., Int. Ed.* **1990**, *29*, 138.
(33) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665.
(34) Grayson, S. M.; Fréchet, J. M. J. *Chem. Rev.* **2001**, *101*, 3819.
(35) Carlmark, A.; Hawker, C.; Hult, A.; Malkoch, M. *Chem. Soc. Rev.* **2009**, *38*, 352.
(36) Jansen, J.; De Brabander-van den Berg, E. M. M.; Meijer, E. W. *Science* **1994**, *266*, 1226.
(37) Jain, N. K.; Asthana, A. *Expert Opin. Drug Delivery* **2007**, *4*, 495.
(38) Knapen, J. W. J.; Vandermade, A. W.; Dewilde, J. C.; Vanleeuwen, P.; Wijkens, P.; Grove, D. M.; Vankoten, G. *Nature* **1994**, *372*, 659.
(39) Wiener, E. C.; Brechbiel, M. W.; Brothers, H.; Magin, R. L.; Gansow, O. A.; Tomalia, D. A.; Lauterbur, P. C. *Magn. Reson. Med.* **1994**, *31*, 1.
(40) Barth, R. F.; Adams, D. M.; Soloway, A. H.; Alam, F.; Darby, M. V. *Bioconjugate Chem.* **1994**, *5*, 58.
(41) Tam, J. P. *Proc. Nalt. Acad. Sci. U.S.A.* **1988**, *85*, 5409.
(42) Liu, H. B.; Jiang, A.; Guo, J. A.; Uhrich, K. E. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 703.
(43) Patri, A. K.; Majoros, I. J.; Baker, J. R., Jr. *Curr. Opin. Chem. Biol.* **2002**, *6*, 466.
(44) Gillies, E. R.; Fréchet, J. M. J. *Drug Discovery Today* **2005**, *10*, 35.
(45) Fischer, M.; Vogtle, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 884.
(46) Gingras, M.; Raimundo, J. M.; Chabre, Y. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1010.
(47) Svenson, S.; Tomalia, D. A. *Adv. Drug Delivery Rev.* **2005**, *57*, 2106.
(48) Duncan, R.; Izzo, L. *Adv. Drug Delivery Rev.* **2005**, *57*, 2215.
(49) Boas, U.; Heegaard, P. M. H. *Chem. Soc. Rev.* **2004**, *33*, 43.
(50) Lee, C. C.; MacKay, J. A.; Frechet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, *23*, 1517.
(51) Esfand, R.; Tomalia, D. A. *Drug Discovery Today* **2001**, *6*, 427.
(52) Oosterom, G. E.; Reek, J. N. H.; Kamer, P. C. J.; Van Leeuwen, P. W. N. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1828.
(53) Adronov, A.; Frechet, J. M. J. *Chem. Commun.* **2000**, 1701.
(54) Twyman, L. J.; King, A. S. H.; Martin, I. K. *Chem. Soc. Rev.* **2002**, *31*, 69.
(55) Aulenta, F.; Hayes, W.; Rannard, S. *Eur. Polym. J.* **2003**, *39*, 1741.
(56) Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem.—Eur. J.* **1997**, *3*, 1193.
(57) Lo, S.; Burn, P. L. *Chem. Rev.* **2007**, *107*, 1097.
(58) Trinchi, A.; Muster, T. H. *Supramol. Chem.* **2007**, *19*, 431.
(59) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Macromolecules* **1986**, *19*, 2466.
(60) Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638.
(61) Hawker, C. J.; Frechet, J. M. J. *J. Chem. Soc., Chem. Commun.* **1990**, 1010.
(62) VanHest, J. C. M.; DelNoye, D. A. P.; Baars, M.; VanGenderen, M. H. P.; Meijer, E. W. *Science* **1995**, *268*, 1592.
(63) Zimmerman, S. C.; Zeng, F. W.; Reichert, D. E. C.; Kolotuchin, S. V. *Science* **1996**, *271*, 1095.
(64) Denkewalter, R. G. Macromolecular highly branched homogeneous compound. U.S. Patent 4410688, 1983; p 4688.
(65) Caminade, A. M.; Turrin, C. O.; Majoral, J. P. *Chem.—Eur. J.* **2008**, *14*, 7422.
(66) Wang, J.; Jiang, M.; Nilsen, T. W.; Getts, R. C. *J. Am. Chem. Soc.* **1998**, *120*, 8281.
(67) Li, Y. G.; Tseng, Y. D.; Kwon, S. Y.; D'Espaux, L.; Bunch, J. S.; McEuen, P. L.; Luo, D. *Nat. Mater.* **2004**, *3*, 38.
(68) Um, S. H.; Lee, J. B.; Kwon, S. Y.; Li, Y.; Luo, D. *Nat. Protoc.* **2006**, *1*, 995.
(69) Yin, P.; Choi, H. M. T.; Calvert, C. R.; Pierce, N. A. *Nature* **2008**, *451*, 318.
(70) Seebach, D.; Herrmann, G. F.; Lengweiler, U. D.; Bachmann, B. M.; Amrein, W. *Angew. Chem., Int. Ed.* **1996**, *35*, 2795.
(71) Miao, Z. M.; Cheng, S. X.; Zhang, X. Z.; Wang, Q. R.; Zhuo, R. X. *J. Biomed. Mater. Res.* **2007**, *81B*, 40.
(72) Lee, C. C.; Grayson, S. M.; Frechet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 3563.
(73) Grinstaff, M. W. *Chem.—Eur. J.* **2002**, *8*, 2838.
(74) Tang, M. X.; Redemann, C. T.; Szoka, F. C. *Bioconjugate Chem.* **1996**, *7*, 703.
(75) Kevwitch, R. M.; McGrath, D. V. *New J. Chem.* **2007**, *31*, 1332.
(76) Li, S.; Szalai, M. L.; Kevwitch, R. M.; McGrath, D. V. *J. Am. Chem. Soc.* **2003**, *125*, 10516.
(77) Szalai, M. L.; Kevwitch, R. M.; McGrath, D. V. *J. Am. Chem. Soc.* **2003**, *125*, 15688.
(78) Amir, R. J.; Shabat, D. *Chem. Commun.* **2004**, 1614.
(79) Shabat, D. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 1569.
(80) Gopin, A.; Ebner, S.; Attali, B.; Shabat, D. *Bioconjugate Chem.* **2006**, *17*, 1432.
(81) Haba, K.; Popkov, M.; Shamis, M.; Lerner, R. A.; Barbas, C. F.; Shabat, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 716.
(82) De Groot, F. M. H.; Albrecht, C.; Koekkoek, R.; Beusker, P. H.; Scheeren, H. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 4490.
(83) Malik, N.; Wiwattanapatapee, R.; Klopsch, R.; Lorenz, K.; Frey, H.; Weener, J. W.; Meijer, E. W.; Paulus, W.; Duncan, R. *J. Controlled Release* **2000**, *65*, 133.
(84) Fischer, D.; Li, Y. X.; Ahlemeyer, B.; Krieglstein, J.; Kissel, T. *Biomaterials* **2003**, *24*, 1121.
(85) Chen, H. T.; Neerman, M. F.; Parrish, A. R.; Simanek, E. E. *J. Am. Chem. Soc.* **2004**, *126*, 10044.
(86) Perumal, O. P.; Inapagolla, R.; Kannan, S.; Kannan, R. M. *Biomaterials* **2008**, *29*, 3469.
(87) Ihre, H. R.; De Jesus, O. L. P.; Szoka, F. C.; Frechet, J. M. J. *Bioconjugate Chem.* **2002**, *13*, 443.
(88) De Jesus, O. L. P.; Ihre, H. R.; Gagne, L.; Frechet, J. M. J.; Szoka, F. C. *Bioconjugate Chem.* **2002**, *13*, 453.
(89) Boyd, B. J.; Kaminskas, L. M.; Karellas, P.; Krippner, G.; Lessene, R.; Porter, C. J. H. *Mol. Pharm.* **2006**, *3*, 614.
(90) Kaminskas, L. M.; Boyd, B. J.; Karellas, P.; Henderson, S. A.; Giannis, M. P.; Krippner, G. Y.; Porter, C. J. H. *Mol. Pharm.* **2007**, *4*, 949.
(91) Kolhatkar, R. B.; Kitchens, K. M.; Swaan, P. W.; Ghandehari, H. *Bioconjugate Chem.* **2007**, *18*, 2054.
(92) Agashe, H. B.; Dutta, T.; Garg, M.; Jain, N. K. *J. Pharm. Pharmacol.* **2006**, *58*, 1491.
(93) Agrawal, P.; Gupta, U.; Jain, N. K. *Biomaterials* **2007**, *28*, 3349.
(94) Gajbhiye, V.; Kumar, P. V.; Tekade, R. K.; Jain, N. K. *Curr. Pharm. Des.* **2007**, *13*, 415.
(95) Kaminskas, L. M.; Boyd, B. J.; Karellas, P.; Krippner, G. Y.; Lessene, R.; Kelly, B.; Porter, C. J. H. *Mol. Pharm.* **2008**, *5*, 449.
(96) Stasko, N. A.; Johnson, C. B.; Schoenfisch, M. H.; Johnson, T. A.; Holmuhamedov, E. L. *Biomacromolecules* **2007**, *8*, 3853.
(97) Kim, Y.; Klutz, A. M.; Jacobson, K. A. *Bioconjugate Chem.* **2008**, *19*, 1660.
(98) Cloninger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742.
(99) Klajnert, B.; Bryszewska, M. *Acta Biochim. Pol.* **2001**, *48*, 199.
(100) Futaki, S.; Nakase, I.; Suzuki, T.; Zhang, Y. J.; Sugiura, Y. *Biochemistry* **2002**, *41*, 7925.
(101) Jansen, J. F. G. A.; Meijer, E. W.; de Brabander-van den Berg, E. M. M. *J. Am. Chem. Soc.* **1995**, *117*, 4417.
(102) Dutta, T.; Garg, M.; Jain, N. K. *Eur. J. Pharm. Sci.* **2008**, *34*, 181.
(103) Gajbhiye, V.; Kumar, P. V.; Sharma, A.; Jain, N. K. *Curr. Nanosci.* **2008**, *4*, 267.
(104) Kono, K.; Fukui, T.; Takagishi, T.; Sakurai, S.; Kojima, C. *Polymer* **2008**, *49*, 2832.
(105) Kono, K.; Kojima, C.; Hayashi, N.; Nishisaka, E.; Kiura, K.; Wataral, S.; Harada, A. *Biomaterials* **2008**, *29*, 1664.
(106) Klajnert, B.; Appelhans, D.; Komber, H.; Morgner, N.; Schwarz, S.; Richter, S.; Brutschy, B.; Ionov, M.; Tonkikh, A. K.; Bryszewska, M.; Voit, B. *Chem.—Eur. J.* **2008**, *14*, 7030.
(107) Malik, N.; Evagorou, E. G.; Duncan, R. *Anti-Cancer Drugs* **1999**, *10*, 767.
(108) Gillies, E. R.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 14137.
(109) Gillies, E. R.; Dy, E.; Frechet, J. M. J.; Szoka, F. C. *Mol. Pharm.* **2005**, *2*, 129.
(110) Amir, R. J.; Pessah, N.; Shamis, M.; Shabat, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 4494.
(111) Patel, D.; Henry, J.; Good, T. *Biochim. Biophys. Acta, Gen. Subj.* **2006**, *1760*, 1802.
(112) Patel, D. A.; Henry, J. E.; Good, T. A. *Brain Res.* **2007**, *1161*, 95.
(113) Hawthorne, M. F.; Maderna, A. *Chem. Rev.* **1999**, *99*, 3421.
(114) Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F. G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.
(115) Capala, J.; Barth, R. F.; Bendayan, M.; Lauzon, M.; Adams, D. M.; Soloway, A. H.; Fenstermaker, R. A.; Carlsson, J. *Bioconjugate Chem.* **1996**, *7*, 7.
(116) Yang, W. L.; Wu, G.; Barth, R. F.; Swindall, M. R.; Bandyopadhyaya, A. K.; Tjarks, W.; Tordoff, K.; Moeschberger, M.; Sferra, T. J.; Binns, P. J.; Riley, K. J.; Ciesielski, M. J.; Fenstermaker, R. A.; Wikstrand, C. J. *Clin. Cancer Res.* **2008**, *14*, 883.
(117) Backer, M. V.; Gaynutdinov, T. I.; Patel, V.; Bandyopadhyaya, A. K.; Thirumamagal, B. T. S.; Tjarks, W.; Barth, R. F.; Claffey, K.; Backer, J. M. *Mol. Cancer. Ther.* **2005**, *4*, 1423.
(118) Yang, W. L.; Barth, R. F.; Wu, G.; Kawabata, S.; Sferra, T. J.; Bandyopadhyaya, A. K.; Tjarks, W.; Ferketich, A. K.; Moeschberger, M. L.; Binns, P. J.; Riley, K. J.; Coderre, J. A.; Ciesielski, M. J.; Fenstermaker, R. A.; Wikstrand, C. J. *Clin. Cancer Res.* **2006**, *12*, 3792.
(119) Wu, G.; Yang, W. L.; Barth, R. F.; Kawabata, S.; Swindall, M.; Bandyopadhyaya, A. K.; Tjarks, W.; Khorsandi, B.; Blue, T. E.;

Ferketich, A. K.; Yang, M.; Christoforidis, G. A.; Sferra, T. J.; Binns, P. J.; Riley, K. J.; Ciesielski, M. J.; Fenstermaker, R. A. *Clin. Cancer Res.* **2007**, *13*, 1260.
(120) Kukowska Latallo, J. F.; Bielinska, A. U.; Johnson, J.; Spindler, R.; Tomalia, D. A.; Baker, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4897.
(121) DeLong, R.; Stephenson, K.; Loftus, T.; Fisher, M.; Alahari, S.; Nolting, A.; Juliano, R. L. *J. Pharm. Sci.* **1997**, *86*, 762.
(122) Bielinska, A. U.; Kukowska Latallo, J. F.; Baker, J. R. *Biochim. Biophys. Acta, Gene Struct. Expression* **1997**, *1353*, 180.
(123) Dutta, T.; Garg, M.; Jain, N. K. *Vaccine* **2008**, *26*, 3389.
(124) Eom, K. D.; Kim, J. S.; Park, S. M.; Kim, M. S.; Yu, R.; Jung, H. M.; Kim, S. G.; Yoo, H. *J. Nanosci. Nanotechnol.* **2006**, *6*, 3532.
(125) Kiselev, A. V.; Il'ina, P. L.; Egorova, A. A.; Baranov, A. N.; Guryanov, I. A.; Bayanova, N. V.; Tarasenko, I.; Lesina, E. A.; Vlasov, G. P.; Baranov, V. S. *Russ. J. Genet.* **2007**, *43*, 593.
(126) Kim, B. S.; Choi, J. W. *Biotechnol. Bioprocess Eng.* **2007**, *12*, 323.
(127) Wada, K.; Arima, H.; Tsutsumi, T.; Hirayama, F.; Uekama, K. *Biol. Pharm. Bull.* **2005**, *28*, 500.
(128) Tsutsumi, T.; Hirayama, F.; Uekama, K.; Arima, H. *J. Pharm. Sci.* **2008**, *97*, 3022.
(129) Patil, M. L.; Zhang, M.; Betigeri, S.; Taratula, O.; He, H.; Minko, T. *Bioconjugate Chem.* **2008**, *19*, 1396.
(130) Inoue, Y.; Kurihara, R.; Tsuchida, A.; Hasegawa, M.; Nagashima, T.; Mori, T.; Niidome, T.; Katayama, Y.; Okitsu, O. *J. Controlled Release* **2008**, *126*, 59.
(131) Kang, H. M.; DeLong, R.; Fisher, M. H.; Juliano, R. L. *Pharm. Res.* **2005**, *22*, 2099.
(132) Langereis, S.; Dirksen, A.; Hackeng, T. M.; van Genderen, M. H. P.; Meijer, E. W. *New J. Chem.* **2007**, *31*, 1152.
(133) Wiener, E. C.; Konda, S.; Shadron, A.; Brechbiel, M.; Gansow, O. *Invest. Radiol.* **1997**, *32*, 748.
(134) Swanson, S. D.; Kukowska-Latallo, J. F.; Patri, A. K.; Chen, C. Y.; Ge, S.; Cao, Z. Y.; Kotlyar, A.; East, A. T.; Baker, J. R. *Int. J. Nanomed.* **2008**, *3*, 201.
(135) Kaneshiro, T. L.; Jeong, E. K.; Morrell, G.; Parker, D. L.; Lu, Z. R. *Biomacromolecules* **2008**, *9*, 2742.
(136) Landmark, K. J.; DiMaggio, S.; Ward, J.; Kelly, C.; Vogt, S.; Hong, S.; Kotlyar, A.; Myc, A.; Thomas, T. P.; Penner-Hahn, J. E.; Baker, J. R.; Holl, M. M. B.; Orr, B. G. *ACS Nano* **2008**, *2*, 773.
(137) Boswell, C. A.; Eck, P. K.; Regino, C. A. S.; Bernardo, M.; Wong, K. J.; Milenic, D. E.; Choyke, P. L.; Brechbiel, M. W. *Mol. Pharm.* **2008**, 527.
(138) Fu, Y. J.; Raatschen, H. J.; Nitecki, D. E.; Wendland, M. F.; Novikov, V.; Fournier, L. S.; Cyran, C.; Rogut, V.; Shames, D. M.; Brasch, R. C. *Biomacromolecules* **2007**, *8*, 1519.
(139) Cyran, C. C.; Fu, Y. J.; Raatschen, H. J.; Rogut, V.; Chaopathomkul, B.; Shames, D. M.; Wendland, M. F.; Yeh, B. M.; Brasch, R. C. *J. Magn. Reson. Imaging* **2008**, *27*, 581.
(140) Zhu, W. L.; Mollie, B.; Bhujwalla, Z. M.; Artemov, D. *Magn. Reson. Med.* **2008**, *59*, 679.
(141) Xu, H.; Regino, C. A. S.; Koyama, Y.; Hama, Y.; Gunn, A. J.; Bernardo, M.; Kobayashi, H.; Choyke, P. L.; Brechbiel, M. W. *Bioconjugate Chem.* **2007**, *18*, 1474.
(142) Koyama, Y.; Talanov, V. S.; Bernardo, M.; Hama, Y.; Regino, C. A. S.; Brechbiel, M. W.; Choyke, P. L.; Kobayashi, H. *J. Magn. Reson. Imaging* **2007**, *25*, 866.
(143) Wiener, E. C.; Auteri, F. P.; Chen, J. W.; Brechbiel, M. W.; Gansow, O. A.; Schneider, D. S.; Belford, R. L.; Clarkson, R. B.; Lauterbur, P. C. *J. Am. Chem. Soc.* **1996**, *118*, 7774.
(144) Bryant, L. H.; Brechbiel, M. W.; Wu, C. C.; Bulte, J. W. M.; Herynek, V.; Frank, J. A. *J. Magn. Reson. Imaging* **1999**, *9*, 348.
(145) Lu, Z. R.; Wang, X. H.; Parker, D. L.; Goodrich, K. C.; Buswell, H. R. *Bioconjugate Chem.* **2003**, *14*, 715.
(146) Lu, Z. R.; Mohs, A. M.; Zong, Y.; Feng, Y. *Int. J. Nanomed.* **2006**, *1*, 31.
(147) Amirkhanov, N. V.; Dimitrov, I.; Opitz, A. W.; Zhang, K.; Lackey, J. P.; Cardi, C. A.; Lai, S.; Wagner, N. J.; Thakur, M. L.; Wickstrom, E. *Biopolymers* **2008**, *89*, 1061.
(148) Pikkemaat, J. A.; Wegh, R. T.; Lamerichs, R.; van de Molengraaf, R. A.; Langereis, S.; Burdinski, D.; Raymond, A. Y. F.; Janssen, H. M.; de Waal, B. F. M.; Willard, N. P.; Meijer, E. W.; Grull, H. *Contrast Media Mol. Imaging* **2007**, *2*, 229.
(149) Ali, M. M.; Woods, M.; Caravan, P.; Opina, A. C. L.; Spiller, M.; Fettinger, J. C.; Sherry, A. D. *Chem.—Eur. J.* **2008**, *14*, 7250.
(150) Brinas, R. P.; Troxler, T.; Hochstrasser, R. M.; Vinogradov, S. A. *J. Am. Chem. Soc.* **2005**, *127*, 11851.
(151) Rozhkov, V.; Wilson, D.; Vinogradov, S. *Macromolecules* **2002**, *35*, 1991.
(152) Supattapone, S.; Nguyen, H. O. B.; Cohen, F. E.; Prusiner, S. B.; Scott, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14529.
(153) Gichm, L.; Christensen, C.; Boas, U.; Heegaard, P. A. H.; Otzen, D. E. *Biopolymers* **2008**, *89*, 522.
(154) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755.
(155) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555.
(156) Peerlings, H. W. I.; Nepogodiev, S. A.; Stoddart, J. F.; Meijer, E. W. *Eur. J. Org. Chem.* **1998**, 1879.
(157) Andre, S.; Ortega, P. J. C.; Perez, M. A.; Roy, R.; Gabius, H. J. *Glycobiology* **1999**, *9*, 1253.
(158) Zanini, D.; Roy, R. *J. Org. Chem.* **1998**, *63*, 3486.
(159) Kim, Y.; Hechler, B.; Klutz, A. M.; Gachet, C.; Jacobson, K. A. *Bioconjugate Chem.* **2008**, *19*, 406.
(160) Thomas, T. P.; Shukla, R.; Kotlyar, A.; Liang, B.; Ye, J. Y.; Norris, T. B.; Baker, J. R. *Biomacromolecules* **2008**, *9*, 603.
(161) Tam, J. P.; Zavala, F. *J. Immunol. Methods* **1989**, *124*, 53.
(162) Tam, J. P.; Lu, Y. A.; Yang, J. L. *Eur. J. Biochem.* **2002**, *269*, 923.
(163) Klajnert, B.; Janiszewska, J.; Urbanczyk-Lipkowska, Z.; Bryszewska, M.; Shcharbin, D.; Labieniec, M. *Int. J. Pharm.* **2006**, *309*, 208.
(164) Klajnert, B.; Janiszewska, J.; Urbanczyk-Lipkowska, Z.; Bryszewska, A.; Epand, R. M. *Int. J. Pharm.* **2006**, *327*, 145.
(165) Meyers, S. R.; Juhn, F. S.; Griset, A. P.; Luman, N. R.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2008**, *130*, 14444.
(166) Zaupa, G.; Scrimin, P.; Prins, L. J. *J. Am. Chem. Soc.* **2008**, *130*, 5699.
(167) Trevisiol, E.; Le Berre-Anton, V.; Leclaire, J.; Pratviel, G.; Caminade, A. M.; Majoral, J. P.; Francois, J. M.; Meunier, B. *New J. Chem.* **2003**, *27*, 1713.
(168) Le Berre, V.; Trevisiol, E.; Dagkessamanskaia, A.; Sokol, S.; Caminade, A. M.; Majoral, J. P.; Meunier, B.; Francois, J. *Nucleic Acids Res.* **2003**, *31*, 8.
(169) Rozkiewicz, D. I.; Gierlich, J.; Burley, G. A.; Gutsmiedl, K.; Carell, T.; Ravoo, B. J.; Reinhoudt, D. N. *ChemBioChem* **2007**, *8*, 1997.
(170) Rozkiewicz, D. I.; Brugman, W.; Kerkhoven, R. M.; Ravoo, B. J.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2007**, *129*, 11593.
(171) Grinstaff, M. W. *Biomaterials* **2007**, *28*, 5205.
(172) Sontjens, S. H. M.; Nettles, D. L.; Carnahan, M. A.; Setton, L. A.; Grinstaff, M. W. *Biomacromolecules* **2006**, *7*, 310.
(173) Degoricija, L.; Bansal, P. N.; Sontjens, S. H. M.; Joshi, N. S.; Takahashi, M.; Snyder, B.; Grinstaff, M. W. *Biomacromolecules* **2008**, *9*, 2863.
(174) Duan, X.; Sheardown, H. *Biomaterials* **2006**, *27*, 4608.
(175) Kofoed, J.; Reymond, J. L. *Curr. Opin. Chem. Biol.* **2005**, *9*, 656.
(176) Astruc, D.; Chardac, F. *Chem. Rev.* **2001**, *101*, 2991.
(177) Yemul, O.; Imae, T. *Biomacromolecules* **2005**, *6*, 2809.
(178) Delort, E.; Nguyen-Trung, N. Q.; Darbre, T.; Reymond, J. L. *J. Org. Chem.* **2006**, *71*, 4468.
(179) Javor, S.; Delort, E.; Darbre, T.; Reymond, J. L. *J. Am. Chem. Soc.* **2007**, *129*, 13238.
(180) Tamis, D.; Jean-Louis, R. *Chim. Oggi* **2007**, *25*, 25.
(181) Chi, Y. G.; Scroggins, S. T.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2008**, *130*, 6322.
(182) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2001**, *47*, 113.
(183) Kabanov, A. V.; Batrakova, E. V.; Alakhov, V. Y. *J. Controlled Release* **2002**, *82*, 189.
(184) Lavasanifar, A.; Samuel, J.; Kwon, G. S. *Adv. Drug Delivery Rev.* **2002**, *54*, 169.
(185) Lavasanifar, A.; Samuel, J.; Kwon, G. S. *J. Biomed. Mater. Res.* **2000**, *52*, 831.
(186) Lavasanifar, A.; Samuel, J.; Sattari, S.; Kwon, G. S. *Pharm. Res.* **2002**, *19*, 418.
(187) Lavasanifar, A.; Samuel, J.; Kwon, G. S. *J. Controlled Release* **2002**, *79*, 165.
(188) Hubbell, J. A. *Science* **2003**, *300*, 595.
(189) Lutz, J.-F.; Laschewsky, A. *Macromol. Chem. Phys.* **2005**, *206*, 813.
(190) Ringsdorf, H.; Lehman, P.; Weberskirch, R. Books of abstracts, 217th ACS national meeting, Anaheim, CA, 1999, March 21−25.
(191) Li, Z.; Kesselman, E.; Talmon, Y.; Hillmyer, M. A.; Lodge, T. P. *Science* **2004**, *306*, 98.
(192) Saito, N.; Liu, C.; Lodge, T. P.; Hillmyer, M. A. *Macromolecules* **2008**, *41*, 8815.
(193) Schlaad, H.; Antonietti, M. *Eur. Phys. J. E* **2003**, *10*, 17.
(194) Schlaad, H. *Peptide Hybrid Polymers*; Springer-Verlag Berlin: Berlin, 2006; pp 53−73.
(195) Deming, T. J. *Nature* **1997**, *390*, 386.
(196) Löwik, D. W. P. M.; Ayres, L.; Smeenk, J. M.; Van Hest, J. C. M. *Adv. Polym. Sci.* **2006**, *202*, 19.
(197) Nicolas, J.; Mantovani, G.; Haddleton, D. M. *Macromol. Rapid Commun.* **2007**, *28*, 1083.
(198) Le Droumaguet, B.; Mantovani, G.; Haddleton, D. M.; Velonia, K. *J. Mater. Chem.* **2007**, *17*, 1916.
(199) Hannink, J. M.; Cornelissen, J.; Farrera, J. A.; Foubert, P.; De Schryver, F. C.; Sommerdijk, N.; Nolte, R. J. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4732.

(200) Thordarson, P.; Le Droumaguet, B.; Velonia, K. *Appl. Microbiol. Biotechnol.* **2006**, *73*, 243.
(201) Kwon, G.; Naito, M.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. *Langmuir* **1993**, *9*, 945.
(202) Harada, A.; Kataoka, K. *Science* **1999**, *283*, 65.
(203) Adams, D. J.; Rogers, S. H.; Schuetz, P. *J. Colloid Interface Sci.* **2008**, *322*, 448.
(204) Harada, A.; Katoaka, K. *Soft Matter* **2008**, *4*, 162.
(205) Harada, A.; Kataoka, K. *Macromolecules* **1998**, *31*, 288.
(206) Yuan, X. F.; Harada, A.; Yamasaki, Y.; Kataoka, K. *Langmuir* **2005**, *21*, 2668.
(207) Gillies, E. R.; Frechet, J. M. J. *Chem. Commun.* **2003**, 1640.
(208) Lee, Y.; Bae, Y.; Hiki, S.; Ishii, T.; Kataoka, K. *J. Am. Chem. Soc.* **2008**, *129*, 5362.
(209) Kataoka, K.; Togawa, H.; Harada, A.; Yasugi, K.; Matsumoto, T.; Katayose, S. *Macromolecules* **1996**, *29*, 8556.
(210) Osada, K., Kataoka, K. *Peptide Hybrid Polymers*; Springer-Verlag: Berlin, 2006; pp 113−153.
(211) Harada, A.; Cammas, S.; Kataoka, K. *Macromolecules* **1996**, *29*, 6183.
(212) Chapman, T. M.; Hillyer, G. L.; Mahan, E. J.; Shaffer, K. A. *J. Am. Chem. Soc.* **1994**, *116*, 11195.
(213) Gillies, E. R.; Jonsson, T. B.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 11936.
(214) Gillies, E. R.; Frechet, J. M. J. *Bioconjugate Chem.* **2005**, *16*, 361.
(215) Lübbert, A.; Castelletto, V.; Hamley, I. W.; Nuhn, H.; Scholl, M.; Bourdillon, L.; Wandrey, C.; Klok, H. A. *Langmuir* **2005**, *21*, 6582.
(216) Astafieva, I.; Khougaz, K.; Eisenberg, A. *Macromolecules* **1995**, *28*, 7127.
(217) Gebhardt, K. E.; Ahn, S.; Venkatachalam, G.; Savin, D. A. *Langmuir* **2007**, *23*, 2851.
(218) Reinhard, S.; Losik, M. *Langmuir* **2007**, *23*, 7196.
(219) Cho, C. S.; Cheon, J. B.; Jeong, Y. I.; Kim, I. S.; Kim, S. H.; Akaike, T. *Macromol. Rapid Commun.* **1997**, *18*, 361.
(220) Cheon, J. B.; Kim, B. C.; Park, Y. H.; Park, J. S.; Moon, J. Y.; Nahm, J. H.; Cho, C. S. *Macromol. Chem. Phys.* **2001**, *202*, 395.
(221) Meyer, M.; Schlaad, H. *Macromolecules* **2006**, *39*, 3967.
(222) Kuo, S. W.; Lee, H. F.; Huang, C. F.; Huang, C. J.; Chang, F. C. *J. Polym. Sci., A: Polym. Chem.* **2008**, *46*, 3108.
(223) Lin, J.; Zhu, G.; Zhu, S.; Lin, S.; Nose, T.; Dinge, W. *Polymer* **2008**, *49*, 1132.
(224) Chécot, F.; Lecommandoux, S.; Klok, H. A.; Gnanou, Y. *Eur. Phys. J. E* **2003**, *10*, 25.
(225) Chécot, F.; Brlet, A.; Oberdisse, J.; Gnanou, Y.; Mondain-Monval, O.; Lecommandoux, S. *Langmuir* **2005**, *21*, 4308.
(226) Rodriguez-Hernandez, J.; Babin, J.; Zappone, B.; Lecommandoux, S. *Biomacromolecules* **2005**, *6*, 2213.
(227) Babin, J.; Rodriguez-Hernandez, J.; Lecommandoux, S.; Klok, H. A.; Achard, M. F. *Faraday Discuss.* **2005**, *128*, 179.
(228) Tian, H.; Deng, C.; Lin, H.; Sun, J.; Deng, M.; Chen, X.; Jing, X. *Biomaterials* **2005**, *26*, 4209.
(229) Tian, L.; Hammond, P. T. *Chem. Mater.* **2006**, *18*, 3976.
(230) Tian, H. Y.; Chen, X. S.; Lin, H.; Deng, C.; Zhang, P. B.; Wei, Y.; Jing, X. B. *Chem.—Eur. J.* **2006**, *12*, 4305.
(231) Guan, H.; Xie, Z.; Zhang, P.; Deng, C.; Chen, X.; Jing, X. *Biomacromolecules* **2005**, *6*, 1954.
(232) Sun, J.; Chen, X. S.; Deng, C.; Yu, H. J.; Xie, Z. G.; Jing, X. B. *Langmuir* **2007**, *23*, 8308.
(233) Deng, C.; Chen, X.; Yu, H.; Tian, H.; Sun, J.; Jing, X. *Biomacromolecules* **2007**, *8*, 1013.
(234) Thünemann, A. F.; Kubowicz, S.; von Berlepsch, H.; Mohwald, H. *Langmuir* **2006**, *22*, 2506.
(235) Sugimoto, H.; Nakanishi, E.; Yamauchi, F.; Yasumura, T.; Inomata, K. *Polymer* **2005**, *46*, 10800.
(236) Agut, W.; Taton, D.; Lecommandoux, S. *Macromolecules* **2007**, *40*, 5653.
(237) Park, J. S.; Akiyama, Y.; Yamasaki, Y.; Kataoka, K. *Langmuir* **2007**, *23*, 138.
(238) Rao, J. Y.; Luo, Z. F.; Ge, Z. S.; Liu, H.; Liu, S. Y. *Biomacromolecules* **2007**, *8*, 3871.
(239) Deng, L.; Shi, K.; Zhang, Y.; Wang, H.; Zeng, J.; Guo, X.; Du, Z.; Zhang, B. *J. Colloid Interface Sci.* **2008**, *323*, 169.
(240) Huang, C.; Chang, F. *Macromolecules* **2008**, *41*, 7041.
(241) Le Hellaye, M.; Fortin, N.; Guilloteau, J.; Soum, A.; Lecommandoux, S.; Guillaume, S. M. *Biomacromolecules* **2008**, *9*, 1924.
(242) Rodriguez-Hernandez, J.; Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026.
(243) Xu, J. S. *Soft Matter* **2008**, *4*, 1745.
(244) Liu, S.; Armes, S. P. *Langmuir* **2003**, *19*, 4432.
(245) Rao, J. Y.; Zhang, Y. F.; Zhang, J. Y.; Liu, S. Y. *Biomacromolecules* **2008**, *9*, 2586.
(246) Agut, W.; Agnaou, R.; Lecommandoux, S.; Taton, D. *Macromol. Rapid Commun.* **2008**, *29*, 1147.
(247) Lee, E.; Shin, H.; Na, K.; Bae, Y. *J. Controlled Release* **2003**, *90*, 363.
(248) Lee, E.; Gao, Z.; Bae, Y. *J. Controlled Release* **2008**, *132*, 164.
(249) Kim, G. M.; Bae, Y. H.; Jo, W. H. *Macromol. Biosci.* **2005**, *5*, 1118.
(250) Geng, Y.; Discher, D. E.; Justynska, J.; Schlaad, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 7578.
(251) Abraham, S.; Kim, I.; Batt, C. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 5720.
(252) Sinaga, A.; Hatton, T. A.; Tam, K. C. *Macromolecules* **2007**, *40*, 9064.
(253) Goh, S. L.; Platt, A. P.; Rutledge, K. E.; Lee, I. *J. Polym. Sci., A: Polym. Chem.* **2008**, *46*, 5381.
(254) Zhou, C.; Leng, B.; Yao, J.; Qian, J.; Chen, X.; Zhou, P.; Knight, D. P.; Shao, Z. *Biomacromolecules* **2006**, *7*, 2415.
(255) Arimura, H.; Ohya, Y.; Ouchi, T. *Macromol. Rapid Commun.* **2004**, *25*, 743.
(256) Arimura, H.; Ohya, Y.; Ouchi, T. *Biomacromolecules* **2005**, *6*, 720.
(257) Nottelet, B.; El Ghzaoui, A.; Coudane, J.; Vert, M. *Biomacromolecules* **2007**, *8*, 2594.
(258) Motala-Timol, S.; Jhurry, D.; Zhou, J.; Bhaw-Luximon, A.; Mohun, G.; Ritter, H. *Macromolecules* **2008**, *41*, 5571.
(259) Wang, Y.; Zou, S.; Kim, K. T.; Manners, I.; Winnik, M. A. *Chem.—Eur. J.* **2008**, *14*, 8624.
(260) Kim, K. T.; Vandermeulen, G. W. M.; Winnik, M. A.; Manners, I. *Macromolecules* **2005**, *38*, 4958.
(261) Sutthasupa, S.; Sanda, F.; Masuda, T. *Macromolecules* **2008**, *41*, 305.
(262) Lutz, J. F. *Polym. Int.* **2006**, *55*, 979.
(263) Mori, H.; Matsuyama, M.; Endo, T. *Macromol. Chem. Phys.* **2008**, *209*, 2100.
(264) McCormick, C. L.; Sumerlin, B. S.; Lokitz, B. S.; Stempka, J. E. *Soft Matter* **2008**, *4*, 1760.
(265) Klok, H. A. *J. Polym. Sci., A: Polym. Chem.* **2005**, *43*, 1.
(266) Skey, J.; O'Reilly, R. K. *J. Polym. Sci., A: Polym. Chem.* **2008**, *46*, 3690.
(267) Lokitz, B. S.; York, A. W.; Stempka, J. E.; Treat, N. D.; Li, Y. T.; Jarrett, W. L.; McCormick, C. L. *Macromolecules* **2007**, *40*, 6473.
(268) Lokitz, B. S.; Convertine, A. J.; Ezell, R. G.; Heidenreich, A.; Li, Y. T.; McCormick, C. L. *Macromolecules* **2006**, *39*, 8594.
(269) Liu, J.; Zhang, Q.; Remsen, E. E.; Wooley, K. L. *Biomacromolecules* **2001**, *2*, 362.
(270) Huang, H.; Kowalewski, T.; Remen, E. E.; Gertzmann, R.; Wooley, K. L. *J. Am. Chem. Soc.* **1997**, *119*, 11653.
(271) Thurmond, K. B., II; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1996**, *118*, 7239.
(272) Wooley, K. L. *Chem.—Eur. J.* **1997**, *3*, 1397.
(273) Morris, M. C.; Deshayes, S.; Heitz, F.; Divita, G. *Biol. Cell* **2008**, *100*, 201.
(274) Becker, M. L.; Remsen, E. E.; Pan, D.; Wooley, K. *Bioconjugate Chem.* **2004**, *15*, 699.
(275) Lim, Y. B.; Lee, E.; Lee, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 9011.
(276) Becker, M. L.; Liu, J.; Wooley, K. L. *Biomacromolecules* **2005**, *6*, 220.
(277) Futaki, S. *Adv. Drug Delivery Rev.* **2005**, *57*, 547.
(278) ten Cate, M. G. J.; Börner, H. G. *Macromol. Chem. Phys.* **2007**, *208*, 1437.
(279) Dunehoo, A. L.; Anderson, M.; Majumdar, S.; Kobayashi, N.; Berkland, C.; Siahaan, T. J. *J. Pharm. Sci.* **2006**, *95*, 1856.
(280) Xiong, X.; Mahmud, A.; Uluda, H.; Lavasanifar, A. *Biomacromolecules* **2007**, *8*, 874.
(281) Deng, C.; Chen, X.; Sun, J.; Lu, T.; Wang, W.; Jing, X. *J. Polym. Sci. A: Polym. Chem.* **2007**, *45*, 3218.
(282) Marsden, H. R.; Korobko, A. V.; van Leeuwen, E. N. M.; Pouget, E. M.; Veen, S. J.; Sommerdijk, N. A. J. M.; Kros, A. *J. Am. Chem. Soc.* **2008**, *130*, 9386.
(283) Liu, H.; Jiang, X.; Fan, J.; Wang, G.; Liu, S. Y. *Macromolecules* **2007**, *40*, 9074.
(284) Nyström, A. M.; Wooley, K. L. *Tetrahedron* **2008**, *64*, 8543.
(285) Shi, M.; Wosnick, J. H.; Ho, K.; Keating, A.; Shoichet, M. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 6126.
(286) Shi, Z. Q.; Zhou, Y. F.; Yan, D. Y. *Macromol. Rapid Commun.* **2008**, *29*, 412.
(287) Dirks, A. J. T.; van Berkel, S. S.; Hatzakis, N. S.; Opsteen, J. A.; van Delft, F. L.; Cornelissen, J. J. L. M.; Rowan, A. E.; van Hest, J. C. M.; Rutjes, F. P. J. T.; Nolte, R. J. M. *Chem. Commun.* **2005**, 4172.
(288) Dirks, A. J.; Nolte, R. J. M.; Cornelissen, J. J. L. M. *Adv. Mater.* **2008**, *20*, 3953.
(289) Velonia, K.; Rowan, A. E.; Nolte, R. J. M. *J. Am. Chem. Soc.* **2002**, *124*, 4224.
(290) Wright, E. R.; Conticello, V. P. *Adv. Drug Delivery Rev.* **2002**, *54*, 1057.

(291) Sallach, R. E.; Wei, M.; Biswas, N.; Conticello, V. P.; Lecommandoux, S.; Dluhy, R. A.; Chaikof, E. L. *J. Am. Chem. Soc.* **2006**, *128*, 12014.
(292) Dreher, M. R.; Simnick, A. J.; Fischer, K.; Smith, R. J.; Patel, A.; Schmidt, M.; Chilkoti, A. *J. Am. Chem. Soc.* **2008**, *130*, 687.
(293) Okada, M. *Prog. Polym. Sci.* **2001**, *26*, 67.
(294) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431.
(295) Spain, S. G.; Gibson, M. I.; Cameron, N. R. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 2059.
(296) Hayashi, M.; Loykulnant, S.; Hirao, A.; Nakahama, S. *Macromolecules* **1998**, *31*, 2057.
(297) Li, Z. C.; Liang, Y. Z.; Li, F. M. *Chem. Commun.* **1999**, 1557.
(298) Loykulnant, S.; Hirao, A. *Macromolecules* **2001**, *34*, 8434.
(299) Yamada, K.; Minoda, M.; Fukuda, T.; Miyamoto, T. *J. Polym. Sci., A: Polym. Chem.* **2001**, *39*, 459.
(300) Narumi, A.; Matsuda, T.; Kaga, H.; Satoh, T.; Kakuchi, T. *Polymer* **2002**, *43*, 4835.
(301) Narain, R.; Armes, S. P. *Macromolecules* **2003**, *36*, 4675.
(302) Lu, F. Z.; Meng, J. Q.; Du, F. S.; Li, Z. C.; Zhang, B. Y. *Macromol. Chem. Phys.* **2005**, *206*, 513.
(303) Suriano, F.; Coulembier, O.; Degee, P.; Dubois, P. *J. Polym. Sci., A: Polym. Chem.* **2008**, *46*, 3662.
(304) Dai, X. H.; Dong, C. M. *J. Polym. Sci., A: Polym. Chem.* **2008**, *46*, 817.
(305) Dai, X. H.; Dong, C. M.; Yan, D. *J. Phys. Chem. B* **2008**, *112*, 3644.
(306) Murphy, J. J.; Nomura, K. *Chem. Commun.* **2005**, 4080.
(307) Murphy, J. J.; Furusho, H.; Paton, R. M.; Nomura, K. *Chem.—Eur. J.* **2007**, *13*, 8985.
(308) Ramiah, V.; Matahwa, H.; Weber, W.; McLeary, J. B.; Sanderson, R. D. *Macromol. Symp.* **2007**, *255*, 70.
(309) Simon Ting, S. R.; Granville, A. M.; Quemener, D.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *Aust. J. Chem.* **2007**, *60*, 405.
(310) Cameron, N. R.; Spain, S. G.; Kingham, J. A.; Weck, S.; Albertin, L.; Barker, C. A.; Battaglia, G.; Smart, T.; Blanazs, A. *Faraday Discuss.* **2008**, *139*, 359.
(311) Stenzel, M. H. *Chem. Commun.* **2008**, 3486.
(312) Xiao, N. Y.; Li, A. L.; Liang, H.; Lu, J. *Macromolecules* **2008**, *41*, 2374.
(313) Zhang, L.; Bernard, J.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. A. *Macromol. Rapid Commun.* **2008**, *29*, 123.
(314) Yasugi, K.; Nakamura, T.; Nagasaki, Y.; Kato, M.; Kataoka, K. *Macromolecules* **1999**, *32*, 8024.
(315) Bes, L.; Angot, S.; Limer, A.; Haddleton, D. M. *Macromolecules* **2003**, *36*, 2493.
(316) Joralemon, M. J.; Murthy, K. S.; Remsen, E. E.; Becker, M. L.; Wooley, K. L. *Biomacromolecules* **2004**, *5*, 903.
(317) Hu, Y. C.; Pan, C. Y. *Macromol. Rapid Commun.* **2005**, *26*, 968.
(318) Rieger, J.; Stoffelbach, F.; Cui, D.; Imberty, A.; Lameignere, E.; Putaux, J. L.; Jérôme, R.; Jérôme, C.; Auzély-Velty, R. *Biomacromolecules* **2007**, *8*, 2717.
(319) Lim, Y. B.; Moon, K. S.; Lee, M. *J. Mater. Chem.* **2008**, *18*, 2909.
(320) Kim, B. S.; Hong, D. J.; Bae, J.; Lee, M. *J. Am. Chem. Soc.* **2005**, *127*, 16333.
(321) Haddleton, D. M.; Ohno, K. *Biomacromolecules* **2000**, *1*, 152.
(322) Kakuchi, T.; Narumi, A.; Miura, Y.; Matsuya, S.; Sugimoto, N.; Satoh, T.; Kaga, H. *Macromolecules* **2003**, *36*, 3909.
(323) Felici, M.; Marzá-Pérez, M.; Hatzakis, N. S.; Nolte, R. J. M.; Feiters, M. C. *Chem.—Eur. J.* **2008**, *14*, 9914.
(324) Narumi, A.; Miura, Y.; Otsuka, I.; Yamane, S.; Kitajyo, Y.; Satoh, T.; Hirao, A.; Kaneko, N.; Kaga, H.; Kakuchi, T. *J. Polym. Sci., A: Polym. Chem.* **2006**, *44*, 4864.
(325) Houga, C.; Meins, J.-L.; Borsali, R.; Taton, D.; Gnanou, Y. *Chem. Commun.* **2007**, 3063.
(326) Loos, K.; Stadler, R. *Macromolecules* **1997**, *30*, 7641.
(327) Loos, K.; Boker, A.; Zettl, H.; Zhang, A. F.; Krausch, G.; Muller, A. H. E. *Macromolecules* **2005**, *38*, 873.
(328) Hernandez, O. S.; Soliman, G. M.; Winnik, F. M. *Polymer* **2007**, *48*, 921.
(329) Zeng, J. G.; Shi, K. Y.; Zhang, Y. Y.; Sun, X. H.; Zhang, B. L. *Chem. Commun.* **2008**, 3753.
(330) Dong, H.; Li, Y.; Cai, S.; Zhuo, R.; Zhang, X.; Liu, L. *Angew. Chem., Int. Ed.* **2008**, *47*, 5573.
(331) Chen, D.; Jiang, M. *Acc. Chem. Res.* **2005**, *38*, 494.
(332) Noro, A.; Nagata, Y.; Tsukamoto, M.; Hayakawa, Y.; Takano, A.; Matsuhita, Y. *Biomacromolecules* **2005**, *6*, 2328.
(333) Sivakova, S.; Rowan, S. J. *Chem. Soc. Rev.* **2005**, *34*, 9.
(334) Spijker, H. J.; Dirks, A. J.; Van Hest, J. C. M. *J. Polym. Sci., A: Polym. Chem.* **2006**, *44*, 4242.
(335) Lutz, J. F.; Pfeifer, S.; Chanana, M.; Thunemann, A. F.; Bienert, R. *Langmuir* **2006**, *22*, 7411.
(336) Mather, B. D.; Baker, M. B.; Beyer, F. L.; Berg, M. A. G.; Green, M. D.; Long, T. E. *Macromolecules* **2007**, *40*, 6834.
(337) Thibault, R. J.; Galow, T. H.; Turnberg, E. J.; Gray, M.; Hotchkiss, P. J.; Rotello, V. M. *J. Am. Chem. Soc.* **2002**, *124*, 15249.
(338) Ishihara, Y.; Bazzi, H. S.; Toader, V.; Godin, F.; Sleiman, H. F. *Chem.—Eur. J.* **2007**, *13*, 4560.
(339) Teixeira, F., Jr.; Rigler, P.; Verbert-Nardin, C. *Chem. Commun.* **2007**, *11*, 1130.
(340) Börner, H. G.; Schlaad, H. *Soft Matter* **2007**, *3*, 394.
(341) Lutz, J. F.; Börner, H. G. *Prog. Polym. Sci.* **2008**, *33*, 1.
(342) Watson, K. J.; Park, S.; Im, J. H.; Mirkin, C. A. *J. Am. Chem. Soc.* **2001**, *123*, 5592.
(343) Jeong, J.; Park, T. *Bioconjugate Chem.* **2001**, *12*, 917.
(344) Li, Z.; Zhang, Y.; Fullhart, P.; Mirkin, C. A. *Nano Lett.* **2004**, *4*, 1055.
(345) Alemdaroglu, F. E.; Herrmann, A. *Org. Biomol. Chem.* **2007**, *5*, 1311.
(346) Alemdaroglu, F. E.; Ding, K.; Berger, R.; Herrmann, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4206.
(347) Alemdaroglu, F. E.; Wang, J.; Borsch, M.; Berger, R.; Herrmann, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 974.
(348) Wang, J.; Alemdaroglu, F. E.; Prusty, D. K.; Herrmann, A.; Berger, R. *Macromolecules* **2008**, *41*, 2914.
(349) Ding, K.; Alemdaroglu, F. E.; Börsch, M.; Berger, R.; Herrmann, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 1172.
(350) Alemdaroglu, F. E.; Alemdaroglu, N. C.; Langguth, P.; Herrmann, A. *Macromol. Rapid Commun.* **2008**, *29*, 326.
(351) Alemdaroglu, F. E.; Alemdaroglu, N. C.; Langguth, P.; Herrmann, A. *Adv. Mater.* **2008**, *20*, 899.
(352) Shi, X. Y.; Shen, M. W.; Mohwald, H. *Prog. Polym. Sci.* **2004**, *29*, 987.
(353) Decher, G.; Hong, J. D.; Schmitt, J. *Thin Solid Films* **1992**, *210*, 831.
(354) Möhwald, H.; Lichtenfeld, H.; Moya, S.; Voigt, A.; Sukhorukov, G.; Leporatti, S.; Dähne, L.; Antipov, A.; Gao, C. Y.; Donath, E. *Stud. Surf. Sci. Catal.* **2001**, *132*, 485.
(355) Decher, G.; Maclennan, J.; Sohling, U.; Reibel, J. *Thin Solid Films* **1992**, *210—211*, 504.
(356) Decher, G. *Science* **1997**, *277*, 1232.
(357) Lvov, Y.; Decher, G.; Sukhorukov, G. *Macromolecules* **1993**, *26*, 5396.
(358) Lvov, Y.; Ariga, K.; Ichinose, I.; Kunitake, T. *J. Am. Chem. Soc.* **1995**, *117*, 6117.
(359) Onda, M.; Lvov, Y.; Ariga, K.; Kunitake, T. *Biotechnol. Bioeng.* **1996**, *51*, 163.
(360) Sukhorukov, G. B.; Donath, E.; Lichtenfeld, H.; Knippel, E.; Knippel, M.; Budde, A.; Mohwald, H. *Colloids Surf., A* **1998**, *137*, 253.
(361) Caruso, F.; Mohwald, H. *J. Am. Chem. Soc.* **1999**, *121*, 6039.
(362) Caruso, F.; Schuler, C. *Langmuir* **2000**, *16*, 9595.
(363) Schüler, C.; Caruso, F. *Macromol. Rapid Commun.* **2000**, *21*, 750.
(364) Lvov, Y.; Caruso, F. *Anal. Chem.* **2001**, *73*, 4212.
(365) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Mohwald, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2202.
(366) Sukhorukov, G. B.; Donath, E.; Davis, S.; Lichtenfeld, H.; Caruso, F.; Popov, V. I.; Mohwald, H. *Polym. Adv. Technol.* **1998**, *9*, 759.
(367) Shenoy, D. B.; Antipov, A. A.; Sukhorukov, G. B.; Mohwald, H. *Biomacromolecules* **2003**, *4*, 265.
(368) Ma, Y. J.; Dong, W. F.; Hempenius, M. A.; Mohwald, H.; Vancso, G. J. *Nat. Mater.* **2006**, *5*, 724.
(369) Antipov, A. A.; Shchukin, D.; Fedutik, Y.; Petrov, A. I.; Sukhorukov, G. B.; Mohwald, H. *Colloids Surf., A* **2003**, *224*, 175.
(370) Caruso, F.; Lichtenfeld, H.; Giersig, M.; Mohwald, H. *J. Am. Chem. Soc.* **1998**, *120*, 8523.
(371) Caruso, F.; Caruso, R. A.; Mohwald, H. *Science* **1998**, *282*, 1111.
(372) Shchukin, D. G.; Sukhorukov, G. B.; Mohwald, H. *Chem. Mater.* **2003**, *15*, 3947.
(373) Shchukin, D. G.; Sukhorukov, G. B.; Mohwald, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4472.
(374) Dai, Z. F.; Voigt, A.; Leporatti, S.; Donath, E.; Dähne, L.; Mohwald, H. *Adv. Mater.* **2001**, *13*, 1339.
(375) Fang, M.; Grant, P. S.; McShane, M. J.; Sukhorukov, G. B.; Golub, V. O.; Lvov, Y. M. *Langmuir* **2002**, *18*, 6338.
(376) Lvov, Y.; Antipov, A. A.; Mamedov, A.; Mohwald, H.; Sukhorukov, G. B. *Nano Lett.* **2001**, *1*, 125.
(377) Sukhorukov, G. B.; Fery, A.; Brumen, M.; Mohwald, H. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4078.
(378) Caruso, F.; Trau, D.; Mohwald, H.; Renneberg, R. *Langmuir* **2000**, *16*, 1485.
(379) Balabushevitch, N. G.; Sukhorukov, G. B.; Moroz, N. A.; Volodkin, D. V.; Larionova, N. I.; Donath, E.; Mohwald, H. *Biotechnol. Bioeng.* **2001**, *76*, 207.
(380) Volodkin, D. V.; Balabushevitch, N. G.; Sukhorukov, G. B.; Larionova, N. I. *Biochemistry (Moscow)* **2003**, *68*, 236.
(381) Antipov, A. A.; Sukhorukov, G. B.; Mohwald, H. *Langmuir* **2003**, *19*, 2444.

(382) Volodkin, D. V.; Petrov, A. I.; Prevot, M.; Sukhorukov, G. B. *Langmuir* **2004**, *20*, 3398.
(383) Volodkin, D. V.; Larionova, N. I.; Sukhorukov, G. B. *Biomacromolecules* **2004**, *5*, 1962.
(384) Sukhorukov, G. B.; Volodkin, D. V.; Gunther, A. M.; Petrov, A. I.; Shenoy, D. B.; Mohwald, H. *J. Mater. Chem.* **2004**, *14*, 2073.
(385) Ibarz, G.; Dähne, L.; Donath, E.; Mohwald, H. *Adv. Mater.* **2001**, *13*, 1324.
(386) Sukhorukov, G. B.; Fery, A.; Möhwald, H. *Prog. Polym. Sci.* **2005**, *30*, 885.
(387) Kohler, K.; Biesheuvel, P. M.; Weinkamer, R.; Mohwald, H.; Sukhorukov, G. B. *Phys. Rev. Lett.* **2006**, 97.
(388) Dejugnat, C.; Sukhorukov, G. B. *Langmuir* **2004**, *20*, 7265.
(389) Shchukin, D. G.; Ustinovich, E. A.; Sukhorukov, G. B.; Mohwald, H.; Sviridov, D. V. *Adv. Mater.* **2005**, *17*, 468.
(390) Mauser, T.; Dejugnat, C.; Mohwald, H.; Sukhorukov, G. B. *Langmuir* **2006**, *22*, 5888.
(391) Mauser, T.; Dejugnat, C.; Sukhorukov, G. B. *Macromol. Rapid Commun.* **2004**, *25*, 1781.
(392) Glinel, K.; Sukhorukov, G. B.; Mohwald, H.; Khrenov, V.; Tauer, K. *Macromol. Chem. Phys.* **2003**, *204*, 1784.
(393) Petrov, A. I.; Gavryushkin, A. V.; Sukhorukov, G. B. *J. Phys. Chem. B* **2003**, *107*, 868.
(394) Kohler, K.; Mohwald, H.; Sukhorukov, G. B. *J. Phys. Chem. B* **2006**, *110*, 24002.
(395) Kohler, K.; Sukhorukov, G. B. *Adv. Funct. Mater.* **2007**, *17*, 2053.
(396) De Geest, B. G.; Skirtach, A. G.; De Beer, T. R. M.; Sukhorukov, G. B.; Bracke, L.; Baeyens, W. R. G.; Demeester, J.; De Smedt, S. C. *Macromol. Rapid Commun.* **2007**, *28*, 88.
(397) Tiourina, O. P.; Antipov, A. A.; Sukhorukov, G. B.; Larionova, N. L.; Lvov, Y.; Mohwald, H. *Macromol. Biosci.* **2001**, *1*, 209.
(398) Sukhorukov, G. B.; Antipov, A. A.; Voigt, A.; Donath, E.; Mohwald, H. *Macromol. Rapid Commun.* **2001**, *22*, 44.
(399) Shchukin, D. G.; Shutava, T.; Shchukina, E.; Sukhorukov, G. B.; Lvov, Y. M. *Chem. Mater.* **2004**, *16*, 3446.
(400) An, Z. H.; Mohwald, H.; Li, J. B. *Biomacromolecules* **2006**, *7*, 580.
(401) Krishna, G.; Shutava, T.; Lvov, Y. *Chem. Commun.* **2005**, 2796.
(402) Gao, C. Y.; Donath, E.; Mohwald, H.; Shen, J. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 3789.
(403) Gao, C. Y.; Liu, X. Y.; Shen, J. C.; Mohwald, H. *Chem. Commun.* **2002**, 1928.
(404) Sukhorukov, G. B.; Dähne, L.; Hartmann, J.; Donath, E.; Mohwald, H. *Adv. Mater.* **2000**, *12*, 112.
(405) Donath, E.; Moya, S.; Neu, B.; Sukhorukov, G. B.; Georgieva, R.; Voigt, A.; Baumler, H.; Kiesewetter, H.; Mohwald, H. *Chem.—Eur. J.* **2002**, *8*, 5481.
(406) Kreft, O.; Georgieva, R.; Baumler, H.; Steup, M.; Muller-Rober, B.; Sukhorukov, G. B.; Mohwald, H. *Macromol. Rapid Commun.* **2006**, *27*, 435.
(407) Voigt, A.; Buske, N.; Sukhorukov, G. B.; Antipov, A. A.; Leporatti, S.; Lichtenfeld, H.; Baumler, H.; Donath, E.; Mohwald, H. *J. Magn. Magn. Mater.* **2001**, *225*, 59.
(408) Estrela-Lopis, I.; Leporatti, S.; Typlt, E.; Clemens, D.; Donath, E. *Langmuir* **2007**, *23*, 7209.
(409) Liu, L.; Duan, X.; Liu, H.; Wang, S.; Li, Y. *Chem. Commun.* **2008**, 5999.
(410) Krol, S.; Diaspro, A.; Magrassi, R.; Ballario, P.; Grimaldi, B.; Filetici, P.; Ornaghi, P.; Ramoino, P.; Gliozzi, A. *IEEE Trans. Nanobiosci.* **2004**, *3*, 32.
(411) Svaldo Lanero, T.; Cavalleri, O.; Krol, S.; Rolandi, R.; Gliozzi, A. *J. Biotechnol.* **2006**, *124*, 723.
(412) Svaldo-Lanero, T.; Krol, S.; Magrassi, R.; Diaspro, A.; Rolandi, R.; Gliozzi, A.; Cavalleri, O. *Ultramicroscopy* **2007**, *107*, 913.
(413) De Geest, B. G.; Van Camp, W.; Du Prez, F. E.; De Smedt, S. C.; Demeester, J.; Hennink, W. E. *Macromol. Rapid Commun.* **2008**, *29*, 1111.
(414) De Geest, B. G.; Van Camp, W.; Du Prez, F. E.; De Smedt, S. C.; Demeester, J.; Hennink, W. E. *Chem. Commun.* **2008**, 190.
(415) Tong, W.; Gao, C. *J. Mater. Chem.* **2008**, *18*, 3799.
(416) Peyratout, C. S.; Dähne, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 3762.
(417) Shchukin, D. G.; Sukhorukov, G. B. *Adv. Mater.* **2004**, *16*, 671.
(418) Shchukin, D. G.; Radtchenko, I. L.; Sukhorukov, G. B. *J. Phys. Chem. B* **2003**, *107*, 86.
(419) Dähne, L.; Leporatti, S.; Donath, E.; Mohwald, H. *J. Am. Chem. Soc.* **2001**, *123*, 5431.
(420) Radtchenko, I. L.; Giersig, M.; Sukhorukov, G. B. *Langmuir* **2002**, *18*, 8204.
(421) Antipov, A. A.; Shchukin, D.; Fedutik, Y.; Zanaveskina, I.; Klechkovskaya, V.; Sukhorukov, G.; Mohwald, H. *Macromol. Rapid Commun.* **2003**, *24*, 274.
(422) Ghan, R.; Shutava, T.; Patel, A.; John, V. T.; Lvov, Y. *Macromolecules* **2004**, *37*, 4519.
(423) Shutava, T.; Zheng, Z. G.; John, V.; Lvov, Y. *Biomacromolecules* **2004**, *5*, 914.
(424) Balabushevich, N. G.; Sukhorukov, G. B.; Larionova, N. I. *Macromol. Rapid Commun.* **2005**, *26*, 1168.
(425) Mak, W. C.; Bai, J.; Chang, X. Y.; Trau, D. *Langmuir* **2009**, *25*, 769.
(426) Mak, W. C.; Cheung, K. Y.; Trau, D. *Adv. Funct. Mater.* **2008**, *18*, 2930.
(427) Kreft, O.; Prevot, M.; Mohwald, H.; Sukhorukov, G. B. *Angew. Chem., Int. Ed.* **2007**, *46*, 5605.
(428) Kreft, O.; Skirtach, A. G.; Sukhorukov, G. B.; Mohwald, H. *Adv. Mater.* **2007**, *19*, 3142.
(429) Krol, S.; Gliozzi, A.; Diaspro, A. *Front. Drug Des. Discovery* **2006**, *2*, 333.
(430) De Geest, B. G.; De Koker, S.; Sukhorukov, G. B.; Kreft, O.; Parak, W. J.; Skirtach, A. G.; Demeester, J.; De Smedt, S. C.; Hennink, W. E. *Soft Matter* **2009**, *5*, 282.
(431) Liu, X.; Yu, W.; Wang, W.; Xiong, Y.; Ma, X.; Yuan, Q. *Prog. Chem.* **2008**, *20*, 126.
(432) Sukhorukov, G. B.; Rogach, A. L.; Garstka, M.; Springer, S.; Parak, W. J.; Munoz-Javier, A.; Kreft, O.; Skirtach, A. G.; Susha, A. S.; Ramaye, Y.; Palankar, R.; Winterhalter, M. *Small* **2007**, *3*, 944.
(433) Sukhorukov, G. B.; Mohwald, H. *Trends Biotechnol.* **2007**, *25*, 93.
(434) Sukhorukov, G. B.; Rogach, A. L.; Zebli, B.; Liedl, T.; Skirtach, A. G.; Kohler, K.; Antipov, A. A.; Gaponik, N.; Susha, A. S.; Winterhalter, M.; Parak, W. J. *Small* **2005**, *1*, 194.
(435) Andreeva, D. V.; Gorin, D. A.; Mohwald, H.; Sukhorukov, G. B. *Langmuir* **2007**, *23*, 9031.
(436) Antipov, A. A.; Sukhorukov, G. B.; Donath, E.; Mohwald, H. *J. Phys. Chem. B* **2001**, *105*, 2281.
(437) Borodina, T.; Markvicheva, E.; Kunizhev, S.; Moehwald, H.; Sukhorukov, G. B.; Kreft, O. *Macromol. Rapid Commun.* **2007**, *28*, 1894.
(438) De Geest, B. G.; Sanders, N. N.; Sukhorukov, G. B.; Demeester, J.; De Smedt, S. C. *Chem. Soc. Rev.* **2007**, *36*, 636.
(439) Gaponik, N.; Radtchenko, I. L.; Gerstenberger, M. R.; Fedutik, Y. A.; Sukhorukov, G. B.; Rogach, A. L. *Nano Lett.* **2003**, *3*, 369.
(440) Antipov, A. A.; Sukhorukov, G. B.; Fedutik, Y. A.; Hartmann, J.; Giersig, M.; Mohwald, H. *Langmuir* **2002**, *18*, 6687.
(441) Skirtach, A. G.; Javier, A. M.; Kreft, O.; Kohler, K.; Alberola, A. P.; Mohwald, H.; Parak, W. J.; Sukhorukov, G. B. *Angew. Chem., Int. Ed.* **2006**, *45*, 4612.
(442) Skirtach, A. G.; De Geest, B. G.; Mamedov, A.; Antipov, A. A.; Kotov, N. A.; Sukhorukov, G. B. *J. Mater. Chem.* **2007**, *17*, 1050.
(443) De Geest, B. G.; Skirtach, A. G.; Mamedov, A. A.; Antipov, A. A.; Kotov, N. A.; De Smedt, S. C.; Sukhorukov, G. B. *Small* **2007**, *3*, 804.
(444) Lentacker, I.; De Geest, B. G.; Vandenbroucke, R. E.; Peeters, L.; Demeester, J.; De Smedt, S. C.; Sanders, N. N. *Langmuir* **2006**, *22*, 7273.
(445) De Geest, B. G.; Vandenbroucke, R. E.; Guenther, A. M.; Sukhorukov, G. B.; Hennink, W. E.; Sanders, N. N.; Demeester, J.; De Smedt, S. C. *Adv. Mater.* **2006**, *18*, 1005.
(446) De Koker, S.; De Geest, B. G.; Cuvelier, C.; Ferdinande, L.; Deckers, W.; Hennink, W. E.; De Smedt, S.; Mertens, N. *Adv. Funct. Mater.* **2007**, *17*, 3754.
(447) Bédard, M. F.; Munoz-Javier, A.; Mueller, R.; Del Pino, P.; Fery, A.; Parak, W. J.; Skirtach, A. G.; Sukhorukov, G. B. *Soft Matter* **2009**, *5*, 148.
(448) Jewell, C. M.; Lynn, D. M. *Adv. Drug Delivery Rev.* **2008**, *60*, 979.
(449) Moya, S.; Donath, E.; Sukhorukov, G. B.; Auch, M.; Baumler, H.; Lichtenfeld, H.; Mohwald, H. *Macromolecules* **2000**, *33*, 4538.
(450) Ge, L.; Mohwald, H.; Li, J. *Biochem. Biophys. Res. Commun.* **2003**, *303*, 653.
(451) Troutier, A.-L.; Ladavière, C. *Adv. Colloid Interface Sci.* **2007**, *133*, 1.
(452) Tiourina, O. P.; Radtchenko, I.; Sukhorukov, G. B.; Mohwald, H. *J. Membr. Biol.* **2002**, *190*, 9.
(453) De Rose, R.; Zelikin, A. N.; Johnston, A. P. R.; Sexton, A.; Chong, S.-F.; Cortez, C.; Mulholland, W.; Caruso, F.; Kent, S. J. *Adv. Mater.* **2008**, *20*, 4698.
(454) De Geest, B. G.; Jonas, A. M.; Demeester, J.; De Smedt, S. C. *Langmuir* **2006**, *22*, 5070.
(455) Levy, T.; Dejugnat, C.; Sukhorukov, G. B. *Adv. Funct. Mater.* **2008**, *18*, 1586.
(456) De Geest, B. G.; Dejugnat, C.; Sukhorukov, G. B.; Braeckmans, K.; De Smedt, S. C.; Demeester, J. *Adv. Mater.* **2005**, *17*, 2357.
(457) De Geest, B. G.; Dejugnat, C.; Verhoeven, E.; Sukhorukov, G. B.; Jonas, A. M.; Plain, J.; Demeester, J.; De Smedt, S. C. *J. Controlled Release* **2006**, *116*, 159.
(458) De Geest, B. G.; Dejugnat, C.; Prevot, M.; Sukhorukov, G. B.; Demeester, J.; De Smedt, S. C. *Adv. Funct. Mater.* **2007**, *17*, 531.

(459) De Geest, B. G.; Stubbe, B. G.; Jonas, A. M.; Van Thienen, T.; Hinrichs, W. L. J.; Demeester, J.; De Smedt, S. C. *Biomacromolecules* **2006**, *7*, 373.
(460) Kiser, P. F.; Wilson, G.; Needham, D. *Nature* **1998**, *394*, 459.
(461) De Geest, B. G.; Mehuys, E.; Laekeman, G.; Demeester, J.; De Smedt, S. C. *Expert Opin. Drug Delivery* **2006**, *3*, 459.
(462) De Geest, B. G.; De Koker, S.; Immesoete, K.; Demeester, J.; De Smedt, S. C.; Hennink, W. E. *Adv. Mater.* **2008**, *20*, 3687.
(463) De Geest, B. G.; McShane, M. J.; Demeester, J.; De Smedt, S. C.; Hennink, W. E. *J. Am. Chem. Soc.* **2008**, *130*, 14480.
(464) Kreft, O.; Javier, A. M.; Sukhorukov, G. B.; Parak, W. J. *J. Mater. Chem.* **2007**, *17*, 4471.
(465) Derveaux, S.; De Geest, B. G.; Roelant, C.; Braeckmans, K.; Demeester, J.; De Smedt, S. C. *Langmuir* **2007**, *23*, 10272.
(466) Derveaux, S.; Stubbe, B. G.; Roelant, C.; Leblans, M.; De Geest, B. G.; Demeester, J.; De Smedt, S. C. *Anal. Chem.* **2008**, *80*, 85.
(467) Vignali, D. A. A. *J. Immunol. Methods* **2000**, *243*, 243.
(468) Stubbe, B. G.; Gevaert, K.; Derveaux, S.; Braeckmans, K.; De Geest, B. G.; Goethals, M.; Vandekerckhove, J.; Demeester, J.; De Smedt, S. C. *Adv. Funct. Mater.* **2008**, *18*, 1624.
(469) Sinha, V. R.; Aggarwal, A.; Trehan, A. *Am. J. Drug Delivery* **2004**, *2*, 157.
(470) Heuberger, R.; Sukhorukov, G.; Voros, J.; Textor, M.; Mohwald, H. *Adv. Funct. Mater.* **2005**, *15*, 357.
(471) Germain, M.; Balaguer, P.; Nicolas, J.-C.; Lopez, F.; Esteve, J.-P.; Sukhorukov, G. B.; Winterhalter, M.; Richard-Foy, H.; Fournier, D. *Biosens. Bioelectron* **2006**, *21*, 1566.
(472) Krol, S.; Nolte, M.; Diaspro, A.; Mazza, D.; Magrassi, R.; Gliozzi, A.; Fery, A. *Langmuir* **2005**, *21*, 705.
(473) Fluri, D. A.; Kemmer, C.; Daoud-El Baba, M.; Fussenegger, M. *J. Controlled Release* **2008**, *131*, 211.
(474) Discher, B. M.; Hammer, D. A.; Bates, F. S.; Discher, D. E. *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 125.
(475) Dimova, R.; Seifert, U.; Pouligny, B.; Forster, S.; Dobereiner, H. G. *Eur. Phys. J. E* **2002**, *7*, 241.
(476) Discher, B. M.; Won, Y. Y.; Ege, D. S.; Lee, J. C. M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143.
(477) Discher, D. E.; Ahmed, F. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 323.
(478) Olsen, B. D.; Segalman, R. A. *Mater. Sci. Eng., R* **2008**, *62*, 37.
(479) Cameron, N. S.; Corbierre, M. K.; Eisenberg, A. *Can. J. Chem.* **1999**, *77*, 1311.
(480) Förster, S.; Antonietti, M. *Adv. Mater.* **1998**, *10*, 195.
(481) Srinivas, G.; Discher, D. E.; Klein, M. L. *Nat. Mater.* **2004**, *3*, 638.
(482) Lee, J. C. M.; Santore, M.; Bates, F. S.; Discher, D. E. *Macromolecules* **2002**, *35*, 323.
(483) Zhu, J. T.; Jiang, Y.; Liang, H. J.; Jiang, W. *J. Phys. Chem. B* **2005**, *109*, 8619.
(484) Dobereiner, H. G. *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 256.
(485) Förster, S.; Konrad, M. *J. Mater. Chem.* **2003**, *13*, 2671.
(486) Battaglia, G.; Ryan, A. J. *J. Am. Chem. Soc.* **2005**, *127*, 8757.
(487) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967.
(488) Lee, J. C. M.; Bermudez, H.; Discher, B. M.; Sheehan, M. A.; Won, Y. Y.; Bates, F. S.; Discher, D. E. *Biotechnol. Bioeng.* **2001**, *73*, 135.
(489) Bermudez, H.; Brannan, A. K.; Hammer, D. A.; Bates, F. S.; Discher, D. E. *Macromolecules* **2002**, *35*, 8203.
(490) Luo, L. B.; Eisenberg, A. *Langmuir* **2001**, *17*, 6804.
(491) Meng, F. H.; Hiemstra, C.; Engbers, G. H. M.; Feijen, J. *Macromolecules* **2003**, *36*, 3004.
(492) Discher, B. M.; Bermudez, H.; Hammer, D. A.; Discher, D. E.; Won, Y. Y.; Bates, F. S. *J. Phys. Chem. B* **2002**, *106*, 2848.
(493) Choucair, A.; Eisenberg, A. *Eur. Phys. J. E* **2003**, *10*, 37.
(494) Cheng, Z. L.; Tsourkas, A. *Langmuir* **2008**, *24*, 8169.
(495) Christian, N. A.; Milone, M. C.; Ranka, S. S.; Li, G. Z.; Frail, P. R.; Davis, K. P.; Bates, F. S.; Therien, M. J.; Ghoroghchian, P. P.; June, C. H.; Hammer, D. A. *Bioconjugate Chem.* **2007**, *18*, 31.
(496) Duncan, T. V.; Ghoroghchian, P. P.; Rubtsov, I. V.; Hammer, D. A.; Therien, M. J. *J. Am. Chem. Soc.* **2008**, *130*, 9773.
(497) Ghoroghchian, P. P.; Frail, P. R.; Susumu, K.; Blessington, D.; Brannan, A. K.; Bates, F. S.; Chance, B.; Hammer, D. A.; Therien, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 2922.
(498) Ahmed, F.; Discher, D. E. *J. Controlled Release* **2004**, *96*, 37.
(499) Kukula, H.; Schlaad, H.; Antonietti, M.; Forster, S. *J. Am. Chem. Soc.* **2002**, *124*, 1658.
(500) Pang, Z. Q.; Lu, W.; Gao, H. L.; Hu, K. L.; Chen, J.; Zhang, C. L.; Gao, X. L.; Jiang, X. G.; Zhu, C. Q. *J. Controlled Release* **2008**, *128*, 120.
(501) Arifin, D. R.; Palmer, A. F. *Biomacromolecules* **2005**, *6*, 2172.
(502) Najafi, F.; Sarbolouki, M. N. *Biomaterials* **2003**, *24*, 1175.
(503) Choi, H. J.; Montemagno, C. D. *Nano Lett.* **2005**, *5*, 2538.
(504) Vriezema, D. M.; Garcia, P. M. L.; Oltra, N. S.; Hatzakis, N. S.; Kuiper, S. M.; Nolte, R. J. M.; Rowan, A. E.; van Hest, J. C. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 7378.
(505) Opsteen, J. A.; Cornelissen, J.; van Hest, J. C. M. *Pure Appl. Chem.* **2004**, *76*, 1309.
(506) Park, C.; Yoon, J.; Thomas, E. L. *Polymer* **2003**, *44*, 6725.
(507) Yow, H. N.; Routh, A. F. *Soft Matter* **2006**, *2*, 940.
(508) Kita-Tokarczyk, K.; Grumelard, J.; Haefele, T.; Meier, W. *Polymer* **2005**, *46*, 3540.
(509) Mecke, A.; Dittrich, C.; Meier, W. *Soft Matter* **2006**, *2*, 751.
(510) Dirks, A. J.; Cornelissen, J.; van Delft, F. L.; van Hest, J. C. M.; Nolte, R. J. M.; Rowan, A. E.; Rutjes, F. *QSAR Comb. Sci.* **2007**, *26*, 1200.
(511) Becker, M. L.; Liu, J. Q.; Wooley, K. L. *Chem. Commun.* **2003**, 180.
(512) Bromley, E. H. C.; Channon, K.; Moutevelis, E.; Woolfson, D. N. *ACS Chem. Biol.* **2008**, *3*, 38.
(513) Kimura, S.; Kim, D. H.; Sugiyama, J.; Imanishi, Y. *Langmuir* **1999**, *15*, 4461.
(514) Kimura, S.; Muraji, Y.; Sugiyama, J.; Fujita, K.; Imanishi, Y. *J. Colloid Interface Sci.* **2000**, *222*, 265.
(515) Deming, T. J. *Peptide Hybrid Polymers*; Springer-Verlag Berlin: Berlin, 2006; pp 1−18.
(516) Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. *Nat. Mater.* **2004**, *3*, 244.
(517) Holowka, E. P.; Pochan, D. J.; Deming, T. J. *J. Am. Chem. Soc.* **2005**, *127*, 12423.
(518) Iatrou, H.; Frielinghaus, H.; Hanski, S.; Ferderigos, N.; Ruokolainen, J.; Ikkala, O.; Richter, D.; Mays, J.; Hadjichristidis, N. *Biomacromolecules* **2007**, *8*, 2173.
(519) Koide, A.; Kishimura, A.; Osada, K.; Jang, W. D.; Yamasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2006**, *128*, 5988.
(520) Kishimura, A.; Liamsuwan, S.; Matsuda, H.; Dong, W. F.; Osada, K.; Yamasaki, Y.; Kataoka, K. *Soft Matter* **2009**, *5*, 529.
(521) Sun, J.; Shi, Q.; Chen, X. S.; Guo, J. S.; Jing, X. B. *Macromol. Chem. Phys.* **2008**, *209*, 1129.
(522) Nardin, C.; Bolikal, D.; Kohn, J. *Langmuir* **2004**, *20*, 11721.
(523) Makino, A.; Yamahara, R.; Ozeki, E.; Kimura, S. *Chem. Lett.* **2007**, *36*, 1220.
(524) Chécot, F.; Lecommandoux, S.; Gnanou, Y.; Klok, H. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 1339.
(525) Gebhardt, K. E.; Ahn, S.; Venkatachalam, G.; Savin, D. A. *J. Colloid Interface Sci.* **2008**, *317*, 70.
(526) Rijcken, C. J. F.; Soga, O.; Hennink, W. E.; van Nostrum, C. F. *J. Controlled Release* **2007**, *120*, 131.
(527) McCormick, C. L.; Kirkland, S. E.; York, A. W. *J. Macromol. Sci., Polym. Rev.* **2006**, *46*, 421.
(528) Lee, H. J.; Yang, S. R.; An, E. J.; Kim, J. D. *Macromolecules* **2006**, *39*, 4938.
(529) Kros, A. K.; Jesse, W. J.; Metselaar, G. A. M.; Cornelissen, J. J. L. M. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 4349.
(530) Cornelissen, J. J. L. M.; Donners, J. J. J. M.; de Gelder, R.; Graswinckel, W. S.; Metselaar, G. A.; Rowan, A. E.; Sommerdijk, N. A. J. M.; Nolte, R. J. M. *Science* **2001**, *293*, 676.
(531) Cornelissen, J. J. L. M.; Fischer, M.; Sommerdijk, N. A. J. M.; Nolte, R. J. M. *Science* **1998**, *280*, 1427.
(532) Vriezema, D. M.; Hoogboom, J.; Velonia, K.; Takazawa, K.; Christianen, P. C. M.; Maan, J. C.; Rowan, A. E.; Nolte, R. J. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 772.
(533) De Hoog, H. P. M.; Vriezema, D. M.; Nallani, M.; Kuiper, S.; Cornelissen, J.; Rowan, A. E.; Nolte, R. J. M. *Soft Matter* **2008**, *4*, 1003.
(534) Vriezema, D. M.; Kros, A.; de Gelder, R.; Cornelissen, J.; Rowan, A. E.; Nolte, R. J. M. *Macromolecules* **2004**, *37*, 4736.
(535) Zhang, J. X.; Li, X. D.; Yan, M. Q.; Qiu, L. Y.; Jin, Y.; Zhu, K. J. *Macromol. Rapid Commun.* **2007**, *28*, 710.
(536) Hordyjewicz-Baran, Z.; You, L. C.; Smarsly, B.; Sigel, R.; Schlaad, H. *Macromolecules* **2007**, *40*, 3901.
(537) You, L. C.; Schlaad, H. *J. Am. Chem. Soc.* **2006**, *128*, 13336.
(538) Seeberger, P. H. *Nature* **2005**, *437*, 1239.
(539) Seeberger, P. H.; Werz, D. B. *Nature* **2007**, *446*, 1046.
(540) Doores, K. J.; Gamblin, D. P.; Davis, B. G. *Chem.—Eur. J.* **2006**, *12*, 656.
(541) Kim, B. S.; Yang, W. Y.; Ryu, J. H.; Yoo, Y. S.; Lee, M. *Chem. Commun.* **2005**, 2035.
(542) Wenz, G. *Angew. Chem., Int. Ed.* **1994**, *33*, 803.
(543) Guo, M. Y.; Jiang, M.; Zhang, G. Z. *Langmuir* **2008**, *24*, 10583.
(544) Jing, B.; Chen, X.; Wang, X. D.; Yang, C. J.; Xie, Y. Z.; Qiu, H. Y. *Chem.—Eur. J.* **2007**, *13*, 9137.
(545) Pasparakis, G.; Alexander, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 4847.
(546) Li, Z. C.; Shen, Y.; Liang, Y. Z.; Li, F. M. *Chin. J. Polym. Sci.* **2001**, *19*, 297.
(547) Zhou, W.; Dai, X. H.; Dong, C. M. *Macromol. Biosci.* **2008**, *8*, 268.
(548) Wang, C. Q.; Li, G. T.; Tao, S. Y.; Guo, R. R.; Yan, Z. *Carbohydr. Polym.* **2006**, *64*, 466.

(549) Gao, K. J.; Li, G. T.; Shi, H. W.; Lu, X. P.; Gao, Y. B.; Xu, B. Q. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 4889.
(550) Gao, K. J.; Li, G.; Lu, X.; Wu, Y. G.; Xu, B. Q.; Fuhrhop, J. H. *Chem. Commun.* **2008**, 1449.
(551) Inaki, Y. *Prog. Polym. Sci.* **1992**, *17*, 515.
(552) Smith, W. T. *Prog. Polym. Sci.* **1996**, *21*, 209.
(553) Spijker, H. J.; Dirks, A. J.; van Hest, J. C. M. *Polymer* **2005**, *46*, 8528.
(554) Khan, A.; Haddleton, D. M.; Hannon, M. J.; Kukulj, D.; Marsh, A. *Macromolecules* **1999**, *32*, 6560.
(555) Tang, H. D.; Radosz, M.; Shen, Y. Q. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 5995.
(556) Sivakova, S.; Wu, J.; Campo, C. J.; Mather, P. T.; Rowan, S. J. *Chem.—Eur. J.* **2006**, *12*, 446.
(557) Feldkamp, U.; Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 1856.
(558) Kim, J.; Liu, Y.; Ahn, S. J.; Zauscher, S.; Karty, J. M.; Yamanaka, Y.; Craig, S. L. *Adv. Mater.* **2005**, *17*, 1749.
(559) Socher, E.; Bethge, L.; Knoll, A.; Jungnick, N.; Herrmann, A.; Seitz, O. *Angew. Chem., Int. Ed.* **2008**, *47*, 9555.
(560) Ilhan, F.; Galow, T. H.; Gray, M.; Clavier, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 5895.
(561) Lutz, J. F.; Thunemann, A. F.; Rurack, K. *Macromolecules* **2005**, *38*, 8124.
(562) Uzun, O.; Xu, H.; Jeoung, E.; Thibault, R. J.; Rotello, V. M. *Chem.—Eur. J.* **2005**, *11*, 6916.
(563) Thibault, R. J.; Hotchkiss, P. J.; Gray, M.; Rotello, V. M. *J. Am. Chem. Soc.* **2003**, *125*, 11249.
(564) Thibault, R. J.; Uzun, O.; Hong, R.; Rotello, V. M. *Adv. Mater.* **2006**, *18*, 2179.
(565) Drechsler, U.; Thibault, R. J.; Rotello, V. M. *Macromolecules* **2002**, *35*, 9621.
(566) Lutz, J. F.; Thunemann, A. F.; Nehring, R. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 4805.
(567) Yoshikawa, I.; Sawayama, J.; Araki, K. *Angew. Chem., Int. Ed.* **2008**, *47*, 1038.
(568) Letchford, K.; Burt, H. *Eur. J. Pharm. Biopharm.* **2007**, *65*, 259.
(569) Qiu, L. Y.; Bae, Y. H. *Pharm. Res.* **2006**, *23*, 1.
(570) Zhong, Z. Y.; Dijkstra, P. J.; Birg, C.; Westerhausen, M.; Feijen, J. *Macromolecules* **2001**, *34*, 3863.
(571) Middleton, J. C.; Tipton, A. J. *Biomaterials* **2000**, *21*, 2335.
(572) Meng, F. H.; Engbers, G. H. M.; Feijen, J. *J. Controlled Release* **2005**, *101*, 187.
(573) Lee, Y.; Chang, J. B.; Kim, H. K.; Park, T. G. *Macromol. Res.* **2006**, *14*, 359.
(574) Rameez, S.; Alosta, H.; Palmer, A. F. *Bioconjugate Chem.* **2008**, *19*, 1025.
(575) Ghoroghchian, P. P.; Frail, P. R.; Li, G. Z.; Zupancich, J. A.; Bates, F. S.; Hammer, D. A.; Therien, M. J. *Chem. Mater.* **2007**, *19*, 1309.
(576) Ghoroghchian, P. P.; Lin, J. J.; Brannan, A. K.; Frail, P. R.; Bates, F. S.; Therien, M. J.; Hammer, D. A. *Soft Matter* **2006**, *2*, 973.
(577) Wittemann, A.; Azzam, T.; Eisenberg, A. *Langmuir* **2007**, *23*, 2224.
(578) Ghoroghchian, P. P.; Li, G. Z.; Levine, D. H.; Davis, K. P.; Bates, F. S.; Hammer, D. A.; Therien, M. J. *Macromolecules* **2006**, *39*, 1673.
(579) Shum, H. C.; Kim, J. W.; Weitz, D. A. *J. Am. Chem. Soc.* **2008**, *130*, 9543.
(580) Mai, Y. Y.; Zhou, Y. F.; Yan, D. Y. *Small* **2007**, *3*, 1170.
(581) Nardin, C.; Widmer, J.; Winterhalter, M.; Meier, W. *Eur. Phys. J. E* **2001**, *4*, 403.
(582) Broz, P.; Benito, S. M.; Saw, C.; Burger, P.; Heider, H.; Pfisterer, M.; Marsch, S.; Meier, W.; Hunziker, P. *J. Controlled Release* **2005**, *102*, 475.
(583) Opsteen, J. A.; Brinkhuis, R. P.; Teeuwen, R. L. M.; Löwik, D. W. P. M.; van Hest, J. C. M. *Chem. Commun.* **2007**, 3136.
(584) Van Dongen, S. F. M.; Nallani, M.; Schoffelen, S.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; van Hest, J. C. M. *Macromol. Rapid Commun.* **2008**, *29*, 321.
(585) Monnard, P. A.; DeClue, M. S.; Ziock, H. J. *Curr. Nanosci.* **2008**, *4*, 71.
(586) Napoli, A.; Boerakker, M. J.; Tirelli, N.; Nolte, R. J. M.; Sommerdijk, N.; Hubbell, J. A. *Langmuir* **2004**, *20*, 3487.
(587) Battaglia, G.; Ryan, A. J.; Tomas, S. *Langmuir* **2006**, *22*, 4910.
(588) Axthelm, F.; Casse, O.; Koppenol, W. H.; Nauser, T.; Meier, W.; Palivan, C. G. *J. Phys. Chem. B* **2008**, *112*, 8211.
(589) Nardin, C.; Winterhalter, M.; Meier, W. *Langmuir* **2000**, *16*, 7708.
(590) Nardin, C.; Thoeni, S.; Widmer, J.; Winterhalter, M.; Meier, W. *Chem. Commun.* **2000**, 1433.
(591) Nallani, M.; Benito, S.; Onaca, O.; Graff, A.; Lindemann, M.; Winterhalter, M.; Meier, W.; Schwaneberg, U. *J. Biotechnol.* **2006**, *123*, 50.
(592) Broz, P.; Driamov, S.; Ziegler, J.; Ben-Haim, N.; Marsch, S.; Meier, W.; Hunziker, P. *Nano Lett.* **2006**, *6*, 2349.
(593) Onaca, O.; Sarkar, P.; Roccatano, D.; Friedrich, T.; Hauer, B.; Grzelakowski, M.; Guven, A.; Fioroni, M.; Schwaneberg, U. *Angew. Chem., Int. Ed.* **2008**, *47*, 7029.
(594) Sauer, M.; Haefele, T.; Graff, A.; Nardin, C.; Meier, W. *Chem. Commun.* **2001**, 2452.
(595) Graff, A.; Sauer, M.; Van Gelder, P.; Meier, W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5064.
(596) Ben-Haim, N.; Broz, P.; Marsch, S.; Meier, W.; Hunziker, P. *Nano Lett.* **2008**, *8*, 1368.
(597) Van Dongen, S. F. M.; Nallani, M.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; Van Hest, J. C. M. *Chem.—Eur. J.* **2009**, *15*, 1107.
(598) Nehring, R.; Palivan, C. G.; Casse, O.; Tanner, P.; Tuxen, J.; Meier, W. *Langmuir* **2009**, *25*, 1122.
(599) Arnau, J.; Lauritzen, C.; Petersen, G. E.; Pedersen, J. *Protein Expression Purif.* **2006**, *48*, 1.
(600) Nallani, M.; de Hoog, H. P. M.; Cornelissen, J.; Palmans, A. R. A.; van Hest, J. C. M.; Nolte, R. J. M. *Biomacromolecules* **2007**, *8*, 3723.
(601) Nallani, M.; Woestenenk, R.; De Hoog, H. P. M.; Van Dongen, S. F. M.; Boezeman, J.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; Van Hest, J. C. M. *Small* **2009**, *5*, 1138.
(602) Kuiper, S. M.; Nallani, M.; Vriezema, D. M.; Cornelissen, J. J. L. M.; Hest, J. C. M. V.; Nolte, R. J. M.; Rowan, A. E. *Org. Biomol. Chem.* **2008**, *6*, 4315.
(603) Reiner, J. E.; Wells, J. M.; Kishore, R. B.; Pfefferkorn, C.; Helmerson, K. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 1173.
(604) Jofre, A.; Hutchison, J. B.; Kishore, R.; Locascio, L. E.; Helmerson, K. *J. Phys. Chem. B* **2007**, *111*, 5162.
(605) Photos, P. J.; Bacakova, L.; Discher, B.; Bates, F. S.; Discher, D. E. *J. Controlled Release* **2003**, *90*, 323.
(606) Ahmed, F.; Photos, P. J.; Discher, D. E. *Drug Dev. Res.* **2006**, *67*, 4.
(607) Ghoroghchian, P. P.; Frail, P. R.; Susumu, K.; Park, T. H.; Wu, S. P.; Uyeda, H. T.; Hammer, D. A.; Therien, M. J. *J. Am. Chem. Soc.* **2005**, *127*, 15388.
(608) Ahmed, F.; Pakunlu, R. I.; Brannan, A.; Bates, F.; Minko, T.; Discher, D. E. *J. Controlled Release* **2006**, *116*, 150.
(609) Lin, J. J.; Silas, J. A.; Bermudez, H.; Milam, V. T.; Bates, F. S.; Hammer, D. A. *Langmuir* **2004**, *20*, 5493.
(610) Lin, J. J.; Ghoroghchian, P.; Zhang, Y.; Hammer, D. A. *Langmuir* **2006**, *22*, 3975.
(611) Hammer, D. A.; Robbins, G. P.; Haun, J. B.; Lin, J. J.; Qi, W.; Smith, L. A.; Ghoroghchian, P. P.; Therien, M. J.; Bates, F. S. *Faraday Discuss.* **2008**, *139*, 129.
(612) Choucair, A.; Soo, P. L.; Eisenberg, A. *Langmuir* **2005**, *21*, 9308.
(613) Lomas, H.; Canton, I.; MacNeil, S.; Du, J.; Armes, S. P.; Ryan, A. J.; Lewis, A. L.; Battaglia, G. *Adv. Mater.* **2007**, *19*, 4238.
(614) Lomas, H.; Massignani, M.; Abdullah, K. A.; Canton, I.; Lo Presti, C.; MacNeil, S.; Du, J. Z.; Blanazs, A.; Madsen, J.; Armes, S. P.; Lewis, A. L.; Battaglia, G. *Faraday Discuss.* **2008**, *139*, 143.
(615) Cerritelli, S.; Velluto, D.; Hubbell, J. A. *Biomacromolecules* **2007**, *8*, 1966.
(616) Li, B.; Martin, A. L.; Gillies, E. R. *Chem. Commun.* **2007**, 5217.

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