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# Poly(2-oxazolines) in Biological and Biomedical Application Contexts

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## Abstract

Polyoxazolines of various architectures and chemical functionalities can be prepared in a living and therefore controlled manner *via* cationic ring-opening polymerisation. They have found widespread applications, ranging from coatings to pigment dispersants. Furthermore, several polyoxazolines are water-soluble or amphiphilic and relatively non-toxic, which makes them interesting as biomaterials.

This paper reviews the development of polyoxazoline-based polymers in biological and biomedical application contexts since the beginning of the millennium. This includes nanoscalar systems such as membranes and nanoparticles, drug and gene delivery applications, as well as stimuli-responsive systems.

***Keywords:*** polyoxazoline, biomaterials, self-assembly, drug delivery, gene delivery, stimuli-responsive polymers

**Contents**

## Introduction

Polyoxazolines have been the subject of a considerable amount of research since the 1960ies,[1-7] with a significant number of papers focusing on the polymerisation of 2-substituted oxazolines.[8-11] Monomers substituted in the 4- or 5- position are more difficult to polymerise, due to steric crowding.[11] Polymerisations are usually carried out *via* a living cationic ring opening mechanism (CROP), which yields well-defined polymers of narrow average molecular weight distributions (Figure 1).[4, 6] Furthermore, a large property space can theoretically be accessed, as the synthesis of 2-substituted monomers can be conveniently accomplished *via* condensation of a primary nitrile and 2-aminoethanol.[9, 12] Commercially, however, only 2-methyl, 2-ethyl, 2-isopropyl and 2-phenyl oxazoline are currently available.

Although the use of poly(2-oxazolines) in adhesive and coating formulations,[13-15] as pigment dispersants in inks,[16] and drug delivery applications[17] has been documented, the polymers have not found widespread commercial application, as the (batch) polymerisations times range from several hours to several days.[18-25] Recent advances in synthetic technology, notably the advent of microwave reactors, which allow easy access to high-temperature/high-pressure synthesis conditions, have allowed acceleration of polymerisations by factors of up to 350, when compared with conventional reflux conditions, while reducing the occurrence of side reactions and maintaining the living character of the reaction.[26]

Another attractive feature of polyoxazolines is the ease of preparation of co-polymers, notably block copolymers,[27-30] but also of star-shaped[29, 31, 32] and hyperbranched[33, 34] motives as well as cross-linked networks.[35] Block copolymers generally provide easy access to amphiphiles, which, provided the blocks

are judiciously chosen, are capable of self-assembly to form a variety of complex structures, such as (multicompartment) micelles[36] and vesicles.[37, 38]

Due to the versatility of this class of polymer and their ability to form functional materials and nanostructures, the interest in poly(2-acyl-2-oxazoline)-based materials is rising. Furthermore, this class of materials could be promising for use in biomedical applications, but is under-researched and under-utilized in this context at present. The aim of this paper is to provide a general overview over "the state of the art" in poly(oxazoline) research in biological and biomedical application areas, roughly covering the last decade of research.

## 2. Poly(2-oxazolines) in biological application contexts

One of the principal application areas for polymers in biological and medicinal contexts is drug and gene delivery.[39, 40] In the simplest case, a drug is enclosed in a polymeric matrix and released over time through diffusion.[41] Alternatively, complex multifunctional polymers with covalently attached drug moieties are constructed.[42] The combination of a polymer with a drug molecule has several main advantages over the use of a pure drug molecule: (1) potentially increased solubility of insoluble drugs or drugs of low aqueous solubility, (2) improved pharmacogenetics, (3) protection against deactivation and degradation during transport and circulation, (4) reduced antigenic activity and (5) the possibility to combine a drug with other functional components such as contrast agents etc..[43] The majority of polymers that have so far been investigated for use in drug and gene delivery applications have focused on linear polymers such as poly(ethylene oxide) (PEO),[44] poly(*N*-2-hydroxypropyl methacrylamide) (HPMA) and others.[45]

However, a significant amount of attention is now focusing on other structural (nanoscalar) motives such as micelles and vesicles formed by lipopolymers[46, 47] or amphiphilic block copolymers,[48, 49] as well as on dendrimers[50, 51] and star-shaped polymers[52, 53] or microgels.[54]

### 2.1 Poly(2-oxazoline)-based lipopolymers

As discussed above, drugs are usually enclosed in a polymeric matrix or covalently attached to a polymer moiety for delivery purposes. Enclosure in a matrix either entails compounding the drug molecule into an amorphous polymer (as used in stents[55] and polymeric implants) or encapsulating it in a vehicle formed using amphiphilic block copolymers. These vehicles can have different organizational shapes, but all rely on the principle that amphiphiles self-assemble in water to form nanostructures composed of a hydrophobic core and a hydrophilic shell. As such, lipopolymers have received considerable attention, with the most intensively investigated lipopolymers being poly(ethylene glycol) based moieties (PEG-lipids).[56-59] However, a number of lipo-polyoxazolines have also been prepared. Woodle *et al*. reported the synthesis of poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline)-based lipid conjugates as an alternatives to PEG-based materials.[60] The conjugates were prepared by linking the glutarate esters of the pre-formed polymers to disteaorylphosphatidyl ethanolamine (DSPE) or *via* termination of the polymerisation with this reagent (Figure 2). The lipopolymers were used to prepare $^{67}$Ga-labelled liposomes, which were subsequently injected into the bloodstream of rats in order to monitor their progress and distribution *via* blood-level and tissue measurements. The experiments showed that the behavior of the poly(oxazoline)-

based liposomes is similar to that of the corresponding PEG-based materials, both in terms of their circulation in the bloodstream (showing long circulation lifetimes) as well as in terms of uptake by liver and spleen. This behaviour can be attributed to factors similar to those responsible for the "PEG-effect"[61], *i.e*. the high mobility of chains and the water-binding ability, both of which contribute to the steric stabilization observed in polymer-lipid liposomes. Furthermore, the "stealth effect" may also be operational.[62] Similar results were obtained by Zalipsky *et al*. in another study.[63] Lipids can also be used as initiators rather than terminating agents: functional tosylates such as 1,2-di-dodecanoylpropyl-p-toluene sulfonate can be employed to initiate the polymerisation of either 2-methyl or 2-phenyl-1,3-oxazoline.[64] Jordan *et al.* subsequently reported the preparation of poly(*N*-propionyloxazolines), end functionalized with a number of different lipophilic moieties (methyl-, *N*-hexadecyl-, (C16-) and 1,2-*O*-dioctadecyl-sn-glyceryl-).[65] The polymerisations were initiated using the lipid trifluoromethane sulfonate derivatives and proceed *via* the usual cationic ring-opening mechanism. This method of preparing lipopolymers has subsequently been branded the "initiator method". The polymers were end-capped with 4-aminobutyl dimethyl siloxane, which allows for the possibility of forming brush-type polymer layers by grafting onto a solid support. Another example of lipo-polyoxazoline was provided by Volet *et al*. who prepared telechelic PMOXA polymers containing one long-chain alkyl end-cap *via* cationic ring-opening polymerisation of 2-methyl-2-oxazoline, initiated by either 1-iodo dodecane or 1-iodo octadecane.[66] Critical micellar concentrations in these systems can be controlled *via* the hydrophilic/lipophilic balance.

Infrared reflection absorption spectroscopy (IRRAS) studies on dioctadecyl-glyceryl-substituted poly(2-methyl-2oxazoline) monolayers at the air/water interface were

carried out in order to elucidate the phase behavior of these polymers.[67] The results provided some first evidence, that at high lateral surface pressures, considerable ordering of the $CH_2$ groups of the lipopolymer occurs. These findings were subsequently confirmed by investigating the rheological properties of Langmuir monolayers of both PEG-based and polyoxazoline-based lipopolymers at the air-water interface.[68] A high-film-pressure transition for mixtures containing between 40 % and 100 % of lipopolymers was observed, which is a requirement for the existence of a rheological transition. At this transition, both the PEG-based and the oxazoline-based materials show dramatic increases in both their storage and loss moduli as the area per polymer is decreased. This observation can be interpreted in terms of the formation of a gel network: at zero surface pressure, the polymer molecules lie in the plain of the air-water interface without showing a significant amount of interaction. As the film is compressed, the polymers desorb into the water sub-phase. At very high pressures, the lipids move close together and an alkyl chain condensation occurs to form a lipid microdomain, which consists of alkyl chains from two to four lipopolymers, which do not cluster extensively. The hydrophilic part of the polymer is considered to be fully hydrated at low pressures; as the pressure is increased, the water is squeezed out from the polymer water complex, giving rise to hydrogen-bonded bridges between the chains (Figure 3).

Nuyken and others subsequently refined this picture even further by using X-ray and neutron reflectometry.[69] Experiments showed, that the phase transition observed at high surface pressures is indeed due to alkyl chain condensation, triggered by strain exerted by the poly(2-methyl-2-oxazoline) units on the lipid moieties. The strain originates from the confinement of the polyoxazoline to the interface, which, in turn, leads to a reduction of the conformational entropy of the chains below that of the free

chains. Passing the phase transition, the strain leads to partial immersion of the lipids in the aqueous phase.

A number of groups have reported the formation of stripe-like micropatterns when transferring a Langmuir monolayer onto a solid substrate. Jordan *et al.* reported the use of mixed monolayers of lipids and lipopolyoxazolines to confine cell adhesion receptors in those micropatterns.[70] Micropattern formation is generally explained in terms of the periodic oscillations of the contact angle and the water meniscus height of the aqueous subphase during the transfer.[71] There are three physical parameters that seem to influence the stripe pattern formation, namely the transfer velocity, the interlayer viscosity and the "hydrophobic mismatch". Experiments indicate that, as the transfer velocity is increased, the spacing between individual stripes decreases. Beyond a certain threshold, the stripe patterns start to branch and eventually disappear altogether to give rise to an optically homogeneous film. The viscosity of the film at the three-phase contact line is directly linked to the length of the polyoxazoline chains. Short chains could be shown to give rise to well-defined stripes, whereas longer chains give branched patterns (Figure 4). Further experiments suggest, that the possibility of pattern formation as a consequence of phase separation can be excluded. Furthermore, platelet integrin $\alpha_b\beta_3$ could be incorporated into the micropattern. Experiments showed, that the integrins are preferentially incorporated into the lipopolymer rich regions of the pattern.

Building on this work, Jordan also reported the preparation of stable lipid membranes with controlled lipid membrane spacings, by using lipopolymers as a tether.[72] Using the systems described above, a monolayer was preorganized onto a substrate in a first step. Subsequently lipid vesicles were fused on top of the layer, thus generating

supported lipid membranes. Control over the substrate-membrane spacing was achieved *via* the degree of polymerisation (DP) of the polyoxazoline moiety.

In a further study, Jordan and Nuyken reported the synthesis of novel 2-substituted lipopolyoxazolines capable of hydrogen bonding in a bid to gain further understanding of the gelation phenomena discussed above.[73] The lipopolymers were prepared using the initiator method. The oxazoline monomers (2-(2'-*N*-pyrrolidonyl-ethyl-)2-oxazoline, 2-(3'-methoxymonoethyleneglycol)propyl-2-oxazoline, and 2-(3'-methoxytriethyleneglycol)propyl-2-oxazoline) are capable of hydrogen bonding and if hydrogen bonds do have a stabilizing effect on 2D gel formation, the introduction of these side chains should have a marked effect on, for example, the surface rheology of the lipopolymers. However, when examining the storage moduli of lipo-PEGs, lipo-poly-2-methyl- and lipo-poly-2-ethyl oxazoline as well as those of the side chain-modified polyoxazolines as a function of film pressure, the authors observed that while the lipo-PEGs and lipo-polyoxazolines showed a 2D gel transition, the latter was absent for the side chain modified polymers and the storage modulus is completely unaffected by the compression of the monolayer. This indicates that lipopolymer gels are not stabilized by hydrogen bonding between the polymer units and that alkyl chain condensation is indeed necessary for gel formation.

## 2.2 Amphiphilic Polyoxazolines other than Lipopolymers

### 2.2.1 Membrane Technology

Lipids, of course, are not the only co-monomers that can be used in combination with poly(oxazolines) to generate self-assembled systems; chitosan-derivatized systems have also been investigated. Next to cellulose, chitin is the most abundant biopolymer

and is essentially composed of *N*-actyl-*D*-glucosamine repeating units, which, similar to the bonding situation in cellulose, are linked by a 1,4-β bond.[74] Furthermore, chitin is of potential interest for its possible use in functional biomaterials, although its comparative insolubility in most organic solvents has so far limited its application.

Chitin-derivatized polymers can be synthesized by reacting deacetylated chitin (chitosan) with living poly-(2-alkyl-oxazoline) chains, where the alkyl group is either a methyl or an ethyl group (Figure 5).[75] This results in the isolation of polymers in medium yields, which are soluble in both water as well as N,N-dimethylformamide and DMSO and partially soluble in methanol, acetonitrile and chloroform. Graft co-polymer of this type therefore improves on some properties of both the pure oxazoline as well as pure chitin. $^{1}$H NMR studies on the chitin-grafted poly(2-ethyl-2-oxazoline) in both $D_2O$ and DMSO-$d_6$ indicate highly restricted motion of the chitin skeleton, which can be interpreted to arise from a tightly coiled micellar conformation of this polymer in solution. This behavior is consistent with previous experimental observations on polysaccharide substituted polystyrenes.[76]

Studies on block poly(2-oxazoline) co-polymers have already shown that the activity of several enzymes such as horseradish peroxidase, catalase and lipase in organic solvents can be significantly increased, when these enzymes are lyophilized in the presence of amphiphilic poly(2-oxazolines).[77-80] Studies using the chitin-*graft*-poly(2-oxazoline) polymers described above gave similar results. Catalase, which has been lyophilized with chitin-graft-poly(2-methyl-2-oxazoline), showed rates of hydrogen peroxide consumption in chloroform, which were ten-fold higher than those observed in the absence of polymer.[81]

In another attempt to improve the usability of chitins, the miscibility of commodity polymers such as poly(vinyl alcohol) (PVA) with chitin derivatives using poly(oxazolines) as compatibilizers was investigated.[82] Series of thin films of PVA/chitin-*graft*-poly(2-ethyl-2-oxazoline) as well as poly(2-ethyl-2-oxazoline)/chitin-*graft*-poly(2-ethyl-2-oxazoline) blends were prepared *via* solvent casting from aqueous solution, with the chitin-*graft*-poly(2-ethyl-2-oxazoline) content ranging from 10 to 90 wt. % for both types of blends. These showed a single glass transition temperature across the whole composition range, indicating that there is intimate mixing of the two components. IR analysis indicated hydrogen bonding interactions between the hydroxyl groups of the PVA and the carbonyl groups of the poly(2-ethyl-2-oxazoline). Thermogravimetric analysis showed, that the interaction of the two polymers as well as the good thermal stability both lead to an improved stability of the PVA blends. Subsequent work detailed the synthesis of chitin derivatives containing amphiphilic di-block copolymers, such as chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] as well as the corresponding [poly(2-methyl-2-oxazoline)-*block*-poly(2-butyl oxazoline)] and [poly(2-methyl oxazoline)-*block*-(tert-butyl oxazoline)] polymers using the reaction procedure outlined above.[83] Studies of the solution behavior of chitin-*graft*-[poly(2-methyl-2-oxazoline)-*block*-poly(2-phenyl-2-oxazoline)] showed that the polymer forms micelles in aqueous solution above the critical micellar concentration (0.01 wt % to 0.02 wt %). Cryogenic temperature transmission electron microscopy (Cryo-TEM) of 0.5 wt % solutions of the polymer revealed globular structural units with diameters of about 40 nm as well as cylindrical aggregates of approximately the same diameter and a length of between 80 and 200 nm. The aggregates could furthermore be shown to

complex small molecules such as pyrene and magnesium 1-anilinonaphtalene-8-sulfonate (ANS).

The ease of synthesis of amphiphilic polyoxazoline-based block copolymers and their easy self-assembly into micelles[84] and other vesicles make them attractive objects for the formation of nanocontainers for a number of different applications. One such application is the polymer-assisted mineralization of calcium ions, which is used by nature to design biological composite materials. Giant containers prepared from [poly(2-methyl-2-oxazoline)-*block*-polydimethylsiloxane-*block*-poly(2-methyl-2-oxazoline)] (PMOXA-PDMS-PMOXA) have been used as completely synthetic vesicles for the precipitation of calcium phosphate.[85] The vesicles were prepared by phoresing a thin film of the PMOXA-PDMS-PMOXA polymer, deposited on conductive glass or platinum electrodes in phosphate buffer, leading to vesicles with phosphate ions in the core. Subsequent incubation of the latter in a calcium chloride solution in the presence of an ionophore led to the precipitation of calcium phosphate inside the vesicle over a period of time (Figure 6).

Work by Meier *et al*. demonstrated the feasibility of generating giant free-standing membranes, composed of the PMOXA-PDMS-PMOXA polymer.[86] Self-assembly of the polymer was used to generate membranes with an average thickness of 10 nm, which is significantly larger than most lipid membranes. Moreover, charge pulse experiments showed, that the polymeric membranes are significantly more cohesive than lipid membranes: the voltages required for membrane rupture are significantly larger than those usually needed for lipid membranes. If a telechelic PMOXA-PDMS-PMOXA polymer, functionalized by methacrylate groups is used, this effect can be amplified even further by UV cross-linking of the methacrylate endgroups, which causes a covalent stabilization of the membrane.

Given the high stability of synthetic polymer membranes, channels need to be created in order to make them permeable for ions or small molecules. One possible strategy is the inclusion of porins, transmembrane proteins that allow passive diffusion of molecules into the membranes. Meier and colleagues studied the inclusion of OmpF and maltoporin in membranes of telechelic PMOXA-PDMS-PMOXA, functionalized with methacrylate end groups.[87] These porins form trimeric channels in the outer membranes of gram-negative bacteria. The proteins can be integrated into the membrane by depositing drops of a stock solution on top of a pre-formed membrane, followed by the application of a current. The successful incorporation of the protein into the synthetic polymer could be shown by conductance measurements across the membrane. These indicated, that although the synthetic polymer membrane is significantly thicker than the lipid membranes occurring in living cells, the porins can be functionally reconstituted and the conformation of the protein is not significantly influenced. When the methacrylate groups were photopolymerised to stabilize the membrane, the conductivity decreased, which was interpreted to indicate the closure or expulsion of some of the porins from the membrane due to a reorganization and tighter packing of the polymer.

This work was subsequently expanded to nanocapsules, prepared from the same polymer (Figure 7).[88] The capsules were prepared by first casting a film of a methacrylate-capped PMOXA-PDMS-PMOXA polymer, followed by addition of a solution of the OmpF porin. Subsequently, the protein/polymer film was dispersed in buffer and unilamellar capsules of homogenous sizes were obtained *via* rapid extrusion through polycarbonate filters. The capsules were stabilized by photopolymerisation of the methacrylate end-groups. Consistent with the results for the membrane system, experiments showed, that the porins incorporated into the

capsules maintained their activity, despite a mismatch in the hydrophilic/hydrophobic balance of the copolymer membrane system. When enzymes are incorporated into the capsule, the rate and selectivity of substrate penetration into the capsule and thus enzyme reaction kinetics could be controlled. This was demonstrated by incorporating β-lactamase into the nanocapsules and monitoring the rate of hydrolysis of ampicilin, which is a β-lactam antibiotic. The results indicate, that the relative activity of the nanocapsules increases linearly with an increasing concentration of protein channels.[89]

A closer examination of the interaction between PMOXA-PDMS-PMOXA triblocks and lipids shows, that the formation of hybrid polymer/lipid nanocapsules is possible.[90] The formation of the composites is independent of the method used to prepare the nanocapsules (film hydration, dispersion, detergent removal) and the lipid distribution in the membranes is homogeneous both at low as well as high lipid/polymer ratios. Furthermore, a monomer exchange between polymersomes and liposomes was observed, which, again can give rise to possible mixed structures and can be used to stabilize pre-formed and loaded liposomes.

As already indicated in the discussion so far, the loading of polymeric nanocontainers with functional molecules is also of significant scientific interest. A recent study showed, that nanocontainers consisting of PMOXA-PDMS-PMOXA polymer can be charged with the small molecule fluorophore sulforhodamine B as well as a labeled avidin.[91] Fluorescence correlation and fluorescence cross-correlation spectroscopy subsequently revealed, that the containers have sizes ranging from 140 to 172 nm and that both the dye as well as the protein could be loaded into the containers. However, the determination of the loading of the containers with the small molecules is

challenging, as a significant difference between the experimentally determined and theoretically expected loadings, was observed. This could be ascribed to self-quenching of the dye molecules inside the container. By contrast, there was a good agreement between experimental and theoretical loadings for the avidin complex, with approximately 13 avidin molecules per sphere of 70 nm radius. Furthermore, the researchers prepared biotinylated nanocapsules by mixing the PMOXA-PDMS-PMOXA polymer with the biotin-substituted analogue. The intrinsic binding constant of streptavidin to the labeled nanocapsules was determined to be $1.7 \pm 0.4 \times 10^{-8}$ M, with approximately $1921 \pm 357$ streptavidin molecules bound to each nanocontainer.

So far, all of the discussed vesicles were loaded during the preparation/assembly phase. However, the feasibility of "post-loading" pre-formed containers using bacteriophages has also been demonstrated.[92] Apart from facilitating the transport of maltose and maltodextrin across cell membranes, maltoporin (LamB) also serves as a receptor for the λ-phage. When incorporated into the walls of a PMOXA-PDMS-PMOXA nanocontainer, the phages are clearly able to bind to the porin embedded in the vesicle walls (Figure 8). Furthermore, experiments using fluorescently labeled dye interacting with DNA have shown that the phage can translocate DNA across the synthetic membrane. The nanoparticles are stable and protect the encapsulated material from degradation by DNAse.

One problem generally encountered when attempting to reconstitute membrane proteins in synthetic membranes by essentially random incorporation, is the question of their orientation. Most membrane proteins have distinct domains, depending on whether they are in contact with the extracellular medium or the cytoplasm. When reconstituting proteins in synthetic membranes through simple mixing of protein and

polymer (as discussed so far), the orientation of the membrane proteins is essentially random. This, in turn, means that only a fraction of the porins incorporated in such systems is functional and active.

This problem has been addressed by preparing both a symmetric PMOXA-PDMS-PMOXA as well as asymmetric poly(ethylene oxide)-*block*-poly(dimethyl siloxane)-*block*-poly(2-methyl-2-oxazoline) (PEO-PDMS-PMOXA) polymers in an attempt to induce a directed insertion of proteins into a polymer membrane.[93, 94] The latter was prepared in two forms containing both a large ($PEO_{25}$-$PDMS_{40}$-$PMOXA_{110}$) and a small ($PEO_{67}$-$PDMS_{40}$-$PMOXA_{45}$) poly(2-methyl-2-oxazoline) block. Upon dissolution in water, the polymers form vesicles, with the hydrophobic block being covered by the hydrophilic blocks on both sides of the vesicle wall. The more voluminous hydrophilic block is usually located on the outside of the vesicle. The $PEO_{25}$-$PDMS_{40}$-$PMOXA_{110}$ polymer, therefore, gives rise to an ABC motive, whereas the $PEO_{67}$-$PDMS_{40}$-$PMOXA_{45}$ triblock results in a CBA orientation. Aquaporin 0, labeled with a histidine tag on its amino terminus, was subsequently embedded into all of the capsules.[94] To determine the amount of incorporated protein as well as the proteins' orientation, antibodies targeting the histidine residue were used. This allowed the determination of the proteins' orientation, as the amino residue would normally be located in the cytoplasm, *i.e.* on the inside of the cell, under physiological conditions. Incubation of the polymer/protein conjugates derived from the symmetric PMOXA-PDMS-PMOXA with antibodies reveals a statistical incorporation of the aquaporin into the vesicles, as an equal distribution of histidine tags at the outer and inner surface of the vesicle wall was observed. The ABC motif, by contrast, induces a "physiological" orientation of the aquaporins, with approximately 80% of the histidine labels on the inside of the vesicle (corresponding to the cytoplasm in real

cells). The CBA motif results in a “non-physiological” orientation of the protein, with approximately 70 % of the label being located on the outside of the vesicle. In this way, the authors have clearly demonstrated, that breaking the symmetry of the membrane system results in a directed orientation of the porin protein into the membrane, which is consistent with results obtained for natural membranes.[95]

The behavior of Langmuir-Blodgett films of the PMOXA-PDMS-PMOXA polymer at two different lengths and with or without the inclusion of alamethicin was subsequently reported.[96] Experiments showed, that the block lengths play an important role in the organization of polymers in Langmuir-Blodgett films with the larger polymers having greater flexibility and therefore access to a larger number of conformations, which allow it to accommodate hosts such as the alamethicin peptide in the membrane with greater ease than would be the case for shorter polymers. Furthermore, it was observed that alamethicin promotes expanded phases of the co-polymer membranes, giving rise to partial polymer/peptide miscibility.

Further evidence for the observation that membrane proteins can be incorporated into synthetic structures without compromising their ability to self-assemble and maintain fluidity and function was provided by Schmidt and co-workers.[97] The researchers created artificial membranes of the PMOXA-PDMS-PMOXA triblock copolymer of defined thickness (5.7 nm) and subsequently incorporated α-haemolysin, OmpG and alamethicin. Conductance measurements revealed, that the polymer membranes have seal resistances of tens of giga-ohms (GΩ). Furthermore, the conductance of single channels is reduced for proteins in synthetic polymer membranes with respect to polymers in lipid membranes, which could be explained by the greater cohesion of the

polymer systems. The voltage gating ability as well as the thresholds of the voltage gated channels were similar for both lipid and polymer membranes.

Recently, micellar systems composed of poly(2-ethyl-2-oxazoline)-*block*-poly(ε-caprolactone) (PEOXA-PCL) or –poly(L-lactide) have become of interest. Jeong and colleagues reported the preparation of PEOXA-*block*-PCL micelles from a number of different organic solvents in an effort to determine the influence of the organic solvent on micelle formation.[98] The experiment established a correlation between the Hansen solubility parameter of the organic solvent and both the size of the micelle as well as the rigidity of its core. In particular, the polar contribution to the solubility parameter seemed to be the dominating factor. It was found that the higher the polar component, the lower the solubility of the amphiphilic polymer and the smaller the micelle sizes. Furthermore, an increasing polar contribution also led to increasing rigidity of the micellar core.

### 2.2.2 Multi-compartment systems

Although micelles and self-assembled nanocontainers are fascinating objects and the subject of much past and ongoing work,[99] they are intrinsically limited in the sense that they create only one core environment inside one surface. One of the reasons, biological systems are successful at carrying out complex tasks is the fact that they are essentially granular, *i.e*. they consist of separate but cooperative subdomains, which accomplish specialized tasks. To approximate this phenomenon in synthetic systems, Ringsdorf proposed the concept of “multicompartmentation” in the mid-1990ies.[100] However, only very little work has been carried out so far, with most of the reports focusing on either surfactants[101, 102] or polysoaps.[102] As far as

polyoxazolines are concerned, Nuyken *et al.* reported the synthesis of a two-compartment micellar system based on the self-assembly of fluorocarbon and hydrocarbon endcapped polyoxazolines.[103] The authors produced ABC triblock copolymers, consisting of a well-defined perfluoro-octyl group, a poly(2-methyl-2-oxazoline) block and a hydrocarbon end group of varying length, ranging from 6 to 18 carbon atoms. Using a number of experimental techniques, it was demonstrated, that at low concentrations the polymers exist as unimers, which assemble into micelles above the critical micellar concentration. The micellar core of these systems consists of the hydrocarbon end-groups. At higher concentrations, the fluorocarbon endgroups start to associate into oligomers and ultimately into a network of star-like micelles, such that micelles containing a hydrocarbon and a fluorocarbon core are present (Figure 9). Furthermore, the degree of association increases linearly with the concentration of the polymer in solution. A further study expanded this work by investigating the size and shape of the micelles formed by this polymer system, using a combination of small-angle X-ray scattering (SAX), analytical ultracentrifugation, surface tension measurements and isothermal titration calorimetry.[103] Polymers of varying size (degree of polymerisation n = 35, 57, 72 w.r.t. poly(2-methyl-2-oxazoline)) were investigated. The polymer was found to0 form cylindrical micelles of different radii (3.0 nm for n = 35, 3.8 nm for n = 57, 40 nm for n = 72) and of approximately 20 nm length. Micelles prepared from the two longer polymers can be doped with 1,4-diiodofluorobutane, which the authors interpret as evidence for the formation of distinct fluorocarbon domains.

## 2.3 Poly(oxazoline)-based Vectors

The delivery of DNA and RNA into cells is a highly topical and non-trivial problem and has been the subject of ongoing research for the last 20 years. A number of attempts have been made to use polymers for the delivery of functional DNA.[104, 105] While synthetic vectors generally have a much lower transfection efficiency than, for example, a virus, their use is nevertheless attractive, given their usually well-defined chemistry, the significant amount of available molecular diversity and the absence of problems that are concomitant with the use of natural transfection agents. Polymer vectors are typically cationic and form polyplexes with anionically charged nucleic acids. Commonly used polymers are poly(L-lysine),[106] polyethylene imine,[107] polyamido amine dendrimers[108] and chitosan.[109] Amongst these, polyethylene imine (PEI) is by far the most popular, due to its high charge density – every third atom is a nitrogen atom, which can be protonated. While PEI is normally produced *via* the acid-catalyzed polymerisation of aziridine, which gives rise to highly branched species,[110] a number of papers have recently reported the preparation of the polymer starting from poly(oxazolines). An example of this is the synthesis of a linear poly(ethylene glycol)-*block*-poly(ethylene imine) *via* a polyoxazoline.[111] In a first step of the synthesis, a heterotelechelic poly(ethylene oxide) polymer, functionalized with an acetal group on one end and a sulphonate group on the other, was prepared. This polymer was subsequently used as a macroinitiator in the cationic ring-opening polymerisation of 2-methyl-2-oxazoline, to give an Acetal-PEG-PMOXA polymer (Figure 10), which, in a final step was hydrolyzed using strong aqueous base to give the desired Acetal-PEG-PEI species. NMR spectroscopy showed a complete disappearance of the methyl group of the poly-2-oxazoline side chain after hydrolysis, indicating that the reaction is in essence

complete or at least proceeds to high conversion. Soon after, Park *et al*. described the synthesis of random co-polymers of poly(2-ethyl-2-oxazoline)-*co*-poly(ethylene imine) through partial acid hydrolysis of poly(2-ethyl-2-oxazoline).[112] Varying the acid concentration afforded control over the degree of hydrolysis of the polymer and therefore the charge density on the backbone. It could be shown that the partially hydrolyzed poly(2-ethyl-2-oxazoline) formed very compact complexes with DNA and that the condensation capacity of the polymer was suitable for transfection. The cell cytotoxicity was found to depend on the charge density, the molecular weight and the degree of branching. Generally speaking, polymers with lower charge densities were less cytotoxic than those with higher ones and branched polymers were found to be significantly more cytotoxic than linear ones. High molecular weights, too, gave rise to high cytotoxicities, which is consistent with previous observations for PEI.[113] The transfection efficiency was highest for polymers with an 88 % degree of hydrolysis and was found to be comparable to that of commonly used PEI. The manufacture of polyethylene imines *via* the partial hydrolysis of polyoxazolines is therefore highly desirable, as it leads to well-defined and benign linear polymers as opposed to branched and cytotoxic species normally obtained from the aziridine synthesis route.

Partial acid hydrolysis of poly(2-ethyl-2-oxazoline) was also used to prepare polymer/DNA nanoparticles based on folate-poly(ethylene imine)-*block*-poly(L-lactide) copolymers.[114] Folate receptors are normally overexpressed on tumor cells and it was hoped that folic acid substitution on the polymer will lead to an increase in specific targeting. Poly(lactic acid) was added to the polymer for improved biocompatibility and availability. The block copolymer forms polyplexes with DNA at a polymer/DNA ratio (PD ratio) of about 10. AFM experiments showed, that the

complexes are spherical particles of approximately 100 nm size. *In vitro* transfection and cell viability studies indicated, that polymers containing high amounts of poly(lactic acid) showed low transfection efficiencies. However, it was also found, that folate-PEI-PLLA polymers are less cytotoxic than pure PEI.

A further study by Kilbanov et al showed, that commercial PEI contained significant amounts of residual N-propionyl groups, which are only incompletely removed during the manufacturing process. Amide groups are generally non-basic; their presence should therefore reduce the condensation ability of DNA. This is supported by the results of titration experiments and fluorescence spectrometry using ethidium bromide displacement assays. When commercial products were fully deacetylated, a significant increase in both DNA binding ability and transfection efficiency were observed. PEIs produced *via* the hydrolysis of poly(2-ethyl-2-oxazoline) was found to give even better results than the deacetylated commercial product, thus underlining the importance of the linearity of the polymer.

Low molecular weight PEI-PEG-PEI triblock co-polymers were also synthesized from the corresponding PMOXA-PEG-PMOXA polymers, *via* acid hydrolysis.[115] The PEG block was introduced in a bid to reduce cytotoxicity of the PEI as well as to improve the colloidal stability of the polymer/DNA polyplexes, similar to the introduction of poly(lactic acid) in the example above. When screened w.r.t. transfection efficiency *in vitro*, the triblock showed transfection efficiencies similar to those of high-molecular weight commercial polyethylene imine together with a decreased cytotoxicity.

Guis *et al.* subsequently reported the synthesis of high molecular weight poly(ethylene glycol-*block*-2-ethyl-2-oxazoline-*co*-2-methyl-2-oxazoline).[116] The synthesis of this species was achieved by random polymerisation of 2-methyl-2-oxazoline and 2-ethyl-2-oxazoline using a tosylated poly(ethylene glycol) macroinitiator. This gave rise to polymers with degrees of polymerisation of around 500 in the PEOXA block. The material was subsequently hydrolyzed using alkaline hydrolysis to give large linear PEG-*block*-PEI polymers. Transfection essays showed that the polymer has good gene transfer efficiencies, which is consistent with the results discussed above.

While all studies presented so far have only used poly(oxazolines) as a precursor material for the synthesis of linear poly(ethylene imine), only one paper has considered the use of unmodified poly(oxazolines) in gene transfer applications. Hsiue *et al.* describe the synthesis of poly(2-ethyl-2-oxazoline)-*block*-(polyethylene imine) using a convergent route (Figure 11).[117] The poly(2-ethyl-2-oxazoline) block was prepared *via* conventional cationic ring-opening polymerisation and linear poly(ethylene imine) *via* partial hydrolysis of PEOXA. The target polymer is then formed via a thiol-disulphide exchange reaction. A polymer/DNA weight ratio of 12 is required to form stable polyplexes of a mean diameter of 190 nm. Furthermore, it was observed that the DNA binding ability of the PEI fragment increased with increasing degrees of hydrolysis, which is consistent with previous results. The polyplexes are pH sensitive and transfection efficiency assays as well as cytotoxicity assays show that these polymers have low toxicity and high transfection efficiency *in vitro*.

## 2.4 Stimuli-responsive Systems

By definition, stimuli responsive polymers show large changes in their properties in response to small changes in their environment and can be used for the development of smart drug delivery systems. Typical stimuli include temperature,[118] pH,[119] electric[120] and magnetic fields,[121] light[122, 123] and concentration. Polymers usually respond with changes in chemical, mechanical, electrical, optical, shape, surface and permeability properties as well as with phase separation effects.

An early example of stimulus-responsive polyoxazolines are poly(2-ethyl-2-oxazoline)-*block*-poly(ε-caprolactone) (PCL) or poly(2-ethyl-2-oxazoline)-*block*-poly(L-lactide) copolymers.[124] When placed in water, the polymers form micelles with critical micellar concentrations in the range of 1.0 – 8.1 mg/L. The micelles have an outer shell of hydrophilic poly(2-ethyl-2-oxazoline) which, at pH values below 3.5, forms a hydrogen-bonded complex with poly(methacrylic acid), which precipitates out of solution. The complexes were found to be stable over several month. When the pH is raised above 3.8, the micelles can be redispersed. Similar behaviour was subsequently observed for analogous polymers containing poly(1,3-trimethylene carbonate) blocks.[125] Furthermore, PEOXA-*block*-PCL is water insoluble, but can be swollen in water and retains its shape, thus showing the typical characteristics of a hydrogel.[126] Swollen gels show two phase transitions: the first transition takes place upon heating from room temperature to a temperature range from about 30 to 35 °C, indicated by an increasing transmittance of the gel and a second one in the range between 36 to 45 °C, which causes a decrease in transmittance. Furthermore, the swelling behavior of the gels itself is temperature dependent, with gels showing reversible thermonegative swelling. At 15 °C, the observed swelling ratios were

higher than at 35 °C. These observations can be interpreted in terms of the relative hydrophilicities/hydrophobicities. At low temperatures, the swelling is entirely driven by hydration of the hydrophilic PEOXA block *via* hydrogen bonding with water. As the temperature increases, hydrogen bonding is weakened, while hydrophobic interactions within the gel (arising from the poly(caprolactone) moiety) increase. Furthermore, the block size of the PEOXA could be shown to have an effect on the swelling ratio: increasing the block size also led to an increase in swelling ratio. This affords control over the swelling behavior *via* the PEOXA block length. Dried gels and hydrogels of copolymers prepared from poly(caprolactone) blocks of $M_n$ = 2500 showed a maximum tensile strength in the range of 10.6 to 12.5 MPa in the dried state and 3.2 to 7.3 MPa in the swollen state and an ultimate elongation in the range of 880 – 930 % (dried) and 320 – 1000 % (swollen). Hydrogels, which have experienced a temperature cycle, were found not to retain these materials properties, presumably due to a loss of crystallinity in the poly(caprolactone) domain.

In order to investigate the micellization as well as the phase behavior of poly(2-ethyl-2-oxazoline)-*block*-poly(caprolactone) in greater detail, a number of PEOXA-PCL diblock and PEOXA-PCL-PEOXA triblock copolymers were synthesized.[127] Critical micellar concentrations were found to be in the range of 4.6 to 35.5 mg/L for the diblocks and 4.7 to 9.0 mg/L for the triblocks, depending on the PCL block lengths. Aqueous solutions of the both the di- and triblocks exhibited thermally reversible sol-gel transitions. The temperature, at which the transition as well as the precipitation from sol occurred, is dependent on the PCL block length. Furthermore, the addition of inorganic salts was shown to have an effect on the transition temperatures.

The addition of sodium chloride to an aqueous solution of the polymer resulted in a shift of the phase transition temperature to a lower regime, which leads to a lower solubility. Addition of sodium thiocyanate was found to have the opposite effect: thiocyanate anions accumulate at the polymer/water interface,[128] which moves the stability region of the gel and the cloud point to a higher temperature, effectively leading to a solubility increase. Furthermore, the addition of saccharides also led to a depression of the transition temperature, as did the addition of carboxylic acids.

The addition of (multifunctional) carboxylic acids was found to induce aggregation in aqueous solutions of PEOXA-PCL copolymers.[129] As the molar concentration ratios of COOH groups with respect to poly(2-ethyl-2-oxazoline) repeat units is increased, micelles formed by the PEOXA-PCL polymer in aqueous solution start to agglomerate, leading to large aggregates with sizes of between 96 and 146 nm. If the pH value of the system is subsequently increased, the agglomerations can be destroyed and the polymers redispersed as micelles. However, micelle release is not only dependent on the pH of the medium, but also on the molecular weight of the polycarboxylic acid (polycarboxylic acids with higher molecular weight show slower release than those of smaller molecular weight) as well as the chemical structure of the polycarboxylic acid. When taken together with the fact that the release of micelles from the complexes proceeds over the space of several hours, it is easy to imagine, that this has potentially interesting applications in drug delivery.

Micelles formed by PEOXA-PCL have also been investigated as carriers for paclitaxel, an anti-cancer drug.[130] Micelles with paclitaxel loading contents of between 0.5 – 7.6 wt % could be prepared using a dialysis method,[131] with the loading being dependent on the block composition of the polymers, the organic solvent used in the dialysis experiment and the weight ratio of drug to polymer. In

experiments, the toxicity of the polymer was found to be insignificant when compared to other matrices such as Tween 80 and comparable to Cremophore EL (polyoxyethylated castor oil, CreEL). Paclitaxel containing micelles were shown to inhibit the growth of KB cells (cell line derived from a human carcinoma of the nasopharynx) to the same extent as comparable CreEL formulations (but with reduced toxicity of the carrier).

Hsiue *et al*. reported the synthesis of a series of hydrogels based on poly(2-ethyl-2-oxazoline) and three-armed poly(D,L-lactide).[132] The polymers were prepared *via* photochemical cross-linking of three-arm poly(D,L-lactide-trimethacrylate) (3PLA-TMA) and poly(2-ethyl-2-oxazoline) dimethacrylate (PEOXA-DMA) (Figure 12). All of the resulting hydrogels were sensitive to both pH as well as temperature stimuli and showed a high degree of water retention. Consistent with the corresponding linear systems (see above), gels derived from the crosslinked polymer show thermonegative swelling behavior and low swelling at low pH. Scanning electron micrographs showed, that the degree of cross-linking has a significant effect on the particle and pore size. Polymers consisting only of cross-linked polyoxazoline give rise to uniformly spherical particles and porous structures. Introduction of the poly(caprolactone) units lead to smaller particles in the case of low PCL content and to uniform surfaces without discernible pores at higher concentrations.

The photochemical crosslinking of telechelic PEOXA bearing acrylate groups on both chain ends leads to networks, which have a highly soluble component.[133] The observed cloud point for the cross-linked species is, as expected, somewhat lower than that for a completely linear PEOXA of similar molecular weight. When attempting to reduce the amount of soluble fraction *via* the addition of an equimolar amount of ethyleneglycol dimethacrylate with respect to the macromonomer, a

hydrogel with a significantly decreased cloud point (when compared to the pure PEOXA gels) was obtained. Furthermore, significantly different swelling behaviors were observed. In a further attempt to control the LCST behavior, the researchers prepared segmented networks by free-radical copolymerisation of PEOXA macromonomer with hydroxyethyl methacrylate (HEMA), 2-hydroxypropyl acrylate (HPA) or methyl methacrylate (MMA). As a general rule, the LCST behavior of the resulting networks depends on the hydrophilicity/hydrophobicity of the co-monomer and the fraction of PEOXA present in the networks.

Like the cross-linked systems described above, linear poly(L-lactide)-*block*-poly(2-ethyl-2-oxazoline)-*block*-poly(L-lactide) polymers show thermonegative behavior in aqueous solution and even precipitate from solution in the temperature range between 33 to 38 °C.[134] The latter is explained by a combination of dehydration of the PEOXA segments and the aggregation of the hydrophobic PLLA segments. Furthermore, acid-base titrations indicated, that the triblocks show considerable buffering capacity over the whole pH range. At room temperature, they are protonated and exist as polycations, which could make them potentially suitable candidates for gene delivery applications.

PLLA-*block*-PEOXA-*block*-PLLA has also been shown to be a promising candidate for the delivery of the anti-cancer drug doxorubicin (DOX).[135] In aqueous solution the polymer forms flower-like micelles, which can be loaded with the DOX active ingredient. At physiological pH (7.4) and *in vitro*, the release of doxorubicin is essentially suppressed, while at pH 5 the drug is released from the micelles, due to micellar deformation: micelles are taken up by cells *via* endocytosis with the endocytosed particles usually being transported to the lysosome. V-ATPases subsequently pump protons into the particle, which lowers the pH to 5.[136] As

PLLA-*block*-PEOXA-*block*-PLLA is pH sensitive, this leads to a deformation of the micellar structure and the release of DOX inside the cell, which causes apoptosis. PLLA-*block*-PEOXA-*block*-PLLA is both biocompatible and shows virtually no cytotoxicity even at high concentration, thus allowing for high doses of the polymer to be administered. The somewhat easier-to-prepare PLLA-*block*-PEOXA diblock copolymer showed very similar behavior, although it was found to be more cytotoxic than the corresponding triblock as well as showing a higher release of doxorubicin at physiological pH.[137] PLLA-*block*-PEOXA-*block*-PLLA has recently been shown to be degradable by proteinase K.[138]

Poly(2-isopropyl-2-oxazoline) (PIOXA), whose repeat unit is isomeric to that of poly(*N*-isopropyl acrylamide), has also attracted attention as a smart material. Like the other polyoxazolines it shows a thermonegative behavior in aqueous solution and undergoes reversible phase separation in the temperature range from 45 to 63 °C, depending on the molecular weight of the polymer. Differential scanning calorimetry showed that the phase transition is endothermic, with the enthalpy of transition ranging from 1.51 kJ/mol to 5.64 kJ/mol depending on molecular weight.[139] Consistent with previous observations, the presence of sodium chloride ions lowers the phase transition temperature and increases the transition enthalpy. Pressure perturbation calorimetry demonstrates the extreme sensitivity of the solvation volume w.r.t. the polymer chain length, nearly doubling in value for a polymer with a DP of 50 compared with a poly(2-isopropyl-2-oxazoline) oligomer with a DP of 17. This, in turn, indicates that the number of hydrogen-bonding positions along the chain increases with increasing molecular weight. Further studies using telechelic and heterotelechelic poly(2-isopropyl-2-oxazolines) end-functionalized by hydroxy, amine or acetal groups furthermore show that the cloud points of these polymers are

highly concentration dependent.[140] However, when comparing the cloud point temperatures for 1.0 M solutions of acetal-PIOXA-OH ($M_n$ = 9,600, $M_n/M_w$ = 1.15) with that of Me-PIOXA-OH ($M_n$ = 9,700, $M_n/M_w$ = 1.02), nearly identical values are found, indicating that under these circumstances, the molecular weight is the determining factor for the thermal response of these polymer.

Further work by Kataoka *et al*. showed, that the LCST of amphiphilic poly(2-isopropyl-2-oxazoline) can be accurately tuned by copolymerisation with a hydrophilic 2-ethyl-2-oxazoline comonomer.[141] The researchers prepared simple gradient copolymers of the two components in such a way as to gradually decrease the ethyl oxazoline content along the chain, while increasing the isopropyl oxazoline content from the α-terminal to the ω-terminal chain end. Experiments investigating the thermosensitive behavior of the resulting gradient copolymers showed, that the LCST increases linearly with the mole percentage of ethyl oxazoline over a broad temperature range from 38.7 to 67.3 °C. Consistent with previous observations, the presence of sodium chloride lowers the LCST temperature. The tuning of LCST behavior *via* compositional variation has also been demonstrated for poly(chloromethylstyrene-*co*-*N*-isopropylacrylamide-*graft*-2-alkyl-2-oxazoline) polymers.[142] The materials were synthesized by cationic ring-opening polymerisation of 2-methyl or 2-ethyl-2-oxazoline initiated by random copolymers of chloromethylstyrene and *N*-isopropyl acrylamide, acting as macroinitiators. The polymers showed transition temperatures in the range of 28 to 40 °C, with the increase in the observed transition temperature being proportional to the amount of alkyl oxazoline units in the side chain, relative to the amount of NIPAAm units in the main chain, provided the same macroinitiator is used. The observed transition can be explained in terms of the initial intramolecular collapse of the polymer backbone,

followed by an intermolecular aggregation of the polymer as the solution approaches the transition temperature, ultimately leading to precipitation of the polymer. If the content of long polyoxazoline grafts is sufficiently high, stabilized aggregates with thermoresponsive cores are formed at the transition temperature and no precipitation is observed.

Recently, Schlaad and Meyer reported the synthesis of poly(2-isopropyl-2-oxazoline)-*block*-poly(L-glutamate) in an effort to combine the temperature sensitive properties of the poly(2-isopropyl-2-oxazoline) with the pH sensitivity of the poly(L-glutamate).[143] Unfortunately, no data on the stimulus-sensitive behavior of these polymers is currently available. In a similar fashion, Winnik and colleagues report the synthesis of poly(2-ethyl-2-oxazoline)-*block*-hyaluronan copolymers and speculate that these should have temperature-sensitive properties. However, no actual data has been provided.[144]

### 2.5 Antimicrobial polymers

Antimicrobial polymers are becoming increasingly important materials in the face of spreading microbial infections and increasing microbial resistance to antibiotics[145] and research in this area has been conducted since the late 1970ies. Most systems tend to be based on quarternary ammonium salts[146] which are covalently attached to a polymer, although biguanide,[147] phosphonium[148] and sulphonium[149] salts have also been used. The proposed mode of action of all of these cationic species is a disruption of the cytoplasmic membrane, which leads to a release of potassium ions as well as constituents of the membrane and subsequent cell death.

Waschinski *et al*. recently reported the synthesis of a series of PMOXA and PEOXA polymers, terminated with quarternary ammonium groups.[150] The polymers were prepared *via* standard cationic ring-opening polymerisation and terminated using a series of *N*-alkyl-*N,N*-dimethyl amines as well as pyridine. The materials were subsequently evaluated w.r.t. their antimicrobial properties by determining the minimal inhibitory concentration against *Staphylococcus aureus*. The screening showed, that only poly(2-methyl-2-oxazoline)-based polymers containing alkyl ammonium functions with alkyl chains of twelve carbon atoms or longer have any antimicrobial activity – hydroxy or pyridyl functions at the chain end were found to be inactive. Furthermore, the antimicrobial activity of the polymers appeared to be independent of the molecular weight of the materials. A pronounced effect of the head group on antibacterial properties was also observed: polymers containing a proton as the headgroup and a dodecyldimethyl ammonium end group were found to be less bactericidal than the analogous polymer with a methyl headgroup. Poly(2-methyl-2-oxazoline) containing a BOC-protected $NH_2$ headgroup, by contrast, showed very high antimicrobial activities, although this effect is not observed in poly(2-ethyl-2-oxazoline)-based polymers.

A closer investigation of the influence of the end-groups subsequently revealed, that headgroups consisting of simple alkyl chains of between 4 and 10 carbon atoms in length are most effective in increasing the antimicrobial properties of the ammonium-functionalized polymers.[151] To explain this effect, the authors hypothesize that the polymers exist as unimolecular micelles in solution below the critical micellar concentration. If this is the case, the endgroups of the polymer would be aggregated and could collaboratively penetrate the cell wall at the same point, which could be

more disruptive than the insertion of a single ammonium group. However, more work is required to elucidate the observed effect.

## 3. Summary and Conclusion

Polyoxazoline-based or polyoxazoline-derived polymers clearly have a significant application potential in a large number of technological contexts, whether this be the formation of stealth liposomes, or of membrane structures and containers which allow the incorporation of functional proteins, thus mimicking natural systems, or whether it is the use of polyoxazoline-based polymers as carriers of drugs or as synthetic vectors and antimicrobial materials. When this broad application range is coupled with properties such as responsiveness to external stimuli, this class of polymers becomes a prime candidate for use in "smart" materials. Furthermore, the fact, that poly(oxazolines) can be prepared *via* living polymerisation processes, affords extraordinary control and definition, a factor that is tremendously important, particularly when dealing with regulatory authorities.

However, while the research literature concerning the fundamental properties and applications of polymers such as poly(ethylene oxide) or poly(ethylene imine) in biological application contexts is vast, polyoxazolines are only now beginning to be explored by the scientific community. Furthermore, the structural variation encountered in the research literature so far is small and usually limited to polymers, which can be derived from commercially available 2-oxazolines (2-methyl, 2-ethyl, 2-isopropyl, 2-phenyl). However, the synthesis of 2-substituted oxazoline monomers is comparatively straightforward. This should provide polymer chemists with tremendous opportunity, as the accessible chemical space should be significantly

larger than the one, which has been explored so far. The research presented in this review already indicates that poly(oxazolines) are in many cases equivalent to or even exceed more traditionally used polymers, in areas such as solubility control, toxicity etc.. One might therefore speculate that a further exploration of "poly(oxazoline) chemical space", particularly in combination with other classes of polymers, might lead to interesting new materials and could hold a great deal of promise.

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**References**

[1] A. Levy, Polymerisation of cyclic imino ethers III. Effect of ring substituents. J. Polym. Sci., Part A: Polym Chem. 6 (1968) 57-62.

[2] A. Levy, M. Litt, Polymerisation of cyclic iminoethers. V. 1,3-Oxazolines with hydroxy-, acetoxy-, and carboxymethyl-alkyl groups in the 2 position and their polymers. J. Polym. Sci., Part A: Polym Chem. 6(7) (1968) 1883-1894.

[3] M. Litt, A. Levy, J. Herz, Polymerisation of cyclic imino ethers. X. Kinetics, chain transfer, and repolymerisation. J. Macromol. Sci., Chem. A9(5) (1975) 703-727.

[4] D.A. Tomalia, D.P. Sheets, Homopolymerisation of 2-alkyl- and 2-aryl-2-oxazolines. J. Polym. Sci., Part A: Polym Chem. 4 (1966) 2253-2265.

[5] T. Bassiri, A. Levy, M. Litt, Polymerisation of cyclic imino ethers I. Oxazolines. J. Polym. Sci., Polym. Lett. Ed. 5 (1967) 871-879.

[6] W. Seeliger, E. Aufderhaar, W. Diepers, R. Feinauer, R. Nehring, W. Thier, H. Hellmann, Neuere Synthesen und Reaktionen cyclischer Imidsaeureester. Angew.Chem. 78 (1966) 913-952.

[7] T. Kagiya, S. Narisawa, T. Maeda, K. Fukui, Ring-opening polymerisation of 2-substituted 2-oxazolines. J. Polym. Sci., Part B: Polym. Phys. 4 (1966) 441.

[8] J.A. Frump, Oxazolines. Their preparation, reactions and applications. Chem. Rev. 71 (1971) 483-505.

[9] K.Aoi, M.Okada, Polymerisation of oxazolines. Prog.Polym.Sci. 21 (1996) 151-208.

[10] S. Kobayashi, Ethyleneimine polymers. Prog. Polym. Sci. 15 (1990) 751-823.

[11] T. Saegusa, S. Kobayashi, Polymerisation of cyclic imino ethers. Int. Rev. Sci., Phys.Chem. Series 2 8 (1975) 153-190.

[12] D. Delorme, Y. Ducharme, C. Brideau, C.C. Chan, N. Chauret, S. Desmarais, D. Dube, J.-P. Falgueyret, R. Fortin, J. Guay, P. Hamel, T.R. Jones, C. Lepine, C. Li, M. McAuliffe, C.S. McFarlane, D.A. Nicoll-Griffith, D. Riendeau, J.A. Yergey, Y. Girard, Dioxabicyclooctanyl naphthalenenitriles as nonredox 5-lipoxygenase Inhibitors: structure-activity relationship study directed toward the improvement of metabolic stability. J. Med. Chem. 38(19) (1996) 3951-3970.

[13] J.F. Warchol, C.D. Walton, Creping adhesives containing oxazoline polymer blends and use in paper product applications, US 97-795911 (1998).

[14] J.M.J. Frechet, K. Yui, Polymerizable polyoxazolines and hyperbranched polymers prepared them, US 96-660684 (1997).
[15] R.H.G. Brinkhuis, Hyperbranched ester-oxazoline polymers as binders for coatings, inks and adhesives, WO 2002-EP13898 (2003).
[16] S.-H. Ma, J.N. Rodriguez-Parada, Preparation of block copolymers of oxazolines and/or oxazines and their use in ink-jet inks as pigment dispersants, US 97-963839 (1998).
[17] A.M. Ansari, P.V. Scaria, M.C. Woodle, Polymers for delivering peptides and small molecules in vivo, WO 2003-US2710 (2003).
[18] R. Hoogenboom, M.W.M. Fijten, U.S. Schubert, Parallel kinetic investigation of 2-oxazoline polymerisations with different initiators as basis for designed copolymer synthesis. J. Polym. Sci., Part A: Polym. Chem. 42(8) (2004) 1830-1840.
[19] R. Hoogenboom, M.W.M. Fijten, U.S. Schubert, Parallel kinetic investigation of 2-oxazoline polymerisations utilizing different initiators as basis for designed copolymer synthesis. PMSE Preprints 90 (2004) 477-478.
[20] R. Hoogenboom, M.W.M. Fijten, C.H. Abeln, U.S. Schubert, Automated parallel investigations of polymerisation kinetics by online monitoring of GC and GPC. PMSE Preprints 90 (2004) 342-343.
[21] R. Hoogenboom, M.W.M. Fijten, U.S. Schubert, The effect of temperature on the living cationic polymerisation of 2-phenyl-2-oxazoline explored utilizing an automated synthesizer. Macromol. Rapid Commun. 25(1) (2004) 339-343.
[22] R. Hoogenboom, M.W.M. Fijten, C.H. Abeln, U.S. Schubert, High-throughput investigation of polymerisation kinetics by online monitoring of GPC and GC. Macromol. Rapid Commun. 25(1) (2004) 237-242.
[23] F. Wiesbrock, R. Hoogenboom, M. Leenen, S.F.G.M. Van Nispen, M. Van der Loop, C.H. Abeln, A.M.J. Van den Berg, U.S. Schubert, Microwave-assisted synthesis of a 42-membered library of diblock copoly(2-oxazoline)s and chain-extended homo poly(2-oxazoline)s and their thermal characterization. Macromolecules 38(19) (2005) 7957-7966.
[24] H. Huang, R. Hoogenboom, M.A.M. Leenen, P. Guillet, A.M. Jonas, U.S. Schubert, J.F. Gohy, Solvent-induced morphological transition in core-cross-linked block copolymer micelles. J. Am. Chem. Soc. 128(11) (2006) 3784-3788.
[25] R. Hoogenboom, F. Wiesbrock, H. Huang, M.A.M. Leenen, H.M.L. Thijs, S.F.G.M. Van Nispen, M. Van der Loop, C.-A. Fustin, A.M. Jonas, J.-F. Gohy, U.S.

Schubert, Microwave-assisted cationic ring-opening polymerisation of 2-oxazolines: a powerful method for the synthesis of amphiphilic triblock copolymers. Macromolecules 39(14) (2006) 4719-4725.

[26] F. Wiesbrock, R. Hoogenboom, C.H. Abeln, U.S. Schubert, Single-mode microwave ovens as new reaction devices: accelerating the living polymerisation of 2-ethyl-2-Oxazoline. Macromol.Rapid.Commun. 25(22) (2004) 1895-1899.

[27] M.H. Litt, B.R. Hsieh, I.M. Krieger, T.T. T.T.Chen, H.L. Lu, Low surface energy polymers and surface-active block polymers : II. Rigid microporous foams by emulsion polymerisation. J. Colloid Interface Sci. 115(2) (1986) 312.

[28] M.H. Litt, B.R. Hsieh, I.M. Krieger, T.T. Chen, H.L. Lu, Low surface energy polymers and surface-active block polymers. II. Rigid microporous foams by emulsion polymerisation. Journal of Colloid and Interface Science 115(2) (1987) 312-329.

[29] G.Cai, M.H.Litt, Preparation and Characterization of phenyl and undecyl oxazoline block copolymers. J. Polym. Sci., Part A: Polym Chem. 27(11) (1989) 3603-3618.

[30] M.H. Litt, C.S. Lin, Selective hydrolysis of oxazoline block copolymers. J. Polym. Sci., Part A: Polym Chem. 30(5) (1992) 779-786.

[31] J.E.McAlvin, C.L.Fraser, Metal-centered star block copolymers: amphiphilic iron tris(bipyridine)-centered polyoxazolines and their chemical fragmentation to bipyridine-centered BAB triblock copolymers. Macromolecules 32(5) (1999) 1341-1347.

[32] R.-H. Jin, Water soluble star block poly(oxazoline) with porphyrin label: a unique emulsion and its shape direction. J. Mater. Chem. 14(3) (2004) 320-327.

[33] R. Weberskirch, R. Hettich, O. Nuyken, D. Schmaljohann, B. Voit, Synthesis of new amphiphilic star polymers derived from a hyperbranched macroinitiator by the cationic "grafting from" method. Macromol. Chem. Phys. 200 (1999) 863.

[34] C. Lach, H. Hanselmann, R. Muelhaupt, Hyperbranched carbosilane oxazoline-macromonomers. Polymerisation and coupling to a trimesic acid core. Macromol. Rapid Commun. 19(9) (1998) 461-465.

[35] D. Christova, R. Velichkova, E. Goethals, F.E. du Prez, Amphiphilic segmented polymer networks based on poly(2-alkyl-2-oxazoline) and poly(methyl methacrylate). Polymer 43 (2002) 4585.

[36] O. Nuyken, R. Weberskirch, T. Kotre, D. Schoenfelder, A. Woerndle, Polymers for micellar catalysis. Polym. Mater. Org. Synth. Catal. (2003) 277-304.
[37] R. Jordan, A. Foertig, O. Purrucker, M. Tanaka, R. Gleixner, Tailored polymers for the construction of biomimetic cell membranes. Polym Prepr. 47(2) (2006) 197-198.
[38] H.-J. Choi, E. Brooks, C.D. Montemagno, Synthesis and characterization of nanoscale biomimetic polymer vesicles and polymer membranes for bioelectronic applications. Nanotechnology 16(5) (2005) S143-S149.
[39] M. Malmsten, Soft drug delivery systems. Soft Matter 2 (2006) 760-769.
[40] K. Osada, K. Kataoka, Drug and gene delivery based on supramolecular assembly of PEG-polypeptide hybrid block copolymers. Prog. Polym. Sci. 202 (2006) 113-153.
[41] G.J.R. Charrois, T.M. Allen, Drug release rate influences the pharmacokinetics, biodistribution, therapeutic activity, and toxicity of pegylated liposomal doxorubicin formulations in murine breast cancer. Biochim. Biophys. Acta 1663 (2004) 167-177.
[42] M. Przybylski, E. Fell, H. Ringsdorf, D.S. Zaharko, Pharmacologically active polymers, 17. Syntheses and characterization of polymeric derivatives of the antitumor agent methotrexate. Makromol. Chem. 179(7) (1978) 1719-1733.
[43] J. Khandare, T. Minko, Polymer-drug conjigates: progress in polymeric drugs. Prog. Polym. Sci. 31 (2006) 359-397.
[44] I. Stachurek, K. Pielichowski, Ethylene oxide-containing (co)polymers in controlled drug delivery. Archives Mater. Sci. 26(4) (2005) 303-327.
[45] C.M. Peterson, J.-G. Shiah, Y. Sun, P. Kopeckova, T. Minko, R.C. Straight, J. Kopecek, HPMA copolymer delivery of chemotherapy and photodynamic therapy in ovarian cancer. Adv. Experim. Med. Biol. 519 (2003) 101-123.
[46] R.I. Mahato, Water insoluble and soluble lipids for gene delivery. Adv. Drug Deliv. Rev. 57(5) (2005) 699-712.
[47] R.I. Mahato, S.W. Kim, Water soluble lipopolymers for gene delivery. Polym. Gene Deliv. (2005) 175-186.
[48] G.S. Kwon, M.L. Forrest, Amphiphilic block copolymer micelles for nanoscale drug delivery. Drug Developm. Res. 67(1) (2006) 15-22.
[49] M.L. Adams, A. Lavasanifar, G.S. Kwon, Amphiphilic block copolymers for drug delivery. Journal Pharm. Sci. 92(7) (2003) 1343-1355.

[50] T. Darbre, J.-L. Reymond, Peptide Dendrimers as Artificial Enzymes, Receptors, and Drug-Delivery Agents. Acc. Chem. Rev. 39(12) (2006) 925-934.
[51] D. Shabat, Self-immolative dendrimers as novel drug delivery platforms. J. Polym. Sci., Part A: Polym Chem. 44(5) (2006) 1569-1578.
[52] L.Y. Qiu, Y.H. Bae, Polymer architecture and drug delivery. Pharm. Res. 23(1) (2006) 1-30.
[53] P.J. Lutz, Free radical homopolymerisation, in heterogeneous medium, of linear and star-shaped polymerizable amphiphilic poly(ethers): a new way to design hydrogels well suited for biomedical applications. Macromol. Symp. 164(Reactive Polymers) (2001) 277-292.
[54] V.C. Lopez, M.J. Snowden, The role of colloidal microgels in drug delivery. Drug Deliv. Syst. & Sci. 3(1) (2003) 19-23.
[55] H.M. Burt, W.L. Hunter, Drug-eluting stents: A multidisciplinary success story. Adv. Drug Deliv. Rev. 58(3) (2006) 350-357.
[56] A.N. Lukyanov, V.P. Torchilin, Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs. Adv. Drug Deliv. Rev. 56(9) (2004) 1273-1289.
[57] V.P. Torchilin, PEG-based micelles as carriers of contrast agents for different imaging modalities. Adv. Drug Deliv. Rev. 54(2) (2002) 235-252.
[58] P. Laverman, O.C. Boerman, W.J.G. Oyen, F.H.M. Corstens, G. Storm, In vivo applications of PEG liposomes: unexpected observations. Crit. Rev. Ther. Drug 18(6) (2001) 551-566.
[59] D. Lasic, D. Needham, The stealth liposome: a prototypical biomaterial. Chem. Rev. 95 (1995) 2601-2628.
[60] M.C. Woodle, New amphiphatic polymer-lipid conjugates forming long-circulating reticuloendothelial system-evading liposomes. Bioconjugate Chem. 5 (1994) 493.
[61] D.D. Lasic, F.J. Martin, A. Gabizon, S.K. Huang, D. Papahadjopoulos, Sterically stabilized liposomes: a hypothesis on the molecular origin of extended circulation times. Biochim. Biophys. Acta: Biomembranes 1070 (1991) 187-192.
[62] S.M. Moghimi, J. Szebeni, Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. Prog. Lipid Res. 42 (2003) 463-478.

[63] S. Zalipsky, C.B. Hansen, J.M. Oaks, T.M. Allen, Evaluation of blood clearance rates and biodistribution of poly(2-oxazoline)-grafted liposomes J.Pharm.Sci. 85 (1996) 133.
[64] M. Einzmann, W.H. Binder, Novel functional initiators for oxazoline polymerisation. J. Polym. Sci., Part A: Polym Chem. 39(16) (2001) 2821-2831.
[65] R. Jordan, K. Martin, H.J. Rader, K.K. Unger, Lipopolymers for surface functionalizations. 1. Synthesis and characterization of terminal functionalized poly(N-propioylethyleneimine)s. Macromolecules 34(26) (2001) 8858-8865.
[66] G. Volet, V. Chanthavong, V. Wintgens, C. Amiel, Synthesis of monoalkyl end-capped poly(2-methyl-2-oxazoline) and its micelle formation in aqueous solution. Macromolecules 38(12) (2005) 5190-5197.
[67] T.R. Baekmark, T. Wiesenthal, P. Kuhn, T.M. Bayerl, O. Nuyken, R. Merkel, New insights into the phase behavior of lipopolymer monolayers at the air/water interface. IRRAS study of a polyoxazoline lipopolymer. Langmuir 13(21) (1997) 5521.
[68] C.A. Naumann, C.F. Brooks, G.G. Fuller, T. Lehmann, J. Ruehe, W. Knoll, P. Kuhn, O. Nuyken, C.W. Frank, Two-dimensional physical networks of lipopolymers at the air/water interface: correlation of molecular structure and surface rheological behavior. Langmuir 17(9) (2001) 2801-2806.
[69] A. Wurlitzer, E. Politsch, S. Huebner, P. Krueger, M. Weygand, K. Kjaer, P. Hommes, O. Nuyken, G. Cevc, M. Loesche, Conformation of polymer brushes at aqueous surfaces determined with X-ray and neutron reflectometry. 2. High-density phase transition of lipopolyoxazolines. Macromolecules 34(5) (2001) 1334-1342.
[70] O. Purrucker, A. Foertig, K. Luedtke, R. Jordan, M. Tanaka, Confinement of transmembrane cell receptors in tunable stripe micropatterns. J. Am. Chem. Soc. 127(4) (2005) 1258-1264.
[71] M. Gleiche, L.F. Chi, H. Fuchs, Nanoscopic channel lattices with controlled anisotropic wetting. Nature 403 (2000) 173-175.
[72] A. Foertig, R. Jordan, K. Graf, G. Schiavon, O. Purrucker, M. Tanaka, Solid-supported biomimetic membranes with tailored lipopolymer tethers. Macromol.Symp. 210 (2004) 329-338.
[73] K. Luedtke, R. Jordan, P. Hommes, O. Nuyken, C.A. Naumann, Lipopolymers from new 2-substituted-2-oxazolines for artificial cell membrane constructs. Macromol. Biosci. 5(5) (2005) 384-393.

[74] M. Zhang, A. Haga, H. Sekiguchi, S. Hirano, Structure of insect chitin isolated from beetle larva cuticle and silkworm (Bombyx mori) pupa exuvia. Int. J. Biol. Macromol. 27(1) (2000) 99-105.

[75] K. Aoi, A. Takasu, M. Okada, Synthesis of novel chitin derivatives having poly(2-alkyl-2-oxazoline) side chains. Macromol. Chem. Phys. 195 (1994) 3835-3844.

[76] K. Kobayashi, H. Sumitomo, T. Itoigawa, Maltopentaose and maltoheptaose-carrying styrene macromers and their homopolymers. Macromolecules 20 (1987) 906.

[77] K. Naka, A. Ohki, S. Maeda, Chem. Lett. (1991) 1303-1306.

[78] K. Naka, T. Nakamura, A. Ohki, S. Maeda, Aggregates of amphiphilic block copolymers derived from poly[(N-acylimino)ethylene]s and their complexes with lipase in water. Polym. J. 27 (1995) 269-274.

[79] K. Naka, Y. Kubo, A. Ohki, S. Maeda, Aggregate of amphiphilic block copolymer derived from poly[(N-acylimino)ethylene]s and guest-binding properties for 8-anilino-1-naphthalenesulfonic acid, pyrene, and enzymes. Polym. J. 26 (1994) 243-249.

[80] K. Naka, R. Yamashita, T. Nakamura, A. Ohki, S. Maeda, K. Aoi, K. Tsutsumiuchi, M. Okada, Aggregate of a peptide-containing block copolymer with a lipase. Macromol.Rapid Commun. 17 (1996) 269-274.

[81] K. Naka, R. Yamashita, T. Nakamura, A. Ohki, S. Maeda, K. Aoi, A. Takasu, M. Okada, Chitin-graft-poly(2-methyl-2-oxazoline) enhanced solubility and activity of catalase in organic solvent. Int. J. Biol. Macromol. 21 (1998) 259-262.

[82] K. Aoi, A. Takasu, M. Tsuchiya, M. Okada, New chitin based polymer hybrids, 3. Miscibility of chitin-graft-poly(2-ethyl-2-oxazoline) with poly(vinyl alcohol). Macromol. Chem. Phys. 199 (1998) 2805-2811.

[83] K. Aoi, A. Takasu, M. Okada, Y. Imae, Synthesis and assembly of novel chitin derivatives having amphiphilic polyoxazoline block copolymer as a side chain. Macromol. Chem. Phys. 200 (1999) 1112-1120.

[84] W.H. Binder, Block copolymers derived from photoreactive 2-oxazolines. Synthesis and micellization behaviour. Macromol. Chem. Phys. 201(9) (2000) 949-957.

[85] M. Sauer, T. Haefele, A. Graff, C. Nardin, W. Meier, Ion-carrier controlled precipitation of calcium phosphate in giant ABA triblock copolymer vesicles. Chem. Commun.(23) (2001) 2452-2453.

[86] C. Nardin, M. Winterhalter, W. Meier, Giant free-standing ABA triblock copolymer membranes. Langmuir 16 (2000) 7708-7712.
[87] W. Meier, C. Nardin, M. Winterhalter, Reconstitution of channel proteins in (polymerised) ABA triblock copolymer membranes. Angew. Chem. Int. Ed. 39(24) (2000) 4599.
[88] M. Winterhalter, C. Hilty, S.M. Bezrukov, C. Nardin, W. Meier, D. Fournier, Controlling membrane permeability with bacterial porins: application to encapsulated enzymes. Talanta 55(5) (2001) 965-971.
[89] C. Nardin, J. Widmer, M. Winterhalter, W. Meier, Amphiphilic block copolymer nanocontainers as bioreactors. Eur. Phys. J. E: Soft Matter 4(4) (2001) 403-410.
[90] T. Ruysschaert, A.F.P. Sonen, T. Haefele, W. Meier, M. Winterhalter, D. Fournier, Hybrid nanocapsules: interactions of ABA block copolymers with liposomes. J. Am. Chem. Soc. 127 (2005) 6242-6247.
[91] P. Rigler, W. Meier, Encapsulation of fluorescent molecules by functionalized polymeric nanocontainers: investigation by confocal fluorescence imaging and fluorescence correlation spectroscopy. J. Am. Chem. Soc. 128(1) (2006) 367-373.
[92] A. Graff, M. Sauer, P. van Gelder, W. Meier, Virus-assisted loading of polymer nanocontainer. Proc.Natl.Acad.Sci. 99(8) (2002) 5064-5068.
[93] R. Stoenescu, A. Graff, W. Meier, Asymmetric ABC-triblock copolymer membranes induce a directed insertion of membrane proteins Macromol. Biosci. 4 (2004) 930-935.
[94] R. Stoenescu, W. Meier, Vesicles with asymmetric membranes from amphiphilic ABC triblock copolymers. Chem. Commun. (2002) 3016-3017.
[95] J.E. Rothman, J. Lenard, Membrane Asymmetry. Science 195(4280) (1977) 743-753.
[96] T. Haefele, K. Kita-Tokarczyk, W. Meier, Phase behaviour of mixed Langmuir monolayers from amphiphlic block copolymers and an antimicrobial peptide. Langmuir 22 (2006) 1164-1172.
[97] D. Wong, T.J. Jeon, J. Schmidt, Single molecule measurements of channel proteins incorporated into biomimetic polymer membranes. Nanotechnology 17 (2006) 3710-3717.

[98] K.Y. Lee, I.C. Kwon, S.Y. Jeong, Physicochemical characteristics of poly(2-ethyl-2-oxazoline)/poly(e-caprolactone) block copolymer micelles in water. Polym. Bull. 56 (2006) 385-393.

[99] D.M. Vriezema, M.C. Arragones, J.A.A.W. Elemans, J.J.L.M. Cornelissen, A.E. Rowan, R.J.M. Nolte, Self-assembled nanoreactors. Chem. Rev. 105(4) (2005) 1445-1489.

[100] H. Ringsdorf, P. Lehmann, R. Weberskirch, Multicompartmentation - A concept for the molecular architecture of life. Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25 (1999) BTEC-001.

[101] K. Matsuoka, Y. Moroi, Micellization of fluorinated amphiphiles. Curr. Opin. Colloid Interf. Sci. 8 (2003) 227-235.

[102] A. Laschewsky, Polymerised micelles with compartments. Curr. Opin. Colloid Interf. Sci. 8 (2003) 274-281.

[103] R. Weberskirch, J. Preuschen, H.W. Spiess, O. Nuyken, Design and synthesis of a two-compartment micellar system based on the self-association behaviour of poly(N-acylethylene imine) end-capped with a fluorocarbon and a hydrocarbon chain. Macromol. Chem. Phys. 201(10) (2000) 995-1007.

[104] D. Putnam, Polymers for gene delivery across length scales. Nature Materials 5 (2006) 439-451.

[105] E. Wagner, J. Kloeckner, Gene delivery using polymer therapeutics. Adv. Polym. Sci. 192 (2006) 135-173.

[106] E. Wagner, M. Cotten, R. Foisner, M.L. Birnstil, Transferrin-polycation-DNA complexes: the effect of polycations on the structure of the complex and DNA delivery to cells. PNAS 88 (1991) 4255-4259.

[107] O. Boussif, F. Lezoualc'h, M.A. Zanta, M.D. Mergny, D. Scherman, B. Demeneix, J. Behr, Polyethylenimine - a versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo. PNAS 92 (1995) 7297-7301.

[108] J. Haensler, F.C.J. Szoka, Polyamidoamine cascade polymers mediate efficient transfection of cells in culture. Bioconj. Chem. 4 (1993) 372.

[109] S. Mansouri, P. Lavigne, K. Corsi, M. Benderdour, E. Beaumont, J.C. Fernandes, Chitosan-DNA nanoparticles as non-viral vectors in gene therapy: strategies to improve transfection efficacy. Eur. J. Pharm. Biopharm. 57 (2004) 1-9.

[110] I.M. Klotz, G.P. Royer, A.R. Sloniewsky, Macromolecule - small molecule interactions. Strong binding and cooperativity in a model synthetic polymer. Biochemistry 8 (1969) 4752-4756.
[111] Y. Akiyama, A. Harada, Y. Nagasaki, K. Kataoka, Synthesis of poly(ethylene glycol)-block-poly(ethylenimine) possessing an acetal group at the PEG end. Macromolecules 33(16) (2000) 5841-5845.
[112] J.H. Jeong, S.H. Song, D.W. Lim, H. Lee, T.G. Park, DNA transfection using linear poly(ethylenimine) prepared by controlled acid hydrolysis of poly(2-ethyl-2-oxazoline). J. Controlled Release 73(2-3) (2001) 391-399.
[113] B. Abdalla, A. Hassan, D. Goula, C. Benoist, J.P. Behr, B. Demeneix, A powerful non-viral vector for in vivo gene transfer in the adult mammalian brian: polyethyleneimine. Hum. Gene Ther. 7 (1996) 1947-1954.
[114] C.-H. Wang, G.-H. Hsiue, Polymer-DNA hybrid nanoparticles based on folate-polyethylenimine-block-poly(L-lactide). Bioconjugate Chemistry 16(2) (2005) 391-396.
[115] Z. Zhong, J. Feijen, M.C. Lok, W.E. Hennink, L.V. Christensen, J.W. Yockman, Y.-H. Kim, S.W. Kim, Low molecular weight linear poly(ethyleneimine)-b-poly(ethylene glycol)-b-poly(ethyleneimine) triblock copolymers: synthesis, characterization and in vitro gene transfer properties. Biomacromolecules 6(6) (2005) 3440-3448.
[116] B. Brissault, A. Kichler, C. Leborgne, O. Danos, H. Cheradame, J. Gau, L. Auvray, C. Guis, Synthesis, characterization and gene transfer application of poly(ethylene glycol-b-ethylenimine) with high molar mass polyamine block. Biomacromolecules 7(10) (2006) 2863-2870.
[117] G.H. Hsiue, H.Z. Chiang, C.H. Wang, T.M. Juang, Nonviral gene carriers based on diblock copolymers of poly(2-ethyl-2oxazoline) and linear polyethyleneimine. Bioconj. Chem. 17 (2006) 781-786.
[118] T. Tanaka, Collapse of gels and the critrical endpoint. Phys.Rev.Lett. 40(12) (1978) 820-823.
[119] D. Schmaljohann, Thermo- and pH-responsive polymers in drug delivery. Adv. Drug Deliv. Rev. 58(15) (2006) 1655-1670.
[120] S.J. Kim, S.G. Yoon, S.M. Lee, S.H. Lee, S.I. Kim, Electrical sensitivity behavior of a hydrogel composed of poly(methacrylic acid)/poly(vinyl alcohol). J. Appl. Polym. Sci. 91(6) (2004) 3613-3617.

[121] J. Chatterjee, Y. Haik, C.-J. Chen, pH-reversible magnetic gel with a biodegradable polymer. Journal of Applied Polymer Science 91(5) (2004) 3337-3341.
[122] G.D. Jaycox, Stimuli-responsive polymers. VII. Photomodulated chiroptical switches: periodic copolyaramides containing azobenzene, phenylene, and chiral binaphthylene main-chain linkages. J. Polym. Sci., Part A: Polym Chem. 42(3) (2004) 566-577.
[123] S.R. Lustig, G.J. Everlof, G.D. Jaycox, Stimuli-responsive polymers. 5. Azobenzene modified polyaramides containing atropisomeric binaphthyl linkages: tuning chiroptical behavior with light and heat. Macromolecules 34(7) (2001) 2364-2372.
[124] S.C. Lee, Y. Chang, Y. Yoon, C. Kim, I.C. Kwon, Y.H. Kim, S.Y. Jeong, Synthesis and micellar characterization of amphiphilic diblock copolymers based on poly(2-ethyl-2-oxazoline) and aliphatic polyesters. Macromolecules 32(6) (1999) 1847-1852.
[125] C. Kim, S.C. Lee, J.H. Shin, J.S. Yoon, I.C. Kwon, S.Y. Jeong, Amphiphilic diblock copolymers based on poly(2-ethyl-2-oxazoline) and poly(1,3-trimethylene carbonate): synthesis and micellar characteristics. Macromolecules 33(20) (2000) 7448.
[126] S.C. Lee, S.W. Kang, C. Kim, I.C. Kwon, S.Y. Jeong, Synthesis and characterization of amphiphilic poly(2-ethyl-2-oxazoline)/poly(e-caprolactone) alternating multiblock copolymers. Polymer 41 (2000) 7091-7097.
[127] C. Kim, S.C. Lee, S.W. Kang, I.C. Kwon, S.Y. Jeong, Phase transition characteristics of amphphilic poly(2-ethyl-2-oxazoline)/poly(e-caprolactone) block copolymers in aqueous solution. J. Polym. Sci., Part B: Polym Phys. 38 (2000) 2400-2408.
[128] M. Malmsten, B. Lindman, Self-assembly in aqueous block copolymer solutions. Macromolecules 25(20) (1992) 5440-5445.
[129] C. Kim, S.C. Lee, I.C. Kwon, H. Chung, S.Y. Jeong, Complexation of poly(2-ethyl-2-oxazoline)-block-poly(e-caprolactone) micelles with multifunctional carboxylic acids. Macromolecules 35(1) (2002) 193-200.
[130] S.C. Lee, C. Kim, I.C. Kwon, H. Chung, S.Y. Jeong, Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(e-caprolactone) copolymer as a carrier for paclitaxel. J. Controlled Release 89(3) (2003) 437.

[131] B.G. Yu, T. Okano, K. Kataoka, G. Kwon, Polymeric micelles for drug delivery: solubilization and haemolytic activity of amphotericin B. J. Controlled Release 53 (1998) 1998.
[132] C. Wang, G.H. Hsiue, Synthesis and characterization of temperature- and pH-sensitive hydrogels based on poly(2-ethyl-2-oxazoline) and poly(D,L-lactide). J.Polym.Sci.,Part A: Polym.Chem. 40 (2002) 1112.
[133] D. Christova, R. Velichkova, W. Loos, E.J. Goethals, F.E. du Prez, New thermo-responsive polymer materials based on poly(2-ethyl-2-oxazoline) segments. Polymer 44 (2003) 2255-2261.
[134] C.H. Wang, G.H. Hsiue, New amphiphilic poly(2-ethyl-2-oxazoline)/poly(L-lactide) triblock copolymers. Biomacromolecules 4(6) (2003) 1487-1490.
[135] C.-H. Wang, C.-H. Wang, G.-H. Hsiue, Polymeric micelles with a pH-responsive structure as intracellular drug carriers. J. Controlled Release 108(1) (2005) 140-149.
[136] W.T. Godbey, K.K. Wu, A.G. Mikos, Poly(ethylenimine) and its role in gene delivery. J. Controlled Release 60(2-3) (1999) 149-160.
[137] G.H. Hsiue, C.H. Wang, C.L. Lo, C.H. Wang, J.P. Li, J.L. Yang, Environmental sensitive micelles based on poly(2-ethyl-2-oxazoline)-b-poly(L-lactide) diblock copolymer for application in drug delivery. Int. J. Pharm. 317 (2006) 69-75.
[138] C.H. Wang, K.R. Fan, G.H. Hsiue, Enzymatic degradation of PLLA-PEOz-PLLA triblock copolymers. Biomaterials 26 (2005) 2803-2811.
[139] C. Diab, Y. Akiyama, K. Kataoka, F.M. Winnik, Microcalorimetric study of the temperature-induced phase separation in aqueous solutions of poly(2-isopropyl-2-oxazolines). Macromolecules 37(7) (2004) 2556-2562.
[140] J.S. Park, Y. Akiyama, F.M. Winnik, K. Kataoka, Versatile synthesis of end-functionalized thermosensitive poly(2-isopropyl-2-oxazolines). Macromolecules 37(18) (2004) 6786.
[141] J.S. Park, K. Kataoka, Precise control of lower critical solution temperature of thermosensitive poly(2-isopropyl-2-oxazoline) via gradient copolymerisation with 2-ethyl-2-oxazoline as a hydrophilic comonomer. Macromolecules 39(19) (2006) 6622-6630.
[142] J.C. Rueda, S. Zschoche, H. Komber, D. Schmaljohann, B. Voit, Synthesis and characterization of thermoresponsive graft copolymers of NIPAAm and 2-alkyl-

2-oxazolines by the "grafting from" method. Macromolecules 38(17) (2005) 7330-7336.
[143] M. Meyer, H. Schlaad, Poly(2-isopropyl-2-oxazoline)-poly(L-glutamate) block copolymers through ammonium-mediated NCA polymerisation. Macromolecules 39 (2006) 3967-3970.
[144] Y. Yang, K. Kataoka, F.M. Winnik, Synthesis of diblock copolymers consisting of hyaluronan and poly(2-ethyl-2-oxazoline). Macromolecules 38(6) (2005) 2043.
[145] D.J. Diekema, B.J. BootsMiller, T.E. Vaughn, R.F. Woolson, J.W. Yankey, E.J. Ernst, S.D. Flach, M.M. Ward, C.L.J. Franciscus, M.A. Pfaller, B.N. Doebelling, Antimicrobial resistance trands and outbreak frequency in United States Hospitals. Clin. Inf. Dis. 38 (2004) 78-85.
[146] T. Ikeda, S. Tazuke, Biologically active polycations: antimicrobial activities of poly[trialkyl(vinylbenzy1)ammonium chloride]-type polycations
. Macromol Chem., Rapid Commun. 4(7) (1983) 459-461.
[147] P. Broxton, P.M. Woodcock, J. Gilbert, Assessment of resistance towards biocides following the attachment of micro-organisms to, and growth on, surfaces. J. Appl. Bacteriol. 91(2) (1983) 248-.
[148] A. Kanazawa, T. Ikeda, T. Endo, Polymeric phosphonium salts as a novel class of cationic biocides. III. Immobilization of phosphonium salts by surface photografting and antibacterial activity of the surface-treated polymer films. J. Polym. Sci., Part A: Polym Chem. 31(6) (1993) 1467-1472.
[149] A. Kanazawa, T. Ikeda, T. Endo, Antibacterial activity of polymeric sulfonium salts. J. Polym. Sci., Part A: Polym Chem. 31(11) (1993) 2873-2876.
[150] C.J. Waschinski, J.C. Tiller, Poly(oxazoline)s with telechelic antimicrobial functions. Biomacromolecules 6(1) (2005) 235-243.
[151] C.J. Waschinski, V. Herdes, F. Schueler, J.C. Tiller, Influence of satellite groups on telechelic antimicrobial functions of polyoxazolines. Macromol. Biosci. 5(2) (2005) 149-156.

## Figure Captions

**Figure 1:** Schematic representation of the mechanism for the cationic ring opening polymerisation of 2-oxazolines.

**Figure 2:** Schematic representation of the synthesis of poly(2-oxazoline)/DSPE conjugates (DSPE = di-stearoylphosphatidylethanolamin, TEA = triethanolamine, DCC = N,N'-dicyclohexylcarbodiimide, HOSu = N-hydroxysuccinimide).[60]

**Figure 3:** Possible model to explain network formation in lipopolymers. (**A**) Hydrated chain with water molecules at locations where H-bonding sites are exposed to bulk water, (**B**) hydrated water molecules acting as intercalates.[68] (Reprinted with permission from ref [68]. Copyright 2007, American Chemical Society)

**Figure 4:** Fluorescence images of mixed lipid/lipopolyoxazoline monolayers with different degrees of polymerisation (a) n = 14 (DS-$PMOx_{14}$-Si), (b) n = 33 (DS-$PMOx_{33}$-Si), (c) n = 104 (DS-$PMOx_{104}$-Si), where DS = distearoyl lipid moiety, PMOx = poly(2-methyl-2-oxazoline), Si = trimethyl silane).[70] (Reprinted with permission from ref [70]. Copyright 2005, American Chemical Society.)

**Figure 5:** Synthesis of chitin derivatives containing poly(2-alkyl-2-oxazoline) side chains (R = Me, Et).[75]

**Figure 6:** Transmission electron micrograph of phosphate loaded PMOXA-PDMS-PMOXA vesicles after 1 h (left) and 24 h (right) incubation with calcium chloride in the presence of an ionophore.[85] (Reproduced by permission of the Royal Society of Chemistry from ref 85.)

**Figure 7:** Schematic representation of a polymer nanoreactor with porins incorporated in the membrane and enclosed enzymes (enzymes = boxes, substrate = small circle, reaction product = small triangles).[88]

**Figure 8:** Electron micrograph of a complex between λ phage and maltoporin-bearing PMOXA-PDMS-PMOXA nanocontainers. (A) λ Phage attached to a single vesicle via its tail, (B) λ phage bound to an aggregate of vesicles.[92] (Reproduced with permission from ref [92]. Copyright National Academy of Sciences of the United States.)

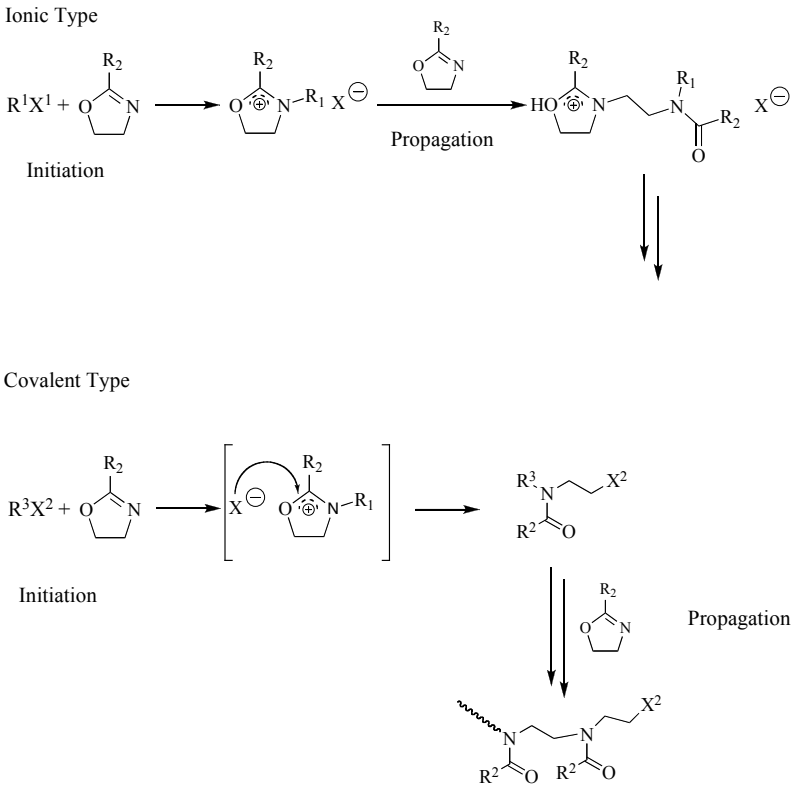
**Figure 9:** Aggregation of FPMOXA25L16 versus concentration in aqueous solution.[103] (Copyright Wiley-VCH Verlang GmbH & Co KG aA. Reproduced with permission from ref 103.)

**Figure 10:** Synthesis of a poly(ethylene glycol)-block-poly(ethylene imine) copolymer *via* a poly(2-methyl-2-oxazoline) species.[111]

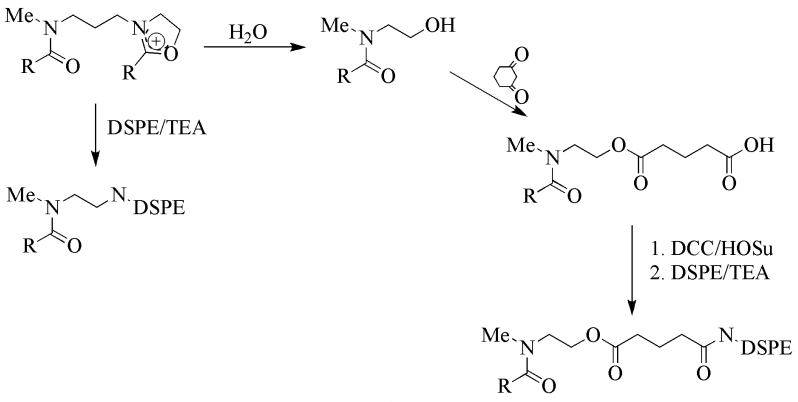
**Figure 11:** Synthesis of poly(2-ethyl-2-oxazoline)-block-poly(ethylene imine).[117]

**Figure 12:** Synthesis of (A) 3-arm poly(D,L-lactide) trimethacrylate and (B) poly(2-ethyl-2-oxazoline) dimethacrylate.[132]

## Figure 1


Ionic Type
Initiation
Propagation
Covalent Type
Initiation
Propagation

## Figure 2




R = Me, Et; n = 50

**Figure 3**

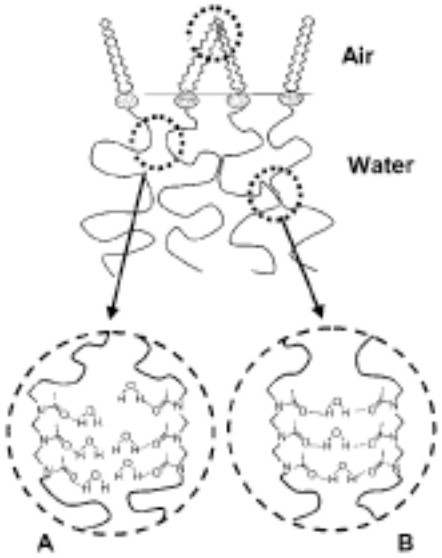


**Figure 4**

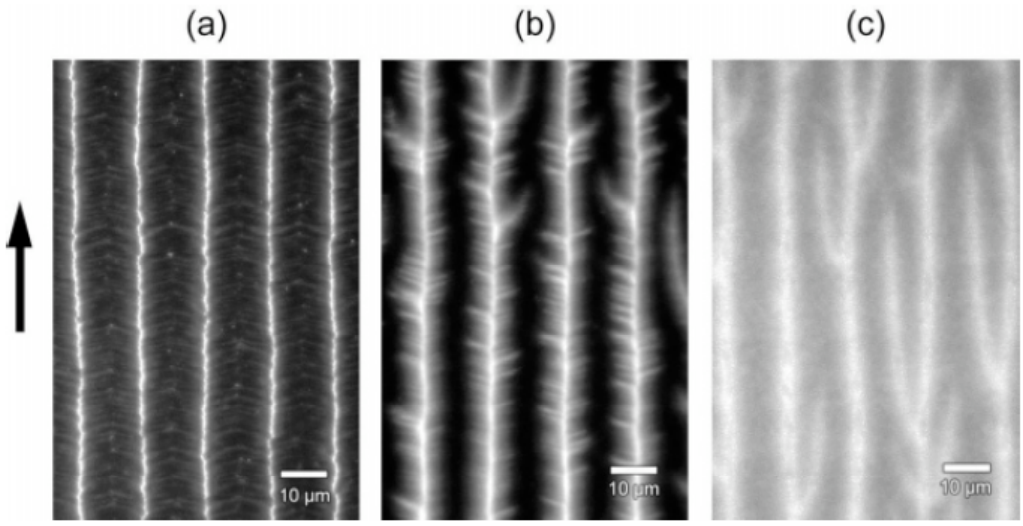


**Figure 5**

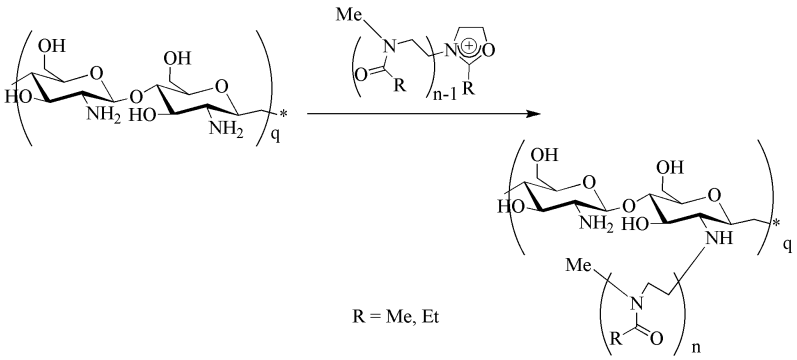

OH
OH
HO
NH2
HO
NH2
q
Me
N
O
R
n-1
N
R
O
OH
OH
HO
NH2
HO
NH
q
Me
N
R
O
n
R = Me, Et

**Figure 6**

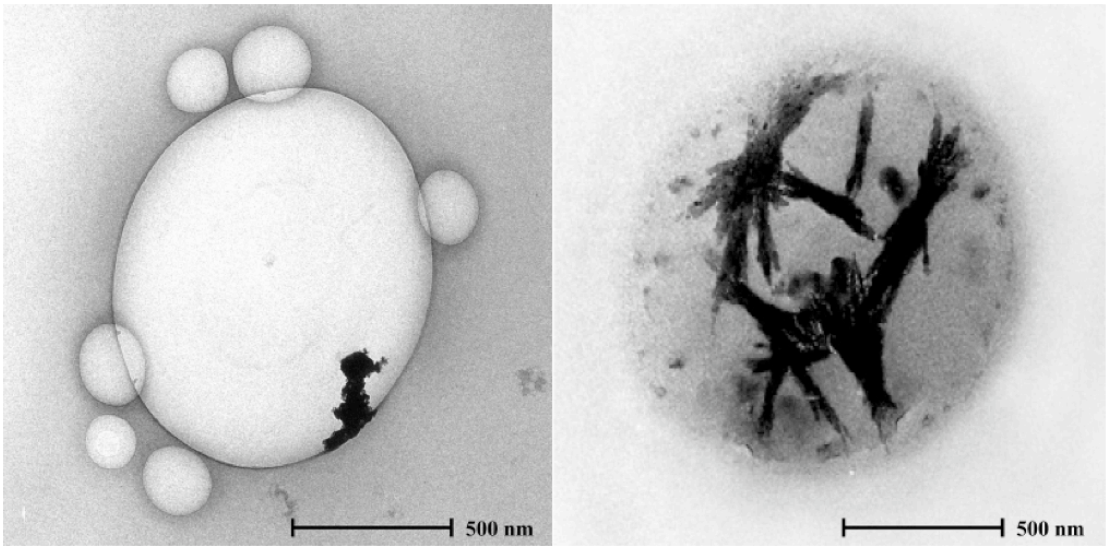


**Figure 7**

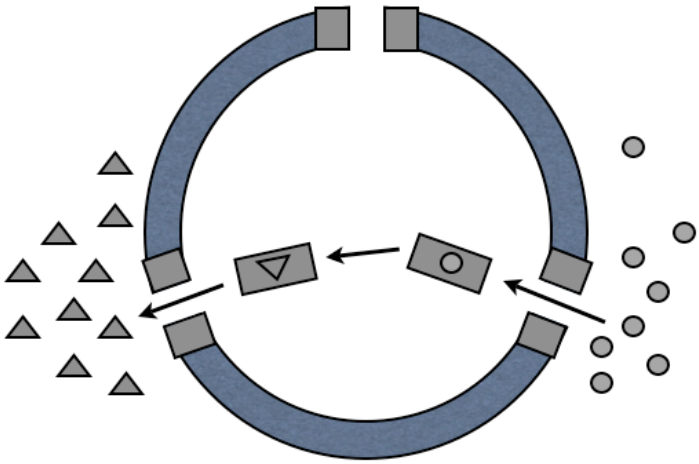


**Figure 8**

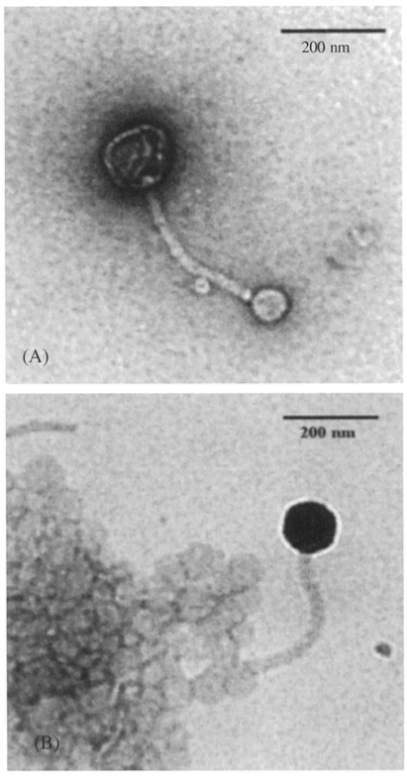


**Figure 9**

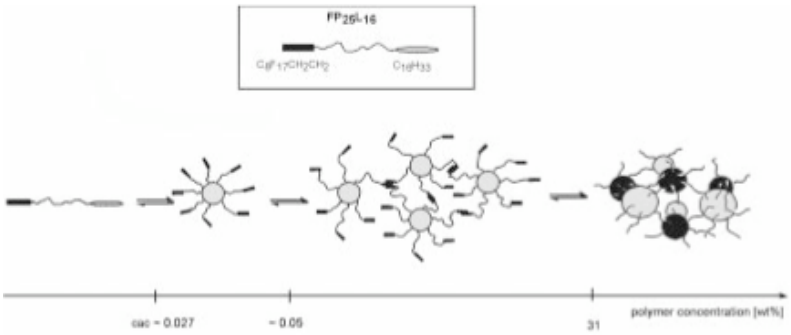
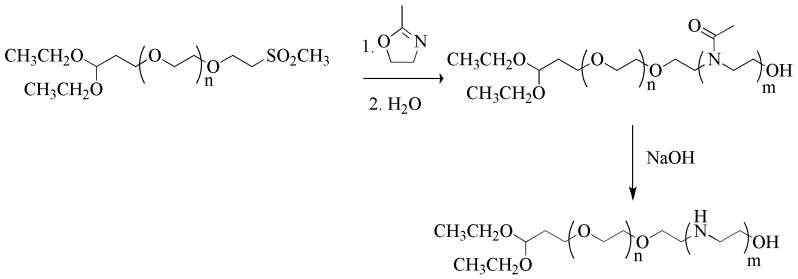


**Figure 10**



## Figure 11

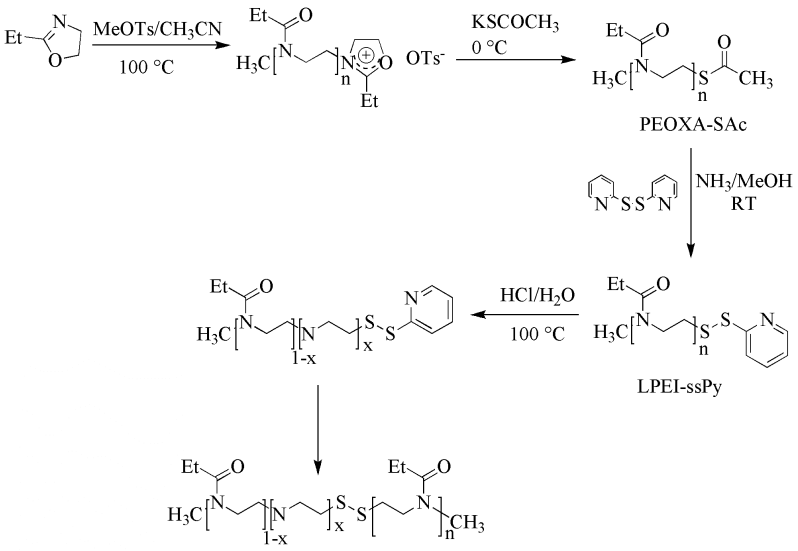
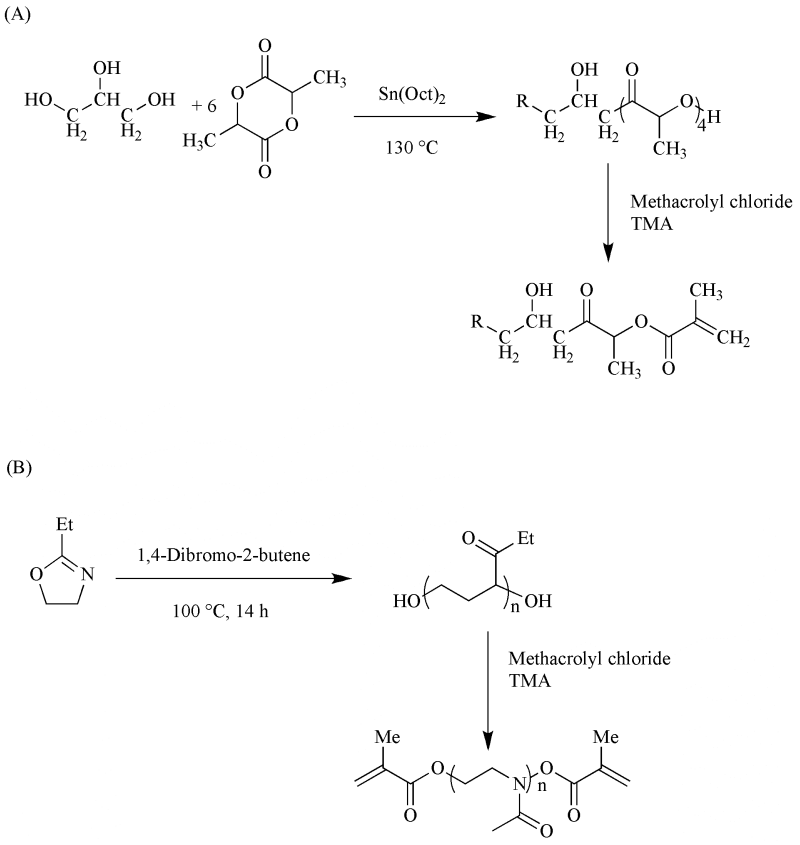

MeOTs/CH3CN
100 °C
OTs-
KSCOCH3
0 °C
PEOXA-SAc
NH3/MeOH
RT
HCl/H2O
100 °C
LPEI-ssPy

**Figure 12**


(A)
Sn(Oct)2
130 °C
Methacrolyl chloride
TMA
(B)
1,4-Dibromo-2-butene
100 °C, 14 h
Methacrolyl chloride
TMA