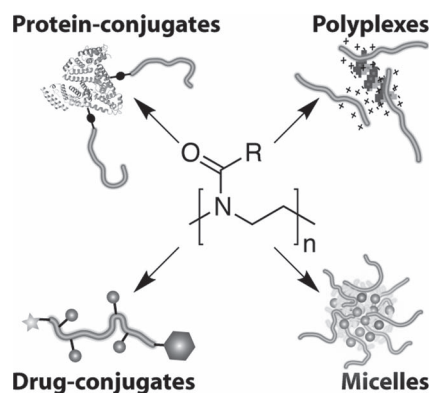




# Poly(2-oxazoline)s as Polymer Therapeutics

Robert Luxenhofer,\* Yingchao Han, Anita Schulz, Jing Tong, Zhijian He, Alexander V. Kabanov, Rainer Jordan\*

Poly(2-oxazoline)s (POx) are currently discussed as an upcoming polymer platform for biomaterials design and especially for polymer therapeutics. POx meet specific requirements needed for the development of next-generation polymer therapeutics such as biocompatibility, high modulation of solubility, variation of size, architecture as well as chemical functionality. Although in the early 1990s first and promising POx-based systems were presented, the field lay dormant for almost two decades. Only very recently, POx-based polymer therapeutics came back into the focus of very intensive research. In this review, we give an overview on the chemistry and physicochemical properties of POx and summarize the research of POx–protein conjugates, POx–drug conjugates, POx-based polyplexes and POx micelles for drug delivery.



## 1. Introduction

Poly(2-oxazoline) (POx) is a prominent member of pseudo-polypeptides. They are accessible via living cationic ring-opening polymerization (LCROP) of 2-oxazolines (Figure 1). The five-membered heterocyclic monomers are either commercially available [2-methyl-2-oxazoline (MeOx), 2-ethyl-2-oxazoline (EtOx), 2-isopropenyl-2-oxazoline (iPrOx) 2-phenyl-2-oxazoline (PheOx)] or accessible via – typically

facile – few step syntheses from nitriles,<sup>[1]</sup> carboxylic acids,<sup>[2]</sup> aldehydes,<sup>[3]</sup> or from MeOx.<sup>[4,5]</sup>

Thus, a large variety of monomers and polymers have been realized giving access to a broad range of physical and chemical properties. It should be noted that many functional groups are likely to interfere with the LCROP and, therefore, need to be protected during polymerization.

Although POx have been known to have significant potential as biomaterials for decades,<sup>[6–9]</sup> so far, no real breakthrough has been achieved in this respect. When POx was first discussed for the use as biomaterials, polyethylene glycol (PEG) became highly recognized as the polymer of choice as a hydrophilic part of biomaterials and was already commercially available in sufficient quality. In contrast, to date, no POx-based polymers are commercially available in high quality (dispersity  $\overline{M}_w/\overline{M}_n = \mathcal{D} \leq 1.2$ ).

In this review, we try to motivate why in particular POx is an interesting platform for biomaterials development and for polymer therapeutics in particular, and summarize what has been realized (preclinically) to date. For this, we classify polymer therapeutics into polymer protein conjugates, polyplexes, polymer–drug conjugates and polymeric micelles, aggregates and polymer nanoparticles (Figure 2).<sup>[10,11]</sup>

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## 2. Background

Biomaterials for drug delivery applications need to cope with a variety of complex problems, depending – simply speaking – on the route of administration, the drug that needs to be delivered, the location of the target site of the drug and potentially the need for a specific release of the payload. To address these issues, biomaterials tend to become increasingly complex, whereas from a pharmaceutical and regulatory point of view, the polymer therapeutics should be as simple as possible.

In this respect, POx is interesting as it represents a versatile synthetic polymer platform. Thus, even complex structures are accessible using straightforward approaches, however, if necessary, combination with other polymers such as polyesters, polypeptides, and polyether can easily be realized.

A particularly important aspect of biomaterials is their interaction with biological systems. Typically, the constituting biomaterial of drug delivery systems is expected to be well tolerated (i.e., to be biocompatible) with low toxicity, little protein interaction, minimal unspecific organ accumulation, and low to none immunogenicity. The synthetic and structural versatility will be discussed in the following chapters. Thereafter, available data regarding biocompatibility of POx will be summarized.

### 2.1. Synthetic Versatility of POx

#### 2.1.1. Initiators

To ensure a high degree of functionality and well-defined products, the initiation reaction should be both quantitative and fast. Both criteria are fulfilled, that is, triflate-based initiators as the initiation reaction is fast with respect to the propagation ( $k_i \gg k_p$ ) and typically quantitative even below room temperature.<sup>[12]</sup> It should be noted, however, that many reports can be found in the literature, in which other classes of initiators, such as tosylates, alkylhalides, and nosylates, have been successfully used to obtain well-defined POx. In the case of alkylhalides, iodine is typically preferred and often obtained in situ by halogen exchange.<sup>[13]</sup>

Functional initiators have been used to realize, among others, alkyne,<sup>[14]</sup> amine,<sup>[15]</sup> hydroxyl,<sup>[16]</sup> or carboxylic acid<sup>[17]</sup> bearing POx (with protection groups where needed) to allow further functionalization (Figure 3a). In a number of other accounts, functionality by structure was also introduced via the initiators such as lipids,<sup>[18,19]</sup> porphyrins,<sup>[20]</sup> or saccharides.<sup>[21]</sup>

For triflate and possibly tosylate initiators, the initiation step is virtually quantitative at or below room temperature, whereas propagation only takes place above  $\approx 40^\circ\text{C}$ .<sup>[12,13,22]</sup> Thus, the so-called initiator salt can be



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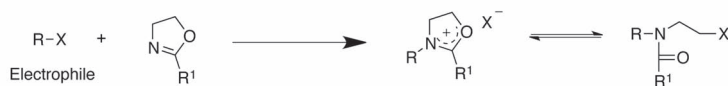
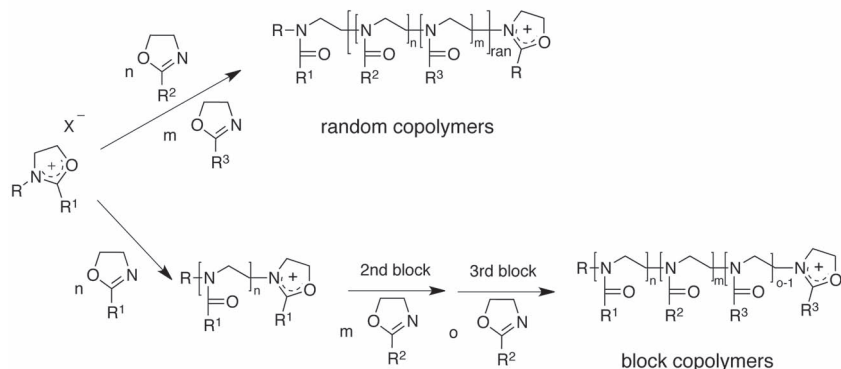
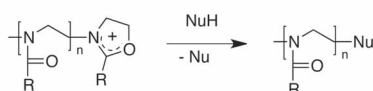


**Rainer Jordan** studied chemistry at the University of Mainz (Germany) and as a IAS fellow at Kyoto University, Japan with Prof. Saegusa. He joined the group of Prof. Unger in Mainz and worked as a PROCOPE fellow at the C.N.R.S. in Paris. In 1996, he obtained his doctoral degree in Chemistry with Prof. Unger (University of Mainz). After a postdoctoral stay with Prof. Ulman at Polytechnic University in Brooklyn, NY, USA, he was appointed as Assistant Professor. He returned to Germany and joined the group of Prof. Nuyken at the Technische Universität München for habilitation. Since 2008/2009, he is Full Professor, Chair of Macromolecular Chemistry at the Technische Universität Dresden, Germany. His research interests include polymer chemistry, surface chemistry, self-assembly, biomimetic systems, nanoscience, and nanomedicine.

isolated and a polymer terminus bearing two functionalities (from triflate and from monomer) can be realized (Figure 3b).

#### 2.1.2. Terminating Agents

The LCROP is of highly living nature and can be specifically terminated to introduce additional functionalities.  $\alpha,\omega$ -Homo- and heterofunctional telechelics can be obtained by combining the initiator<sup>[23]</sup> and termination<sup>[24]</sup> method. The majority of researchers typically terminate using water or  $\text{OH}^-$ , which leaves a  $-\text{OH}$  terminus. However, for many modifications a terminus which can be readily modified, may be desirable. With this respect, azide,<sup>[25]</sup> thiol,<sup>[26]</sup> or amines<sup>[22,27,28]</sup> provide for interesting alternatives (Figure 3c). However, it should be kept in mind that two different possibilities have been described at times for the termination reaction to occur – through 2- or 5-position of the ring, of which typically the 5-position product is

**Initiation****Propagation****Termination**

**Figure 1.** Schematic representation of the initiation, propagation, and termination of the living cationic ring-opening polymerization of 2-oxazolines.

similar water solubility as PEG; however, although PMeOx is purely hydrophilic, PEtOx displays a slight amphiphilic character similar to PEG<sup>[30]</sup> and displays a lower critical solution temperature. As the hydrophobic nature of the 2-substitution increases, the LCST decreases until water insolubility. The series of water solubility from PMeOx to the first water insoluble PBUOx can be given as: PMeOx > PEtOx ≈ PEG > PiPrOx > poly(2-cyclopropyl-2-oxazoline) (PcPrOx) > PnPrOx > PBUOx (see Figure 3d). Recently, Viegas et al.<sup>[31]</sup> essentially confirmed this order for PMeOx, PEtOx, and PEG for low molar mass polymers by RP-HPLC (see Figure 4a). Most interestingly, they also found that for increasing molar masses, PEG and PMeOx become more hydrophilic whereas PEtOx gets more hydrophobic. Rehfeldt et al.<sup>[32]</sup> measured the hydration in static and dynamic swelling experiments of low molar mass PMeOx and PEtOx brushes. For both, PMeOx and PEtOx, very similar dis-

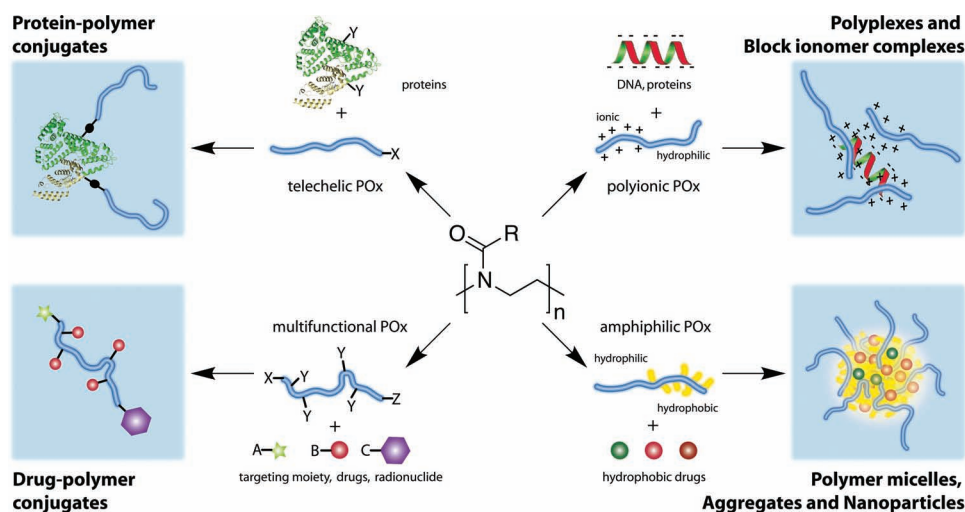
joining pressures were found, while again, the polymer chain length had a stronger effect upon the polymer hydration (Figure 4b,c).

**2.1.3. Non-Reactive Monomers**

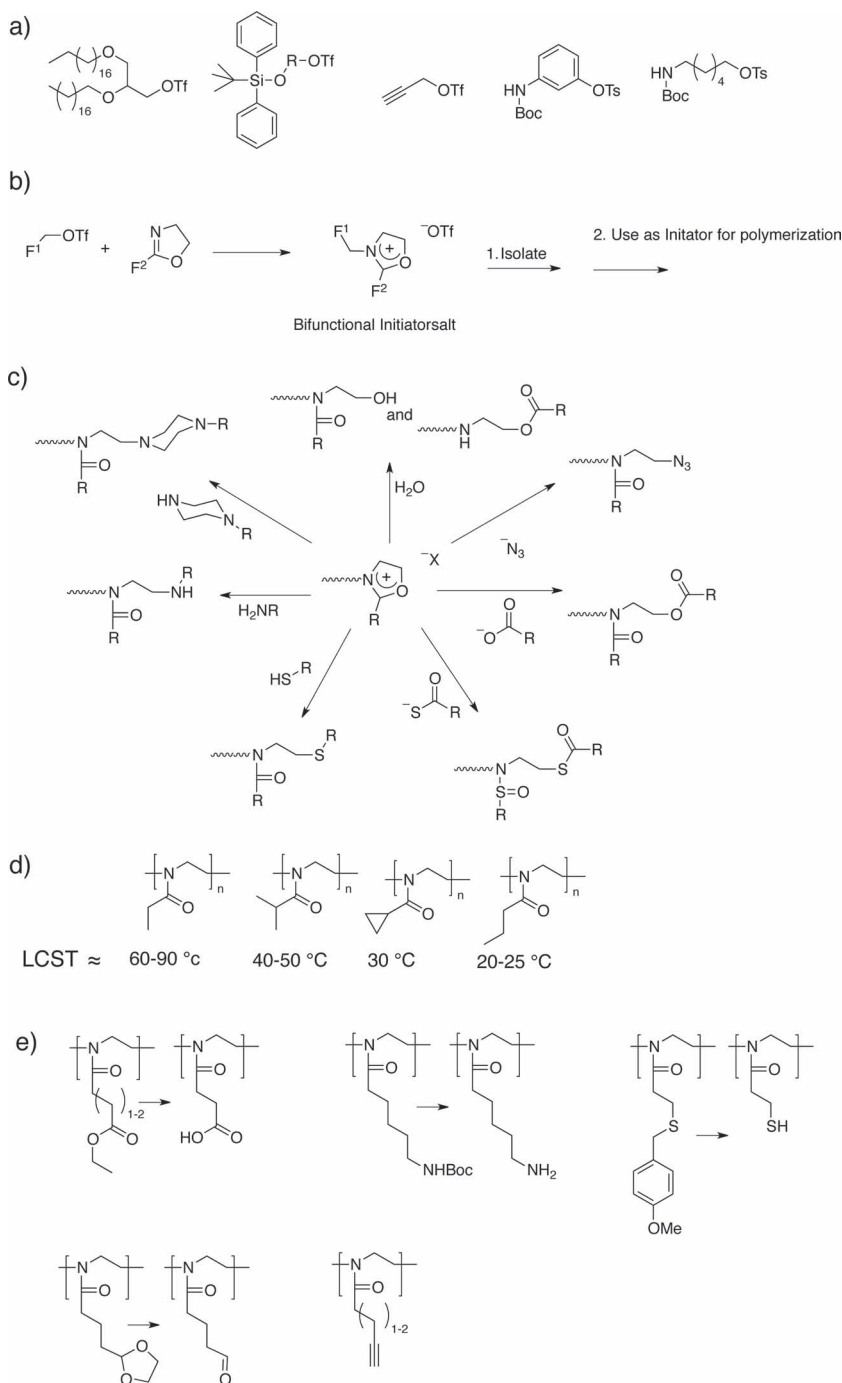
desired.<sup>[29]</sup> This potentially gives alternative termini and subquantitative functionalization.

A wide range of aliphatic or aromatic 2-oxazoline monomers are available for LCROP. These give the possibility to tailor the solution and aggregation properties of POx. For example, such with short aliphatic side chains (C2–C4) exhibit lower critical solution temperature (LCST) in aqueous solutions (Figure 3d), whereas longer non-polar side chains give essentially water-insoluble polymers. It should be noted that in POx the amide group links the pendant moiety to the main chain and thus, increasingly non-polar substituents result into an amphiphilic motive for each monomer unit. Consequently, a “hydrophobic” POx is actually a non-ionic polysoap with a polymerized polar head group and hydrophobic tails. PMeOx and PEtOx as the most hydrophilic polymers are miscible with water at all ratios. Both polymers show a

Amphiphilic POx can be readily obtained by the sequential block copolymerization of MeOx or EtOx with 2-oxazolines having non-polar 2-substituents such as longer 2-*n*-alkyl- or 2-phenyl groups, yielding defined block copolymers of low dispersity. Since the first work by Kobayashi et al.,<sup>[33]</sup> the various combinations and self-assembly have been described in several reviews.<sup>[8,13,34]</sup> The temperature-dependent solubility of POx can be



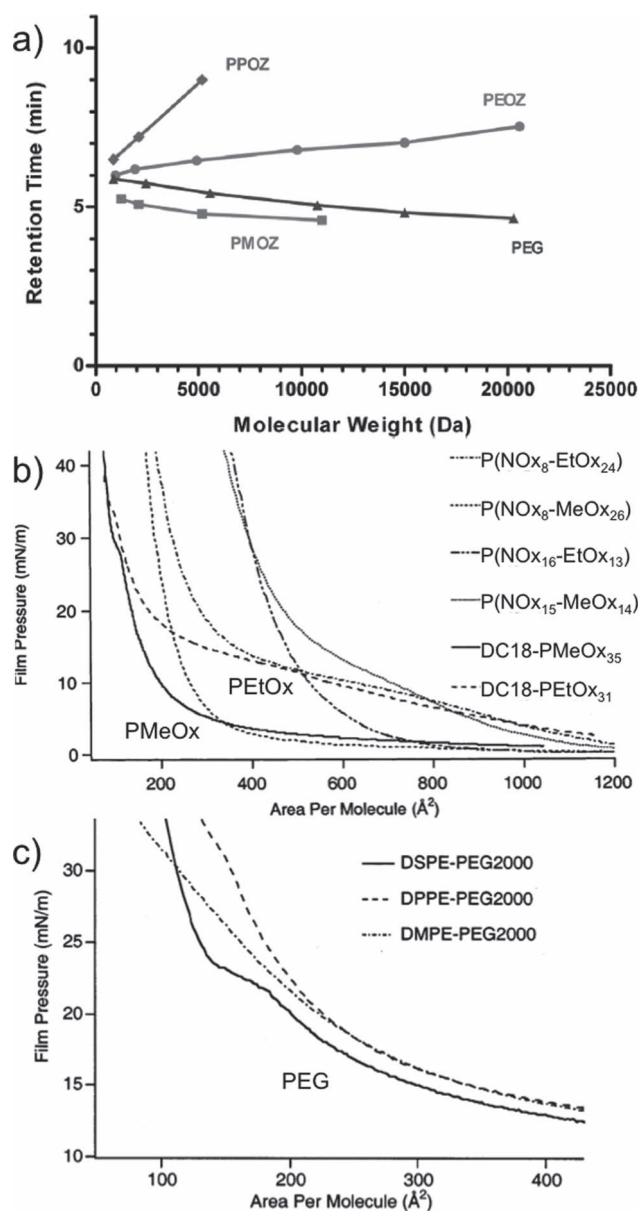
**Figure 2.** Polymer therapeutics based on poly(2-oxazoline)s.



**Figure 3.** Overview on the chemical versatility available by poly(2-oxazolines). (a) Selection of initiators that allow introduction of functional  $\alpha$ -termini of POx. (b) The initiator salt method available for the synthesis of POx offers the unique opportunity to incorporate two functionalities at their  $\alpha$ -terminus. (c) A great variety of nucleophiles can be utilized to terminate the living cationic ring-opening polymerization of POx to obtain  $\omega$ -functionalized POx. A selection of common O-, N-, and S-nucleophiles are presented. (d) Changes in the side chain structure allows for the synthesis of polymers with a wide range of thermal responsiveness of their aqueous polymer solutions. Further fine-tuning can be obtained by copolymerization of different 2-oxazoline monomers. (e) Selection of reported POx structures that allow post-polymerization modification through the polymer side chains before and after deprotection.

modulated over a wide range by copolymerization using EtOx, iPrOx, and nPrOx with either hydrophilic or hydrophobic 2-oxazoline comonomers.<sup>[35–48]</sup> The thermosensitive solubility behavior of POx- and PEG-based materials were recently summarized and compared.<sup>[49]</sup> Because of the specific chemical structure of POx, i.e. bearing only hydrogen-bonding acceptors, the cloud points of thermosensitive POx are well-defined, and the soluble–insoluble transition typically occurs within  $<1$  K with a minimal hysteresis. Thus, for “smart” POx systems that take advantage of the temperature sensitivity, specifically tailored POx material can be realized. Importantly, amphiphilic POx of sufficient amphiphilic contrast self-assemble into defined and small, usually spherical micellar aggregates as analyzed by dynamic light scattering (DLS), fluorescence correlation spectroscopy (FCS), and small-angle neutron scattering for PNOx–PMeOx, PNOx–PEtOx, and PNOx–iPrOx as well as PNOx–nPrOx block copolymers,<sup>[27,35,50–52]</sup> or by AFM on surface deposited micelles for PMeOx/PEtOx with soy-based 2-oxazolines (SoyOx), PhOx, and NOx.<sup>[53–58]</sup> The living ring-opening copolymerization of 2-oxazolines even allows for the synthesis of defined tetrablock POx.<sup>[54]</sup> For the design of micelles for drug delivery, amphiphilic gradient copolymers provide an interesting alternative, as the solubility of gradient and block copolymers differ substantially,<sup>[59]</sup> the resulting aggregate structures are significantly different,<sup>[51]</sup> the surface activity of gradient copolymers is much higher<sup>[60]</sup> which considerably modulate the drug encapsulation efficiency,<sup>[61]</sup> the cell uptake,<sup>[62]</sup> and possibly trafficking of drug loaded micelles.

Alternatively, hydrophilic POx can be combined with hydrophobic moieties such as long alkyl chains or lipids by the initiation<sup>[23]</sup> or termination<sup>[24]</sup> method to yield defined non-ionic surfactants. This has been used frequently for the design of lipopolymers of defined hydrophilic–lipophilic balance for model membrane constructs.<sup>[18,19,63–77]</sup>



**Figure 4.** (a) Relative hydrophilicity of PEG, PMeOx (PMOZ), PEtOx (PEOZ), and PnPrOx (PZOZ) as studied by reversed-phase high-performance liquid chromatography. (Reprinted with permission from ref.<sup>[31]</sup> Copyright 2012 American Chemical Society.) The higher the elution time, the lower the hydrophilic character of the polymer. (b) POx amphiphilic copolymers and lipopolymers along with (c) PEG-lipopolymers at the air-water interphase showing the reversible adsorption of PEtOx and PEG at the interface. (Reprinted after modification with permission from ref.<sup>[30]</sup> Copyright 2003, American Chemical Society.)

In a systematic study Volet et al.<sup>[78]</sup> investigated the micelle formation, critical micelle concentration (CMC), viscosity, and so on of PMeOx-monoalkyl amphiphiles. Interestingly, the micelles readily disintegrate upon addition of  $\beta$ -cyclodextrin.

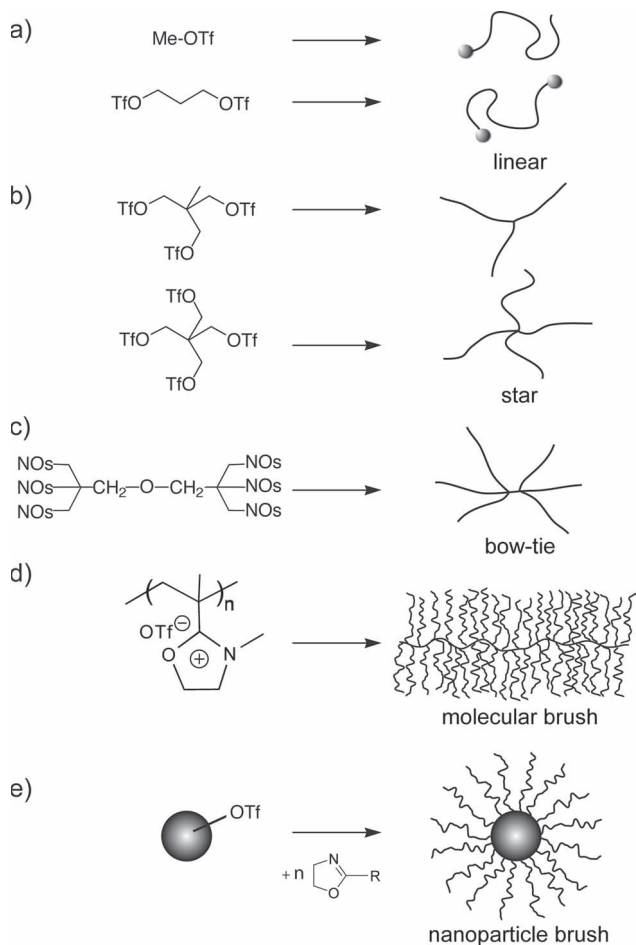
#### 2.1.4. Reactive Monomers

The relatively simple monomer synthesis via a variety of routes has facilitated the preparation of a large variety of monomers that can be utilized for subsequent modifications, typically with moieties which are incompatible with LCROP (Figure 3e). For chemoselective and/or highly efficient modification, monomers bearing alkyne,<sup>[12]</sup> alkene,<sup>[79]</sup> thiols,<sup>[80,81]</sup> or aldehyde<sup>[5]</sup> moieties may be particularly noteworthy. Also reactive but rather interesting for their properties to introduce pending charges along the backbone are monomers with protected amines<sup>[82]</sup> or carboxylic acids.<sup>[2]</sup>

## 2.2. Structural Versatility of POx

One important aspect of a polymer system used in therapeutics is the possibility to specifically tailor the polymer architecture. In particular, numerous studies with other polymers have shown, the polymer architecture critically influences the pharmacokinetics of a polymer and thus, potentially, the performance of a polymer-based drug delivery system.<sup>[83–86]</sup> Here, the living polymerization of 2-oxazolines offers a powerful and yet easy method to vary the resulting polymer architecture by various methods. A direct approach is to use initiator multiplicity to control the polymer architecture which also allows to add terminal functionalities, such as attachment of drug-targeting moieties at the chain ends by the termination method.<sup>[24,87]</sup> Mono- and difunctional initiators yield linear symmetric or asymmetric telechelic polymers,<sup>[16,88]</sup> whereas higher plurifunctional initiators give tri- and tetra- arm star polymers,<sup>[89]</sup> bow-tie multiarm stars,<sup>[36]</sup> and, ultimately, macroinitiators result in comb copolymers or at high grafting densities in molecular brushes (Figure 5).<sup>[90,91]</sup>

In all cases, high initiator efficiency and rapid initiation versus propagation is crucial to obtain defined structures. This can be realized by (pluri)triflates,<sup>[89]</sup> nosylates,<sup>[36]</sup> or oxazolinium macroinitiator salts.<sup>[90,91]</sup> For example, oligohalides have been used for the preparation of star-like polymers, but as initiation is slow compared to propagation it has to be expected that different arms of a star polymer differ significantly in their length.<sup>[93–95]</sup> Alternatively to the *grafting from* approach, star, dendritic, or comb polymers can be synthesized by *grafting through*<sup>[96–99]</sup> and *grafting onto*<sup>[14,100–103]</sup> approach. Although these methods involve additional preparative steps, they allow a direct combination of POx with other polymers including additional functions by main chain copolymerization or conjugation. As complex polymer architectures are notoriously difficult to characterize, the latter two methods enable a better analysis of the side-chain length, composition, functionality, and distribution *prior* grafting. Moreover, defined and thoroughly characterized batches of POx and



**Figure 5.** A straightforward possibility to vary the architecture of POx. (a) Mono- and difunctional initiators yield linear homo- or block copolymers and optionally homo as well as heterofunctional telechelic. (b) Tri-, tetra-, and plurifunctional initiators give rise to stars<sup>[89]</sup> and (c) “bow-tie” polymers.<sup>[36]</sup> (d) Macroinitiators yield molecular brushes.<sup>[90,91]</sup> (e) Initiator functionalized nanoparticles give polymer brush-coated nanocomposites.<sup>[92]</sup>

other polymers of different length and functions may be combined into a final structure to yield complex, yet defined polymer constructs. This may be of advantage in the approval and final application in different therapeutic situations. However, the limiting factor is the definition of the last synthetic step. Here, the recent developments in click chemistry might be an answer to this problem and various groups have recently demonstrated the versatility of combining POx chemistry with various click reactions for functionalization as well as assembly of complex polymer constructs.<sup>[8,12,14,25,28,34,38,79,101,104–108]</sup> In this context, the POx system shows potential for polymer therapeutics development; however, only first steps have been made to utilize this modular toolbox. Moreover, a modular approach may be one possibility to overcome one of the most significant limitations of this polymer, namely,

the limited molar mass of POx realizable by direct LCROP. Although the LCROP is of highly living nature, the cationic propagating species show typical side reactions such as chain transfer which becomes more prominent at high degrees of polymerization.<sup>[109]</sup>

### 2.3. Biocompatibility of POx

The biocompatibility of a particular material is highly complex and correlates with the interaction of the material with a variety of biological entities such as proteins and barrier membranes. Such interactions can be hydrophobic, electrostatic, or hydrogen bonding or any combination thereof. Accordingly, the ability to tailor the physicochemical characteristics of a biomaterial is highly desirable. As discussed above, POx appears to be an ideal candidate in this respect.

In vitro cytotoxicity has been studied for a wide range of POx-based polymers and, generally, was found to be rather low in a range of cell lines such as human neural progenitor cells, Madin–Darby canine kidney cells, MCF7, HEP G2, and CATH.a. Linear, hydrophilic, amphiphilic, star-like POx,<sup>[110–112]</sup> and POx-based particles<sup>[107]</sup> have been studied.

Also hybrid systems, such as poly(L-lactide)-*b*-PEtOx-*b*-poly(L-lactide) (PLA–PEtOx–PLA) were found non-cytotoxic at 50 g L<sup>-1</sup> and exhibit even at 15%wt only moderate reduction in cell viability (human skin fibroblast).<sup>[113]</sup> In contrast, others found marked cytotoxicity at rather low concentrations of below 1 g L<sup>-1</sup> of a PEtOx-poly( $\epsilon$ -caprolactone) (PEtOx–PCL) block copolymer in KB cells<sup>[114]</sup> or PEtOx-*b*-poly(L-lactide) in human fibroblasts (HFW) or non-small-cell lung carcinoma (CL3).<sup>[115]</sup> Similar triblock copolymers (PEtOx-*b*-PCL-*b*-PEtOx) were also found to be non-cytotoxic at up to 10 g L<sup>-1</sup>.<sup>[116]</sup> Moreover, macrophages were not influenced in their metabolic and phagocytic activity after incubation with PEtOx-based polymers.<sup>[117]</sup>

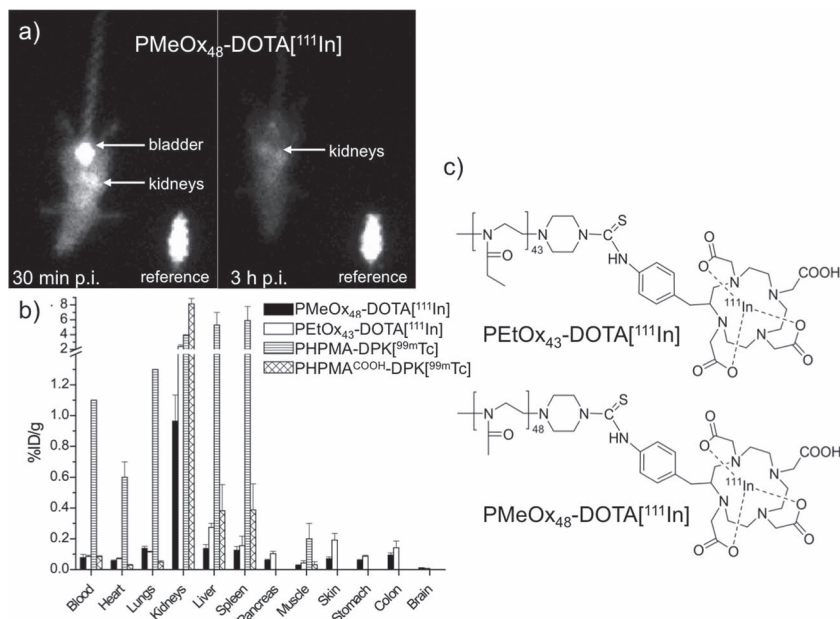
Not surprisingly, POx was found to be cytotoxic when cationic charges are incorporated, either along the backbone or the polymer termini.<sup>[110,118–120]</sup> This was used for the development of antimicrobial POx.<sup>[118,119,121]</sup> Hemocompatibility studies showed no hemolytic effects for pure PEtOx at 20 g L<sup>-1</sup>,<sup>[31]</sup> similarly PEtOx-*b*-PCL block copolymers were non-hemolytic at 10 g L<sup>-1</sup>.<sup>[114]</sup>

With respect to in vivo toxicity, PEtOx has been studied most thoroughly to date, followed by PMeOx. Tomalia and Killat<sup>[122]</sup> described in an early encyclopedia entry that PEtOx is very well tolerated per oral (rats) and dermal (rabbits) administration with LD<sub>50</sub> values exceeding 4 g kg<sup>-1</sup>. It was also described that PEtOx is non-irritating to eye (rabbits) and skin (guinea pigs). Unfortunately, no primary report could be found for these claims. More recently, Viegas et al.<sup>[31]</sup> reported that repeated intravenous injections (rats) of doses as high as 2 g kg<sup>-1</sup>

( $\bar{M}_n = 10 \text{ kg mol}^{-1}$ ) had no adverse effects on the animals and that histological examinations of kidney, liver, and spleen did not produce any difference as compared to control animals. For  $20 \text{ kg mol}^{-1}$  PEtOx, apparently only lower concentrations of  $50 \text{ mg kg}^{-1}$  were studied and found to give no adverse effects. However, only lower doses were studied. Although, to the best of our knowledge, no comparable study has been performed with PMeOx, it may be assumed that similar low toxicity may be observed, as judged from cytotoxicity<sup>[110]</sup> and biodistribution<sup>[123]</sup> studies. More recently, a POx-based hydrogel was investigated in rabbit for intravitreal antibody delivery. Histology after 2 months found no changes in morphology of the retina. Moreover, electroretinography demonstrated that retinal function was normal after gel injection.<sup>[116]</sup>

Interaction of POx with proteins has been studied also by a number of researchers. In an early report, Naka et al.<sup>[124]</sup> investigated the interaction between human serum albumin (HSA) with amphiphilic POx block copolymers. It was found that polymers with 2-butyl-2-oxazoline (BuOx) as the hydrophobic constituent adsorbed less albumin compared to polymers with 2-nonyl (NOx) or 2-phenyl-2-oxazoline (PhOx) as the hydrophobic monomer units.

Other groups studied protein interaction with surface grafted POx [PMeOx, PEtOx, and Poly(2-*n*-propyl-2-oxazoline) (PnPrOx)] in a variety of setups. Generally, the tendency for protein adsorption, cell or bacterial adhesion was found to be low for hydrophilic POx.<sup>[100,121,125–127]</sup> While the most hydrophilic POx such as PMeOx and PEtOx effectively prevent protein adsorption, the more hydrophobic POx (PnPrOx) promotes protein adsorption and subsequent cell adhesion. Additionally, an influence of the polymer architecture and presence of hydrogen-bonding donors was found.<sup>[126]</sup> A direct comparison with analog PEG-based systems was recently carried out by Textor and co-workers.<sup>[128]</sup> Their main finding is that, under physiological conditions, PMeOx-based coatings were significantly more stable than PEG coatings and kept their non-fouling properties while PEG suffered from substantial degradation over time. As PEG degradation results in reactive species (radicals, peroxides, superoxides), the more stable POx seems to be a better, but not yet established alternative for the coatings of implants.



**Figure 6.** Biodistribution of PMeOx and PEtOx. (a) Single photon emission computed tomography (SPECT) images of mice injected with  $^{111}\text{In}$ -labeled PMeOx ( $5 \text{ kg mol}^{-1}$ ) at different time points after injection. Comparison of biodistribution data of poly(2-hydroxypropyl methacrylamide) (PHPMA) (from ref.<sup>[131]</sup>) shows that POx give much lower uptake in the organs of the reticuloendothelial system (liver, spleen).<sup>[132]</sup> Although introduction of negative charges to the HPMA can decrease RES uptake to some extent, extremely high kidney uptake may limit feasibility of this approach. (c) Structures of the radiolabeled POx used in the biodistribution study.

Low unspecific protein adsorption is important in the context of blood circulation and polymer biodistribution. Indeed, liposomes containing POx lipopolymers exhibited prolonged blood circulation, very similar to PEG-coated liposomes. It should be noted that PMeOx-liposomes exhibited higher blood levels and lower liver accumulation 24 h after administration.<sup>[17,129]</sup>

Hydrophilic POx alone show, depending on the molar mass, a biodistribution profile that is consistent with the so-called stealth behavior. PMeOx and PEtOx of  $5 \text{ kg mol}^{-1}$  and low dispersity ( $\mathcal{D} < 1.2$ ) were excreted very rapidly (essentially at first renal passage) and uptake in organs of the reticuloendothelial system was very low (PMeOx: Liver and Spleen  $\approx 0.1\% \text{ ID/g}$  0.5–24 h p.i.; PEtOx: Liver and Spleen  $\approx 0.2\%–0.3\% \text{ ID/g}$  0.5–24 h p.i.) (Figure 6).<sup>[123]</sup> In contrast, PMeOx of 15 and  $30 \text{ kg mol}^{-1}$  circulated much longer [blood levels 48 h p.i.: 4% ID ( $15 \text{ kg mol}^{-1}$ ), 15% ID ( $30 \text{ kg mol}^{-1}$ )]. In the same study, very low liver and spleen uptake was observed but uptake in skin was found to be significant.<sup>[130]</sup>

Formation of antibodies and complement activation are major concerns regarding polymer therapeutics. To the best of our knowledge, antibodies against POx are currently unknown, but ELISA has shown some complement activation. The levels of C3a-desArg fragments of the complement cascade were studied after serum incubation



with a series of PMeOx, PEtOx, and PBuOx block copolymers.<sup>[133]</sup> The levels were moderate but significantly above negative control. Similarly, amphiphilic block copolymers of EtOx and 2-phenyl-2-oxazoline (PheOx) were shown to induce relatively high levels of SC5b-9 in a concentration and structure-dependent manner.<sup>[111]</sup>

In summary, although much more needs to be done to verify the safety of POx for clinical use, in particular, with respect to polymer genomics and immunogenicity, the available data look very promising that POx may eventually be recognized as a safe material in general (GRAS).

In the context of POx for the use as polymer therapeutics, the equally low protein interaction compared to PEG along with a significantly better chemical stability of POx in conjunction with low unspecific organ deposition<sup>[123]</sup> are good arguments to look into the substitution of PEG with hydrophilic POx, for example, currently used PEGylated drugs that are constantly administered over long time or are to remain in the body.

### 3. Polymer–Protein Conjugates

It is widely recognized that many issues need to be addressed for efficient therapeutic protein delivery. They include but are not limited to poor physicochemical stabilities, problematic pharmacokinetics, immunogenicity, and inability to cross biological barriers such as the blood brain barrier and cellular membranes.<sup>[134]</sup> PEGylation with

linear or branched PEG successfully enhanced in vivo stability and circulation time and reduced immunogenicity of many protein therapeutics, and is now widely used in pharmaceutical and biotechnology industry with several very successful products in the market.<sup>[10,135,136]</sup> However, it is also evident that PEGylation does not help too much to enhance the delivery of proteins across cellular membranes and biological barriers to reach their target sites. Therefore, alternative polymer modification strategies to enhance the transport and intracellular delivery of proteins are necessary. At the same time, it would be desirable to retain the advantages of PEG and utilize the experience with PEGylation in protein pharmacokinetics.

POx has been studied for protein modification (POxylation) and delivery for some time. In analogy to the PEGylation, most of these studies used the hydrophilic PEtOx and PMeOx homopolymers.

Various proteins such as trypsin, catalase, RNase, insulin, serum albumin, and few others have been conjugated to PMeOx or PEtOx (Table 1), and these protein–POx conjugates generally performed similarly to PEGylated proteins both in vitro and in vivo. Early work was performed in the 1990s, but research later ceased for almost two decades.

In a two-step synthesis Farkas et al.<sup>[137]</sup> prepared POx–bovine serum albumin (BSA) conjugates and successively POx–BSA–antigen conjugates for vaccination against *vibrio cholerae*. For this, a non-protected aniline-functionalized 2-oxazoline was used for the copolymerization with

■ Table 1. Overview of POxylated proteins.

Polymer	Protein	Linking chemistry	Reference
PMeOx (1–10 kDa), PEtOx (3.5–6.5 kDa)	Catalase	P–COOH/DCC/NHS + Protein–NH <sub>2</sub>	[31,138]
PEtOx (5 kDa)	Trypsin	P–COOH/DCC/NHS + Protein–NH <sub>2</sub>	[139]
PMeOx (6 kDa), P(MeOx- <i>b</i> -BuOx) (12 kDa), P(EtOx- <i>b</i> -BuOx) (12 kDa), P(EtOx- <i>co</i> -BuOx) (9 kDa)	HRP	P–NH <sub>2</sub> + diNHS + Protein–NH <sub>2</sub>	[146]
PEtOx (5 kDa and 10 kDa)	BSA	P–COOH/DCC/NHS + Protein–NH <sub>2</sub>	[31]
PEtOx (5 kDa)	Ribonuclease	P–COOH/DCC/NHS + Protein–NH <sub>2</sub>	[31]
PEtOx (5 kDa)	Uricase	P–COOH/DCC/NHS + Protein–NH <sub>2</sub>	[31]
PEtOx (5 kDa)	Insulin	P–COOH/DCC/NHS + Protein–NH <sub>2</sub>	[31]
P(IPOx- <i>g</i> -PEtOx) (surface bound protein density gradients)	GFP	P–COOH/EDC/NHS + Protein–NH <sub>2</sub>	[141]
PMeOx (5.8, 6.4 kDa) PEtOx (4.8, 9.3 kDa)	Q $\beta$ virus coat protein	P–alkyne (CuAAc) + Protein–N <sub>3</sub>	[106]
PEtOx (5, 10, 20 kDa)	G-CSF	P–COH + Protein–NH <sub>2</sub> and P–NH <sub>2</sub> + TGase + Protein–CONH <sub>2</sub>	[140]

MeOx and later polymer analog coupling. Although it was shown that both, BSA and the antigen was coupled, the free amino group interfered strongly with the LCROP and rather undefined polymers were obtained.

Saegusa and co-workers<sup>[138]</sup> studied POxylated catalase in vitro and compared the enzymatic activity in dependence of the nature and molar mass of the attached polymer (PMeOx and PEtOx; 1–10 kg mol<sup>-1</sup>). It was found that while the molar mass and extend of modification had a major effect on the enzymatic activity of PMeOx-modified catalase such influence was not observed for PEtOx.<sup>[138]</sup> The results were recently confirmed by others.<sup>[31]</sup> Both groups realized conjugation by NHS activated, carboxylate terminated POx. Using the same chemistry, PEtOx (5 and 10 kg mol<sup>-1</sup>) was also conjugated to ribonuclease (RNase), insulin, BSA, and uricase. Depending on the extent of modification and the enzyme, low (RNase), moderate (uricase) to high (catalase) residual enzymatic activities were observed. In, PEtOx-insulin vivo, PEtOx–insulin conjugate lowered blood glucose level for 8 h compared to 2 h for insulin alone in rats and PEtOx–BSA conjugate exhibited significantly attenuated immunogenic properties compared to BSA alone or PEG–BSA conjugates in rabbits.<sup>[31]</sup>

Veronese and co-workers<sup>[139]</sup> have used similar chemistry to conjugate trypsin to PEtOx (5 kg mol<sup>-1</sup>). Again, it was found that enzymatic activity depended on the extent of the conjugation and was similar to PEGylated trypsin.

Using a different chemistry, Mero et al.<sup>[140]</sup> prepared PEtOx conjugates with granulocyte colony growth stimulating factor (G-CSF). No significant loss of protein function was observed in while vitro, whereas in vivo a pronounced increase in the pharmacodynamic area under the curve was found for neutrophil and white blood cell counts.<sup>1</sup> Interestingly, not only the POx molar mass but also the attachment site appeared to have an effect on the pharmacokinetic and–dynamic response. These results highlight the potential of POx as ideal alternatives to PEG for protein conjugation to modulate the delivery, protein activity and stability. Hutter et al.<sup>[141]</sup> prepared patterned bottle-brush brushes (BBBs) of poly(2-isopropenyl-2-oxazoline-*g*-PEtOx) on diamond by carbon templating<sup>[142]</sup> and coupled green fluorescence protein (GFP) onto the pendant PEtOx chain ends. Besides the surprisingly high conjugation efficiency onto the BBBs, it was found that for polymer–brush–GFP conjugate the protein keeps its native state and the protein stability significantly improves as compared to the free GFP.

<sup>1</sup>Pharmacodynamics may be simplified as what the drug does to the organism, as opposed to pharmacokinetics which may be defined as what the organism does to the drug (e.g., metabolism). Please see: L. Z. Benet, Pharmacokinetics: basic principles and its use as a tool in drug metabolism, page 199 in: Drug Metabolism and Drug Toxicity (Eds: J. R. Mitchell and M. G. Horning), Raven Press, New York 1984.

However, as mentioned above, PEGylation is not necessarily helpful to carry therapeutic proteins across biological barriers. Similarly, hydrophilic POx are expected to convey the same problems. Some of us have reported that modification with amphiphilic block copolymers such as Pluronics can efficiently enhance the cellular delivery and transport across the blood brain barrier of various proteins and increase their in vivo circulation time at the same time.<sup>[143–145]</sup> This strategy can also be successfully applied using amphiphilic block copolymers based on POx. Especially, variation in side chain length and architecture of POx provides a powerful tool to modulate the polymer hydrophilic/hydrophobic balance, which may be critical for enhancing protein delivery, for example, to the brain.

We prepared conjugates of horseradish peroxidase (HRP) with two block copolymers P(MeOx-*b*-BuOx) and P(EtOx-*b*-BuOx), one random copolymer P(EtOx-*co*-BuOx) and a PMeOx homopolymer using biodegradable or non-biodegradable linkers and a NHS-based synthetic strategy.<sup>[146]</sup> These conjugates contained on average one to two polymer chains per protein molecule and retained 70% to 90% of enzymatic activities. Significantly enhanced cellular uptake in both MDCK cells and Caco-2 cells was found in the conjugates of P(MeOx-*b*-BuOx) and P(EtOx-*b*-BuOx), but not in the conjugates of P(EtOx-*co*-BuOx) and PMeOx.

Recently, Finn et al.<sup>[106]</sup> used the highly efficient copper-catalyzed azide-alkyne cycloaddition (CuAAC) to combine functionalized POx (PMeOx, PEtOx) with virus-like particles (VLPs). The alkyne-function on the POx, placed either as a pendant or as an end function, was reacted with the VLPs (Q $\beta$ ) bearing azide groups. The CuAAC click reaction gave defined core-shell nanocomposites with the hydrophilic POx forming the shell. The combination of VLPs with POx bearing pendant alkyne groups per chain resulted in near to quantitative click reaction because of the cooperative binding effect. Most interestingly, the temperature stability of the POx–VLP conjugate was significantly enhanced if the capsid subunits were interconnected via the side chain functionalized POx. This strategy of a protein complex in a polymer net should also work for other functional protein complexes such as enzymes, to enhance or modulate their stability and function and may open new modes for their use. The potential use of POx-virus biohybrids spans from the modulation of the immunosystem to drug delivery, taken advantage of the virus inner volume and the chemistry of the POx shell to stealth the virus and to attach specific binding motifs for targeting.

#### 4. Polyplexes

In the context of gene delivery, POx have long been of great interest, albeit as a very convenient source of defined and linear poly(ethylene imine) (PEI), which can be obtained

by hydrolysis of POx. Apart from PEI, also platforms comprising POx have been investigated for gene delivery. Park and co-workers<sup>[120]</sup> used the well controllable hydrolysis of PEtOx to investigate the effect of partial hydrolysis. It should be noted that rather poorly defined, commercially available POx ( $\bar{M}_n = 3-4$ ) was utilized in this study. Degrees of hydrolysis were about 50% to 90%. Clearly, cytotoxicity of the polymers was strongly dependent on the degree of hydrolysis with PEtOx-co-PEI (8/92 mol/mol) being as toxic as branched PEI. In general, polycations are toxic because of their strong interaction potential with cell membranes (negatively charged phospholipids) and proteins. Hence, in gene delivery, toxicity has to be balanced with transfection efficiency. In this field, many questions remain, but for POx-PEI copolymers it could be demonstrated that the same transfection efficiency could be obtained while lowering the cytotoxicity.<sup>[120]</sup> Klivanov et al.<sup>[147]</sup> showed that full deacylation of POx dramatically boosts the gene delivery efficiency and specificity.

Hsiue and co-workers used an extension of this approach to obtain block copolymers of POx and PEI. Analog to Park's work, starting from a linear PEtOx, the side chains were partially saponified. Subsequently these copolymers were coupled to another PEtOx via a disulfide linkage. Interestingly, although much higher ratios of polymer/DNA were necessary to efficiently condense DNA compared to branched PEI, HeLa cells could be very well transfected with luciferase encoding plasmid DNA. As the toxicity of the non-ionic/cationic copolymers was strongly attenuated as compared to branched or linear PEI, the strategy of lowering the charge density in non-viral vectors appears promising. However, to date no further development on this platform has been reported.<sup>[26]</sup>

Finally, Lühmann and co-workers<sup>[148]</sup> studied brushes comprising a poly(L-lysine) (PLL) backbone with PMeOx grafted onto the side chains (PLL-*g*-PMeOx). These

materials were previously discussed in the context of non-fouling surfaces.<sup>[100,121,128]</sup> Here, only graft copolymers with comparably short PMeOx (4 kg/mol) and rather low grafting densities of  $\approx 10\%$  could effectively transfect cells (COS-7). In part, this was attributed to the fact that more densely grafted brushes could not condense DNA as well (steric hindrance). In addition, when larger PMeOx (8 kg mol<sup>-1</sup>) was grafted onto PLL, the resulting DNA condensates were larger and found to be less effectively taken up into cells, which would also prevent transfection. Also, and similar to analogous PLL-*g*-PEG brushes, DNA was protected from degradation by serum proteins.

In summary, only few groups have utilized POx-based platforms for gene delivery purposes to date. However, considering the promising first results and the versatility of POx chemistry and the possibility of structural variations, more systems can be expected in the near future.

## 5. Polymer-Drug and Polymer-Peptide Conjugates

Only very few examples can be found for this category which are summarized in Table 2. In fact, only two peer-reviewed papers have been published, the other examples were presented at conferences or published online otherwise.

One of the earliest papers of POx in a biomedical category falls in this context. In a pioneering study, Riffle and co-workers<sup>[87]</sup> used living POx chains to directly attach the polymer to a short dodecapeptide (EDQVDPRLIDGK). The recovered yields were rather low. Subsequently, the avidity of the peptide to a specific antibody was tested. It was reported that PMeOx and PEtOx in the range of 1-10 kDa did diminish the binding to the antibody to some extent. The increase in half-maximal inhibitory

■ Table 2. POxylated targeting or therapeutic small molecules.

Polymer	Peptide/drug	Linking chemistry	Reference
PMeOx PEtOx (1-10 kDa)	HCPC	Direct termination with protein-NH <sub>2</sub>	[87]
PMeOx, PEtOx	RGD	POx-alkyne + Peptide-azide, POx-aldehyde + Peptide-aminoxy	[28,132]
PEtOx	CREKA	Native ligation	[132]
PMeOx	MTII	POx-aldehyde + Peptide-aminoxy	[132]
PEtOx	Ara-C	P-COOH/DCC/NHS + Protein-NH <sub>2</sub>	[139]
Unknown, presumably PEtOx	Irinotecan/folate (SER203)	Presumably POx-alkyne + drug azide	[149]
Unknown, presumably PEtOx	Irinotecan (SER201)	Presumably POx-alkyne + drug azide	[149]
Unknown, presumably PEtOx	Unknown/folate (SER207)	Presumably POx-alkyne + drug azide	[149]

concentrations ( $IC_{50}$ ) were increased by a factor of 25 at maximum.<sup>[87]</sup> After this work, a large gap of about 15 years occurred before attachment of small peptides to POx was investigated again.

Luxenhofer et al.<sup>[28,132]</sup> prepared conjugates of various POx with a variety of small peptides, namely, cyclic RGD binding integrin  $\alpha_v\beta_3$ , the phage-display identified CREKA sequence and MTII, known to bind melanocortin receptor. All conjugations were performed using chemoselective reactions using POx with appropriate side chains. CREKA, bearing an N-terminal cysteine was an ideal candidate for native ligations, while the other peptides were attached using aminoxy-aldehyde ligation or click chemistry (Cu-catalyzed 1,3 dipolar cycloaddition of azides and alkynes). Most conjugates contained also radionuclide chelators and were designed for radionuclide therapy or diagnosis. This work presented a proof-of-principle with respect to the preparation of such conjugates but unfortunately not comprehensive biological evaluation was performed. Preliminary work using the RGD and MTII conjugates showed that the peptides led to some specific binding and in vitro as well as in vivo.

Similarly preliminary, POx conjugates to cytosine arabinose (Ara-C) exhibited a biological effect, albeit somewhat attenuated as compared to a corresponding PEG conjugate.<sup>[139]</sup>

Finally, Harris and coworkers presented data on POx conjugates to irinotecan (with and without folate targeting), a potent drug plagued by severe side effects (most importantly diarrhea and immunosuppression). Although no details are available for this commercially developed platform (Serina Therapeutics Inc.), it was suggested that the conjugation chemistry utilized is similar or equal to the strategy employed by Luxenhofer et al. before.<sup>[149,150]</sup> The first results are promising and the company plans to move forward into clinical trials in 2013.<sup>[149]</sup>

## 6. Polymeric Micelles, Aggregates, and Nanoparticles

A common problem for the application of many potent drugs and drug candidates especially in cancer therapy is the often very limited water solubility of these molecules and hence, their poor bioavailability. One possibility is to link the drug to a hydrophilic polymer (see Section 5), which however, results in the formation of a “new chemical entity” which complicates regulatory approval. Another possibility that does not involve chemical modification of the drug, is to solubilize the drugs in polymeric micelles. Here, formulations with polymer amphiphiles are most suitable, as polymeric micelles are of higher thermodynamic stability as compared to micelles from low molar mass amphiphiles, no ionic motives are needed to realize

a strong amphiphilic contrast, their critical micelle concentration is very low ( $10^{-8}$ – $10^{-2}$  mol L<sup>-1</sup>) and thus polymeric micelles are less likely to disassemble upon dilution, that is, when administered intravenously.<sup>[151,152]</sup> Moreover, the hydrophilic polymer shell of the micelles can automatically provide the stealth effect known from naturally occurring transport systems and liposomal formulations to prolong the blood circulation time. At the same time it offers the possibility to attach specific binding sites for targeted drug delivery.<sup>[153]</sup> Especially for anti-cancer agents, polymeric micelles are suitable for selective delivery through a passive targeting mechanism,<sup>[154]</sup> and formulations using Pluronics (PEG-*b*-PPG-*b*-PEG) have been studied to a great extent as drug delivery systems.<sup>[155–157]</sup>

For micellar drug delivery, POx is ideally suited. Analog to the polyethers, POx is synthesized by living polymerization, allowing for high structural and compositional definition and quantitative endgroup functionalization. However, in contrast to polyethers, the water solubility of POx can be specifically fine-tuned and also spans a broader range as discussed above.

Combination of hydrophilic POx with (biocompatible) hydrophobic polymers yields polymer amphiphiles suitable for drug delivery by compartmentation into polymersomes as nanocarrier systems for drug and gene delivery.<sup>[158]</sup> A prominent example is the work by Meier using (giant) polymersomes assembled from PEG–PDMS–PMeOx<sup>[159,160]</sup> or PMeOx–PDMS–PMeOx<sup>[158,161–164]</sup> for encapsulations of trypsin,<sup>[165]</sup> pravastin,<sup>[166]</sup> and *trypanosoma vivax* nucleoside hydrolase (TvNH) combined with the bacterial outer membrane protein OmpF (a porine) for transmembrane transport of prodrugs and drugs.<sup>[167]</sup> The biomimetic nature of the polymersome makes a cell specific integration and trafficking of such constructs as artificial organelles possible and they could even be used for a virus-assisted loading with DNA.<sup>[168]</sup> As outlined above, the outer POx layer can be used for specific attachment of cell targeting moieties while keeping the stealth effect of the hydrophilic POx shell of the assembly. Meier et al.<sup>[169]</sup> used the biotin-streptavidin system to conjugate ligands that target scavenger receptor A1 from macrophages. In human and transgenic cell lines, receptor-specific binding was followed by vesicular uptake while otherwise non-specific binding and cytotoxicity was not observed. Recently, the same group attached antibodies onto the amine or hydroxyl-terminated PMeOx shell for specific targeting of SKBR3 breast cancer cells.<sup>[170]</sup> Montemagno and co-workers<sup>[171]</sup> used the second alternative, PETox-PDMS-PETox, for the self-assembly of polymersomes to realize the biosynthesis of ATP.<sup>[172,173]</sup> Very recently, Tiller and co-workers<sup>[174]</sup> reported on PMeOx–PPhOx–PMeOx ABA block copolymers that self-assemble in aqueous solutions into unimolecular micelles, micellar aggregates and, analog to the POx–PDMS–POx system, into form-stable

polymersomes. Although no drug loading of the polymersomes were shown yet, it can be expected that first drug-delivery systems will be realized soon.

Combination of POx with other hydrophobic polymers result in polymer amphiphiles that can combine advantageous properties of POx in terms of the stealth effect with already established polymer systems. Obeid and Scholz<sup>[15]</sup> used  $\alpha,\omega$ -amino,hydroxyl-PMeOx as macroinitiators for the ROP of *N*-carboxyanhydrides (NCAs) to yield defined POx-poly(amino acid)s (PAA) copolymers.<sup>[175]</sup> In water, the amphiphilic POx-PAA block copolymers assembled into particles with a diameter around 70–130 nm depending on the block sizes and ratio, a suitable size range to use such particles as polymer therapeutics. Hsiue et al.<sup>[176]</sup> prepared polyion complex micelles (PIC) from PEtOx-poly(aspartic acid) (PAsp) from amino-terminated PEtOx by the same route. Under neutral or alkaline conditions both polymers are hydrophilic, only under acidic conditions (pH < 5.2) core-shell micelles with a PEtOx shell are formed. Via the PAsp segment, an antifungal drug, amphotericin B (AmB), was associated into PICs in high contents (up to 47 wt%). The formulation of AmB into micelles significantly increased the solubility of the drug as well as its efficacy (against fungal cells). This was attributed to a release of AmB in its monomeric form. In vitro the cytotoxicity with respect to human fibroblasts HT-1080 of loaded PIC was found to be less than a comparable commercial formulation (Fungizone) used in clinics. Chang et al.<sup>[177]</sup> prepared amphiphilic comb copolymers with a polyphosphazene backbone and PMeOx side chains by the *grafting from* method using benzylbromide initiators along the main chain. The final amphiphilic poly(4-methylphenoxyphosphazene)-*g*-PMeOx formed defined micelles that solubilized pyrene efficiently. Despite its structure, polyphosphazene is a highly biocompatible and non-toxic polymer and thus its combination with POx into amphiphiles has some potential. Recently, Guillerme et al.<sup>[101]</sup> combined  $\alpha$ -alkyne-PMeOx with poly( $\epsilon$ -caprolactone) (PCL) bearing azide pendant functions to form comb copolymers via *grafting onto* using Cu-catalyzed click chemistry. In water, the PCL-*g*-PMeOx copolymers assembled into defined spherical nanoparticles of 40 nm ( $R_h$ ). Jeong and co-workers<sup>[178]</sup> used hydroxyl-end-functionalized PEtOx to prepare linear block copolymers with poly(L-lactide) (PLA) and PCL. PEtOx-PCL polymeric micelles were used to solubilize paclitaxel (PTX) by the dialysis method.<sup>[114]</sup> PTX is one of the most potent anti-cancer drugs known for various cancers including ovarian, breast, and lung cancer but is unfortunately almost insoluble ( $1\mu\text{g mL}^{-1}$ ). The loading content was in the range of 0.5–7.6 wt% depending on the copolymer composition and preparation conditions with a decreasing loading efficiency for increasing PTX feed ratio. The PTX-loaded micelles with a hydrodynamic diameter around 20 nm showed no significant hemolysis

and a membrane toxicity comparable to Cremophor EL. In vitro studies revealed that the inhibition of KB human epidermoid carcinoma cells was similar to commercial PTX formulations based on Cremophor EL. Following the same synthetic route, PEtOx-*b*-PLA diblock<sup>[115,179]</sup> as well as triblock<sup>[180,181]</sup> copolymers were synthesized by Hsiue et al. and their pH- and temperature responsiveness investigated. The PEtOx-*b*-PLA micelles were used for the solubilization of doxorubicin (DOX) in neutral pH, DOX release could be triggered by acidic conditions. The system is proposed as an intracellular drug carrier releasing the payload in acidic cellular organelles by means of micelle disintegration. Later on, the PEtOx-*b*-PLA copolymer was used in combination with poly(*N*-isopropyl acrylamide-co-methacrylic acid)-*g*-PLA graft copolymer and PEG-*b*-PLA<sup>[179]</sup> to form mixed micelles for DOX transport with optimized properties in terms of higher drug efficiency and lower material cytotoxicity (in vitro studies with HeLa cells) as compared to one component micelles. PEtOx-PLA-based micelles can also be used for a different therapeutic approach, namely for photodynamic therapy (PDT). In PDT, light-induced chemical reactions are used to cause localized tissue damage for the treatment of cancerous or other non-malignant conditions with the help of photosensitizers and specific irradiation. One problem of PDT is that patients have to stay basically in the dark during the therapy to avoid skin phototoxicity. Lai and co-workers<sup>[182]</sup> incorporated meta-tetra(hydroxyphenyl)chlorin (mTHPC), into pH-sensitive PEtOx-*b*-PLA micelles and investigated the cytotoxicity and antitumor effect in vitro and in vivo. It was found that formulated mTHPC was as effective as free mTHPC, however, the photosensitivity of the skin (mice) could be significantly reduced which indicates a possible improvement of PDT by the use of POx-based micelles as the photosensitizer carrier system.

As outlined above, different amphiphilic POx have been investigated with respect to their self-assembly behavior into defined micelles or nanoparticles and their interaction with proteins and cells. Naka et al.<sup>[124]</sup> studied various amphiphilic POx with PMeOx as the hydrophilic and PBuOx, (poly(2-*n*-octyl-2-oxazoline) POcOx and PPhOx as the hydrophobic block in the range of  $\bar{M}_n = 4.7\text{--}7.1$  kDa. Depending on the block copolymer nature and composition, the formation of spherical as well as cylindrical micelles were found by DLS and TEM. A detailed study of their interaction with HSA revealed that adsorption of HSA mainly occurs at the surface or via the hydrophilic shell of POx micelles and not at the hydrophobic core. Donev et al.<sup>[111]</sup> studied the complement activation, neurotoxicity and neurodevelopmental gene expression of P(EtOx-co-PhOx) (mass range: 6.5–12.5 kDa,  $R_h$ : 110–150 nm) using human neural progenitor cells (ReNcell VM expanded and differentiated in presence of POx). For high micellar concentrations, a significant complement

activation was found for P(EtOx-co-PhOx) particles of  $R_H = 122$  nm based on the resulting C5b-9 concentration (a membrane attack complex responsible for cellular lysis.) However, at low concentrations and for slightly smaller or bigger micelles no significant activation of the complement system was found. In cytotoxicity assays with human neurons no neurotoxic effect was observed for all P(EtOx-co-PhOx) micelles. Finally, the effect of the micelles upon gene expression during differentiation was studied. Using a panel of thirty relevant genes only the smallest P(EtOx-co-PhOx) micelles may cause some neuropsychiatric disorder because of down-regulation of two genes during neurogenesis. For differentiated neurons, no adverse effects could be observed for all POx micelles. This first study, however, allows no clear answer to a possible structure–property relationship with respect to the block copolymer, amphiphilic contrast as well as micellar structure. Luxenhofer et al.<sup>[110]</sup> synthesized a series of POx homopolymers (PMeOx, PEtOx;  $\overline{M}_n = 6.4$ – $6.7$  kDa), amphiphilic diblock copolymers (PMeOx-PEtOx- with -PBuOx, -PiPrOx, -PnPOx;  $\overline{M}_n = 10$ – $13$  kDa) as well as 14 different ABA and ABC triblocks ( $\overline{M}_n = 5.8$ – $14.7$  kDa) including a variation of endfunctions and introduction of an alkyne side functionality for polymer analogue click reactions. The cytotoxicity of all polymers was studied using various cancer or immortalized cell lines (human breast cancer: MCF7, MCF7-ADR, immortalized canine kidney cells: MDCK). For all hydrophilic homopolymers as well as all amphiphilic di- and triblock copolymers no cytotoxicity even at high concentrations could be found. The only exceptions were found for POx equipped with quaternized endgroups bearing a long *n*-alkyl chain. Such compounds are known as antimicrobial polymers from the studies of Tiller and co-workers.<sup>[118,119,183–185]</sup> Furthermore, Luxenhofer studied the cellular uptake (endocytosis) of the hydrophilic and amphiphilic di and triblock POx by flow cytometry and confocal fluorescence microscopy. A clear relationship between the amphiphilic structure and cell uptake was found. The more hydrophobic the copolymer structure is, the more readily it enters the cell. The cell uptake appears to be similar to pluronics or amphiphilic poly(2-hydroxypropylmethacrylamide) (PHPMA) copolymers,<sup>[62]</sup> however, endocytosis was found to be unusually rapid for several amphiphilic POx, for example, for PEtOx-*b*-PBuOx block copolymers with 100% gated cells within only 10 min. After internalization of the POx micelles by the cells, the polymer was found to be distributed throughout the entire cell with increasing concentration in the perinuclear region and possibly enriched in vesicular compartments. No remaining POx was found in the cell membranes. The efficiency and speed of cell gating is a very promising indicator that POx micelles as a drug-delivery vehicle for conjugated or incorporated drugs. Along this line, Schubert and co-

workers<sup>[107]</sup> presented a first study of the cellular uptake of random P(EtOx-co-(2-decenyl-2-oxazoline)) (P(EtOx-co-DecOx)) into mouse fibroblasts. The pendant double-bond allows for later polymer analog functionalization by, for example, thio-click reactions. The water-insoluble PEtOx-PDecOx copolymer ( $\overline{M}_n = 5.2$  kDa) gave particles of 0.25 and 0.66  $\mu\text{m}$  diameter by a nano-precipitation method. Cell uptake was observed by confocal microscopy. The last section of this paper will present work on micellar drug-delivery systems using amphiphilic POx which started to appear only since 2010.

In 2010, Hruby et al.<sup>[186]</sup> reported on PMeOx-P(iPrOx-co-BuOx)-PMeOx triblock copolymers ( $\overline{M}_n = 4.7$  kDa) as a radionuclide delivery system. They used the temperature dependent solubility of the intermediate block to induce the formation of micelles ( $d = 200$  nm) under physiological conditions. For radiolabeling, some of the BuOx was replaced by 2-butenyl-2-oxazoline (EnOx) and modified by thio-click with sulfanylethyl-2-(4-hydroxyphenyl)acetamide which could be used for the introduction of <sup>125</sup>I as the radionuclide in good yields and sufficient in vitro stability. The haemolytic activity of all polymers as tested using full blood was found to be very low even at high concentrations which would correspond to a (hypothetical) total dose of 5 g per human. Various iodine radioisotopes are suitable for both, diagnosis as well as radionuclide therapy of solid tumors.

Milonaki et al.<sup>[61]</sup> used amphiphilic gradient PMeOx-co-PhOx copolymers for the encapsulation of the hydrophobic drug indomethacin (IND), a nonsteroidal anti-inflammatory drug [nonselective inhibitor of cyclooxygenase (COX) 1 and 2] for the treatment of rheumatoid and osteoarthritis but also present in prescription medication to reduce fever, pain, stiffness, and swelling. Based on DLS and AFM characterization, the PMeOx-co-PhOx copolymers assemble into a mixture of spherical micelles, vesicles, and larger aggregates of different sizes and ratios depending on the copolymer composition and aqueous media used. The IND could be loaded into the copolymer assemblies at very high drug–polymer ratio (0.25:1–0.75:1 w:w), which was attributed to the hydrophobic nature of the drug along with other favorable drug–polymer interactions.

Amphiphilic P(EtOx-co-BuOx) as well as a simple hydrophilic PEtOx homopolymer ( $\overline{M}_n = 8$  kDa and 5 kDa) was used by Kabanov et al.<sup>[112]</sup> to formulate hydrophobic fullerene (C<sub>60</sub>). Fullerene is referred to as a “radical sponge” due to its powerful radical scavenging potential. However, the water insolubility along with its toxicity limits its use as a therapeutic antioxidant. The formulation of fullerene with PEtOx, PEtOx-PBuOx [as well as with poly(*N*-vinylpyrrolidone), PVP for comparison] from a toluene/chloroform solution yields nanoformulations with a fullerene content of up to 1 wt% and defined sizes with a C<sub>60</sub> enriched inner core and a outer shell formed by

the hydrophilic POx. The cytotoxicity of the POx-C<sub>60</sub> nanoformulation using three different cell lines [MDCK, Hep G2, and catecholaminergic (CATH.a) neuronal cells] was evaluated and no cytotoxicity was found at concentrations up to 50  $\mu$ M. Only at high concentrations of 100  $\mu$ M, POx-C<sub>60</sub> caused minor inhibition of cell growth (MDCK) and reduced cell viability (HEP G2 and CATH.a neurons). The cellular uptake into CATH.a neurons of, for example, PEtOx-C<sub>60</sub> was found to be considerably greater compared to PVP-C<sub>60</sub> and no accumulation into mitochondria was found, instead the C<sub>60</sub> was mainly in the cytoplasm. Efficient scavenging of intracellular superoxide induced by Ang II stimulation indicates the therapeutic potential of the POx-C<sub>60</sub> nanoformulation in Ang II-related cardiovascular diseases such as hypertension and heart failure.

Luxenhofer et al.<sup>[133]</sup> used amphiphilic PEtOx-*b*-PBuOx diblock ( $\overline{M}_n = 11.5$  kDa) and a series of PMeOx-PBuOx-PMeOx triblock copolymers ( $\overline{M}_n = 8.5$ –10.4 kDa) for the solubilization of the anti-cancer drug paclitaxel (PTX). The POx-PTX formulation was prepared by a simple, yet highly reproducible and effective thin film method that yielded small and defined PTX-loaded POx-micelles in the range of 20–30 nm. An extraordinary high drug-loading capacity of up to 45% was found (a nearly 1:1 drug:polymer ratio) and the micellar solution remained clear and stable under ambient conditions for weeks. Along with the fact that only POx needs to be used as the formulation excipient, makes this formulation highly competitive to PTX formulations currently used in cancer chemotherapy (see Table 3). The cytotoxicity of the unloaded POx amphiphiles using human breast carcinoma cells (MCF7/ADR) was found to be low and immunogenicity as determined by the activation of the C3a complement fraction was significantly lower compared to Cremophor EL. Even PTX-loaded POx micelles were determined less immunogenic than Cremophor EL or Taxol (commercial formulation of PTX with Cremophor EL). In the *in vivo* test using tumor-bearing mice, the POx-PTX formulation showed superior growth inhibition than Taxol. Interestingly, probing the polar microenvironment of the micelles of POx amphiphiles by pyrene,

the extremely high loading capacity coincides with a high I<sub>1</sub>/I<sub>3</sub> ratio from the pyrene fluorescence spectra. Using the I<sub>1</sub>/I<sub>3</sub> ratio as a measure for the polarity of the micellar core, this would indicate that the solubilized pyrene experiences a higher polar environment as, for example, in water or other polar organic solvents. The unusually high drug-loading is attributed to the PBuOx block forming the micellar core. Here, the pendant alkyl chains are just long enough to render the block hydrophobic but at the same time allow for effective polar interactions of incorporated molecules (pyrene or PTX) with the tertiary amide group of the polymer. In the series of 2-oxazoline monomer units this seems to be unique as analog experiments with pyrene solubilized in PNOx-PMeOx as well as Pluronic P85 gave expectable I<sub>1</sub>/I<sub>3</sub> ratios (1.33 for PNOx-PMeOx, 1.49 for Pluronic P85) in the pyrene spectrum. Accordingly, in PNOx-PMeOx, the PTX loading capacities were significantly lower (7 wt%). In the same study, also solubilization of AmB, cyclosporine A (CyA, an immunosuppressant drug used in organ transplantation), and etoposide (ETO, an anticancer agent that inhibits topoisomerase II) were studied. For ETO, no stable micellar solution could be obtained but AmB as well as CyA could be loaded into POx-micelles at high drug contents (both 17 wt%). The unusually high drug-loading capacity of POx polymers were also observed by Milonaki et al.<sup>[61]</sup> for the solubilization of IND as described above using PhOx as the hydrophobic block. A correlation of the alkyl chain length of a micelle forming surfactant with the taxane loading was also found for small molecule excipients.<sup>[187,188]</sup> Incorporation of drugs into POx micelles appears to be most effective for the incorporation of water-insoluble drugs featuring polar and aromatic groups that can interact with the polymeric carrier not only by hydrophobic but additional polar,  $\pi$ - $\pi$ , and H-bonding interactions. Comparing the high drug-loading capacity of POx, for example, PTX with formulations used in current chemotherapy shows the significant improvement POx could provide to cancer and other chemotherapies. In terms of drug loading as well as the total amount of solubilized drug in a stable formulation, POx micelles

■ Table 3. Comparison of micelles from PMeOx-PBuOx-PMeOx (POxsol) as a drug delivery system for PTX with other formulations.

Formulation	Status	Excipient	PTX payload [wt%]	PTX: excipient	c(PTX) in stable solution [mg mL <sup>-1</sup> ]	Reference
Taxol	Market	Cremophor EL/EtOH	<1	1:100	0.3–1.2	a)
Abraxane	Market	HSA	10	1:10	5	a)
Genexol	Market	PEGylated PLA	17	1:5	6	[189]
OPAXIO <sup>b)</sup>	Phase III	PLGA	26	1:4	9	[190]
NK 105	Phase II/III	PEGylated synthetic peptide	30	1:3	3	[191]
POxsol	Preclinical	PMeOx-PBuOx-PMeOx	45	1:1	40	[192,193]

<sup>a)</sup>Information given in package insert; <sup>b)</sup>Formerly known as XYOTAX.

■ Table 4. Drugs formulated in POx-based micelles or self-assembled aggregates.

Polymer	Payload	d [nm]	Reference
PMeOx-PDMS-PMeOx	OmpF/TvNH	200	[167]
PMeOx-PDMS-PMeOx	Trypsin	<200	[165]
PMeOx-PDMS-PMeOx	Pravastin	97 ± 10	[166]
PMeOx-PDMS-PMeOx	DNA	250	[168]
PEtOx-PCL	PTX	18.3-23.4	[114]
PLA-PEtOx-PLA	DOX	150 (blank) 200 (pH-dependent)	[180]
Mixed: poly( <i>N</i> -isopropyl acrylamide- <i>co</i> -methacrylic acid)- <i>g</i> -poly( <i>D,L</i> -lactide) (P(NIPAAm- <i>co</i> -MAAc)- <i>g</i> -PLA); PEG-PLA; PEtOx-PLA	DOX	180-300 (blank) 165 (pH-dependent)	[179]
PEtOx-PLA	DOX	121-164 (blank) 170-210	[115]
PEtOx-PAsp	AmB	108	[176]
PEtOx-PLA	mTHPC	77	[182]
PMeOx-PiPrOx-PMeOx	<sup>125</sup> I		[186]
PMeOx-grad-PhOx	IND	Micelles, vesicles, and larger aggregates	[61]
PEtOx-PEtOx-PBuOx	Fullerene (C <sub>60</sub> )	156 (formulation) 132 (formulation)	[112]
PEtOx-PBuOx	PTX	n.d.	[133]
PNOx-PMeOx	PTX	n.d.	[133]
PMeOx-PNOx-PMeOx	PTX	n.d.	[193]
PMeOx-PBuOx-PMeOx	PTX	20-30, 36	[133,192]
PMeOx-PBuOx-PMeOx	AmB	145	[133]
PMeOx-PBuOx-PMeOx	CyA	n.d.	[133]
PMeOx-PBuOx-PMeOx	DTX	18	[192-194]
PMeOx-PBuOx-PMeOx	ETO	not stable	[192-194]
PMeOx-PBuOx-PMeOx	17-AAG	33	[192-194]
PMeOx-PBuOx-PMeOx	BTZ	not stable	[192-194]
PMeOx-PBuOx-PMeOx	PTX/17-AAG	43	[192,194]
PMeOx-PBuOx-PMeOx	PTX/ETO	42	[192,194]
PMeOx-PBuOx-PMeOx	PTX/BTZ	36	[192,194]
PMeOx-PBuOx-PMeOx	PTX/17-AAG/ETO	53	[192,194]
PMeOx-PBuOx-PMeOx	PTX/17-AAG/BTZ	99	[192,194]
PMeOx-PBuOx-PMeOx	DTX/17-AAG	56	[192,194]
PMeOx-PBuOx-PMeOx	BTZ/17-AAG	57	[192,194]
PMeOx-PBuOx-PMeOx	ETO/17-AAG	79	[192,194]
PMeOx-PBuOx-PMeOx	BXT	not stable	[193]

17-AAG: 17-allylamino-17-demethoxygeldanamycin; AmB: amphotericin B; BTZ: bortezomib; BXT: beaxarotene; CyA: cyclosporine A; DOX: doxorubicin; DTX: docetaxel; ETO: etoposide; IND: indomethacin; mTHPC: meta-tetra(hydroxyphenyl)chlorin; PTX: paclitaxel; n.d.: not determined.

outperform all formulations by orders of magnitude (see Table 3).

A major issue in cancer (chemo)therapy is the development of drug resistance of tumors which limits the

success rate of single-drug chemotherapies. Moreover, most tumors are not comprised of a single but multiple heterogeneous cell populations including "tumor initiating cells" (TICs) with stem cell characteristics, which



sustain constant tumor growth. Such TICs are notoriously resistant towards administered drugs and may cause tumor progression and recurrence of cancer even after initial successful treatment. Hence, different treatment strategies are combined and/or multiple drugs are administered in cancer chemotherapy. Consequently, in a recent work by Han et al.<sup>[192–194]</sup> POx micelles (PMeOx–PBUOx–PMeOx) were challenged to solubilize multiple anti-cancer drugs along with PTX, including docetaxel (DTX), 17-allylamino-17-demethoxygeldanamycin (17-AAG), etoposide (ETO), and bortezomib (BTZ). The loading capacities of drug combinations were found to be even higher with up to 50 wt% of drugs in the final formulation and multidrug loading further enhanced the stability of drug-incorporated micelles. Drug ratio dependent synergistic effects for the cytotoxicity was observed by in vitro studies for ETO/17-AAG in MCF-7 cancer cells and BTZ/17-AAG in MCF-7, PC3, MDA-MB231, and Hep G2 cells. Finally, the cell uptake and drug release characteristics were studied in detail. We summarized all drugs that have been formulated using POx-based polymers in Table 4 along with the respective polymer.

## 7. Other Drug Delivery Modalities

Hydrogels can serve as local drug delivery depots and hydrogels on POx basis have been studied intensively. POx-based hydrogels can be prepared by chemically reversible or permanent crosslinks and by physical crosslinking.<sup>[132,195–199]</sup>

For drug delivery purposes, only few examples can be found to date, but it can be expected that many more will follow soon. El-Hag Ali and AlArifi prepared PEtOx-poly(acrylic acid) hydrogels by irradiation induced polymerization and crosslinking. The authors use Ibuprofen as a model drug and give a proof on concept on the use of POx-based hydrogels as controlled release modality for drug delivery to the gut.<sup>[200]</sup> Also Wiesbrock and co-workers<sup>[201]</sup> reported on POx hydrogels with intended use stimuli-triggered drug delivery. In this case, a model compound, eosin Y was released over time as the hydrogel decomposes. It should be noted, however, that in such case also the highly toxic PEI would be produced over time. This will need to be taken into account as the safety of such gel for such an application is assessed.<sup>[201]</sup> Hsiue and co-workers<sup>[116]</sup> studied PETOx-PCL-PETOx triblock copolymers which show a sol–gel transition between room temperature (sol) and physiological temperature (gel). The gels were used to incorporate the therapeutic antibody Bevacizumab. In vitro drug release studies showed that the antibody was released in a delayed manner making these materials very promising for sustained drug release depots.<sup>[116]</sup> Very recently, Bender et al.<sup>[202]</sup> suggested to use plain PEtOx in solid dispersions for the formulation of drugs.

## 8. Conclusion

It is apparent that poly(2-oxazoline) combines many crucial aspects into one polymer class. On one hand, numerous studies indicate the good compatibility of the POx with biological systems from proteins to whole organisms. The current limiting factors are the lack of regulatory approval and the commercial availability of monomers and defined polymers. On the other hand, the variability in terms of the polymer structure and function is currently the driving force for research to develop polymer therapeutics based on POx and to challenge the established polymer systems.

Although much remains to be done to get a clearer and more detailed picture of the pharmacokinetics and pharmacodynamics of POx-based materials, first-in-human studies may commence as early as 2013.

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- [1] W. Seeliger, E. Aufderhaar, W. Diepers, R. Nehring, W. Thier, H. Hellmann, *Angew. Chem. Int. Ed.* **1966**, *5*, 875.
- [2] M. T. Zarka, O. Nuyken, R. Weberskirch, *Chem. Eur. J.* **2003**, *9*, 3228.
- [3] K. Schwekendiek, F. Glorius, *Synthesis* **2006**, *18*, 2996.
- [4] P. Persigehl, R. Jordan, O. Nuyken, *Macromolecules* **2000**, *33*, 6977.
- [5] C. Taubmann, R. Luxenhofer, S. Cesana, R. Jordan, *Macromol. Biosci.* **2005**, *5*, 603.
- [6] M. Barz, R. Luxenhofer, R. Zentel, M. J. Vicent, *Polym. Chem.* **2011**, *2*, 1900.
- [7] K. Knop, R. Hoogenboom, D. Fischer, U. S. Schubert, *Angew. Chem. Int. Ed.* **2010**, *49*, 6288.
- [8] H. Schlaad, C. Diehl, A. Gress, M. Meyer, A. L. Demirel, Y. Nur, A. Bertin, *Macromol. Rapid Commun.* **2010**, *31*, 511.
- [9] N. Adams, U. S. Schubert, *Adv. Drug Deliv. Rev.* **2007**, *59*, 1504.
- [10] M. J. Vicent, H. Ringsdorf, R. Duncan, *Adv. Drug Deliv. Rev.* **2009**, *61*, 1117.
- [11] R. Duncan, *Nat. Rev. Drug Discov.* **2003**, *2*, 347.
- [12] R. Luxenhofer, R. Jordan, *Macromolecules* **2006**, *39*, 3509.
- [13] K. Aoi, M. Okada, *Prog. Polym. Sci.* **1996**, *21*, 151.
- [14] M. W. M. Fijten, C. Haensch, B. M. van Lankvelt, R. Hoogenboom, U. S. Schubert, *Macromol. Chem. Phys.* **2008**, *209*, 1887.

- [15] R. Obeid, C. Scholz, *Biomacromolecules* **2011**, *12*, 3797.
- [16] M. Reif, R. Jordan, *Macromol. Chem. Phys.* **2011**, *212*, 1815.
- [17] S. Zalipsky, C. B. Hansen, J. M. Oaks, T. M. Allen, *J. Pharm. Sci.* **1996**, *85*, 133.
- [18] R. Jordan, K. Martin, H. J. Räder, K. K. Unger, *Macromolecules* **2001**, *34*, 8858.
- [19] M. Einzmann, W. H. Binder, *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 2821.
- [20] R. H. Jin, *J. Mater. Chem.* **2004**, *14*, 320.
- [21] K. Aoi, H. Suzuki, M. Okada, *Macromolecules* **1992**, *25*, 7073.
- [22] S. Kobayashi, E. Masuda, S. Shoda, Y. Shimano, *Macromolecules* **1989**, *22*, 2878.
- [23] S. Kobayashi, S. Iijima, T. Igarashi, T. Saegusa, *Macromolecules* **1987**, *20*, 1729.
- [24] S. Kobayashi, H. Uyama, N. Higuchi, T. Saegusa, *Macromolecules* **1990**, *23*, 54.
- [25] G. Volet, T. X. Lav, J. Babinot, C. Amiel, *Macromol. Chem. Phys.* **2011**, *212*, 118.
- [26] G. H. Hsiue, H. Z. Chiang, C. H. Wang, T. M. Juang, *Bioconjug. Chem.* **2006**, *17*, 781.
- [27] T. B. Bonne, K. Lüdtke, R. Jordan, P. Stepanek, C. M. Papadakis, *Colloid Polym. Sci.* **2004**, *282*, 833.
- [28] R. Luxenhofer, M. Lopez-Garcia, A. Frank, H. Kessler, R. Jordan, *PMSE Prepr.* **2006**, *95*, 283.
- [29] O. Nuyken, G. Maier, A. Gross, H. Fischer, *Macromol. Chem. Phys.* **1996**, *197*, 83.
- [30] M. B. Foreman, J. P. Coffman, M. J. Murcia, S. Cesana, R. Jordan, G. S. Smith, C. A. Naumann, *Langmuir* **2003**, *19*, 326.
- [31] T. X. Viegas, M. D. Bentley, J. M. Harris, Z. Fang, K. Yoon, B. Dizman, R. Weimer, A. Mero, G. Pasut, F. M. Veronese, *Bioconjug. Chem.* **2011**, *22*, 976.
- [32] F. Rehfeldt, M. Tanaka, L. Pagnoni, R. Jordan, *Langmuir* **2002**, *18*, 4908.
- [33] S. Kobayashi, T. Igarashi, Y. Moriuchi, T. Saegusa, *Macromolecules* **1986**, *19*, 535.
- [34] R. Hoogenboom, *Angew. Chem. Int. Ed.* **2009**, *48*, 7978.
- [35] S. Salzinger, S. Huber, S. Jaksch, P. Busch, R. Jordan, C. M. Papadakis, *Colloid Polym. Sci.* **2012**, *290*, 385.
- [36] A. Kowalczyk, J. Kronek, K. Bosowska, B. Trzebicka, A. Dworak, *Polym. Int.* **2011**.
- [37] R. Obeid, E. Maltseva, A. F. Thünemann, F. Tanaka, F. M. Winnik, *Macromolecules* **2009**, *42*, 2204.
- [38] C. Diehl, H. Schlaad, *Macromol. Biosci.* **2009**, *9*, 157.
- [39] S. Huber, N. Hutter, R. Jordan, *Colloid Polym. Sci.* **2008**, *286*, 1653.
- [40] S. Huber, R. Jordan, *Colloid Polym. Sci.* **2008**, *286*, 395.
- [41] R. Hoogenboom, H. M. L. Thijs, M. J. H. C. Jochems, B. M. van Lankvelt, M. W. M. Fijten, U. S. Schubert, *Chem. Commun.* **2008**, 5758.
- [42] M. Meyer, M. Antonietti, H. Schlaad, *Soft Matter* **2007**, *3*, 430.
- [43] J. S. Park, K. Kataoka, *Macromolecules* **2007**, *40*, 3599.
- [44] J. S. Park, K. Kataoka, *Macromolecules* **2006**, *39*, 6622.
- [45] J. Park, Y. Akiyama, F. M. Winnik, K. Kataoka, *Macromolecules* **2004**, *37*, 6786.
- [46] C. Diab, Y. Akiyama, K. Kataoka, F. M. Winnik, *Macromolecules* **2004**, *37*, 2556.
- [47] D. Christova, R. Velichkova, W. Loos, E. J. Goethals, F. du Prez, *Polymer* **2003**, *44*, 2255.
- [48] F. P. Chen, A. E. Ames, L. D. Taylor, *Macromolecules* **1990**, *23*, 4688.
- [49] C. Weber, R. Hoogenboom, U. S. Schubert, *Prog. Polym. Sci.* **2011**.
- [50] C. M. Papadakis, R. Ivanova, K. Lüdtke, K. Mortensen, P. K. Pranzas, R. Jordan, *J. Appl. Cryst.* **2007**, *40*, S361.
- [51] T. B. Bonne, K. Lüdtke, R. Jordan, C. M. Papadakis, *Macromol. Chem. Phys.* **2007**, *208*, 1402.
- [52] R. Ivanova, T. Komenda, T. B. Bonne, K. Lüdtke, K. Mortensen, P. K. Pranzas, R. Jordan, C. M. Papadakis, *Macromol. Chem. Phys.* **2008**, *209*, 2248.
- [53] C. A. Fustin, H. Y. Huang, R. Hoogenboom, F. Wiesbrock, A. M. Jonas, U. S. Schubert, J. F. Gohy, *Soft Matter* **2007**, *3*, 79.
- [54] R. Hoogenboom, F. Wiesbrock, M. A. M. Leenen, H. M. L. Thijs, H. Y. Huang, C. A. Fustin, P. Guillet, J. F. Gohy, U. S. Schubert, *Macromolecules* **2007**, *40*, 2837.
- [55] C. A. Fustin, V. Lefevre, R. Hoogenboom, U. S. Schubert, J. F. Gohy, *Macromol. Chem. Phys.* **2007**, *208*, 2026.
- [56] 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. Fustin, A. M. Jonas, J. Gohy, U. S. Schubert, *Macromolecules* **2006**, *39*, 4719.
- [57] H. Y. Huang, R. Hoogenboom, M. A. M. Leenen, P. Guillet, A. M. Jonas, U. S. Schubert, J. F. Gohy, *J. Am. Chem. Soc.* **2006**, *128*, 3784.
- [58] R. Hoogenboom, M. A. M. Leenen, H. Y. Huang, C. A. Fustin, J. F. Gohy, U. S. Schubert, *Colloid Polym. Sci.* **2006**, *284*, 1313.
- [59] H. M. L. Lambermont-Thijs, R. Hoogenboom, C. Fustin, C. Bomal-D'Haese, J. Gohy, U. S. Schubert, *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 515.
- [60] M. Bortenschlager, N. Schöllhorn, A. Wittmann, R. Weberskirch, *Chem. Eur. J.* **2007**, *13*, 520.
- [61] Y. Milonaki, E. Kaditi, S. Pispas, C. Demetzos, *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *50*, 1226.
- [62] M. Barz, R. Luxenhofer, R. Zentel, A. V. Kabanov, *Biomaterials* **2009**, *30*, 5682.
- [63] T. R. Baekmark, T. Wiesenthal, P. Kuhn, A. Albersdörfer, O. Nuyken, R. Merkel, *Langmuir* **1999**, *15*, 3616.
- [64] K. Lüdtke, R. Jordan, P. Hommes, O. Nuyken, C. A. Naumann, *Macromol. Biosci.* **2005**, *5*, 384.
- [65] A. P. Siegel, A. Kimble-Hill, S. Garg, R. Jordan, C. A. Naumann, *Biophys. J.* **2011**, *101*, 1642.
- [66] P. Seitz, M. Reif, K. Yashikawa, R. Jordan, M. Tanaka, *J. Phys. Chem. B* **2011**, *115*, 2256.
- [67] A. P. Siegel, M. J. Murcia, M. Johnson, M. Reif, R. Jordan, J. Rühle, C. A. Naumann, *Soft Matter* **2010**, *6*, 2723.
- [68] P. C. Seitz, M. D. Reif, O. V. Konovalov, R. Jordan, *ChemPhysChem* **2009**, *10*, 2876.
- [69] M. A. Deverall, S. Garg, K. Lüdtke, R. Jordan, J. Rühle, C. A. Naumann, *Soft Matter* **2008**, *4*, 1899.
- [70] K. Lüdtke, R. Jordan, N. Furr, S. Garg, K. Forsythe, C. A. Naumann, *Langmuir* **2008**, *24*, 5580.
- [71] O. Purruicker, S. Gönnerwein, A. Förtig, R. Jordan, M. Rusp, M. Bärmann, L. Moroder, E. Sackmann, M. Tanaka, *Soft Matter* **2007**, *3*, 333.
- [72] O. Purruicker, A. Förtig, R. Jordan, E. Sackmann, M. Tanaka, *Phys. Rev. Lett.* **2007**, *98*, 078102.
- [73] S. Garg, J. Rühle, K. Lüdtke, R. Jordan, C. A. Naumann, *Biophys. J.* **2007**, *92*, 1263.
- [74] O. Purruicker, A. Förtig, K. Lüdtke, R. Jordan, M. Tanaka, *J. Am. Chem. Soc.* **2005**, *127*, 1258.
- [75] A. Förtig, R. Jordan, K. Graf, G. Schiavon, O. Purruicker, M. Tanaka, *Macromol. Symp.* **2004**, *210*, 329.
- [76] O. Purruicker, A. Förtig, R. Jordan, M. Tanaka, *ChemPhysChem* **2004**, *5*, 327.
- [77] R. Jordan, A. Ulman, *J. Am. Chem. Soc.* **1998**, *120*, 243.
- [78] G. Volet, V. Chanthavong, V. Wintgens, C. Amiel, *Macromolecules* **2005**, *38*, 5190.
- [79] A. Gress, A. Voelkel, H. Schlaad, *Macromolecules* **2007**, *40*, 7928.

- [80] Y. Chujo, K. Sada, A. Naka, T. Saegusa, R. Nomura, *Macromolecules* **1993**, *26*, 883.
- [81] S. Cesana, A. Kurek, M. A. Baur, J. Auernheimer, O. Nuyken, *Macromol. Rapid Commun.* **2007**, *28*, 608.
- [82] S. Cesana, J. Auernheimer, R. Jordan, H. Kessler, O. Nuyken, *Macromol. Chem. Phys.* **2006**, *207*, 183.
- [83] E. R. Gillies, E. Dy, J. M. Fréchet, F. C. Szoka, *Mol. Pharmaceutics* **2005**, *2*, 129.
- [84] L. Y. Qiu, Y. H. Bae, *Pharm. Res.* **2006**, *23*, 1.
- [85] C. C. Lee, J. A. MacKay, J. M. Fréchet, F. C. Szoka, *Nat. Biotechnol.* **2005**, *23*, 1517.
- [86] B. Chen, K. Jerger, J. M. Fréchet, F. C. Szoka, *J. Controlled Release* **2009**, *140*, 203.
- [87] W. H. Velander, R. D. Madurawe, A. Subramanian, G. Kumar, G. Sinai-Zingde, J. S. Riffle, C. L. Orthner, *Biotechnol. Bioeng.* **1992**, *39*, 1024.
- [88] Y. Shimano, K. Sato, S. Kobayashi, *J. Polym. Sci., Part A: Polym. Chem.* **1995**, *33*, 2715.
- [89] R. Luxenhofer, M. Bezen, R. Jordan, *Macromol. Rapid Commun.* **2008**, *29*, 1509.
- [90] N. Zhang, R. Luxenhofer, R. Jordan, *Macromol. Chem. Phys.* **2012**, *213*, 973.
- [91] N. Zhang, S. Huber, A. Schulz, R. Luxenhofer, R. Jordan, *Macromolecules* **2009**, *42*, 2215.
- [92] R. Jordan, N. West, A. Ulman, Y. M. Chou, O. Nuyken, *Macromolecules* **2001**, *34*, 1606.
- [93] S. Kobayashi, H. Uyama, Y. Narita, J. Ishiyama, *Macromolecules* **1992**, *25*, 3232.
- [94] O. Nuyken, J. Rueda-Sanchez, B. Voit, *Macromol. Rapid Commun.* **1997**, *18*, 125.
- [95] R. Weberskirch, R. Hettich, O. Nuyken, D. Schmaljohann, B. Voit, *Macromol. Chem. Phys.* **1999**, *200*, 863.
- [96] S. Kobayashi, M. Kaku, S. Sawada, T. Saegusa, *Polym. Bull.* **1985**, *13*, 447.
- [97] S. I. Shoda, E. Masuda, M. Furukawa, S. Kobayashi, *J. Polym. Sci., Part A: Polym. Chem.* **1992**, *30*, 1489.
- [98] H. Uyama, Y. Honda, S. Kobayashi, *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 123.
- [99] A. Groß, G. Maier, O. Nuyken, *Macromol. Chem. Phys.* **1996**, *197*, 2811.
- [100] R. Konradi, B. Pidhatika, A. Mühlebach, M. Textor, *Langmuir* **2008**, *24*, 613.
- [101] B. Guillermin, V. Darcos, V. Lapinte, S. Monge, J. Coudane, J. J. Robin, *Chem. Commun.* **2012**, *48*, 2879.
- [102] C. Weber, C. Remzi Becer, W. Guenther, R. Hoogenboom, U. S. Schubert, *Macromolecules* **2010**, *43*, 160.
- [103] C. Weber, A. Krieg, R. M. Paulus, H. M. L. Lambermont-Thijs, C. R. Becer, R. Hoogenboom, U. S. Schubert, *Macromol. Symp.* **2011**, *308*, 17.
- [104] E. D. Rio, G. Lligadas, J. C. Ronda, M. Galià, V. Cádiz, *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 3069.
- [105] K. Kempe, R. Hoogenboom, M. Jaeger, U. S. Schubert, *Macromolecules* **2011**, *44*, 6424.
- [106] F. Manzenrieder, R. Luxenhofer, M. Retzlaff, R. Jordan, M. G. Finn, *Angew. Chem. Int. Ed.* **2011**, *50*, 2601.
- [107] K. Kempe, A. Vollrath, H. W. Schaefer, T. G. Poehlmann, C. Biskup, R. Hoogenboom, S. Hornig, U. S. Schubert, *Macromol. Rapid Commun.* **2010**, *31*, 1869.
- [108] C. Diehl, H. Schlaad, *Chem. Eur. J.* **2009**, *15*, 11469.
- [109] M. Litt, A. Levy, J. Herz, *J. Macromol. Sci. Chem.* **1975**, *A9*, 703.
- [110] R. Luxenhofer, G. Sahay, A. Schulz, D. Alakhova, T. K. Bronich, R. Jordan, A. V. Kabanov, *J. Controlled Release* **2011**, *153*, 73.
- [111] R. Donev, N. Koseva, P. Petrov, A. Kowalczyk, J. Thome, *World J. Biol. Psychiatr.* **2011**, *12*, 44.
- [112] J. Tong, M. C. Zimmermann, S. Li, X. Yi, R. Luxenhofer, R. Jordan, A. V. Kabanov, *Biomaterials* **2011**, *32*, 3654.
- [113] X. Wang, X. Li, Y. Li, Y. Zhou, C. Fan, W. Li, S. Ma, Y. Fan, Y. Huang, N. Li, Y. Liu, *Acta Biomater.* **2011**, *7*, 4149.
- [114] S. C. Lee, C. Kim, I. C. Kwon, H. Chung, S. Y. Jeong, *J. Controlled Release* **2003**, *89*, 437.
- [115] G. H. Hsiue, C. H. Wang, C. L. Lo, C. H. Wang, J. P. Li, J. L. Yang, *Int. J. Pharm.* **2006**, *317*, 69.
- [116] C. H. Wang, Y. S. Hwang, P. R. Chiang, C. R. Shen, W. H. Hong, G. H. Hsiue, *Biomacromolecules* **2012**, *13*, 40.
- [117] J. Kronek, J. Luston, Z. Kroneková, E. Paulovicová, P. Farkas, N. Petrenciková, L. Paulovicová, I. Janigová, *J. Mater. Sci. Mater. Med.* **2010**, *21*, 879.
- [118] C. J. Waschinski, V. Herdes, F. Schueler, J. C. Tiller, *Macromol. Biosci.* **2005**, *5*, 149.
- [119] C. J. Waschinski, J. C. Tiller, *Biomacromolecules* **2005**, *6*, 235.
- [120] J. H. Jeong, S. H. Song, D. W. Lim, H. Lee, T. G. Park, *J. Controlled Release* **2001**, *73*, 391.
- [121] B. Pidhatika, J. Möller, E. M. Benetti, R. Konradi, E. Rakhmatullina, A. Mühlebach, R. Zimmermann, C. Werner, V. Vogel, M. Textor, *Biomaterials* **2010**, *31*, 9462.
- [122] D. A. Tomalia, G. R. Killat, in *Encyclopedia of Polymer Science and Engineering*, Wiley and Sons, New York **1985**, p. 680.
- [123] F. C. Gaertner, R. Luxenhofer, B. Blechert, R. Jordan, M. Essler, *J. Controlled Release* **2007**, *119*, 291.
- [124] K. Naka, T. Nakamura, A. Ohki, S. Maeda, *Macromol. Chem. Phys.* **1997**, *198*, 101.
- [125] T. Lehmann, J. Rühle, *Macromol. Symp.* **1999**, *142*, 1.
- [126] N. Zhang, I. Amin, T. Pompe, R. Luxenhofer, C. Werner, R. Jordan, *Macromol. Biosci.* **2012**, *12*, 926.
- [127] H. Wang, L. Li, Q. Tong, M. Yan, *ACS Appl. Mater. Interfaces* **2011**, *3*, 3463.
- [128] B. Pidhatika, M. Rodenstein, Y. Chen, E. Rakhmatullina, A. Mühlebach, C. Acikgöz, M. Textor, R. Konradi, *Biointerfaces* **2012**, *7*, 1.
- [129] M. C. Woodle, C. M. Engbers, S. Zalipsky, *Bioconjugate Chem.* **1994**, *5*, 493.
- [130] P. Goddard, L. E. Hutchinson, J. Brown, L. J. Brookman, *J. Controlled Release* **1989**, *10*, 5.
- [131] A. Mitra, A. Nan, H. Ghandehari, E. McNeill, J. Mulholland, B. R. Line, *Pharm. Res.* **2004**, *21*, 1153.
- [132] R. Luxenhofer, *Thesis TU München, Garching* **2007**.
- [133] R. Luxenhofer, A. Schulz, C. Roques, S. Li, T. K. Bronich, E. V. Batrakova, R. Jordan, A. V. Kabanov, *Biomaterials* **2010**, *31*, 4972.
- [134] L. R. Brown, *Expert Opin. Drug Deliv.* **2005**, *2*, 29.
- [135] P. Bailon, C. Y. Won, *Expert Opin. Drug Deliv.* **2009**, *6*, 1.
- [136] G. Pasut, F. Veronese, *Adv. Polym. Sci.* **2006**, *192*, 95.
- [137] P. Farkas, J. Korcová, J. Kronek, S. Bystrický, *Eur. J. Med. Chem.* **2010**, *45*, 795.
- [138] M. Miyamoto, K. Naka, M. Shiozaki, Y. Chujo, T. Saegusa, *Macromolecules* **1990**, *23*, 3201.
- [139] A. Mero, G. Pasut, L. D. Via, M. W. M. Fijten, U. S. Schubert, R. Hoogenboom, F. M. Veronese, *J. Controlled Release* **2008**, *125*, 87.
- [140] A. Mero, Z. Fang, G. Pasut, F. M. Veronese, T. X. Viegas, *J. Controlled Release* **2012**, *159*, 353.
- [141] N. A. Hutter, M. Steenackers, A. Reitingner, O. A. Williams, J. A. Garrido, R. Jordan, *Soft Matter* **2011**, *7*, 4861.
- [142] M. Steenackers, R. Jordan, A. Küller, M. Grunze, *Adv. Mater.* **2009**, *21*, 2921.
- [143] X. Yi, E. Batrakova, W. A. Banks, S. Vinogradov, A. V. Kabanov, *Bioconjug. Chem.* **2008**, *19*, 1071.
- [144] X. Yi, M. C. Zimmerman, R. Yang, J. Tong, S. Vinogradov, A. V. Kabanov, *Free Radic. Biol. Med.* **2010**, *49*, 548.

- [145] T. O. Price, S. A. Farr, X. Yi, S. Vinogradov, E. Batrakova, W. A. Banks, A. V. Kabanov, *J. Pharmacol. Exp. Ther.* **2010**, *333*, 253.
- [146] J. Tong, R. Luxenhofer, X. Yi, R. Jordan, A. V. Kabanov, *Mol. Pharmaceutics* **2010**, *7*, 984.
- [147] M. Thomas, J. J. Lu, Q. Ge, C. Zhang, J. Chen, A. M. Klibanov, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5679.
- [148] T. von Erlach, S. Zwicker, B. Pidhatika, R. Konradi, M. Textor, H. Hall, T. Lühmann, *Biomaterials* **2011**, *32*, 5291.
- [149] <http://serinatherapeutics.com> (date accessed: June 25 2012).
- [150] J. M. Harris, M. D. Bentley, T. X. Viegas, *8th International Symposium on Polymer Therapeutics: From Laboratory to Clinical Practice* **2010**, p. 19.
- [151] K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Deliv. Rev.* **2001**, *47*, 113.
- [152] V. P. Torchilin, *J. Controlled Release* **2001**, *73*, 137.
- [153] V. P. Torchilin, *Cell Mol. Life Sci.* **2004**, *61*, 2549.
- [154] M. Yokoyama, T. Okano, Y. Sakurai, S. Fukushima, K. Okamoto, K. Kataoka, *J. Drug Target.* **1999**, *7*, 171.
- [155] A. V. Kabanov, P. Lemieux, S. Vinogradov, V. Alakhov, *Adv. Drug Deliv. Rev.* **2002**, *54*, 223.
- [156] A. V. Kabanov, E. V. Batrakova, V. Y. Alakhov, *Adv. Drug Deliv. Rev.* **2002**, *54*, 759.
- [157] A. V. Kabanov, V. Y. Alakhov, *CRC Crit. Rev. Ther. Drug Carrier Syst.* **2002**, *19*, 1.
- [158] O. Onaca, R. Enea, D. W. Hughes, W. Meier, *Macromol. Biosci.* **2009**, *9*, 129.
- [159] R. Stoenescu, W. Meier, *Chem. Commun.* **2002**, *2002*, 3016.
- [160] R. Stoenescu, A. Graff, W. Meier, *Macromol. Biosci.* **2004**, *4*, 930.
- [161] M. Kumar, M. Grzelakowski, J. Zilles, M. Clark, W. Meier, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20719.
- [162] A. Taubert, A. Napoli, W. Meier, *Curr. Opin. Chem. Bio.* **2004**, *8*, 598.
- [163] W. Meier, C. Nardin, M. Winterhalter, *Angew. Chem. Int. Ed.* **2000**, *39*, 4599.
- [164] C. Nardin, T. Hirt, J. Leukel, W. Meier, *Langmuir* **2000**, *16*, 1035.
- [165] N. Ben-Haim, P. Broz, S. Marsch, W. Meier, P. Hunziker, *Nano Lett.* **2008**, *8*, 1368.
- [166] P. Broz, N. Ben-Haim, M. Grzelakowski, S. Marsch, W. Meier, P. Hunziker, *J. Cardiovasc. Pharmacol.* **2008**, *51*, 246.
- [167] A. Ranquin, W. Versées, W. Meier, J. Steyaert, P. Van Gelder, *Nano Lett.* **2005**, *5*, 2220.
- [168] A. Graff, M. Sauer, P. Van Gelder, W. Meier, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5064.
- [169] P. Broz, S. M. Benito, C. Saw, P. Burger, H. Heider, M. Pfisterer, S. Marsch, W. Meier, P. Hunziker, *J. Controlled Release* **2005**, *102*, 475.
- [170] S. Egli, M. G. Nussbaumer, V. Balasubramanian, M. Chami, N. Bruns, C. Palivan, W. Meier, *J. Am. Chem. Soc.* **2011**, *133*, 4476.
- [171] H. J. Choi, E. Brooks, C. D. Montemagno, *Nanotechnology* **2005**, *16*, S143.
- [172] H. J. Choi, C. D. Montemagno, *Nano Lett.* **2005**, *5*, 2538.
- [173] H. J. Choi, C. D. Montemagno, *Nanotechnology* **2006**, *17*, 2198.
- [174] C. Krumm, C. P. Fik, M. Meuris, G. J. Dropalla, H. Geltenpoth, A. Sickmann, J. C. Tiller, *Macromol. Rapid Commun.* **2012**, *33*, in this issue (DOI: 10.1002/marc.201200192).
- [175] K. Tsutsumiuchi, K. Aoi, M. Okada, *Macromolecules* **1997**, *30*, 4013.
- [176] C. H. Wang, W. T. Wang, G. H. Hsiue, *Biomaterials* **2009**, *30*, 3352.
- [177] J. Y. Chang, P. J. Park, M. J. Han, *Macromolecules* **2000**, *33*, 321.
- [178] S. C. Lee, Y. Chang, Jin-San Yoon, C. Kim, I. C. Kwon, Y. H. Kim, S. Y. Jeong, *Macromolecules* **1999**, *32*, 1847.
- [179] C. L. Lo, K. M. Lin, C. K. Huang, G. H. Hsiue, *Adv. Funct. Mater.* **2006**, *16*, 2309.
- [180] C. H. Wang, C. H. Wang, G. H. Hsiue, *J. Controlled Release* **2005**, *108*, 140.
- [181] C. H. Wang, G. H. Hsiue, *Biomacromolecules* **2003**, *4*, 1487.
- [182] M.-J. Shieh, C.-L. Peng, W.-L. Chiang, C.-H. Wang, C.-Y. Hsu, S.-J. J. Wang, P.-S. Lai, *Mol. Pharmaceutics.* **2010**, *7*, 1244.
- [183] J. Tiller, *Adv. Polym. Sci.* **2011**, *240*, 193.
- [184] C. J. Waschinski, J. Zimmermann, U. Salz, R. Hutzler, G. Sadowski, J. C. Tiller, *Adv. Mater.* **2008**, *20*, 104.
- [185] C. J. Waschinski, S. Barnert, A. Theobald, R. Schubert, F. Kleinschmidt, A. Hoffmann, K. Saalwächter, J. C. Tiller, *Biomacromolecules* **2008**, *9*, 1764.
- [186] M. Hruby, S. K. Filippov, J. Panek, M. Novakova, H. Mackova, J. Kucka, D. Vetvicka, K. Ulbrich, *Macromol. Biosci.* **2010**.
- [187] P. Kan, Z. B. Chen, C. J. Lee, I. Chu, *J. Controlled Release* **1999**, *58*, 271.
- [188] L. Huynh, J. Grant, J. C. Leroux, P. Delmas, C. Allen, *Pharm. Res.* **2008**, *25*, 147.
- [189] T. Y. Kim, D. W. Kim, J. Y. Chung, S. G. Shin, S. C. Kim, D. S. Heo, N. K. Kim, Y. J. Bang, *Clin. Cancer Res.* **2004**, *10*, 3708.
- [190] J. W. Singer, B. Baker, P. De Vries, A. Kumar, S. Shaffer, E. Vawter, M. Bolton, P. Garzone, *Adv. Exp. Med. Biol.* **2003**, *519*, 81.
- [191] T. Hamaguchi, K. Kato, H. Yasui, C. Morizane, M. Ikeda, H. Ueno, K. Muro, Y. Yamada, T. Okusaka, K. Shirao, Y. Shimada, H. Nakahama, Y. Matsumura, *Br. J. Cancer* **2007**, *97*, 170.
- [192] Y. Han, Z. He, A. Schulz, T. K. Bronich, R. Jordan, R. Luxenhofer, A. V. Kabanov, *Mol. Pharmaceutics* **2012**, *9*, in print (DOI: 10.1021/mp300159u).
- [193] A. Schulz, Y. Han, Z. He, T. K. Bronich, A. V. Kabanov, R. Luxenhofer, R. Jordan, *Polym. Prepr.* **2012**, *53*, 354.
- [194] A. Schulz, Y. Han, Z. He, T. K. Bronich, A. V. Kabanov, R. Jordan, *Polym. Prepr.* **2012**, *53*, 305.
- [195] Y. Chujo, Y. Yoshifuji, K. Sada, T. Saegusa, *Macromolecules* **1989**, *22*, 1074.
- [196] Y. Chujo, K. Sada, R. Nomura, A. Naka, T. Saegusa, *Macromolecules* **1993**, *26*, 5611.
- [197] I. H. Park, I. S. Han, D. K. Kim, T. Saegusa, *Angew. Makromol. Chem.* **1991**, *190*, 165.
- [198] Y. Chujo, K. Sada, T. Saegusa, *Macromolecules* **1990**, *23*, 2636.
- [199] Y. Chujo, K. Sada, K. Matsumoto, T. Saegusa, *Macromolecules* **1990**, *23*, 1234.
- [200] A. El-Hag Ali, A. S. AlArifi, *J. Appl. Polym. Sci.* **2011**, *120*, 3071.
- [201] A. M. Kelly, A. Hecke, B. Wirnsberger, F. Wiesbrock, *Macromol. Rapid Commun.* **2011**, *32*, 1815.
- [202] J. C. M. E. Bender, R. Hoogenboom, P. A. A. Van Vliet, *PCT Int. Appl. WO 2011/002285*, **2011**.