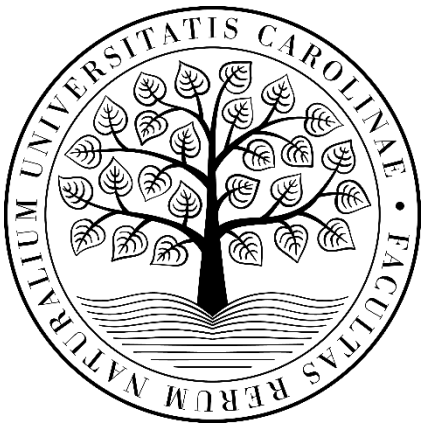
CHARLES UNIVERSITY

FACULTY OF SCIENCE

DEPARTMENT OF PHYSICAL AND MACROMOLECULAR CHEMISTRY



SYNTHESIS OF NOVEL POLY(2-OXAZOLINE)S FOR BIOMEDICAL APPLICATIONS

Habilitation thesis

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Prague 2024

This habilitation thesis is a collection of published work related to the presented topic, supplemented by original comments.

In Prague, 8.2.2024 Ondřej Sedláček

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1. Preface and acknowledgement

This habilitation thesis provides an overview of selected research results obtained by the habilitation candidate Ondřej Sedláček in the field of poly(2-oxazoline) chemistry between 2014 and 2023. The first part introduces the reader to poly(2-oxazoline)s chemistry, emphasizing their potential in biomedical applications. The following parts focus on the summary of own research results, starting with describing a novel method for the synthesis of poly(2-oxazoline)s by acylation of linear polyethylene imine, which provides straightforward access to polymers not achievable by standard cationic ring-opening polymerization methodology. In the following part, we describe our research in poly(2-oxazoline)-based biomaterials, focusing on novel drug delivery systems based on poly(2-oxazoline) drug conjugates and self-assembled amphiphilic copolymers. The final part describes the grafting of surfaces by poly(2-oxazoline)s and poly(2-oxazine)s and the study of the antibiofouling properties of such coatings with possible applications in the biocompatibilization of materials.

I would like to thank all my colleagues and friends who supported me in my scientific and pedagogic career. Among all the excellent coworkers and mentors, I would like to express my special thanks to my doctoral supervisor, prof. Martin Hrubý, who led my steps into the field of polymer chemistry, as well as Prof. Karel Ulbrich, DrSc., head of my former department at the Institute of Macromolecular Chemistry, CAS, for many inspiring consultations; and Prof. Richard Hoogenboom (Ghent University, Belgium), my postdoc supervisor, for providing me the opportunity to professionally grow in his world-class laboratory focusing on poly(2-oxazoline)s. Finally, I thank my wife, Renata, for her patience and support during this work.

2. Introduction

In materials science, the development of well-defined polymer materials is increasingly significant. Such polymers are essential in various areas, including biomedicine, electronics, and environmental technologies. Their defined molecular structure enables the design of tailor-made materials for particular purposes. They possess targeted molar mass, chain architecture, and composition so that they can be tailored for specific applications due to their predictable physicochemical and biological behavior. Such precision in molecular design is crucial for enhancing the performance of advanced polymer materials and pioneering new applications in areas where conventional materials are inadequate.

Living polymerizations represent an important method of synthesizing well-defined polymers. Distinguished from other polymerization methods, living polymerizations maintain chain growth without irreversible termination or chain transfer reactions.[1] Together with fast initiation, the absence of termination ensures uniform growth among all polymer chains, resulting in consistent molecular mass distribution. This method is advantageous as it allows precise control over the polymer's structure. The absence of termination enables the synthesis of block copolymers, the introduction of functional end-groups, and the formation of complex polymer architectures such as dendrimers or stars. As a result, the precision and adaptability in designing polymers are pivotal for achieving desired material properties and opening new pathways for innovative applications, connecting polymer science with other scientific areas. It should be noted that the recent decades witnessed an enormous effort to develop controlled radical polymerization methods, which can offer similar benefits to living polymerizations despite not being truly living (e.g., atom transfer radical polymerization, ATRP, and reversible addition−fragmentation chain-transfer, RAFT, polymerization).[2] The large-scale utilization of these techniques is, however, still in its beginnings.

Ionic polymerizations are typical examples of living polymerizations.[3] In particular, living anionic polymerizations stand out as a widely used tool for commercial polymerization of conjugated dienes, styrene, and their copolymers. Facile synthesis of block copolymers enables the preparation of unique polymer materials, such as thermoplastic elastomers (e.g., styrene-butadiene-styrene produced by Kraton Polymers).[3] Similarly, cationic polymerizations can synthesize otherwise hardly accessible polyisobutylene, polyvinyl ethers, or poly(*N*-vinyl carbazole).[4] Besides cationic polymerizations of vinylic monomers, cationic ring-opening

polymerization of cyclic monomers represents an emerging tool to achieve advanced polymer structures. In particular, it can be utilized to polymerize cyclic ethers, thioethers, aziridine, and 2-oxazolines.[5]

Cationic ring-opening polymerization (CROP) of 2-alkyl-2-oxazolines (AOx) was independently discovered in 1966 by four different research groups (Litt, Tomalia, Seeliger, and Kagiya).[6-9] After extensive research in the early 1970s, research on poly(2-alkyl-2-oxazoline)s (PAOx) nearly stopped for several decades until the new millennium, when PAOx gained interest again, primarily due to their potential in biomedical sciences.[10] The renaissance of PAOx chemistry was further potentiated around 2005, with the discovery of efficient CROP under microwave irradiation, which enabled the rapid synthesis of well-defined PAOx in order of minutes.[11]

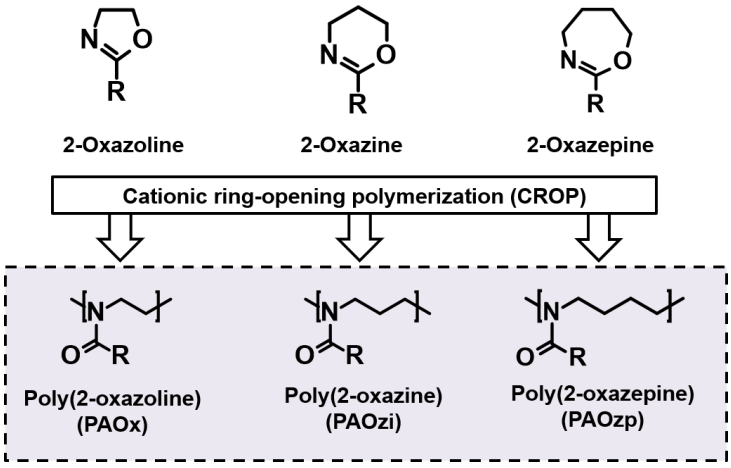

Figure 1. Structures of cyclic imino ether monomers and the corresponding polymers.

The five-membered ring AOx monomers belong to the general class of cyclic imino ether monomers (**Figure 1**), together with six-membered ring 2-alkyl-5,6-dihydro-4*H*-1,3-oxazines (herein and in scientific literature simply referred to as 2-alkyl-2-oxazines, AOzi) and recently discovered seven-membered ring 2-alkyl-4,5,6,7-tetrahydro-1,3-oxazepines (which are, however, challenging to synthesize and polymerize).[12] From this class, the primary attention is devoted to PAOx, with PAOzi gradually gaining popularity in biomedical sciences. The CROP of AOx and AOzi is generally initiated by a strong electrophile, either a strong acid (e.g., sulfuric acid, triflic acid) or, preferably, by nucleophilic substitution of alkyl halide, tosylate, or nosylate by AOx/AOzi monomer (**Figure 2**).[13] The latter method provides a powerful tool for straightforward incorporation of chain end functionalities (**Figure 3**). For example,

initiation with propargyl bromide leads to a polymer with an alkyne chain end group that can be used for conjugation via click chemistry.

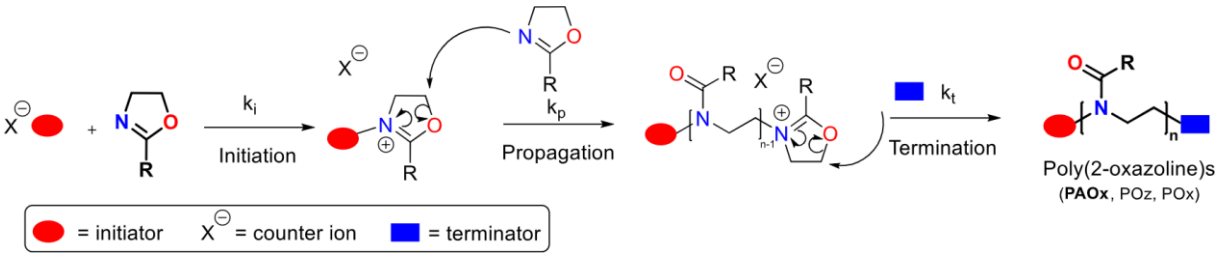

Figure 2. Cationic ring-opening polymerization (CROP) of 2-alkyl-2-oxazolines (AOx): A general polymerization mechanism. Reprinted from reference.[14]

In the absence of nucleophilic/electrophilic impurities, the propagation of cyclic imino ether-based monomers shows all characteristics of living polymerizations without any signs of termination and follows pseudo-first-order propagation kinetics. The driving force of propagation is the transformation of imino ether moiety into a more stable tertiary amide group. CROP of cyclic imino ether monomers proceeds relatively slowly, so high polymerization temperatures (80-140 °C) are typically used, with the advantageous use of microwave irradiation. Five-membered ring AOx show generally faster propagation rates than analogous six-membered ring AOzi due to the difference in ring strain and more favorable stereochemistry of oxazolinium living chain end towards the nucleophilic attack of the following monomer.[15] Nucleophilic termination of CROP is achieved either by exposing the polymerization mixture to adventitious water or by adding a nucleophilic terminator to introduce specific functionality to the chain end.[13] Typical terminating agents are various aliphatic amines (primary or secondary) or sodium azide to introduce an azide chain end group.

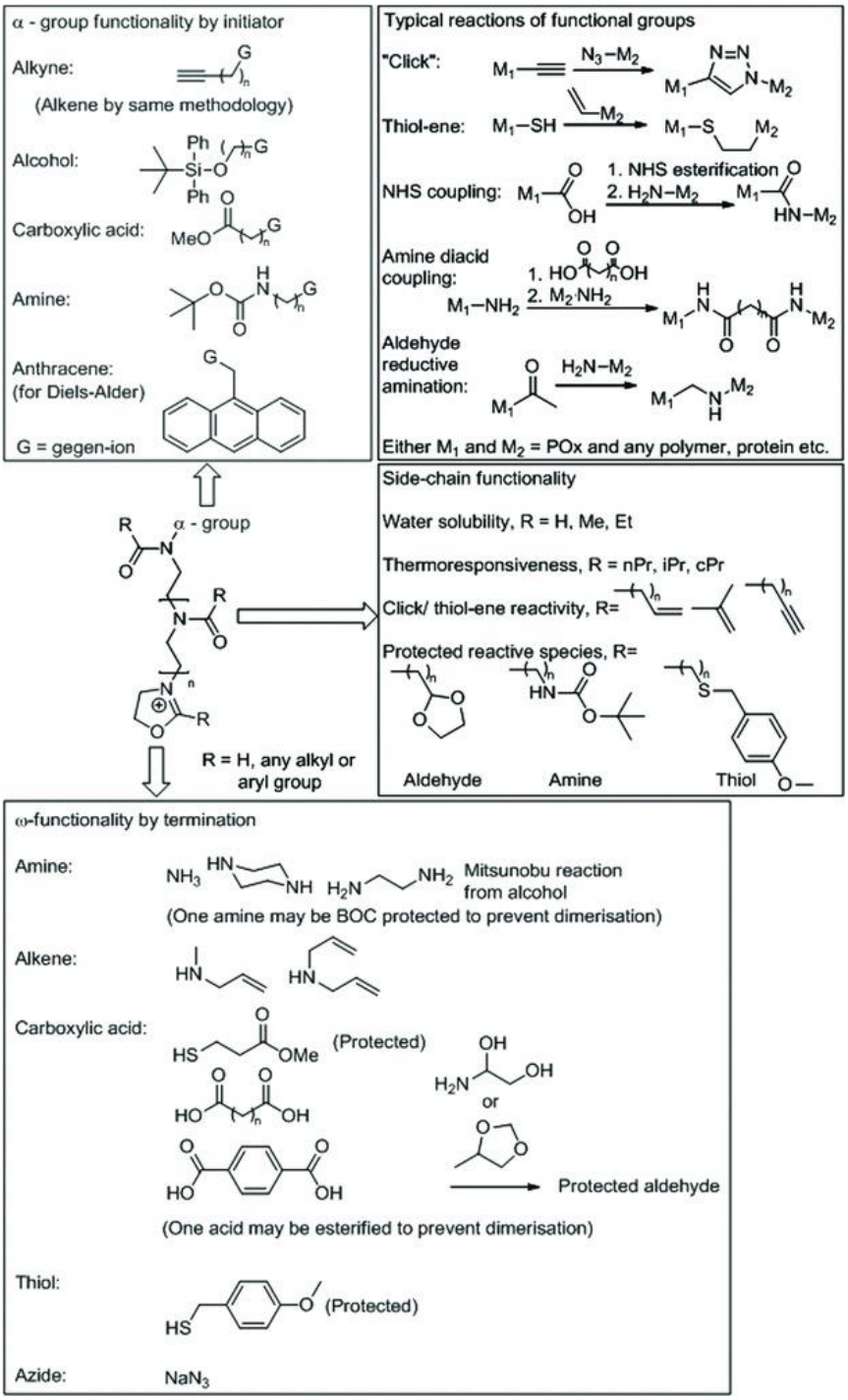

Figure 3. Selected functionalities that can be introduced to PAOx through their synthesis. Reprinted from reference.[16]

PAOx, as well as PAOzi, are generally considered as non-biodegradable. Their thermal properties depend mainly on the structure of side chains.[14] While PAOx with short side chains – methyl to n-butyl - are amorphous polymers with glass transition temperatures (T_g) in the range of 20-70 °C, PAOx with larger aliphatic side chains are semicrystalline with melting point

temperatures around 150 °C. Aromatic poly(2-phenyl-2-oxazoline) (PPhOx) is amorphous with a relatively higher T_g of 105 °C.

Hydrophilic PAOx with the shortest side chains, i.e., poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx), are particularly interesting for biomedical applications, as they show good water-solubility at room/body temperature, negligible toxicity, nonimmunogenicity, and excellent antifouling properties.[10, 17] They can be used for the construction of drug delivery systems,[18] coating of biomaterials,[19] advanced hydrogels,[20] or as excipients for drugs.[21] PAOx with medium-sized side chains show lower critical solution temperature (LCST) behavior in water and can be used to construct various thermoresponsive biomaterials (**Figure 4**).[22] PAOx with long aliphatic or aromatic side chains are hydrophobic and can be used for, e.g., synthesis of hydrophobic block in amphiphilic block copolymers.[23] In the following chapters, we will discuss the detailed synthetic procedures to obtain highly defined PAOx, emphasizing their potential in biomedical applications.

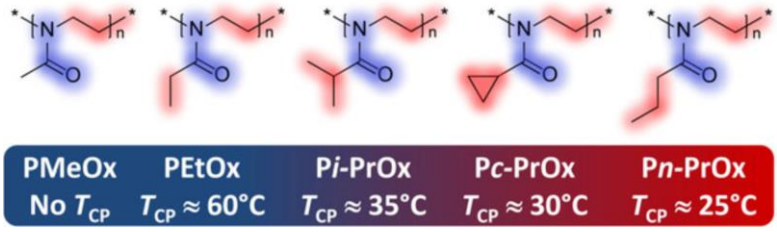

Figure 4. Effect of sidechain substituent on solubility and LCST behavior of PAOx in water, showing the cloud point temperatures (T_{CP}) of polymers. Reprinted from reference.[14]

3. Synthesis of novel poly(2-oxazoline)s by acylation of linear polyethylene imine.

Cationic ring-opening polymerization (CROP) of cyclic imino ether (CIE) monomers represents a robust strategy for the synthesis of a wide range of poly(2-oxazoline)s and poly(2-oxazine)s. However, the ionic character of the process disallows the use of monomers with functional groups that interfere with CROP.[13] CIEs with electrophilic side chain groups (e.g., alkyl halides or tosylate) cannot be polymerized into linear polymers due to the side chain initiation that leads to branched structures.[24] On the other hand, CIEs containing nucleophilic side chain groups would terminate the CROP. In this case, such polymers can be synthesized using appropriate protective group chemistry, which allows the synthesis of polymers with, e.g., pendant hydroxyl[25] or amine groups.[26]

To overcome these difficulties, we developed a new method for the synthesis of poly(2-oxazoline)s by interconversion of side chain acyl groups. Starting from commercially available poly(2-ethyl-2-oxazoline) (PEtOx), complete hydrolysis of its side chains led to well-defined linear polyethylene imine (PEI).[27] The secondary amine groups of PEI can be re-acylated by suitable acyl chlorides, anhydrides, or carboxylic acids using peptide coupling agents that allow the formation of tertiary amides in essentially complete conversion. Furthermore, this method allows us to prepare libraries of different poly(2-oxazoline)s efficiently without synthesis and tedious purification of each monomer.

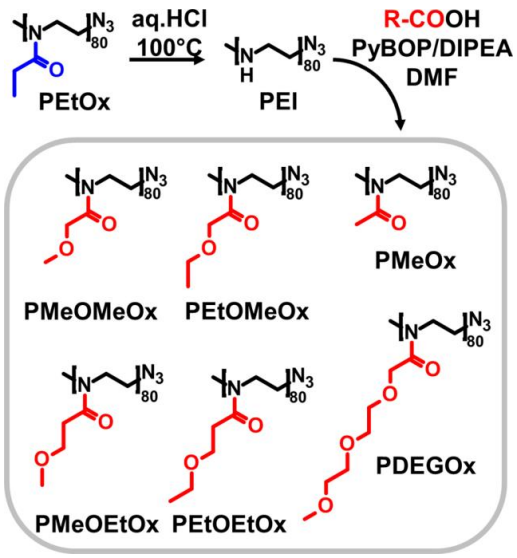

Figure 5. Synthesis of novel PAOx via acylation of linear PEI. Reprinted from Publication P1.

The original motivation for this research was the difficulty in CROP of 2-methoxyethyl-2-oxazoline due to the elimination of methanol at polymerization conditions, yielding 2-vinyl-2-oxazoline (**Publication P1**). On the other hand, we successfully obtained a series of hydrophilic PAOx containing side chain ether groups using the abovementioned PEI acylation protocol. Linear polyethylene imine was acylated by different ether-containing carboxylic acids using the peptide coupling agent PyBOP (**Figure 5**). Within the series, poly(2-methoxymethyl-2-oxazoline) (PMeOMeOx) was identified as the most hydrophilic non-ionic PAOx reported with potential in biomedical sciences. Furthermore, the amorphous poly(2-methoxy-ethoxy-ethoxymethyl-2-oxazoline) (PDEGOx) shows the lowest glass transition temperature ever reported for PAOx (-25 °C). The polymers were not cytotoxic and might be further used in biomedical applications.

We reported the synthesis of poly(2-amino-2-oxazoline)s, a novel class of thermoresponsive polymers (**Publication P2, Figure 6**). These polymers cannot be prepared by CROP of 2-alkylamino-2-oxazolines, as this leads to so-called double isomerization polymerization, where five-membered cyclic urea groups are incorporated into the main chain.[28] Therefore, we utilized our developed protocol to re-acylate linear polyethylene imine by a series of dialkyl carbamoyl chlorides, yielding to the series of PAOx polymers differing in the side chain structure, i.e., 2-dimethylamino-, 2-ethylmethylamino-, 2-diethylamino-, respectively 2-diisopropylamino-group. The structure of the side chains had a significant impact on the polymer hydrophilicity. While poly(2-dimethylamino-2-oxazoline) (PDMAOx) was extremely hydrophilic, poly(2-diisopropylamino-2-oxazoline) (PDiPrAOx) showed water-insoluble hydrophobic character. The polymers with medium-sized side chains, i.e., poly(2-diethylamino-2-oxazoline) (PEDAOx), respectively poly(2-ethylmethylamino-2-oxazoline) (PEMAOx) show thermoresponsive behavior in water, with lower critical solution temperatures (LCST) of around 24 °C, respectively 64 °C, with very sharp phase transition. In particular, as the LCST of PDEAOx is close to room temperature, this polymer can potentially be used to construct a wide range of thermoresponsive biomaterials. The thermal properties of prepared polymers were affected by the side chain structure, as well. While PDEAOx having medium-sized side chains is amorphous with a low glass transition temperature of $T_g \approx$ -10°C, the polymers with short, respectively bulky side chains show semicrystalline behavior with melting point temperatures of T_m = 230 °C (PDiPrAOx), T_m = 170 °C (PDMAOx).

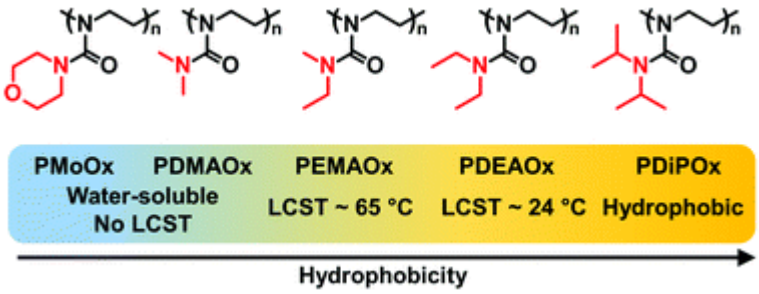

Figure 6. Structures of newly synthesized poly(2-amino-2-oxazoline)s. Reprinted from Publication P2.

The newly developed acylation protocol can also be utilized for the synthesis of high molar mass (HMM) poly(2-methyl-2-oxazoline) (PMeOx), a polymer with excellent hydrophilicity and antifouling properties (**Publication P3**). Traditionally, achieving PMeOx with a molar mass over 10 kg mol^{-1} and low dispersity ($Đ$ < 1.2) has been challenging due to limitations in cationic ring-opening polymerization (CROP), which suffers from negligible chain transfers to monomer, which, however, become important when attempting to reach high molar masses. In the chain transfer, the 2-oxazoline nitrogen serves as a base in addition to being a propagating nucleophile and abstracts a side chain alpha proton of the living oxazolinium chain-end. This protonated monomer then continues in growth, while the proton abstraction leads to a slightly nucleophilic enamine that can react with another living chain, which leads to termination and branching. Due to the lower steric hindrance, MeOx shows more pronounced chain transfers than other 2-oxazoline monomers, making high molar mass polymers challenging to achieve (**Figure 7**). From the polymerization kinetics, we determined the chain transfer coefficient (ratio of chain transfer events to propagation steps) of MeOx COP to be around 1/180, significantly higher than the values reported for, e.g., EtOx (1/2000).[29] In line with this observation, the maximal molar mass of PMeOx obtained by CROP was around M_n = 15 kg mol^{-1}.

On the other hand, the synthesis of HMM PEtOx is well-achievable (**Publication P6**, **Chapter 5.1**). Therefore, we utilized the CROP of EtOx to synthesize low-dispersity HMM PEtOx of different molar masses up to 100 kg mol^{-1}, followed by controlled side chain hydrolysis to a series of linear PEI. The acetylation of PEI with acetic anhydride led to PMeOx with molar masses up to 64 kg mol^{-1} and a $Đ$ = 1.13. The synthesized HMM PMeOx has been used to synthesize PMeOx-doxorubicin conjugates (**Chapter 5.1**).

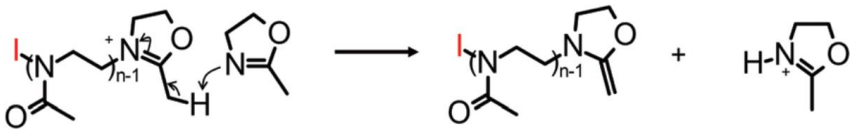

Figure 7. Mechanism of a chain transfer to MeOx monomer hampering the synthesis of HMM PMeOx. Reprinted from Publication P3.

Further, we employed the newly developed protocol for the synthesis of thioether-based poly(2-oxazoline)s for use in reactive oxygen species (ROS)-responsive nanomaterials that can be used in drug delivery applications (**Publication P4, Figure 8**). We aimed to overcome the synthetic challenges associated with incorporating thioether functionalities into PAOx observed by Kempe and coworkers,[30] who succeeded in the synthesis of 2-methylthiomethyl-2-oxazoline monomer but failed to polymerize it by CROP due to the nucleophilicity of thioether group interfering with cationic polymerization. We followed a similar protocol that we used before for the synthesis of the abovementioned ether side chain PAOx. First, linear PEI was re-acylated by a series of thioether-containing carboxylic acids using peptide-coupling agents to obtain a series of thioether-containing PAOx. All polymers were well-defined, with expected M_n of 13 – 15 kg mol^{-1} and narrow molar mass distributions ($Đ \approx 1.2$). All polymers showed amorphous behavior with T_g ranging from 12 to 48 °C. The polymers were insoluble in water in the physiologically relevant temperature range (up to 45 °C).

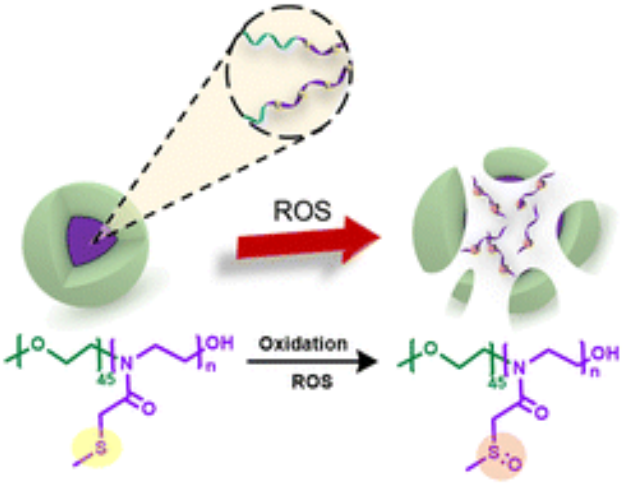

Figure 8. Structure of ROS-responsive block copolymer nanoparticles. Reprinted from Publication P4.

On the other hand, the addition of diluted hydrogen peroxide into the suspension of poly(2-methylthiomethyl-2-oxazoline) (PMTMeOx) led to the immediate dissolution of the polymer.

Therefore, we selected this polymer for the synthesis of ROS-responsive amphiphilic block copolymers poly(ethylene glycol)-*block*-poly(2-methylthiomethyl-2-oxazoline) (PEG-*b*-PMTMeOx). First, we synthesized a series of PEG-b-PEtOx copolymers by chain extension of PEG-based macroinitiator with PEtOx block using standard CROP of EtOx. The side chains of the PEtOx block have been removed, and the PEI block was re-acylated by methylthioacetic acid to achieve amphiphilic PEG-*b*-PMTMeOx. In water, this polymer self-assembles into spherical micelles having hydrophilic PEG shells and hydrophobic ROS-responsive PMTMeOx cores, which could potentially encapsulate and deliver therapeutic agents. The micelles showed oxidation-responsive behavior upon treatment with diluted hydrogen peroxide, indicating their potential for triggering drug release in environments with elevated ROS levels, such as cancerous tissues or inflammation sites.

Finally, we used the protocol to develop a robust method for biomedical visualization of fluorinated polymers by ^{19}F MRI (**Publication P5, Figure 9**). Due to their structural variability and tunable properties, fluorinated polymer materials represent an attractive class of MRI contrast agents. However, the synthesis of fluorinated PAOx is challenging due to the slow propagation rates of the corresponding electron-poor monomers.[31] In our study, we developed new tracers for the ^{19}F MRI based on a series of water-soluble fluorinated PAOx with varying fluorine content. First, we synthesized linear PMeOx followed by controlled partial side chain hydrolysis and re-acylation by difluoroacetic anhydride. As the increasing fluorine content leads to the hydrophobization of copolymers, their composition had to be optimized for maximal ^{19}F MRI signal while retaining water solubility and biocompatibility. The MR properties of fluorinated PAOx polymers were studied *in vitro* by ^{19}F NMR and MRI, revealing their good relaxation properties and imaging sensitivity. All copolymers were found to be non-cytotoxic *in vitro*. Finally, the polymers were successfully visualized by ^{19}F MRI after administration in rats, demonstrating the diagnostic potential of newly synthesized polymers. In conclusion, the newly developed PEI-acylation protocol is a straightforward alternative approach towards PAOx that are not achievable by CROP of the respective monomer, leading to several exciting polymers with potential in biomedical applications.

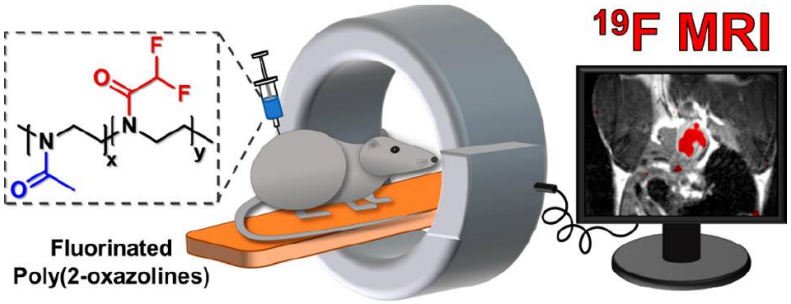

Figure 9. Structure of a fluorinated PMeOx used as a tracer in ^{19}F MRI. Reprinted from Publication P5.

4. Synthesis of biomaterials based on poly(2-oxazoline)s

4.1. Poly(2-oxazoline)-drug conjugates

Polymer-drug conjugates represent an emerging class of therapeutics for treating numerous diseases, including cancer or chronic inflammation. They can be employed for controlled drug delivery and their triggered release, offering significant advantages over the traditionally used small drugs. In cancer therapy, polymer-drug conjugates often employ enhanced accumulation in tumor tissue due to the enhanced permeability and retention (EPR) effect, a unique phenomenon where high-molecular-weight polymers accumulate preferentially in tumor tissues due to their leaky vasculature and impaired lymphatic drainage.[32] The EPR-based tumor accumulation and blood circulation time increase with the conjugate size, motivating researchers to develop protocols for synthesizing well-defined high molar mass (HMM) polymers. On the other hand, in the case of synthetic non-degradable polymer carriers (such as PAOx), their molar mass is targeted to be below approximately 50 kg mol^{-1}, i.e., below the renal filtration threshold, which ensures gradual elimination of conjugate/polymer from an organism by kidneys.

Furthermore, the polymer-drug conjugates are often designed to exhibit triggered drug release mechanisms, ensuring that the cytostatic agents are released specifically at the tumor site, thereby maximizing the therapeutic efficacy while minimizing side effects.[33] Using a water-soluble HMM polymer carrier with a molar mass of 20 – 50 kg mol^{-1} is usually desirable for the synthesis of simple yet efficient polymer-drug conjugates. In particular, such conjugates

based on biocompatible poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) showed excellent results in the therapy of various solid tumors.[34, 35]

Synthesis of high molar mass poly(2-oxazoline)s (PAOx) represents a challenging task once considered impossible to achieve due to the presence of chain transfers to monomer during CROP. Therefore, most previous reports describe the synthesis of PAOx as having relatively short chains (DP < 100, corresponding to ~ 10 kg mol^{-1} for PEtOx). In our work, we focused on optimizing the polymerization protocol for the CROP of EtOx, which led to the synthesis of low-dispersity PEtOx with an unprecedented molar mass of 300 kg mol^{-1} (**Publication P6, Figure 10**). The key to success was using a rigorous purification procedure to obtain monomers without any traces of water and low-temperature polymerization (42 °C) in non-polar solvents (chlorobenzene), suppressing the chain-transfer reactions. Such polymer can then be used to construct HMM drug delivery systems in analogy to other water-soluble polymer carriers synthesized by controlled radical polymerizations (e.g., HPMA) or living anionic polymerization (e.g., PEG).

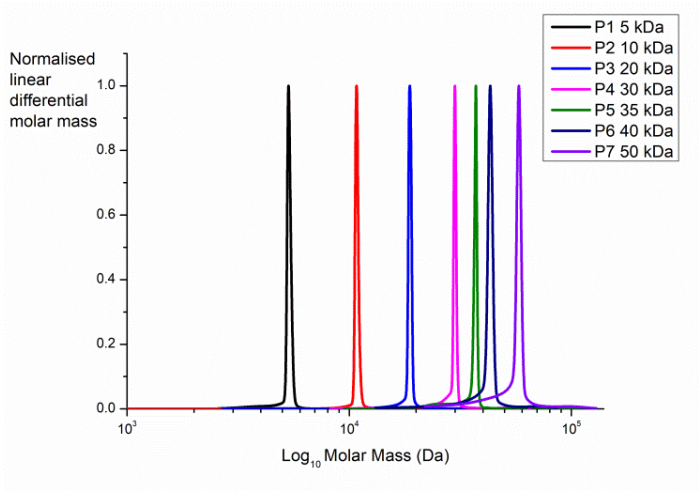

Figure 10. Molar mass distributions of HMM PEtOx synthesized by an optimized method. Reprinted from Publication P6.

In our work, we utilized the newly developed procedure for the first synthesis of HMM PEtOx-drug conjugates for cancer therapy (**Publication P7, Figure 11**). This system consists of a hydrophilic PEtOx carrier with linear architecture and narrow molar mass distribution connected to an anticancer drug, doxorubicin, by an acid-degradable hydrazone linker, which allows its triggered release (activation) in the slightly acidic tumor environment. PEtOx-

doxorubicin conjugates have been prepared in two different molar masses (20 and 40 kg mol^{-1}, respectively) and compared to analogous PHMPA-doxorubicin conjugate (40 kg mol^{-1}).[35] All conjugates show pH-dependent doxorubicin release in buffered conditions. The release was negligible at pH 7.4, mimicking the pH of blood plasma during conjugate circulation. The drug is released rapidly at pH 5.0, mimicking the pH after internalization in endosomes. The *in vitro* studies demonstrate the successful internalization of conjugates in cells while studying the fluorescence lifetimes of doxorubicin during internalization confirms its intracellular release. However, it must be noted that the cellular uptake of PEtOx conjugates was slightly lower compared to the PHPMA analog, presumably due to the lower hydrophilicity of PEtOx. This difference in cellular uptake was also reflected in the somewhat higher in vitro cytotoxicity of PHPMA-based conjugate. *In vivo* distribution experiments in mice with subcutaneous EL4 lymphoma revealed prolonged blood circulation of higher molar mass ^{125}I-labeled conjugates, resulting in increased tumor accumulation. Compared to PHPMA, PEtOx-based conjugate showed slightly longer blood circulation and superior tumor uptake, presumably due to its slightly higher hydrodynamic size. In the therapeutic experiment, all polymer-doxorubicin conjugates were shown to be effective in suppressing the growth of EL4 lymphoma in mice after treatment of a single intravenous dose of conjugates (20 mg of doxorubicin equivalents per kg of animal) compared to untreated controls (**Figure 12**). As expected, higher molar mass conjugates outperformed the 20 kg mol^{-1} conjugate due to more pronounced EPR-based accumulation. The tumor growth suppression by 40 kg mol^{-1} conjugates was somewhat better for PHPMA; this difference was, however, not statistically significant.

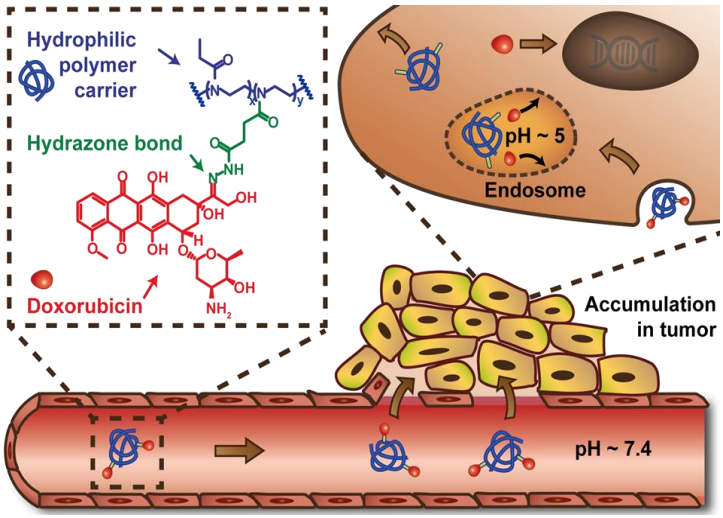

Figure 11. Schematic illustration of the synthesized PEtOx-doxorubicin conjugate and mechanism of its anticancer effectivity. Reprinted from Publication P7.

Similarly, treatment with both 40 kg mol^{-1} PEtOx, respectively, PHPMA conjugates extended the mean survival time of mice from 19 days (untreated controls) to 36 days in both groups. It can be inferred that the superior tumor accumulation of PEtOx conjugate can be counterweighed by its lower cellular uptake, leading to similar in vivo performance of both PEtOx and PHPMA conjugates. On the other hand, the results of this pioneering study confirmed the potential of PAOx for the construction of polymer conjugate drug delivery systems, with significant space for improvement.

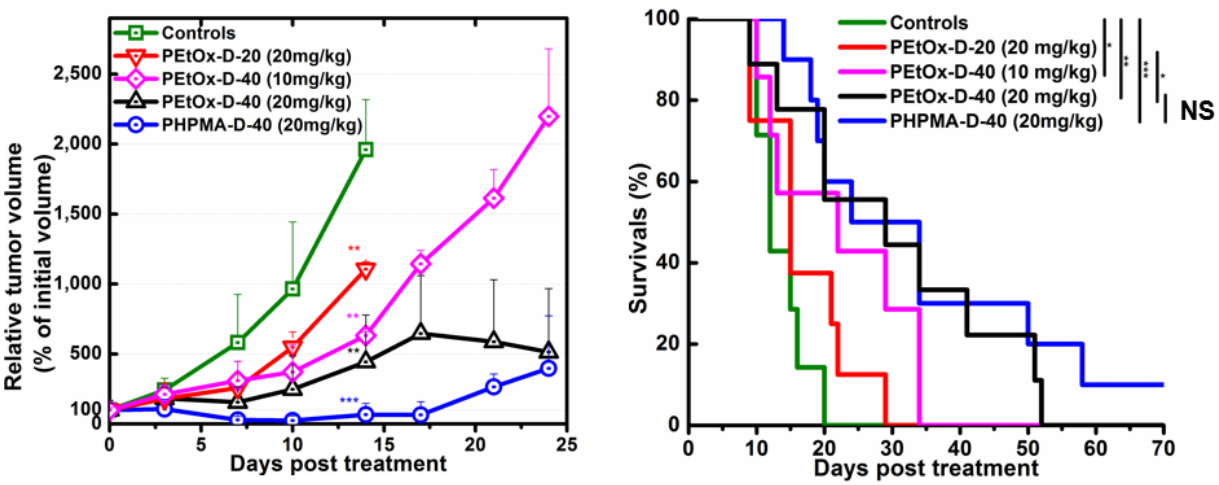

Figure 12. Treatment with a single dose of Dox-polymer conjugates induces therapeutical effect in EL4 T-cell lymphoma-bearing 57BL/6 mice. Mice were s.c. injected with EL4 cells. Eight days afterward, mice were randomized into groups (n = 7-10) and i.v. injected with a single dose of polymer conjugate (Day 0). The doses are expressed in equivalents of Dox per kg of mouse. Left: EL4 tumor growth in mice treated with polymer conjugates. Right: Survival of mice presented as Kaplan-Meier plot.

We hypothesized that the cellular uptake of PAOx polymers can be improved by increasing the hydrophilicity of the polymer. Therefore, we turned our interest from PEtOx to more hydrophilic PMeOx. Compared to PEtOx, PMeOx is more challenging to synthesize (**see Chapter 4**) but shows better antifouling properties (**see Chapter 5.3**) that will positively impact the biocompatibility of PMeOx-based therapeutics. In our work summarized in **Publication P8**, we synthesized polymer-doxorubicin conjugates based on PMeOx carrier and compared them with PEtOx-based analogs. In each case, conjugates of two molar masses, ~10, and ~20,

respectively ~40 kg mol^{-1}, have been synthesized. The higher molar mass PMeOx has been prepared from the corresponding PEtOx by the side chain switch described in **Chapter 4**. To introduce the hydrazone functionality necessary for doxorubicin attachment, an alternative approach to commonly used statistical copolymerization of 2-oxazoline monomer with functional 2-oxazoline monomer has been used to avoid gradient copolymer formation. PMeOx and PEtOx homopolymers were subjected to controlled partial hydrolysis of their side chains to introduce approximately 6 mol% of secondary amine ethylene imine units. These were then reacylated by methyl chlorosuccinate to obtain methyl ester-containing PAOx. The pendant methyl ester groups were then converted to hydrazides, followed by doxorubicin attachment. All conjugates were well-defined, with a molar mass of 10 – 47 kg mol^{-1}.

The antifouling properties of polymer carriers were studied by biolayer interferometry upon coating of streptavidin-containing sensors by biotin-attached polymers, revealing superior blood plasma fouling suppression by PMeOx compared to PEtOx, which is in line with the previously reported data.[36] Higher hydrophilicity of PMeOx was reflected in higher maximal doxorubicin loading in PMeOx conjugates (at least 15.6 wt.%) compared to PEtOx analogs (maximum 10.7 wt.%). The in vitro pH-responsive drug release profiles were comparable for all conjugates, showing fast drug release at pH 5.0 and slow release at pH 7.4. Furthermore, the cellular uptake of fluorescently labeled polymers was studied by confocal microscopy and flow cytometry, showing improved internalization of PMeOx over PEtOx. The study revealed slight cellular uptake pathway differences between the two carriers. While both PEtOx and PMeOx are internalized by clathrin-mediated endocytosis, PMeOx is also internalized by macropinocytosis, which can contribute to its better uptake. The superior uptake was also reflected in the higher cytotoxicity of PMeOx-based doxorubicin conjugates. In summary, a more hydrophilic PMeOx carrier surpasses PEtOx in the majority of parameters and shows higher maximum drug loading, improved cellular uptake, and superior in vitro antitumor efficacy. The reported study demonstrates the potential of PMeOx as an excellent platform for synthesizing advanced drug delivery systems.

Finally, we synthesized novel PEtOx-based conjugates with a multipurpose model drug salicylic acid conjugated to the polymer via hydrolyzable ester liker (**Publication P9**). A series of PEtOx with ~6 mol% of hydroxyl groups differing in their structure (primary, secondary, tertiary) have been synthesized, and salicylic acid has been attached. As expected, the highest conjugation yield was obtained for primary alcohol-containing polymers, while tertiary

hydroxyls were unsuitable for conjugation due to steric effects. Furthermore, the primary hydroxyl-based conjugate showed faster drug release, as well. Given the slow release of the drug (several days), the systems can potentially find applications in the sustained delivery of especially nonsteroidal antiinflamatory drugs (NSAIDs) in the therapy of chronic diseases.

4.2. Self-assembled nanoparticles based on gradient copoly(2-oxazoline)s

Amphiphilic gradient copolymers represent a promising alternative to extensively used block copolymers due to their facile one-step synthesis by statistical copolymerization of monomers of different reactivity. If both monomers differ in hydrophilicity, the amphiphilic gradient copolymer can be prepared in a single step. In case of steep compositional gradient (such polymers are often called "tapered" copolymers), one can achieve sufficient segregation of hydrophilic-unit-rich and hydrophobic-unit-rich segments that can induce self-assembly of copolymers into nanoparticles in an aqueous environment. Unfortunately, widely used radical copolymerizations often provide less steep compositional gradients or alternating copolymers. On the other hand, ionic polymerizations represent an excellent tool for synthesizing steep gradient copolymers, as they allow statistical copolymerization of monomers with significantly different reactivities. As an example, living anionic copolymerization of styrene with isoprene leads to gradient copolymer with steep compositional shift (copolymerization parameters $r_{isoprene}$ = 12.8; $r_{styrene}$ = 0.051),[37] which enables the application of such copolymers as thermoplastic elastomers.[38]

Our research focuses on the synthesis of amphiphilic gradient copolymers by statistical copolymerization of hydrophilic and hydrophobic monomers. In this view, cationic ring-opening copolymerization seems an excellent tool, as it allows straightforward copolymerization of 2-oxazolines of a wide range of chemical compositions and reactivities. In particular, statistical copolymerization of 2-oxazolines with aliphatic side chain with 2-oxazolines with aromatic side chain leads to steeply gradient copolymers due to the lower reactivity of the latter, stemming from the lower nucleophilicity of the nitrogen atom of an aromatic 2-oxazoline, as well as increased ring stability due to the conjugation of the endocyclic double bond. Such copolymers can then be loaded with hydrophobic drugs.[39, 40]

Typically, statistical CROP of 2-methyl-2-oxazoline (MeOx) with 2-phenyl-2-oxazoline (PhOx) leads to amphiphilic gradient copolymers with "quasi-block-like" architecture (reported copolymerization parameters r_{MeOx} >10, r_{PhOx} < 0.1).[41] In this case, PMeOx-grad-PPhOx copolymers self-assemble in water into micellar nanoparticles having PPhOx-rich hydrophobic core with PMeOx-rich hydrophilic shell. Compared to analogous diblock copolymers, which show strong phase segregation between core and shell, gradient copolymers show radially inhomogeneous core distribution, with the outer part of the core being denser compared to the inner core.[42] The higher polymer density at the core-shell interface can be explained by the back-folding of polymer chains resulting from hydrophilic−hydrophobic polymer interactions within the compositional gradient region.

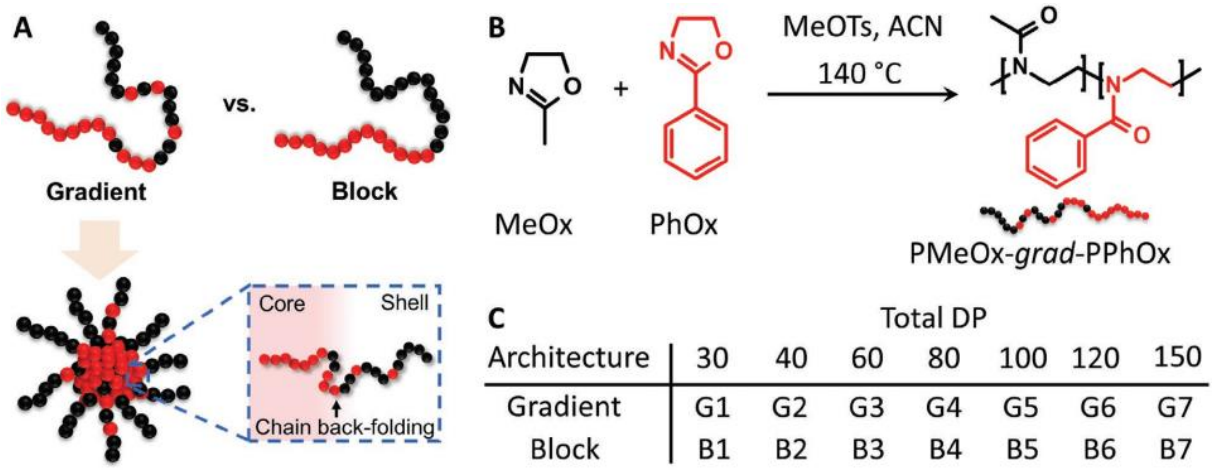

Figure 13. Comparison of amphiphilic gradient and diblock PAOx: A) Schematic illustration of both architectures. B) Synthesis of PMeOxgrad-PPhOx copolymers. C) Overview of the synthesized gradient (G1-G7), respectively diblock (B1-7) copolymers having the same MeOx: PhOx ratio of 70:30. Reprinted from Publication P10.

To further understand this phenomenon, we studied the synthesis and self-assembly of PMeOx-PPhOx gradient copolymers of different total degrees of polymerization (DP = 30 – 150) while keeping the comonomer ratio constant (F_{MeOx} = 0.7) and compared them with their diblock analogs (**Publication P10, Figures 13, 14**). All polymers were self-assembled into polymer nanoparticles in water. The nanoparticle size gradually increased with the total DP of the copolymer. For shorter copolymers (DP ≤ 60), there was no substantial difference in nanoparticle size and spherical morphology. Surprisingly, longer copolymers (DP ≥ 80) showed different nanoparticle sizes and morphology for both architectures. While gradient copolymers

formed smaller micelles with spherical morphology, diblock copolymer-based micelles were bigger and adopted partially worm-like morphology. It can be inferred that at lower total DPs, the gradient copolymers are too short to form efficient back loops at the core-shell interface. On the other hand, back-looping of longer chain copolymers leads to more compact micelles with spherical morphology. On the other hand, gradient copolymer micelles were slightly less stable than diblock analogs, having higher critical aggregation concentrations and faster unimer exchange between micelles.

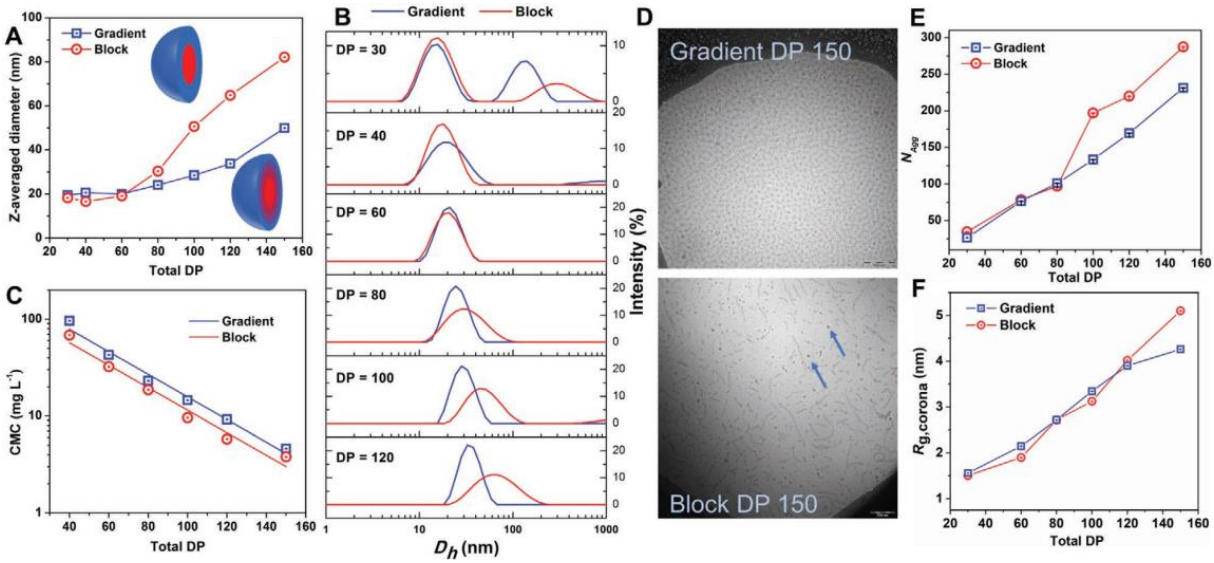

Figure 14. Comparison of self-assembly properties of PMeOx–PPhOx amphiphilic gradient and diblock copolymers in water. A) Hydrodynamic diameters and B) size distributions of copolymers in water. C) Critical micelle concentrations (CMC) of the synthesized copolymers in PBS. D) CryoTEM images of G7 (top) and B7 (bottom) copolymers in water. Arrows indicate ice crystal artifacts. Scale bars represent 200 nm. E) Aggregation number of copolymers in water as a function of DP. F) The gyration radius of a polymer chain in the nanoparticle corona as a function of DP in water. Reprinted from Publication P10.

The obtained nanoparticles were then employed to encapsulate a hydrophobic model drug curcumin using various techniques. In the thin-film technique, the drug and copolymer were first dissolved in a good solvent (ethanol) and evaporated, forming a thin film, which was then re-dissolved with an aqueous buffer.[43] This method was successful only for micelles with short chains (DP 40 and 60). For longer copolymers, the thin film became physically crosslinked by curcumin, making it impossible to dissolve. Therefore, we focused on methods that did not involve any solid polymer/drug film formation, i.e., solvent switch (nanoprecipitation),

respectively direct solubilization of solid curcumin by nanoparticle solution. Both methods yielded similar curcumin loadings of around 15 wt.% for polymers of DP $\geq$ 60. Interestingly, there were no significant differences between drug loading of gradient, respectively block copolymer-based nanoparticles. The drug-loaded micelles were stable in solution at room temperature for at least 30 days. Furthermore, freeze-drying of loaded micelles and their redispersion in buffer did not change the size and drug loading.

In the follow-up study, we sought a synthetic method to alter the gradient steepness. We discovered that changing the copolymerization solvent significantly impacts gradient copolymerization kinetics (**Publication P11**). While statistical copolymerization of MeOx with PhOx in acetonitrile led to a more shallow gradient (copolymerization parameters r_{MeOx} =10, r_{PhOx} = 0.3), copolymerization in more polar sulfolane led to a steeper compositional gradient and almost block-like architecture (r_{MeOx} =22, r_{PhOx} = 0.09). The size of sulfolane-based self-assembled nanoparticles was also between the sizes of analogous diblock acetonitrile-based gradient copolymers. Given the synthetic advantage of gradient copolymers (one-step synthesis) and comparable drug-loading characteristics to diblock analogs, gradient copoly(2-oxazoline)s might represent an exciting alternative to block copolymers in biomedical applications. The slight disadvantage of PMeOx-PPhOx copolymer systems is the relatively high glass transition temperature of rigid PPhOx core (T_g = 103 – 107 °C),[14] which is albeit beneficial for nanoparticle stability; however, it limits the drug loading capacity, drug release rate, and micelle decomposition in vivo.

Currently, the most potent PAOx nanoformulations are based on PMeOx-PBuOx-PMeOx block copolymers developed by Luxenhofer and Kabanov,[44, 45] that show remarkable loading capacity for anticancer drugs (up to 45 wt.%) and excellent anticancer properties in vivo. Unfortunately, amphiphilic gradient PMeOx-PBuOx copolymers cannot be synthesized by simple statistical copolymerization of MeOx and BuOx. The MeOx/BuOx statistical copolymerization parameters are close to 1, providing nearly random copolymer architecture without significant amphiphilic properties. On the other hand, we revealed that statistical copolymerization of aliphatic 2-oxazolines with aliphatic six-membered ring 2-alkyl-oxazines (AOzi) leads to gradient copolymers.

In our work, we focused on the formation of amphiphilic gradient copolymers by statistical copolymerization of hydrophilic 2-methyl-2-oxazine (MeOzi) with 2-n-propyl-2-oxazoline

(PrOx), respectively 2-butyl-2-oxazoline (BuOx) (**Publication P12**). In the homopolymerization kinetic measurements, we observed a significantly lower propagation rate for MeOzi, which is generally explained by the increased steric hindrance of the 6-position in the oxazinium ring resulting from its nonplanar structure.[15] This steric hindrance leads to the slower SN2 substitution by the monomer molecule. Surprisingly, statistical copolymerization of MeOzi with 2-oxazolines led to a complete switch in monomer incorporation rate, where more nucleophilic MeOzi was incorporated faster than 2-oxazoline (**Figure 15**). The copolymerization led to gradient copolymers with a relatively shallow gradient (r_{MeOzi} = 4.44-4.64, r_{PrOx} = 0.19, r_{BuOx} = 0.15). Despite the relatively shallow gradient, the copolymers could self-assemble in water to form nanoparticles.

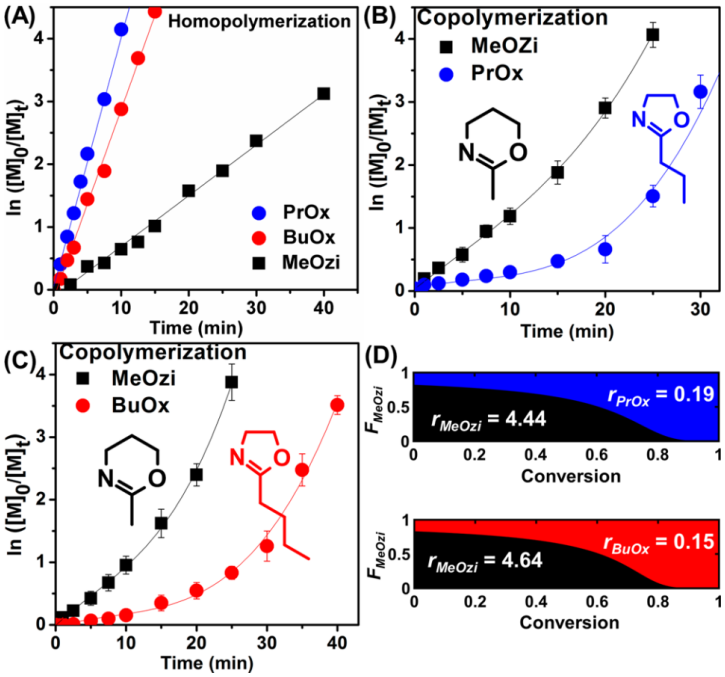

Figure 15. (A) Homopolymerization kinetics for the polymerization of MeOzi, PrOx, and BuOx. (B) Copolymerization kinetics of MeOzi with PrOx. (C) Copolymerization kinetics of MeOzi with BuOx. (D) Gradient microstructure of the copolymers obtained from a 1:1 monomer feed. Reprinted from Publication P12.

Gradient copolymers of PMeOzi-grad-PPrOx showed lower critical solution temperature (LCST) behavior in water. The cloud point temperature, above which the copolymers self-assemble, increased with a fraction of hydrophilic MeOzi in copolymers. Interestingly, the copolymer with 36 mol% of MeOzi showed a cloud point temperature of 33 °C between room and body temperature. Such systems can be thus used in "smart" thermoresponsible

nanomaterials, which can be injected into the body at room temperature as unimers and self-assemble into micelles upon heating in body temperature. On the other hand, PMeOzi-grad-PBuOx polymers with non-thermoresponsive PBuOx segments exhibit a behavior similar to the classical amphiphilic block copolymers, e.g., they were used for encapsulation of drug curcumin. These copolymers represent an interesting alternative to diblock copolymers due to their straightforward single-step synthesis and unique properties. They are currently being studied further as drug delivery systems.

Controlled side chain hydrolysis of PAOx and PAOzi leads to linear polyethylene imine (PEI) and polypropylene imine (PPI), respectively. These polymers were reported as highly efficient synthetic transfection agents for nucleic acids in drug delivery. At physiological pH, PEI and PPI are polycations that form stable polyplex nanoparticles with negatively charged nucleic acids. We hypothesized that incorporating PPI units into linear PPI might lead to decreased cytotoxicity due to the dilution of positive charges (**Publication P13**). We optimized the statistical copolymerizations of 2-oxazolines with 2-oxazines to yield nearly random copolymer architecture, which was achieved by copolymerization of MeOx with 2-isopropyl-2-oxazine (iPrOzi). The random PMeOx-PiPrOzi were synthesized in different comonomer rations and chain lengths. Their controlled hydrolysis led to nearly random PEI-PPI copolymers. These copolymers showed lower cytotoxicity than PEI while keeping high transfection efficiency for plasmid DNA. The in vivo study of copolymers is currently underway.

5. Biocompatibilization of surfaces via polymer brush coating

Coating of material surfaces with hydrophilic biocompatible polymers is a recognized method for enhancing the pharmacological potential of biomaterials.[46] This approach involves coating the materials with water-soluble polymers to shield them from unwanted interactions with blood plasma proteins.[47] The protein interactions often trigger an immune response and lead to the material being rejected and possibly excreted by the mononuclear phagocytic system, thus reducing its effectiveness. This so-called antifouling effect has been documented for a variety of water-soluble polymers, including polyethylene glycol (PEG), poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA), and, importantly also for PAOx.[36] Furthermore, the structural versatility of polymers allows the incorporation of a wide range of functional groups into the coatings, such as drugs or imaging labels. Such versatile coatings can then be utilized to design sophisticated systems for detecting and treating various diseases.

Our work focuses on surfaces coated with water-soluble PAOx and PAOzi. Several routes can be utilized to coat solid surfaces with PAOx/PAOzi. We developed a novel one-pot approach for coating polydopamine-covered gold surfaces with PAOx (**Publication P14, Figure 16**). After the solution CROP, the living oxazolinium chain ends were directly terminated with an amine-containing surface, tethering the polymers to the surface and forming a brush-like architecture. A library of hydrophilic PMeOx, respectively PEtOx homopolymers differing in molar mass ranging from 1.5 to 10 kg mol^{-1} (DP = 16-115) was synthesized, coated to the gold/polydopamine surface, and compared to PEGylated analogs. The structure of coatings was confirmed by X-ray photoelectron spectroscopy (XPS), infrared reflection-absorption spectroscopy (IRRAS), and atomic force microscopy (AFM). At the same time, the polymer surface thickness measured by spectroscopic ellipsometry was 3-5 nm, depending on the type and chain length of the polymer. The antifouling properties of polymer-coated surfaces were studied by surface plasmon resonance spectroscopy (SPR) after challenging the surface with solutions of either single plasma proteins (human serum albumin and fibrinogen) and human blood plasma (whole and diluted to 10 %).

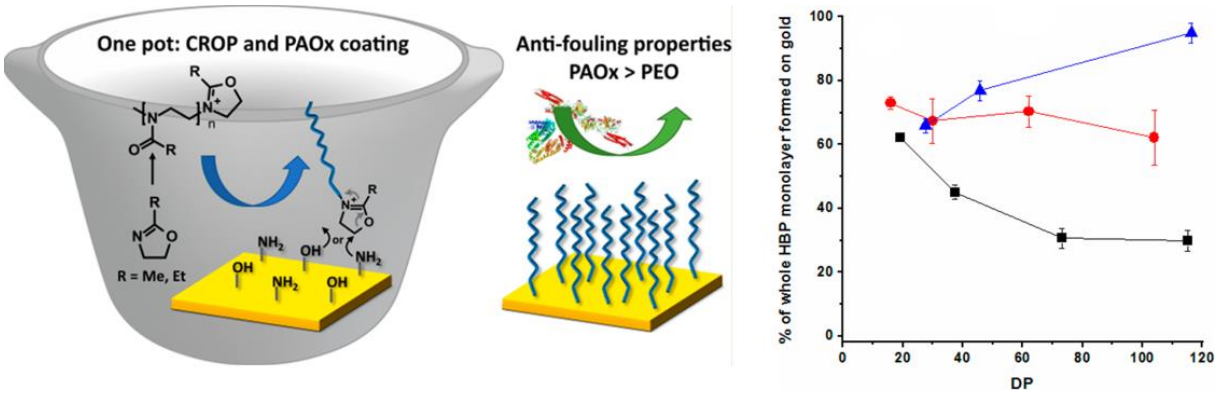

Figure 16. Left: Schematic illustration of polydopamine/gold layer coating with PAOx; Right: Comparison of antifouling properties of PEG (blue), PMeOx (black), and PEtOx (red) against whole blood plasma. Reprinted from Publication P14.

In most cases, the antifouling properties of PAOx outperformed PEGylated surfaces. In the case of the fouling experiments using single blood proteins, PEtOx showed slightly better results than PMeOx; the opposite was true for the more complex fouling experiment with blood plasma, where PMeOx showed superior performance compared to PEtOx, most probably due to the binding of other blood proteins from plasma (e.g., blood complement or immunoglobulins) to the more hydrophobic PEtOx. The antifouling properties of PAOx

surfaces were strongly dependent on the polymer chain length. Coating with short polymers led to thin layers that were not able to efficiently protect the gold/polydopamine surface from plasma fouling, and increasing the chain length led to thicker coatings with better antifouling properties. However, further increasing the PAOx molar mass above ~7 kg mol^{-1} showed hardly any effect on antifouling properties.

PAOx-based antifouling coatings can easily attach various functional molecules thanks to their synthetic versatility. In our recent study, we used PEtOx as a multimodal linker to attach protein molecules to a gold surface (**Publication P15**), which has potential application in developing new sensors with surface-attached antibodies. Furthermore, the antifouling properties of the PEtOx linker provide further benefit to the potential sensor in skipping the traditional surface passivation step. First, a multifunctional statistical PEtOx copolymer was synthesized, containing dithiolane functional groups for gold attachment and amine-reactive pentafluorophenyl ester groups for immobilization of proteins. After the gold surface coating, a model protein (bovine serum albumin, BSA) was attached to the surface. Currently, we are working on applying this system to develop sensors for early detection of osteoporosis.

Recently, Morgese and coworkers reported on superior antifouling properties of poly(2-methyl-2-oxazine) (PMeOzi) compared to PMeOx and PEtOx.[36] On the other hand, somehow contradictory results were reported by Kempe and coworkers,[48] who observed an increased association of poly(2-alkyl-2-oxazine) (PAOzi) nanoparticles with immune cells. To resolve this contradiction, we performed a broad study comparing antifouling properties of water-soluble PAOx (PMeOx and PEtOx), respectively PAOzi (PMeOzi and PEtOzi), each of them in three DPs (25, 50, and 100) (**Publication P16**). The polymers were synthesized by CROP of the respective monomers, followed by termination with potassium ethyl xanthogenate, leading to polymers with terminal xanthate groups used for gold coating. As expected, the hydrophilicity of coatings increased in the following order: PEtOzi < PEtOx < PMeOzi < PMeOx. On the other hand, the measured polymer swelling in water (determined from the ratio of ellipsometric layer thicknesses in a swollen and dry state) showed superior swelling of PAOzi, presumably due to their more flexible backbone.

Strikingly, the antifouling properties increased in the following order: PEtOx < PMeOx ≈ PMeOzi < PEtOzi, not matching the order of polymer hydrophilicity. The superior antifouling characteristics of PAOzi presumably come from their higher polymer chain flexibility and the

additional space occupied by water molecules within the swollen polymer layer. In the future, we will focus on the detailed identification of proteins attached to polymer coatings, which will provide additional insight into this complex topic and help us design new polymers for improved antifouling coatings.

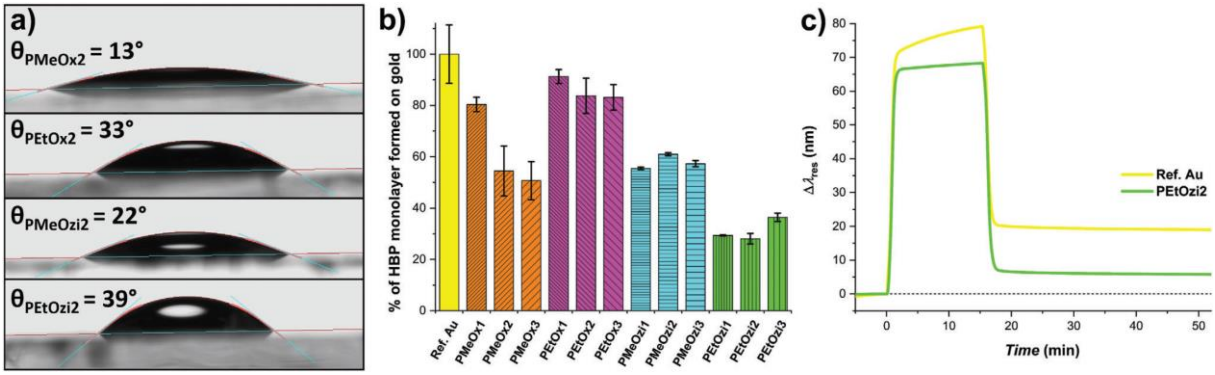

Figure 17: Antifouling properties of hydrophilic PAOx and POzi. a) Representative water contact angles on polymer-coated surfaces (DP 50). b) Comparison between the antifouling properties of PAOx and PAOzi with different DPs (25, 50, and 100) when exposed to human blood plasma. c) Representative SPR sensorgram contrasting the fouling resistance of PEtOzi2 to a bare gold surface. Reprinted from Publication P16.

6. Conclusions and outlook

This thesis summarized the selected achievements of the habilitation candidate in the field of poly(2-oxazoline) chemistry. The presented work led to significant advancement in this area through both synthesis (newly developed high molar mass PAOx, PEI amidation protocol) and biomedical applications (e.g., the first PAOx-drug conjugate for cancer therapy). The indisputable potential of PAOx motivates our further research, focusing on the development of polymer therapeutics based on not only PAOx but, given the excellent antifouling properties of PAOzi (Chapter 5), also conjugates of this polymer type. Several in vivo experiments are underway, namely the anticancer evaluation of PMeOx-doxorubicin conjugates and biodistribution experiments with PAOzi to assess their biomedical potential. We will focus on developing novel PAOx conjugation techniques and cationic PAOx/PAOzi derivatives as potential antimicrobial agents. The ultimate aim is to advance PAOx-based biomaterials toward clinical application and explore avenues for their commercialization, marking a significant stride in polymer chemistry and biomedicine.

7. Included publications

Publication P1

Straightforward route to new poly (2-oxazoline)s via acylation of well-defined polyethyleneimine **Sedlacek, O.**, Janouskova, O., Verbraeken, B., Hoogenboom, R. *Biomacromolecules*, 2019, 20, 1, 222-230

Publication P2

Poly (2-amino-2-oxazoline)s: a new class of thermoresponsive polymers, **Sedlacek, O.*,** Bera, D., Hoogenboom. R. *Polym. Chem.*, 2019, 10, 34, 4683-4689

Publication P3

Synthesis of defined high molar mass poly(2-methyl-2-oxazoline), **Sedlacek, O.*,** Monnery, B., Hogenboom, R. *Polym. Chem.*, 2019, 10, 11, 1276-1290

Publication P4

Thioether-based poly (2-oxazoline) s: from optimized synthesis to advanced ROS-responsive nanomaterials, Semira Bener, Ewa Pavlova, Hynek Beneš, **Ondřej Sedláček***, *Polym. Chem.* 2023, 14 (42), 4838-4847

Publication P5

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Publication P6

Defined High Molar Mass Poly(2-Oxazoline)s, Monnery, B., Jerca, V., **Sedlacek, O.,** Verbraeken, B., Cavill, R., Hoogenboom, R. *Angew. Chem. Int. Ed.*, 2018, 57, 47, 15400-15404

Publication P7

Poly(2-ethyl-2-oxazoline) conjugates with doxorubicin for cancer therapy: in vitro and in vivo evaluation and direct comparison to poly [N-(2-hydroxypropyl) methacrylamide] analogues, **Sedlacek, O.*,** Monnery, B., Mattova, J., Kucka, J., Panek, J., Janouskova, O., Hocherl, A., Verbraeken, B., Vergaelen, M., Zadinova, M., Hoogenboom, R., Hruby, M. *Biomaterials*, 2017, 146, 1-12

Publication P8

Poly (2-methyl-2-oxazoline) conjugates with doxorubicin: From synthesis of high drug loading water-soluble constructs to in vitro anticancer properties, **Sedlacek, O.,*** Van Driessche, A., Uvyn, A., De Geest, B., and Hoogenboom, R. *J. Control. Release* 2020, 326, 53

Publication P9

Poly(2-ethyl-2-oxazoline) Conjugates with Salicylic Acid via Degradable Modular Ester Linkages, Bernhard, Y., **Sedlacek, O**., Van Guyse, J., Bender, J., Zhong, Z., De Geest, B. G., and Hoogenboom, R. *Biomacromolecules* 2020, 21, 8, 3207–3215

Publication P10

Influence of Chain Length of Gradient and Block Copoly(2-oxazoline)s on Self-Assembly and Drug Encapsulation, **Ondrej Sedlacek**, Valentin Bardoula, Elina Vuorimaa-Laukkanen, Lars Gedda, Katarina Edwards, Aurel Radulescu, Grigoriy A Mun, Yong Guo, Junnian Zhou, Hongbo Zhang, Véronique Nardello-Rataj, Sergey Filippov, Richard Hoogenboom, *Small* 2022, 18, 17,2106251

Publication P11

Solvent-control over monomer distribution in the copolymerization of 2-oxazolines and the effect of a gradient structure on self-assembly, Bera, D., **Sedlacek, O**., Hoogenboom, R. *Polym. Chem.*, 2019, 10, 37, 5116-5123

Publication P12

Unexpected Reactivity Switch in the Statistical Copolymerization of 2-Oxazolines and 2-Oxazines Enabling the One-Step Synthesis of Amphiphilic Gradient Copolymers, **Sedlacek, O.,** Lava, K., Verbraeken, B., Kasmi, S, De Geest, B., Hoogenboom, R. J. Am. Chem. Soc., 2019, 141, 24, 9617-9622

Publication P13

Linear Poly(ethylenimine-propylenimine) Random Copolymers for Gene Delivery: From Polymer Synthesis to Efficient Transfection with High Serum Tolerance, M Rachèl Elzes, Ine Mertens, **Ondrej Sedlacek**, Bart Verbraeken, Aniek CA Doensen, Maarten A Mees, Mathias Glassner, Somdeb Jana, Jos MJ Paulusse, Richard Hoogenboom, *Biomacromolecules* 2022, 23, 6, 2459-2470

Publication P14

Poly(2-oxazoline)s One-Pot Polymerization and Surface Coating: From Synthesis to Antifouling Properties Outperforming Poly(ethylene oxide), Svoboda, J., **Sedlacek, O.,** Riedel, T., Hruby, M., Pop-Georgievski, O. *Biomacromolecules*, 2019, 20, 9, 3453-3463

Publication P15

Multifunctional Poly (2-ethyl-2-oxazoline) Copolymers Containing Dithiolane and Pentafluorophenyl Esters as Effective Reactive Linkers for Gold Surface Coatings, **Ondrej Sedlacek**, Tim Egghe, Patricia Khashayar, Martin Purino, Paula Lopes, Jan Vanfleteren, Nathalie De Geyter, Richard Hoogenboom, *Bioconjugate Chem.* 2023, 34, 12, 2311–2318,

Publication P16

Antifouling Properties of Poly (2-Oxazoline) s and Poly (2-Oxazine) s: Direct Comparison of Polymer-Coated Surfaces with the Same Coating Parameters, Jan Svoboda, Niccolo Lusiani, Radoslava Sivkova, Ognen Pop-Georgievski, **Ondrej Sedlacek***, *Macromol. Rapid Commun.* 2023, 2300168

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