



Universiteit
Leiden
The Netherlands

Core cross-linked polymeric micelles based on polypept(o)ides: from secondary structure formation of polypeptides to functional cross-linking strategies for polymeric micelles

Bauer, T.A.

Citation

Bauer, T. A. (2022, June 9). *Core cross-linked polymeric micelles based on polypept(o)ides: from secondary structure formation of polypeptides to functional cross-linking strategies for polymeric micelles*. Retrieved from <https://hdl.handle.net/1887/3307845>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3307845>

Note: To cite this publication please use the final published version (if applicable).



General Introduction

Nanomedicine

Nanomedicine refers to the application of nanotechnology in medicine to improve diagnostic and therapeutic efficacy for patient compliance. In nanomedicine, nanoparticles serve as tools for drug delivery and disease diagnosis.¹⁻⁷ Following the guidelines of the International Organization for Standardization (ISO) and the Commission of the European Union, nanoparticles are objects of any shape with at least one external dimension in the range of 1-100 nm.⁸⁻¹⁰ Nanoparticles offer the potential to modify the pharmacokinetic profile of active pharmaceutical ingredients (APIs) without editing the structural entities required for the mode of action.¹¹⁻¹³ Administration, distribution, metabolism, and excretion (ADME) can be altered and governed by the nanoparticle enhancing specificity and bioavailability of a given drug opening or extending the therapeutic window.^{9,14,15} The nanocarrier design follows the structural demands of the API while referring to the intended application.¹⁶⁻¹⁹ For nucleic acid delivery as required for BioNTech's and Moderna's Covid-19 vaccine, protection of the sensitive cargo to enable release of the intact mRNA into the cytosol of immune cells is of significance and featured by lipid nanoparticles.²⁰⁻²³ Nanomedicine thus opened the therapeutic window for RNA-based therapies.²⁴⁻²⁶ *Vice versa*, for small molecule APIs used in cancer therapy, nanomedicine aims to provide solubility and reduce the large volume distribution caused by the unspecific diffusion of the low molecular weight compounds.^{6,27,28} Despite being highly potent in the mode of action, many small-molecule APIs are hydrophobic and require excipients and high dilutions for administration.^{12,29-31} Approved for medical use in 1993 and 1996, the formulations of paclitaxel using ethanol and Cremophor EL (Taxol), and docetaxel with polysorbate 80 (Taxotere) are among the most successful drugs for adjuvant chemotherapy.^{28,32,33} Nevertheless, anaphylactic hypersensitivity, hemolysis, and peripheral neuropathy are common side effects attributed to the excipients.^{34,35} These restrict the maximum tolerated dose (MTD), while prolonged administration times limit patient compliance.^{34,35} In this case, nanomedicine, therefore, aims to provide solubility and a selective distribution profile to APIs to reduce off-target toxicity and improve therapeutic success.^{4,36,37}

Following the ambitions of nanomedicine shown in Figure 1, a large variety of nanoparticle therapeutics has been developed to improve the pharmacokinetic profiles of APIs. Initial investigations date back to 1954 when Horst Jatzkewitz reported on mescaline coupled to a copolymer of vinylpyrrolidone and acrylic acid *via* an enzymatically cleavable peptide linker.^{19,38-41}

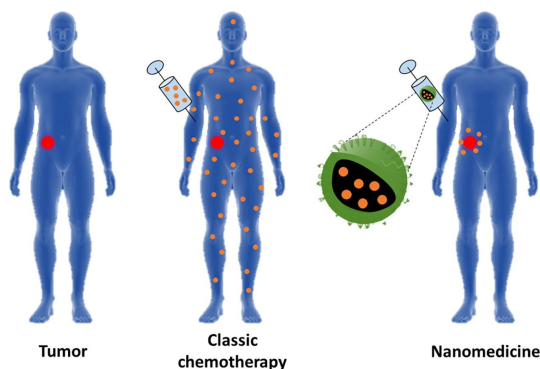


Figure 1. Idealized perspective on chemotherapy by nanomedicine. Figure reprinted from Gonzalez-Valdivieso *et al.* with permission from Elsevier (© 2021).³⁶

In consecutive *in vivo* studies a sustained drug release was observed.³⁹ Mescaline could still be detected in urine after 17 days, compared to 16 h for the free drug, whereas no traces were found after direct conjugation without the cleavable section.³⁹ Jatzkewitz clearly derived the potential of polymer-drug conjugates to alter the pharmacokinetics of APIs.^{38,39} The idea for polymer drug-conjugates was later refined and conceptualized by Helmut Ringsdorf considering solubility, drug conjugation, and targeting.^{19,42} Beyond polymer-drug conjugates many different nanocarriers have been designed and evaluated in (pre-)clinical investigations.^{5,12,13,41,43,44} A schematic overview is shown in Figure 2.^{43,45} The encapsulated or conjugated small molecule APIs are typically in the range of 0.1 to 1.0 nm in hydrodynamic diameter and are displayed as red (hydrophobic drugs) and green (hydrophilic drugs) stars.⁴³

The drug delivery systems can be generally divided into molecular and self-assembled structures and have been optimized for a broad variety of therapeutic cargos and diagnostic probes.^{16,41,47} In particular self-assembled nanoparticles are characterized by their core-shell architecture and can be readily formed from amphiphilic lipids or polymers.^{48–50} Lipophilic drugs can be encapsulated in the hydrophobic membrane or core compartment, while the hydrophilic corona provides steric shielding to reduce or prevent recognition by the immune system.^{50–52} Likewise, hydrophilic polymers are used for shielding of molecular nanocarriers, e.g., to increase the blood circulation half-life and reduce the immunogenicity of proteins.^{41,53,54} Polymersomes and liposomes further allow for encapsulation of hydrophilic drugs in the aqueous core pocket, while additional

stabilization is still essential for membrane-permeable drugs such as doxorubicin.^{13,55–58}

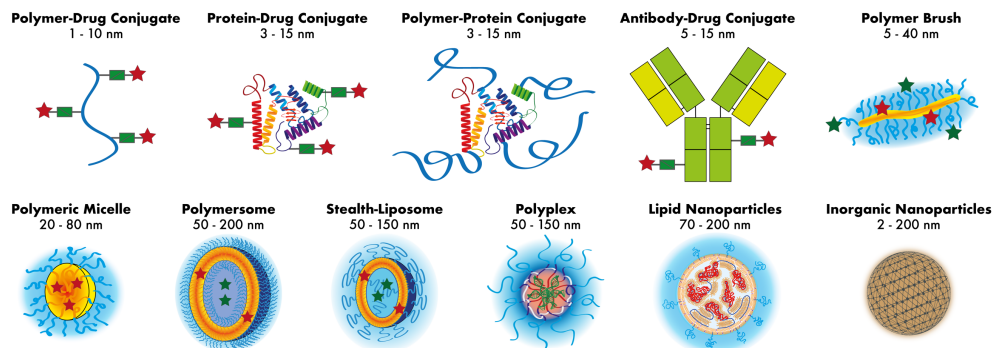


Figure 2. Schematic overview of the most common drug delivery systems in nanomedicine with approximate hydrodynamic diameters.^{45,46} Small molecule drugs are represented by red (hydrophobic) and green (hydrophilic) stars, drug linkers by green rectangles.

In contrast, polyplexes and lipid nanoparticles comprise a cationic lipid or polymer block for complexation and have been designed as non-viral vectors for gene therapy to provide protection and stimuli-responsive release for RNA or DNA.^{22,25,41,59,60} Besides lipid and polymer-based nanocarriers, inorganic nanoparticles, colloids, and metal-organic frameworks have been established for drug delivery and diagnosis.^{45,61–64} As such, iron oxide nanoparticles have been thoroughly investigated for the treatment of iron deficiency anemia, in heat ablation therapy, as well as for their potential as contrast agent in magnetic resonance imaging.^{65–70} Moreover, non-biodegradable electron-dense gold nanoparticles facilitate methodical *in vivo* studies using transmission electron microscopy (TEM) and enhance the sensitivity for surface-enhanced Raman scattering-based imaging.^{3,71–75} For therapeutic inventions, polymeric micelles (PMs) are among the most promising carrier types for hydrophobic APIs.^{52,76–78} Herein, the core compartment allows for high drug loading and can be designed and adjusted for the conjugation of (pro-)drugs featuring stimuli-responsive release.^{79–84} Moreover, PMs can be prepared with hydrodynamic diameters below 100 nm, often between 30 - 50 nm, which facilitate long circulation time and penetration into tumorous or inflamed tissue.^{76,85} As early as 1998, Torchilin and co-workers reported superior tumor accumulation of an ¹¹¹In-labelled protein in murine Lewis lung carcinoma xenograft models when PEG-DSPE micelles were selected over stealth liposomes as the carrier system.⁸⁶ In a detailed investigation,

Cabral *et al.* demonstrated the significance of particle size for deep tissue penetration.⁸⁵ As shown by intravital microscopy using PMs with distinct diameters of 30, 50, 70, and 100 nm, only the 30 nm particles were able to penetrate the poorly permeable pancreatic tumor leading to reduced tumor volumes by the release of the conjugated 1,2-diaminocyclohexane-platin(II) metallodrug.⁸⁵

Physiological Barriers for Nanomedicine

On the journey to the target site, nanomaterials face several barriers and obstacles for successful drug delivery.^{1,87,88} In general, the majority of nanocarriers are applied by parenteral administration routes.^{89–91} Activation of the adaptive immune system typically follows intramuscular injection for addressing transport into the draining lymph node.^{92–94} In contrast, for cancer therapy, nanomedicines are mainly administered by intravenous injection aiming to target metastasis as well as the primary tumor site.^{95–97} Injection into the blood stream exposes the nanocarriers to the blood components, e.g., red blood cells, immune cells, and plasma proteins, as well as to dilution.^{98–101} Stabilization and shielding strategies are thus required to prevent unspecific complement activation, opsonization, and recognition by the mononuclear phagocyte system (MPS).^{1,51,101,102} The MPS consists of bone marrow progenitors, monocytes and tissue macrophages located in organs such as the liver and spleen.^{102,103} Intended to remove foreign material from the blood stream, non-specific accumulation in these organs is a major obstacle for nanomedicines that impedes drug delivery to the diseased target site (Figure 3).^{103,104}

Strategies to avoid rapid clearance comprise the decoration of nanocarriers with hydrophilic polymers, such as poly(ethylene glycol) (PEG) or polysarcosine (pSar), which follow the Whitesides' rules for protein resistant materials and reduce MPS recognition, phagocytosis, and clearance from the circulation.^{106–110} Mechanistically, the enhanced repulsive forces among the hydrated polymer strands form an impermeable coating preventing van der Waals, electrostatic, and hydrophobic interaction with proteins.^{51,111} Following PEG as the gold standard material, the term 'PEGylation' was coined for the surface modification of materials with PEG, attributing reduced recognition properties ('stealth'-effect).^{53,112,113} Herein, the molecular weight of the hydrophilic polymer significantly influences the shielding efficiency.¹¹¹

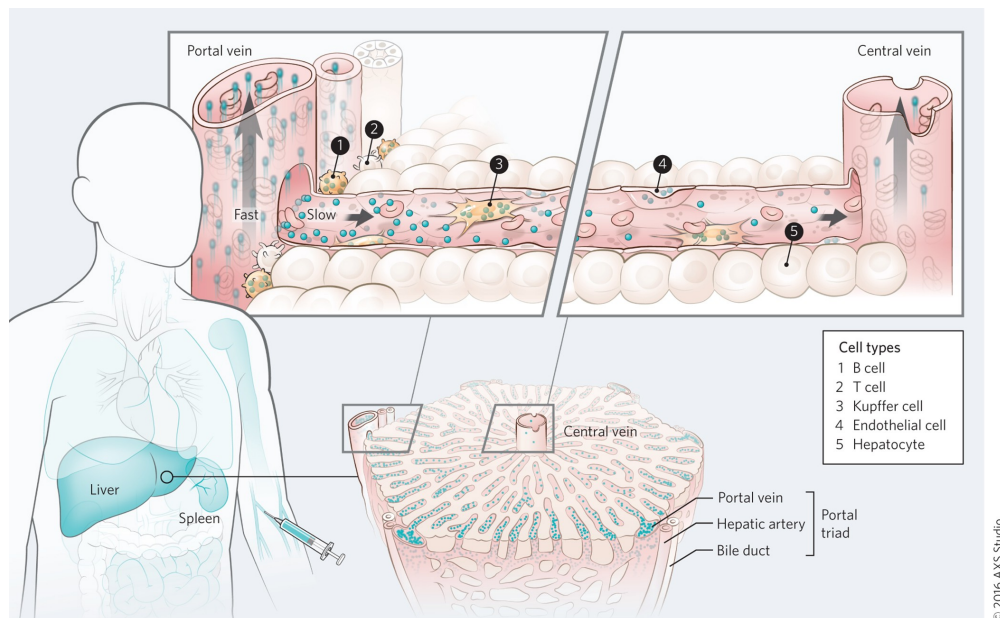


Figure 3. Intravenously administered nanoparticles encounter non-specific interaction with the MPS. The intensity of the turquoise color refers to the nanoparticle uptake within each organ.¹⁰⁵ The reduced flow rates in the liver sinusoid facilitates nanoparticle uptake by the residing immune cells, e.g., Kupffer cells. Figure reprinted from Tsoi *et al.* with permission from Springer Nature (© 2016).¹⁰³

Consequently, the PEG chain length was carefully optimized for the development of Doxil (doxorubicin sulfate nanocrystals, encapsulated in stealth liposomes with a lipid bilayer of a high melting point ($T_m = 53^\circ\text{C}$)), and PEG_{2k}-lipid (lipid: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; DSPE) was selected considering circulation time and lipid metabolism.¹³ For block copolymer micelles, Kwon *et al.* reported that PEG_{12k} significantly reduced the nanoparticle clearance compared to shielding by PEG_{5k}, resulting in a 5-fold increase of nanoparticles still in circulation after 4 h post-injection.^{114,115}

Beyond the surface chemistry, the hydrodynamic diameter is an important parameter affecting the biodistribution of nanoparticles. Large particles with hydrodynamic diameters > 200 nm can be rapidly recognized and cleared *via* the MPS in the liver and spleen.^{17,19,52} Contrariwise, glomerular filtration in the kidneys defines the lower size limit for nanoparticles that aim for long blood circulation.^{1,116} Threshold values for rapid renal excretion were found as ≤ 5.5 nm for quantum dots, approx. 29 kDa for dextran, and around 30 kDa for linear

PEG.^{117–119} Conversely, to avoid long-term side effects such as storage diseases the renal filtration sets an important limit for the maximum size of the individual nanocarrier components if the material is not biodegradable within relevant time frames.^{38,113,120}

Tumor Targeting by the EPR Effect and Beyond

The discovery of the enhanced permeability and retention (EPR) effect supported the field of nanomedicine, giving a rationale for targeting nanoparticles to inflamed and tumorous tissue.^{27,121,122} In 1984, Maeda *et al.* found an increased concentration of neocarzinostatin (NCS) in the tumor tissue of rabbits and mice when NCS was conjugated to a styrene-maleic acid copolymer (SMANCS).¹²³ After detailed elucidating studies in tumor-bearing mice using ⁵¹Cr-labeled proteins of varying molecular weights in 1986, Matsumura and Maeda then accounted the unique vascular characteristics of the tumor tissue for the specific accumulation of macromolecules therein.¹²⁴ Due to extensive and rapid proliferation, the cancer vasculature shows a high tendency for deficient vessel structures leading to fenestrations with higher permeability for nanoparticles compared to normal tissue (Figure 4).^{41,85,124} Moreover, insufficient lymphatic drainage reduces nanoparticle clearance from the tumor retaining the accumulated particles.¹²⁴ Consequently, if unspecific excretion and interaction with the MPS can be prevented, passive accumulation of the nanocarriers can be achieved.^{30,41,125}

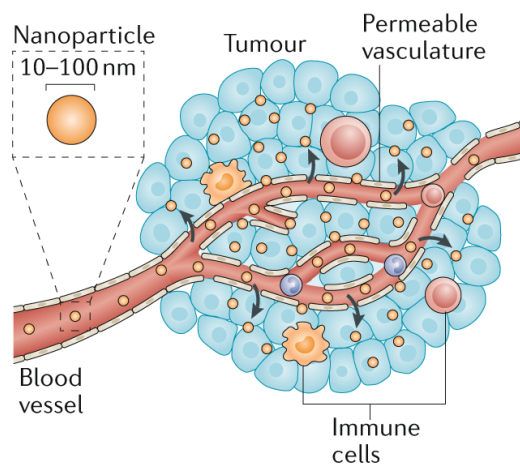


Figure 4. Schematic illustration of the nanoparticle accumulation in cancerous tissue due to the EPR effect. Figure reprinted from Irvine *et al.* with permission from Springer Nature (© 2020).⁷⁸

Nevertheless, the EPR effect has been critically discussed, and cannot be applied as a general concept for all tumor types.^{27,126–129} As such, the EPR effect was reported to be more prominent in murine (xenograft) tumor models compared to human patients.^{130,131} Moreover, a large heterogeneity in EPR susceptibility was observed among cancer patients calling for personalized medicine and patient stratification before applying nanomedicines as a general treatment regimen.^{132,133} Additionally, conjugation of targeting ligands ('active targeting') could improve tissue-selective distribution and enhance cell-specific uptake, while new types of drugs or drug combinations inaccessible for administration without nanomedicine are expected to contribute to therapeutic success.^{31,129,134} The basic requirement for concepts adding specificity yet remains the absence of unspecific interaction since active targeting can only take place when receptor and ligand are already in close proximity.^{135–138}

From the methodical viewpoint, more detailed investigations on the tissue level recently enlightened the actual targeting mechanism providing an deeper understanding and defining the basis for future therapeutic concepts.^{88,139–141} Herein, Chan and co-workers employed PEGylated Gold nanoparticles to investigate the exact mechanism accounting for nanoparticle entry into tumor tissue.^{72,73} Despite minor fractions of particle accumulation *via* passive diffusion, active transport mechanisms were described as the main driving force for nanoparticle entry into tumor tissue for a variety of tumor models.⁷² Moreover, a specific type of endothelial cells, nanoparticle transport endothelial cells (N-TECs), was identified as a gatekeeper for the transport process.⁷³ On the other hand, Biancacci *et al.* combined optical whole-animal imaging by micro-computed tomography-fluorescence tomography (μ CT-FLT) with immunohistochemistry for a detailed biodistribution analysis of dye-labeled core cross-linked polymeric micelles.¹⁴² On the organ level, 18.6% ID/g of the injected dose (ID) were located within the tumor tissue, exceeding the doses found in the liver and spleen (9.1% and 8.9% ID/g). Interestingly, within the tumor microenvironment, 67% of the nanoparticles were found in macrophages and other immune cells, although cancer cells accounted for 71% of the overall cell population.¹⁴² This underlines the potential of core cross-linked polymeric micelles for therapeutic approaches combining chemotherapy and immunomodulation.^{140,142–145}

Polymeric Micelles in Nanomedicine

Considering their small size and the core-shell architecture PMs are ideal carrier systems for hydrophobic small-molecule drugs. PMs can be readily formed by self-assembly of amphiphilic copolymers above the critical micelle concentration (CMC) applying dialysis or microfluidic devices.^{43,48,49,52,146} Self-assembly is an autonomous organization process leading to higher-ordered patterns or structures.¹⁴⁷ For self-assembly of linear block copolymers, spherical or worm-like micelles, lamellae and vesicles, as well as bicontinuous structures, can be obtained depending on the length and flexibility of the individual hydrophilic and hydrophobic segments.^{49,148,149} Compared to surfactant-based micelles, PMs exhibit lower CMC values (typically 10^{-6} - 10^{-8} M vs. 10^{-3} - 10^{-4} M) which refer to the higher number of interaction points.^{150,151} The CMC accounts for the free energy gain after reduction of free surface area by self-assembly and is the benchmark for the thermodynamic stability of a micelle.^{49,101,149,150} At the same time, the CMC represents the concentration of free polymer, so-called unimer, that always remains present in solution (Figure 5).^{43,101} Even after self-assembly, PMs are thus connected to the unimer concentration by a dynamic equilibrium, whereby the rate of exchange refers to the kinetic stability.^{43,101,152} Considering the application of PMs as drug carriers, the equilibrium directly affects therapeutic success, as excretion or adsorption of unimer leads to micelle disassembly.^{43,52,101} Upon injection into the bloodstream PMs are subjected to large dilutions and are exposed to plasma proteins, blood cells, hepatobiliary excretion, and renal filtration.^{1,88,103,116} In combination, these factors can rapidly induce disintegration of the PMs, reducing the nanocarrier to a bare solubilizer incapable to provide target specificity.^{27,37,43,153} In addition, the amphiphilic unimer may be susceptible to eliciting interaction with components of the immune system, e.g., the complement activation, promoting accelerated blood clearance (ABC) and affecting the safety profile of the medicinal product.^{43,154–156}

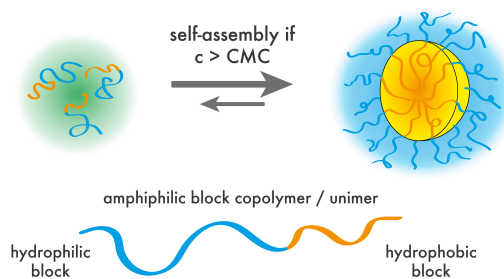


Figure 5. Polymeric micelles and the dynamic equilibrium of unimer and micelle.^{43,101}

While multiple PMs containing hydrophobic small molecule drugs have already been investigated in clinical trials,^{16,89,157–159} e.g., NK105,^{160,161} NK012,¹⁶² BIND-014,¹⁶³ the consequences of limited nanocarrier stability can be exemplified by studying Apealea and Abraxane. Both products have been developed and approved as Cremophor-free formulations of paclitaxel (PTX) to reduce the side effects associated with the excipient (as mentioned above).^{9,32,34,88} In Abraxane, PTX is formulated with human serum albumin forming 130 nm particles in aqueous solutions and was approved by the Food and Drug Administration (FDA) for the treatment of advanced non-small cell lung cancer (NSCLC) in 2005.^{12,164–166} However, upon infusion, the albumin-drug particles dissolve and albumin-PTX complexes are formed, whereby PTX can bind and unbind to readily available proteins within the bloodstream.^{12,166} Compared to Taxol, higher response rates were found for Abraxane in NSCLC patients, while the overall survival (OS) was not significantly improved.^{88,167} Similarly, response rate and progression-free survival (PFS) were higher for metastatic breast cancer patients, but OS was not significantly different.^{88,168} In 2018, Apealea was approved by the European Medicines Agency (EMA) for the treatment of ovarian cancer in combination with carboplatin, and consists of micelles formed from *N*-(13-*cis*-retinoyl)-L-cysteic acid methyl ester sodium salt and *N*-(*all-trans*-retinoyl)-L-cysteic acid methyl ester sodium salt encapsulating PTX.^{169,170} These micelles immediately release PTX into the blood plasma upon administration, yet offer shorter infusion times (1 h for Apealea vs. 3 h for Taxol), higher doses (MTD_{Apealea} 250 mg m⁻² vs. MTD_{Taxol} 175 mg m⁻²) and no mandatory premedication.^{169,170} Nevertheless, Apealea did not improve OS or PFS.^{169,170} Although Apealea and Abraxane both successfully contribute to cancer therapy and patient compliance, passive targeting *via* the EPR effect is not substantiated by the rapidly disintegrating particles, which

exemplifies the need for stable nanocarriers with tumor-specific drug release to exploit the full potential of nanomedicine.^{9,43,88,169}

Core Cross-Linked Polymeric Micelles

Based on the favorable structural properties of PMs, several modification strategies have been developed to suspend the equilibrium between unimer and polymeric micelle to achieve stable nanoparticles.⁷⁹ In general, non-covalent cross-linking strategies using π - π interactions, hydrogen bonds, or the chelation of metal ions, as well as different covalent cross-linking chemistries, have been investigated.^{43,171–175} For the latter, dynamic covalent bonds offer the potential to precisely tailor carrier stability and stimuli-responsive drug release.^{43,81,176} Beyond particle stabilization, only drug conjugation or specific interactions within the core assure to prevent drug leakage during transport and rapid transfer to the hydrophobic domains of surrounding proteins.^{13,37,177}

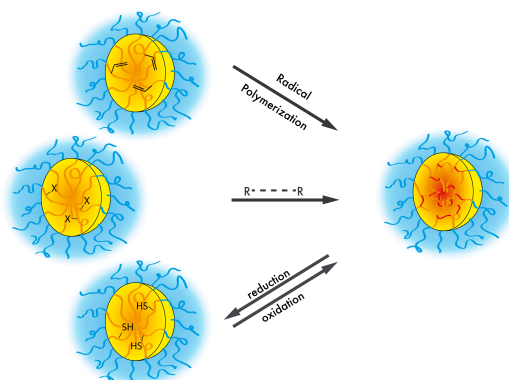


Figure 6. Core cross-linking strategies for polymeric micelles. Figure reproduced from Talelli *et al.* with permission from Elsevier (© 2015).⁴³

Core cross-linked polymeric micelles (CCPMs) have attracted significant interest, and optimization of carrier stability and drug release is a key objective for the delivery of hydrophobic APIs.^{30,43,79,178,179} As shown in Figure 6, the major strategies for core cross-linking include radical polymerization, reactions with bifunctional agents, and reversible oxidation of thiols.⁴³ Besides core cross-linking, shell cross-linking per-se offers a similar potential for stabilization of micelles.^{180,181} Nevertheless, modifications in the nanoparticle shell may easily jeopardize the water-solubility and protein resistance required for effective steric shielding.^{43,98} Early attempts to CCPMs for medicinal applications date back to

1992 when Rolland *et al.* used free radical polymerization for core cross-linking of PMs made from triblock copolymers of PEG-*block*-polyisoprene-*block*-PEG.^{182,183} As a result, the CCPMs retained their structure even in organic solvents and showed a circulation half-life above 50 h after intravenous injection to mice.¹⁸² More recently, Rijcken *et al.* from the Hennink lab developed CCPMs based on free radical cross-linking of thermosensitive PEG-*block*-poly(*N*-(2-hydroxypropyl) methacrylamidelactate) copolymers, culminating in the current evaluation of CPC634 in clinical phase II studies for the treatment of platinum-resistant ovarian cancer (NCT03742713).^{184–188} For the production of CPC634, core cross-linking and drug stabilization are performed simultaneously by a docetaxel pro-drug, which is connected to a methacrylate by a pH-responsive linker gradually releasing the drug at pH 7.4.^{184,186} Surprisingly, cumulative skin toxicity was found as the dose-limiting toxicity in phase I dose-escalation studies likely caused by micronucleation in the skin originating from slow drug release.^{187,189} Nevertheless, for CPC634, in human patients, four-fold higher total docetaxel concentrations were found in the tumor tissue compared to conventional docetaxel.¹⁸⁸ These findings relate to promising preclinical results in which complete tumor regression was observed upon single-dose administration.¹⁸⁵ Besides free radical polymerization, disulfide bonds are frequently implemented as cross-links for PMs.^{43,178,179} Disulfide bonds remain largely intact in extracellular fluids and can be cleaved by the elevated intracellular glutathione concentrations and are considered as the archetype of bio-reversible bonds for drug delivery.^{190,191} Disulfides can either be introduced by the rather unselective oxidation of thiol-groups or by using bifunctional cross-linkers such as 3,3'-dithiodipropionic acid containing a pre-formed disulfide bond.^{43,178,179,192} In addition, the Barz lab recently developed thiol-reactive protecting groups for chemoselective disulfide bond formation.^{193–197} After self-assembly, amphiphilic copolymers containing the *S*-alkylsulfonyl-group can be addressed by thiol-based cross-linkers yielding either CCPMs or cross-linked nanohydrogels.⁸² The combination of this approach with pro-drugs for therapeutic inventions will be elucidated in this thesis.

Among polymer-based nanomedicines, CCPMs have evolved from PMs and are expected to improve small-molecule-based drug delivery. The current challenges for CCPMs still comprise adjusting the intricate connection between carrier stability and stimuli-responsive yet rapid and complete drug release. Implementing multiple or even complex stimuli while accounting for robust and

scalable production thus requires innovative chemistry.^{9,43,88,132,198,199} Focusing on therapeutic success, nanomedicine will always compete with other concepts of therapeutic care.^{200–202} Expanding nanomedicine beyond its horizon implementing or assisting other technologies will thus lead to progress for treating devastating diseases and improving patients' lives.

Polypept(o)ides

Polypept(o)ides are hybrid materials combining the intrinsic functionality and stimuli-responsiveness of polypeptides with polypeptoids, e.g., polysarcosine for solubility and steric shielding.^{108,203,204} Considering peptides and proteins are based on α -amino acids, Bartlett and co-workers defined peptoids as oligomers of *N*-substituted glycines that are connected by amide bonds in the main chain in 1992.^{205,206} The term was later expanded by Zuckerman and co-workers, referring to polypeptoids for larger sequences, and polypeptoids can be considered as structural isomers of polypeptides.^{204,207} Unlike polypeptides, polypeptoids generally lack the acidic hydrogen atom at the amide nitrogen atom and are thus exclusive hydrogen bond acceptors that do not form secondary structures unless specific substituents are introduced.^{208–211} The highly water-soluble pSar, poly(*N*-methyl glycine), is among the most intensively studied polypeptoids.^{212–214} The free amino acid sarcosine can be found in muscle tissue and as a component of creatine (*N*-amidino sarcosine) in tissues with high energy demand.^{215–217} Sarcosine can be synthesized from glycine *via* the enzyme glycine-*N*-methyl transferase and degraded by sarcosine dehydrogenase.^{218–220} Polypept(o)ides thus allow for synthetic polymers entirely based on endogenous amino acids.^{108,203,204}

The term polypept(o)ides was coined by the Barz group in 2014 referring to the new class of polymeric materials combining polypeptides with polypeptoids (Figure 7).^{108,203} Early examples of polypeptide/polypeptoid copolymers date back to the origins of *N*-carboxyanhydride (NCA) and *N*-substituted NCA (NNCA) polymerization when studying fundamental properties and reaction kinetics was the major focus of research.^{221,222} Hanby, Waley, and Watson briefly described the first synthesis of pSar-*block*-poly(DL-phenylalanine) in 1950, yet, comprehensive study design and characterization of the copolymer was provided by Bamford and Ballard in 1956.^{223,224} Besides block copolypept(o)ides, early on, also statistical copolypept(o)ides have been synthesized and studied, aiming to understand the structure and function of the synthetic polypeptides and their natural analogs.^{225–}

227

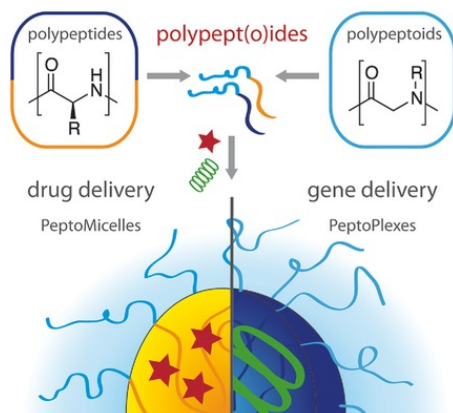
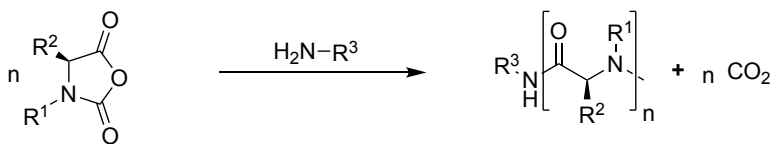


Figure 7. Polypept(o)ides combine the intrinsic functionality and stimuli-responsiveness of polypeptides with the shielding properties of the hydrophilic polypeptoid polysarcosine. Figure reprinted from Klinker *et al.* with permission from John Wiley and Sons (© 2015).¹⁰⁸

Concerning medicinal applications, Kimura and co-workers investigated polypept(o)ides in the 1990s.^{228–231} Among others, microcapsules were prepared from pSar-*b*-poly(ϵ -benzyloxycarbonyl-L-lysine) (pSar-*b*-pLys(Z)), pSar-*b*-poly(γ -methyl-L-glutamate) (pSar-*b*-pGlu(OMe)), and pSar-*b*-poly(L-alanine) (pSar-*b*-pAla), and the release of fluorescein isothiocyanate (FITC)-dextran was determined.²²⁸ Nevertheless, for medical applications, polypept(o)ides have attracted increasing attention only recently since NCA polymerization provided easy access to functional materials with defined polymeric architecture and narrow molecular weight distribution.^{108,204} The latest developments will thus be discussed in the paragraphs *Polysarcosine* and *Polypept(o)ides in Nanomedicine*.

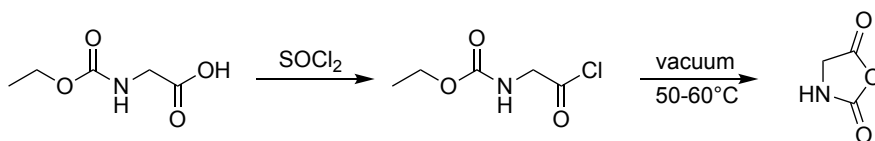
NCA/NNCA Polymerization

Synthetic polypept(o)ides can be conveniently prepared by living amine-initiated ring-opening NCA/NNCA polymerization.^{232–234} Depending on the desired application, polypept(o)ides can be designed with statistical or block-wise primary sequences, linear or branched architectures, and Poisson-like molecular weight distribution.^{108,204} Despite early attempts by Bailey *et al.*,²³⁵ NCA polymerization does not offer control on the primary amino acid sequence, making it a complementary tool distinct from solid-phase peptide synthesis (SPPS) or recombinant peptide expression techniques.^{236–238} A general scheme for the amine-initiated polymerization of NCAs or NNCA ($R^1 \neq H$) is given in Scheme 1.



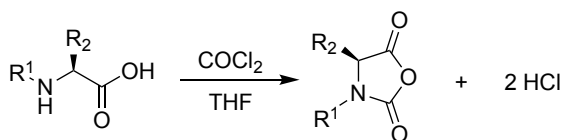
Scheme 1. Nucleophilic amine-initiated polymerization of NCAs and NNCA.

The first NCA synthesis was discovered by Hermann Leuchs in 1906, and NCAs are thus also called Leuchs' anhydrides.^{222,239} In the initial publication, Leuchs obtained glycine NCA upon heating of *N*-ethoxycarbonyl glycine chloride (Scheme 2).²³⁹ Moreover, Leuchs carefully described the release of CO₂ after the reaction of the NCA with water at room temperature, whereby an insoluble product was formed, which was interpreted as a higher anhydride of glycine. Leuchs further applied the methodology to other amino acids, including phenylalanine, leucine, and *N*-phenyl glycine yielding comparable NCAs and reaction products.^{240,241}



Scheme 2. Synthetic pathway to glycine NCA described by Hermann Leuchs.²³⁹

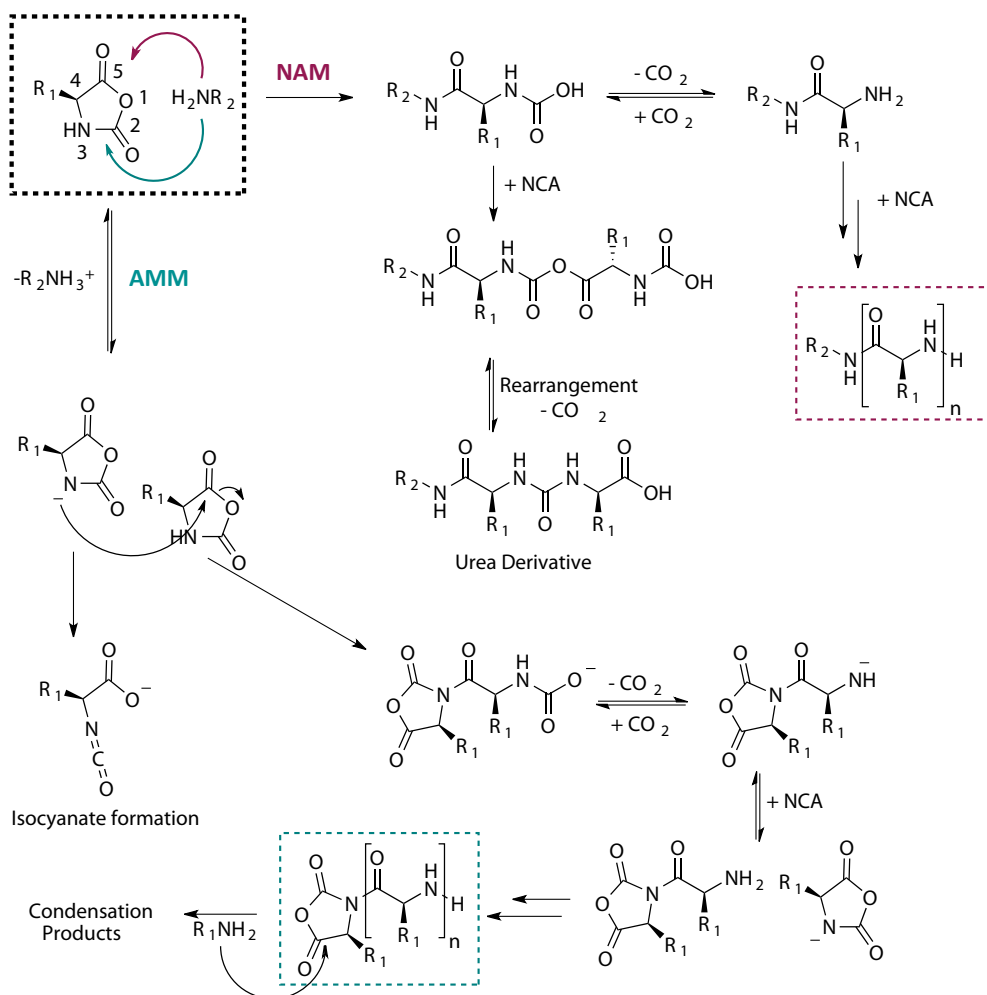
Despite the seminal publication from Hermann Staudinger in 1920, the general concept of polymerization and macromolecules was not completely established and still debated at that time.^{222,242} Early reports on NCAs thus mainly focused on the analysis of the degradation products of NCAs, e.g., diketopiperazines and hydantoin, and referred to so-called higher condensed anhydrides for products with higher molecular weights.^{243–245} Sigmund and Wessely described the first synthesis of sarcosine NCA in 1926.²⁴⁶ Herein, the authors mention polypeptides as possible products of the reaction of sarcosine NCA with pyridine, yet still refuse to describe these as polymers rather referring to higher condensed anhydrides. In 1947, Woodward and Schramm thus claimed the first intended NCA polymerization using traces of water for the initiation of the reaction in benzene-solutions yielding polypeptides as synthetic analogs of proteins.²⁴⁷ In the following, various polypeptides have been synthesized from this methodology and studied for their physicochemical properties.^{214,221,226} The kinetics of the NCA polymerization was first examined by Waley and Watson, revealing a first-order reaction for the polymerization of sarcosine NCA in nitrobenzene.^{248–250}



Scheme 3. NCA synthesis according to the Fuchs-Farthing method.^{251,252}

To generate NCAs and NNCAs, Fuchs suggested the direct reaction amino acids with phosgene, which was refined and improved by Farthing (Scheme 3).^{251,252} Addressing safety concerns and facilitating the application, gaseous phosgene can also be substituted by liquid diphosgene, or solid triphosgene, whereby phosgene is generated *in situ*.^{253,254} The Fuchs-Farthing method is thus the preferred synthetic route to NCA monomers, allowing for high yields and sufficient monomer purity after sublimation and/or repetitive recrystallization.²⁵⁵ Of note, the purity of all reagents, i.e., monomer, solvent, and initiator, is of significance for successful NCA polymerization. Beyond characterization by nuclear magnetic resonance (NMR) spectroscopy, Karl Fischer Titration to determine residual water content, and ion chromatography to detect inorganic contaminations, e.g., chloride ions, complement reagent analysis.^{256,257} In addition, the melting point represents an indicator of monomer purity.²⁵⁵

The mechanistic pathways for the amine-initiated NCA polymerization are displayed in Scheme 4.^{108,258} In general, two competing reaction pathways exist in parallel, namely, the activated monomer mechanism (AMM) and the normal amine mechanism (NAM). According to the NAM, the initiator solely acts as a nucleophile that attacks the NCA at C-5 leading to ring-opening and the formation of the carbamic acid. The carbamic acid then decarboxylates generating a primary amine readily available to attack the next NCA monomer. In case the decarboxylation is too slow, e.g., when the carbamic acid is deprotonated or the solvent is fully saturated with CO₂, a second NCA monomer can already be added at this stage ultimately leading to an urea derivative terminating the chain growth. Based on density functional theory (DFT) calculations, the nucleophilic attack at C-5 is the rate determining step of the NAM pathway using nucleophilic primary amines.²⁵⁹



Scheme 4. Mechanistic pathways for the NCA polymerization. Reprinted from Klinker *et al.* with permission from John Wiley and Sons (© 2015).¹⁰⁸

Contrarily, in the AMM, the initiator acts as a base and abstracts the acidic proton at N-3. In the following, the anionic activated monomer itself acts as the initiator of the reaction. The activated monomer can either undergo direct ring-opening leading to instable isocyanates easily subjected to further side reactions or attack a second NCA monomer at C-5. In the latter case, after decarboxylation, the AMM mechanism can continue in its pure form or in combinations with the NAM procedure. The AMM symptomatically leads to polymers with a reactive oxazolidin-2,5-dione as the end-group. Consequently, condensation products are

frequently observed, and polypeptides derived from AMM processes are characterized by a broad molecular weight distribution. Conversely, Poisson-like molecular weight distributions are obtained for living polymerizations following the NAM process, as the initiation is typically faster than the propagation.^{108,258}

Since NNCAAs do not contain an acidic proton at the nitrogen atom the AMM pathway is generally inhibited for polypeptoids. *Vice versa*, the polymerization of NCAs is easily affected by the nucleophilic or basic character of the initiator.^{108,222,258} In addition and comparable to other living polymerization techniques, also NCA polymerization is highly sensitive to impurities since these may promote the AMM pathway, catalyze side reactions, and initiate or terminate the chain growth.^{255,258,260} In 1997, Deming reported on the first living NCA polymerization by using zero-valent nickel amido-amidate complexes initiating and mediating the chain growth reaction.²⁶¹ Herein, homo- and block copolypeptides of pGlu(Obn) and pLys(Z) could be synthesized with high molecular weights and narrow dispersity ($D < 1.2$). Deming further expanded the concept to cobalt and iron complexes, and synthesized library of functional polypeptides.^{262,263} However, polymerization of NNCAAs was not substantiated until recently since initiation required an acidic proton for β -hydride elimination. The Kramer group thus improved the Ni and Co initiators allowing for polymerization of proline NCA.²⁶⁴ Nevertheless, elaborate synthesis and potentially remaining traces of toxic heavy metal ions hamper this technique.¹⁰⁸ In 2004, the groups of Schu   and Hadjichristidis reported that reaction temperature, sufficient removal of CO₂, and the purity of the components, are the key parameters for the living amine-initiated polymerization of NCAs.^{232,233} In the following, several groups contributed to expanding the living amine-initiated NCA polymerization leading to a well-established type of polymerization.^{203,234,265–}

267

Current investigations aim to accelerate the reaction rates of NCA polymerization and achieve chain lengths beyond 1000.²⁶⁸ In particular organocatalysis techniques were therefore applied to NCA polymerization. In 2019, Zhao *et al.* combined 1,3-bis(2-hydroxyhexafluoroisopropyl)benzene (1,3-bis-HFAB) and *N,N*-dimethyl ethanol amine in dichloromethane to activate the NCA monomer by hydrogen bonds.²⁶⁹ Moreover, Xia *et al.* demonstrated accelerated polymerization in DCM upon addition of crown ether as a catalyst.²⁷⁰ In addition, emulsion techniques have been developed to enable facile synthesis of α -helical multi-block copolypeptides.²⁷¹ Regarding the biomedical applications, large

molecular weights are often not necessarily an advantage, since storage diseases are a realistic threats that also need to be considered for polypept(o)ides.¹²⁰ Nevertheless, fast and robust mechanisms facilitating large-scale production will aid translation of polypept(o)ides.

Polysarcosine

Polysarcosine is a non-ionic and highly water-soluble polymer that adopts random coil conformation in aqueous solution, which is attributed to the equal population of the *cis* and *trans* configuration of the amide bond.^{212,272–274} Comparable to PEG, pSar solely acts as a weak hydrogen bond acceptor without any hydrogen bond donor properties, while being slightly less flexible referring to the respective Kuhn lengths of $l_{k, \text{pSar}} = 1.5 \text{ nm}$ and $l_{k, \text{PEG}} = 1.1 \text{ nm}$.²¹² pSar matches the requirements for protein resistant surfaces summarized by the Whitesides' rules in 2001.¹⁰⁶ Indeed, already Ostuni *et al.* described superior protein resistance of self-assembled monolayers (SAMs) functionalized with tri(sarcosine) since reduced levels of protein adsorption and cell adhesion were found.^{106,275} These results were later confirmed by Messersmith and co-workers reporting excellent resistance of pSar-grafted TiO₂ surfaces toward non-specific adhesion of proteins or any attachment of mammalian or bacterial cells.²¹³ Moreover, Jordan/Luxenhofer and co-workers investigated the resistance of inorganic surfaces to biofouling after modification with polypeptoid brushes.^{276,277} The experimental findings are further supported by molecular dynamics simulations, in which PEG and pSar showed an equally low affinity for interaction with human serum albumin.²⁷⁸ Moreover, both pSar and PEG do not elicit activation of the complement cascade, but acetylation of the amine end-group remains significant.^{109,212} Consequently, pSar can be classified as a 'stealth' material and has emerged as a potential substitute for PEG in medical applications when increased water solubility and reduced immunogenicity and MPS recognition are desired.^{204,212} As an early example, in 1985, Moran and co-workers reported that covalent conjugation of pSar to grass pollen allergens decreased the immunoglobulin E (IgE) formation.^{279,280} More recently, pSar attracted increasing attention as an alternative material to PEG and has been investigated for shielding of antibody-drug-conjugates,²⁸¹ proteins,^{282–284} liposomes,^{109,285} and lipid nanoparticles.²⁸⁶ In 2020, Son *et al.* prepared PEG and pSar functionalized liposomes and compared the immune response after intravenous administration to rats.²⁸⁵ As a result, significantly higher levels of IgM and IgG antibodies were found for the PEGylated liposomes. Hereafter, the second administration revealed the ABC

phenomenon for PEGylated liposomes, yet again identical circulation half-lives for the pSar functionalized liposomes. For LNPs, both, PEG-lipids and pSar-lipids yielded similar mRNA nanocarriers, whereby reduced cytokine secretion and lower immunogenicity were found for the pSar-containing LNPs.²⁸⁶ In summary, pSar has emerged as a biocompatible polymeric material suitable for medicinal applications.

Polypept(o)ides in Nanomedicine

Since 2014, several polypept(o)ide-based polymer and nanoparticle architectures have been established and investigated for therapeutic applications. In these systems, the hydrophilic pSar provides solubility and prevents recognition of the structures by the MPS, while the polypeptide section can be selected and tuned for drug or gene delivery.¹⁰⁸ As polypept(o)ides are easily accessible by the mild chemical conditions of the NCA polymerization, a large variety of polymeric architectures has been synthesized, and functional side- or end groups were introduced and exploited for cross-linking, API incorporation, and addition of targeting motifs.^{108,194,196,204,287,288}

In their seminal publication from 2014, Birke *et al.* reported that for pSar-*b*-poly(γ -benzyl-L-glutamate) (pSar-*b*-pGlu(OBn)) and pSar-*b*-pLys(Z), the succession of the block copolymerization is not relevant, and polymers with narrow dispersity ($\bar{D} < 1.2$) can be obtained in all cases.²⁰³ The amphiphilic copolymers were then applied for stabilization of organic colloids and for encapsulation of the small molecule adenylate cyclase (cAMP) inhibitor MDL-12.330A in polymeric micelles. Herein, MDL-12.330A is stabilized in the micelle core by π - π -interactions with the aromatic benzyl groups.^{203,289} Just recently Johann *et al.* could demonstrate the potential of the cAMP inhibitor-loaded PMs when immune evasion of melanoma cells was successfully inhibited leading to reduced tumor growth after local administration.²⁸⁹ In addition to the stabilization of organic colloids, polypept(o)ides have also been applied for the shielding of metal-organic frameworks (MOFs) and metal-oxide surfaces.^{62,290,291} Besides polymeric micelles for hydrophobic APIs, polymersomes, so-called peptosomes, that allow for encapsulation of hydrophilic and/or hydrophilic cargo have been established by Tanisaka *et al.* using pSar-*b*-pGlu(OMe) and Weber *et al.* for pSar-*b*-pGlu(OBn).^{292,293} Moreover, non-viral transfection agents for gene delivery have been developed based on polypept(o)ides.^{59,60,294,295} For these systems, Heller *et al.* investigated the significance of the block ionomer

microstructure on the formation and transfection efficiency of plasmid DNA (pDNA) polyplexes.⁵⁹ Beyond self-assembled structures, also molecular nanocarriers have been prepared from polypept(o)ides. Compared to their self-assembled counterparts, molecular nanocarriers are chemically synthesized nanoparticles, whereby size and stability can be specified by the synthetic details. In particular, carrier systems with small hydrodynamic diameters, high stability or defined architecture can easily be prepared by these techniques.^{47,108,296} In 2017, Holm *et al.* introduced peptostars with Poisson-like molecular weight distribution and small hydrodynamic diameters of 10 to 30 nm.^{297–299} Interestingly, fluorescence correlation spectroscopy (FCS) in human blood plasma revealed the impact of branching on the shielding efficiency, since significant protein adsorption was observed for the 3-arm peptostars yet not for the 6-arm analog.²⁹⁸ Referring to the defined architecture, Kappel *et al.* recently used cylindrical bottlebrush polymers (peptobrushes) with a dense pSar corona to demonstrate the significance of the number of antibodies for active targeting of nanoparticles to dendritic cells *in vivo*.^{135,136,300} Therefore, fluorescently labeled peptobrushes based on a pLys backbone with grafted pSar₁₀₀ side chains were precisely engineered to bear on average either 2, 6, or 12 antibodies (anti-DEC205) per nanoparticle ($R_h \approx 23$ nm), and the circulation half-life and biodistribution were evaluated in mice. As a result, rapid uptake by liver sinusoidal endothelial cells and decreased circulation times were observed with increasing amounts of ligands per brush, which was attributed to the recognition by the Fc receptor. Conversely, low amounts of anti-DEC205 were efficient for targeting the cells of the lymphoid organs bypassing liver accumulation, making peptobrushes a suitable platform for systemic cancer vaccination strategies.¹³⁶ Aiming to improve nuclear imaging and radionucleotide therapy, Stéen *et al.* designed *trans*-cyclooctene (TCO) functionalized peptobrushes based on pGlu backbones as targeting agents for bio-orthogonal *in vivo* click chemistry.³⁰¹ For diagnosis, the TCO-peptobrush is administered first, whereby the small size (≈ 10 nm) slightly above the value for renal filtration ensures optimal tumor tissue penetration.^{116,302} After 22 to 72 h, when non-accumulated peptobrushes were excreted, a tetrazine-functionalized radiolabeled probe with a short circulation half-life was injected. Consequently, the radioactive probe is only retained at the location of the peptobrush after the successful ultra-fast click reaction between tetrazine and TCO.³⁰³ The seminal pre-targeting approach thus decouples the tumor accumulation of the radiolabeled probe from the imaging step leading to enhanced contrast and reduced radiation exposure. Herein, the microstructure of

the TCO-modified graft copolymers encounters a specific function since the microphase separation of the TCO-modified amino acids improves TCO stability and leads to enhanced rate constants. Accordingly, the peptobrush-assisted TCO/tetrazine click reaction is among the fastest bio-orthogonal ligation techniques directing toward new drug release strategies (click-to-release) for tissue-selective drug release by functional nanomedicines.³⁰³

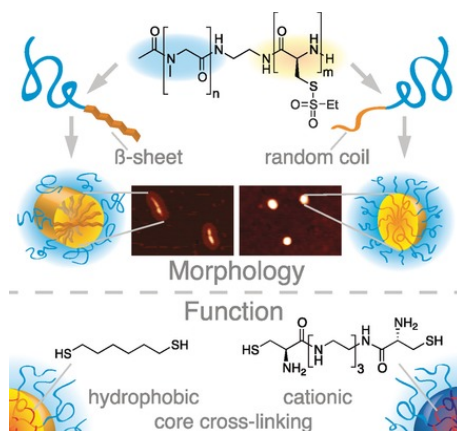


Figure 8. Synthesis of CCPMs and nanohydrogels from thiol-reactive polypeptide(s) using secondary structure formation as a guiding element for self-assembly. Reprinted from Klinker *et al.* with permission from John Wiley and Sons (© 2017).⁸²

Core cross-linked polymeric micelles can be considered as hybrids of self-assembled and molecular nanocarriers. In fact, CCPMs are synthesized from PMs generated by self-assembly, yet the cross-linking reaction leads to a stabilized single-molecule entity.^{46,183} Moreover, a large variety of hydrophobic cargos can be encapsulated and attached to the micellar core, while the release profile can be tuned by stimuli-responsive covalent bonds. To explore the full potential of disulfides as dynamic covalent bonds, Barz and co-workers previously developed reactive protecting groups for thiol-bearing amino acids, e.g., cysteine and homocysteine (Hcy).^{193–195,304,305} As reported by Schäfer *et al.*, *S*-ethylsulfonyl-L-cysteine NCA could be successfully polymerized using nucleophilic amine initiators (hard nucleophiles), and the *S*-ethylsulfonyl group was separately addressed by soft nucleophiles, e.g., thiols yielding disulfides.^{195,197,306} Following up on this, Klinker *et al.* synthesized block copolypeptide(s) of pSar-*block*-poly(*S*-ethylsulfonyl-L-cysteine) (pSar-*b*-pCys(SO₂Et)).⁸² The secondary structure formation of pCys(SO₂Et) was thereby used as a tool to govern the self-assembly

and morphology of PMs, whereby the core polarity could be tuned by hydrophilic or hydrophobic cross-linkers leading to CCPMs or cationic nanohydrogels as the extreme cases (Figure 8). Continuing the pioneering work, secondary structure formation will be further explored as a guiding element, and strategies for drug conjugation and robust production of CCPMs will be developed in this thesis. Furthermore, polymeric architectures that allow for stimuli-responsive cross-linking by pro-drugs based on platinum- or ruthenium-complexes will be designed and evaluated for their potential to overcome drug resistance mechanisms. Moreover, by combining iron oxide nanoparticles with disulfide cross-linked CCPMs, specific delivery of iron to macrophages will be facilitated and the implications for macrophage activation and immunomodulation will be investigated.

Thesis Outline

Envisioning to advance the next generation of nanomedicines this thesis aims to improve core cross-linked polymeric micelles as stimuli-responsive carrier systems for small molecule drugs and co-factors. The emerging potential of polypept(o)ides will be exploited and expanded to facilitate polymer synthesis, understand the relation of secondary structure formation on block copolymer self-assembly, implement disease-related and external stimuli for distinct release profiles, and ensure robust and scalable production of CCPMs. Functional core architectures will be designed by combining polymer science with organic and inorganic chemistry, connecting therapeutic cargo and fine-tuned carriers by dynamic covalent bonds. The discoveries of this thesis may contribute to establishing novel therapeutic approaches to improve patient compliance.

The rationale for nanomedicine and polypept(o)ides as a material class will be introduced in **chapter 1**. Herein, the basic requirements for drug delivery by nanomedicine and the characteristics of CCPMs will be reviewed. Moreover, NCA polymerization and the biomedical application of polypept(o)ides will be discussed.

In **chapter 2**, the synthesis and polymerization of racemic *S*-ethylsulfonyl-DL-cysteine NCA will be investigated to facilitate the production of thiol-reactive copolymers. The reduced tendency for anti-parallel β -sheet formation grants access to higher chain lengths with narrow molecular weight distributions. Increased rate constants and full monomer conversion further enable the synthesis of triblock copolymers (pGlu(OBn)-*b*-p(DL)Cys(SO₂Et)-*b*-pSar) by sequential monomer addition inaccessible *via* the previously established enantiopure L-cysteine analog.

The influence of the secondary structure on the self-assembly of thiol-reactive polypept(o)ides will be discussed in **chapter 3**. Block copolymers of β -sheet-forming enantiopure pSar-*b*-p(L)Cys(SO₂Et), racemic pSar-*b*-p(DL)Cys(SO₂Et), and α -helical pSar-*b*-p(L)Hcy(SO₂Et) will be prepared. The tendency for aggregation will be investigated by dynamic light scattering (DLS) during solvent switch considering various chain lengths of the hydrophobic segment. The significance of α -helix, anti-parallel β -sheet, and racemic β -sheet will be connected to the morphology of the CCPMs by applying TEM and atomic force microscopy (AFM).

For CCPMs based on pSar-*b*-p(L)Cys(SO₂Et), the influence of the core cross-linking on nanocarrier stability will be evaluated in **chapter 4**. Copolypept(o)ides with a short and a long cross-linkable p(L)Cys(SO₂Et) segment will be combined with mono-, bi-, or tri-functional thiol reagents leading to varied cross-linking densities. The cross-linked and non-cross-linked PMs will be subjected to detailed analysis by asymmetrical flow field-flow fractionation (AF4) and fluorescence correlation spectroscopy (FCS) in phosphate-buffered saline (PBS) or human blood plasma. Distinct structure-activity relationships will be examined and related to the circulation half-life and biodistribution of fluorescently labeled CCPMs after intravenous administration to mice.

Within **chapter 5**, the synthetic strategy for CCPMs based on pSar-*b*-p(L)Cys(SO₂Et) will be adjusted for scale-up by a continuous flow process. An optimized setup will be presented that allows for particle synthesis *via* micromixers and online purification by tangential flow filtration. Stimuli-responsive conjugation of paclitaxel (PTX) pro-drugs will be performed by a decoupled drug loading procedure. The prepared PTX-loaded CCPMs will be characterized and investigated for their therapeutic potential in cell culture and zebrafish larvae compared to state-of-the-art treatment by nanoparticle albumin-bound PTX.

Light as an external trigger for drug release of CCPMs will be discussed in **chapter 6**. Polypept(o)ides based on pSar-*b*-pGlu will be combined with polypyridyl ruthenium(II) complexes resembling cytotoxic cisplatin yet granting access to photoinduced ligand exchange reactions. The side chain of pGlu will be functionalized with aromatic nitrile moieties by post-polymerization modification reaction followed by core cross-linking with bifunctional ruthenium(II) complexes. The influence of the nitrile linker on nanoparticle morphology will be evaluated by AFM and TEM, and the practical application will be assessed in cell culture and by analysis in the *in ovo* model.

The significance of drug-resistance mechanisms will be covered in **chapter 7**, whereby polypept(o)ides are applied to overcome cisplatin resistance. Differential expression of the ion channel LRRC8A will be correlated to the survival of head and neck cancer patients under cisplatin therapy. Cisplatin-resistant head and neck cancer cells lacking LRRC8A mediating drug uptake will be generated and sequenced. Polypept(o)ides of pSar-*b*-pGlu(ONa) will be synthesized to reversibly conjugate cisplatin *via* the carboxyl group leading to small-sized polymeric micelles (NP_{Cis}) with narrow polydispersity. The colloidal particle stability and

the potential to bypass LRRC8A-induced drug resistance will be evaluated in zebrafish embryos and cell culture.

In **chapter 8**, the implications of the specific delivery of iron to macrophages will be investigated. Iron oxide nanoparticles (SPIONs) will be combined with CCPMs of pSar-*b*-p(L)Cys(SO₂Et) and connected by surface modification with lipoic acid, simultaneously cross-linking the micellar core (SPION-CCPMs). Applied to primary murine and human macrophages, the substantiated inflammatory responses are evaluated by flow cytometry and quantitative polymerase chain reaction (qPCR) analysis. Further, the results will be correlated to activation of alveolar or interstitial macrophages after intratracheal administration of SPION-CCPMs to mice, directing toward a new class of therapeutic agents for immunomodulation.

The results of this thesis will be briefly summarized and discussed in **chapter 9**, and the relevance and consequences for current and future research will be outlined.

References

- (1) Bertrand, N.; Leroux, J. C. The Journey of a Drug-Carrier in the Body: An Anatomico-Physiological Perspective. *J. Control. Release* **2012**, *161* (2), 152–163.
- (2) Duncan, R. Polymer Therapeutics: Top 10 Selling Pharmaceuticals - What Next? *J. Control. Release* **2014**, *190*, 371–380.
- (3) Kircher, M. F. How Can We Apply the Use of Surface-Enhanced Raman Scattering Nanoparticles in Tumor Imaging? *Nanomedicine* **2017**, *12* (3), 171–174.
- (4) Barz, M.; Luxenhofer, R.; Schillmeier, M. Quo Vadis Nanomedicine? *Nanomedicine* **2015**, *10* (20), 3089–3091.
- (5) Sindhvani, S.; Chan, W. C. W. Nanotechnology for Modern Medicine: Next Step towards Clinical Translation. *J. Intern. Med.* **2021**, *290* (3), 486–498.
- (6) Arranja, A. G.; Pathak, V.; Lammers, T.; Shi, Y. Tumor-Targeted Nanomedicines for Cancer Theranostics. *Pharmacol. Res.* **2017**, *115*, 87–95.
- (7) Duncan, R. Polymer Therapeutics as Nanomedicines: New Perspectives. *Curr. Opin. Biotechnol.* **2011**, *22* (4), 492–501.
- (8) Vert, M.; Doi, Y.; Hellwich, K.-H.; Hess, M.; Hodge, P.; Kubisa, P.; Rinaudo, M.; Schué, F. Terminology for Biorelated Polymers and Applications (IUPAC Recommendations 2012). *Pure Appl. Chem.* **2012**, *84* (2), 377–410.
- (9) Bisso, S.; Leroux, J. C. Nanopharmaceuticals: A Focus on Their Clinical Translatability. *Int. J. Pharm.* **2020**, *578*.
- (10) Soares, S.; Sousa, J.; Pais, A.; Vitorino, C. Nanomedicine: Principles, Properties, and Regulatory Issues. *Front. Chem.* **2018**, *6*, 1–15.
- (11) Lammers, T.; Ferrari, M. The Success of Nanomedicine. *Nano Today* **2020**, *31*, 0–1.
- (12) Ma, W. W.; Hidalgo, M. The Winning Formulation: The Development of Paclitaxel in Pancreatic Cancer. *Clin. Cancer Res.* **2013**, *19* (20), 5572–5579.
- (13) Barenholz, Y. Doxil® - The First FDA-Approved Nano-Drug: Lessons Learned. *J. Control. Release* **2012**, *160* (2), 117–134.
- (14) Markovsky, E.; Baabur-Cohen, H.; Eldar-Boock, A.; Omer, L.; Tiram, G.; Ferber, S.; Ofek, P.; Polyak, D.; Scomparin, A.; Satchi-Fainaro, R. Administration, Distribution, Metabolism and Elimination of Polymer Therapeutics. *J. Control. Release* **2012**, *161* (2), 446–460.
- (15) Hashida, M. Role of Pharmacokinetic Consideration for the Development of Drug Delivery Systems: A Historical Overview. *Adv. Drug Deliv. Rev.* **2020**, *157*, 71–82.
- (16) Mi, P.; Miyata, K.; Kataoka, K.; Cabral, H. Clinical Translation of Self-Assembled Cancer Nanomedicines. *Adv. Ther.* **2021**, *4* (1), 2000159.
- (17) Blanco, E.; Shen, H.; Ferrari, M. Principles of Nanoparticle Design for Overcoming Biological Barriers to Drug Delivery. *Nat. Biotechnol.* **2015**, *33* (9), 941–951.
- (18) Quader, S.; Kataoka, K. Nanomaterial-Enabled Cancer Therapy. *Mol. Ther.* **2017**, *25* (7), 1501–1513.
- (19) Petros, R. A.; DeSimone, J. M. Strategies in the Design of Nanoparticles for Therapeutic Applications. *Nat. Rev. Drug Discov.* **2010**, *9* (8), 615–627.
- (20) Polack, F. P.; Thomas, S. J.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J. L.; Pérez Marc, G.; Moreira, E. D.; Zerbini, C.; Bailey, R.; Swanson, K. A.;

-
- Roychoudhury, S.; Koury, K.; Li, P.; Kalina, W. V.; Cooper, D.; Frenck, R. W.; Hammitt, L. L.; Türeci, Ö.; Nell, H.; Schaefer, A.; Ünal, S.; Tresnan, D. B.; Mather, S.; Dormitzer, P. R.; Şahin, U.; Jansen, K. U.; Gruber, W. C. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* **2020**, *383* (27), 2603–2615.
- (21) Schoenmaker, L.; Witzigmann, D.; Kulkarni, J. A.; Verbeke, R.; Kersten, G.; Jiskoot, W.; Crommelin, D. J. A. mRNA-Lipid Nanoparticle COVID-19 Vaccines: Structure and Stability. *Int. J. Pharm.* **2021**, *601* (April), 120586.
 - (22) Delehedde, C.; Even, L.; Midoux, P.; Pichon, C.; Perche, F. Intracellular Routing and Recognition of Lipid-Based mRNA Nanoparticles. *Pharmaceutics* **2021**, *13* (7), 945.
 - (23) Baden, L. R.; El Sahly, H. M.; Essink, B.; Kotloff, K.; Frey, S.; Novak, R.; Diemert, D.; Spector, S. A.; Rouphael, N.; Creech, C. B.; McGettigan, J.; Khetan, S.; Segall, N.; Solis, J.; Brosz, A.; Fierro, C.; Schwartz, H.; Neuzil, K.; Corey, L.; Gilbert, P.; Janes, H.; Follmann, D.; Marovich, M.; Mascola, J.; Polakowski, L.; Ledgerwood, J.; Graham, B. S.; Bennett, H.; Pajon, R.; Knightly, C.; Leav, B.; Deng, W.; Zhou, H.; Han, S.; Ivarsson, M.; Miller, J.; Zaks, T. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N. Engl. J. Med.* **2021**, *384* (5), 403–416.
 - (24) Cabral, H.; Uchida, S.; Perche, F.; Pichon, C. Nanomedicine-Based Approaches for mRNA Delivery. *Mol. Pharm.* **2020**, *17* (10), 3654–3684.
 - (25) Gómez-Aguado, I.; Rodríguez-Castejón, J.; Vicente-Pascual, M.; Rodríguez-Gascón, A.; Solinís, M. Á.; Del Pozo-Rodríguez, A. Nanomedicines to Deliver mRNA: State of the Art and Future Perspectives. *Nanomaterials* **2020**, *10* (2).
 - (26) Chaudhary, N.; Weissman, D.; Whitehead, K. A. mRNA Vaccines for Infectious Diseases: Principles, Delivery and Clinical Translation. *Nat. Rev. Drug Discov.* **2021**, *0123456789*.
 - (27) Lammers, T.; Kiessling, F.; Hennink, W. E.; Storm, G. Drug Targeting to Tumors: Principles, Pitfalls and (Pre-) Clinical Progress. *J. Control. Release* **2012**, *161* (2), 175–187.
 - (28) Van Eerden, R. A. G.; Mathijssen, R. H. J.; Koolen, S. L. W. Recent Clinical Developments of Nanomediated Drug Delivery Systems of Taxanes for the Treatment of Cancer. *Int. J. Nanomedicine* **2020**, *15*, 8151–8166.
 - (29) Kataoka, K.; Harada, A.; Nagasaki, Y. Block Copolymer Micelles for Drug Delivery: Design, Characterization and Biological Significance. *Adv. Drug Deliv. Rev.* **2001**, *47* (1), 113–131.
 - (30) Ho, K. S.; Shoichet, M. S. Design Considerations of Polymeric Nanoparticle Micelles for Chemotherapeutic Delivery. *Curr. Opin. Chem. Eng.* **2013**, *2* (1), 53–59.
 - (31) Anchordoquy, T. J.; Barenholz, Y.; Boraschi, D.; Chorny, M.; Decuzzi, P.; Dobrovolskaia, M. A.; Farhangrazi, Z. S.; Farrell, D.; Gabizon, A.; Ghandehari, H.; Godin, B.; La-Beck, N. M.; Ljubimova, J.; Moghimi, S. M.; Pagliaro, L.; Park, J. H.; Peer, D.; Ruoslahti, E.; Serkova, N. J.; Simberg, D. Mechanisms and Barriers in Cancer Nanomedicine: Addressing Challenges, Looking for Solutions. *ACS Nano* **2017**, *11* (1), 12–18.
 - (32) Ma, P.; Mumper, R. J. Paclitaxel Nano-Delivery Systems: A Comprehensive Review. *J. Nanomed. Nanotechnol.* **2013**, *4* (2), 1000164.
 - (33) Montero, A.; Fossella, F.; Hortobagyi, G.; Valero, V. Docetaxel for Treatment of Solid
-

- Tumours: A Systematic Review of Clinical Data. *Lancet Oncol.* **2005**, 6 (4), 229–239.
- (34) Gelderblom, H.; Verweij, J.; Nooter, K.; Sparreboom, A. Cremophor EL. *Eur. J. Cancer* **2001**, 37 (13), 1590–1598.
- (35) Sparreboom, A.; Van Teilingen, O.; Nooijen, W. J.; Beijnen, J. H. Nonlinear Pharmacokinetics of Paclitaxel in Mice Results from the Pharmaceutical Vehicle Cremophor EL. *Cancer Res.* **1996**, 56 (9), 2112–2115.
- (36) Gonzalez-Valdivieso, J.; Girotti, A.; Schneider, J.; Arias, F. J. Advanced Nanomedicine and Cancer: Challenges and Opportunities in Clinical Translation. *Int. J. Pharm.* **2021**, 599 (March), 120438.
- (37) Talelli, M.; Rijcken, C. J. F.; Hennink, W. E.; Lammers, T. Polymeric Micelles for Cancer Therapy: 3 C's to Enhance Efficacy. *Curr. Opin. Solid State Mater. Sci.* **2012**, 16 (6), 302–309.
- (38) Luxenhofer, R. A Tutorial Translation of the Description of the Historically First Polymer Drug Conjugate and Its in Vivo Evaluation. *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* **2020**, 75 (910), 303–311.
- (39) Jatzkewitz, H. An Ein Kolloidales Blutplasma-Ersatzmittel (Polyvinylpyrrolidon) Gebundenes Peptamin (Glycyl-L-Leucyl-Mezcalin) Als Neuartige Depotform Für Biologisch Aktive Primäre Amine (Mezcalin). *Zeitschrift fur Naturforsch. - Sect. B J. Chem. Sci.* **1955**, 10 (1), 27–31.
- (40) Jatzkewitz, H. Über Den Einbau Physiologisch Wirksamer Substanzen in Ein Kolloidales Blutplasma-Ersatzmittel. *Hoppe Seyler's Zeitschrift für Physiol. Chemie* **1954**, 297, 149–156.
- (41) Duncan, R. The Dawning Era of Polymer Therapeutics. *Nat. Rev. Drug Discov.* **2003**, 2 (5), 347–360.
- (42) Ringsdorf, H. Structure and Properties of Pharmacologically Active Polymers. *J Polym Sci Polym Symp* **1975**, 153 (51), 135–153.
- (43) Talelli, M.; Barz, M.; Rijcken, C. J. F. F.; Kiessling, F.; Hennink, W. E.; Lammers, T. Core-Crosslinked Polymeric Micelles: Principles, Preparation, Biomedical Applications and Clinical Translation. *Nano Today* **2015**, 10 (1), 93–117.
- (44) Anselmo, A. C.; Mitragotri, S. Nanoparticles in the Clinic: An Update. *Bioeng. Transl. Med.* **2019**, 4 (3), 1–16.
- (45) Mitchell, M. J.; Billingsley, M. M.; Haley, R. M.; Wechsler, M. E.; Peppas, N. A.; Langer, R. Engineering Precision Nanoparticles for Drug Delivery. *Nat. Rev. Drug Discov.* **2021**, 20 (2), 101–124.
- (46) Talelli, M.; Barz, M.; Rijcken, C. J. F. F.; Kiessling, F.; Hennink, W. E.; Lammers, T. Core-Crosslinked Polymeric Micelles: Principles, Preparation, Biomedical Applications and Clinical Translation. *Nano Today* **2015**, 10 (1), 93–117.
- (47) Müllner, M. Molecular Polymer Brushes in Nanomedicine. *Macromol. Chem. Phys.* **2016**, 217 (20), 2209–2222.
- (48) Johnson, B. K.; Prud'homme, R. K. Mechanism for Rapid Self-Assembly of Block Copolymer Nanoparticles. *Phys. Rev. Lett.* **2003**, 91 (11), 1–4.
- (49) Mai, Y.; Eisenberg, A. Self-Assembly of Block Copolymers. *Chem. Soc. Rev.* **2012**, 41 (18), 5969–5985.
- (50) Allen, T. M.; Cullis, P. R. Liposomal Drug Delivery Systems: From Concept to

-
- Clinical Applications. *Adv. Drug Deliv. Rev.* **2013**, *65* (1), 36–48.
- (51) Vonarbourg, A.; Passirani, C.; Saulnier, P.; Benoit, J. P. Parameters Influencing the Stealthiness of Colloidal Drug Delivery Systems. *Biomaterials* **2006**, *27* (24), 4356–4373.
- (52) Cabral, H.; Miyata, K.; Osada, K.; Kataoka, K. Block Copolymer Micelles in Nanomedicine Applications. *Chem. Rev.* **2018**, *118* (14), 6844–6892.
- (53) Davis, F. F. The Origin of Pegnology. *Adv. Drug Deliv. Rev.* **2002**, *54* (4), 457–458.
- (54) Graham, M. L. Pegaspargase: A Review of Clinical Studies. *Adv. Drug Deliv. Rev.* **2003**, *55* (10), 1293–1302.
- (55) Bangham, A. D.; Horne, R. W. Negative Staining of Phospholipids and Their Structural Modification by Surface-Active Agents as Observed in the Electron Microscope. *J. Mol. Biol.* **1964**, *8* (5), 660–IN10.
- (56) Discher, D. E.; Eisenberg, A. Polymer Vesicles. *Science* **2002**, *297* (5583), 967–973.
- (57) Discher, D. E.; Ahmed, F. Polymersomes. *Annu. Rev. Biomed. Eng.* **2006**, *8* (1), 323–341.
- (58) Bleul, R.; Thiermann, R.; Maskos, M. Techniques to Control Polymersome Size. *Macromolecules* **2015**, *48* (20), 7396–7409.
- (59) Heller, P.; Zhou, J.; Weber, B.; Hobernik, D.; Bros, M.; Schmid, F.; Barz, M. The Influence of Block Ionomer Microstructure on Polyplex Properties: Can Simulations Help to Understand Differences in Transfection Efficiency? *Small* **2017**, *13* (17), 1603694.
- (60) Heller, P.; Birke, A.; Huesmann, D.; Weber, B.; Fischer, K.; Reske-Kunz, A.; Bros, M.; Barz, M. Introducing PeptoPlexes: Polylysine-Block-Polysarcosine Based Polyplexes for Transfection of HEK 293T Cells. *Macromol. Biosci.* **2014**, *14* (10), 1380–1395.
- (61) Soenen, S. J.; Parak, W. J.; Rejman, J.; Manshian, B. (Intra)Cellular Stability of Inorganic Nanoparticles: Effects on Cytotoxicity, Particle Functionality, and Biomedical Applications. *Chem. Rev.* **2015**, *115* (5), 2109–2135.
- (62) Zimpel, A.; Al Dana, N.; Steinborn, B.; Kuhn, J.; Höhn, M.; Bauer, T.; Hirschle, P.; Schrimpf, W.; Engelke, H.; Wagner, E.; Barz, M.; Lamb, D. C.; Lächelt, U.; Wuttke, S. Coordinative Binding of Polymers to Metal-Organic Framework Nanoparticles for Control of Interactions at the Biointerface. *ACS Nano* **2019**, *13* (4).
- (63) Cai, W.; Chu, C. C.; Liu, G.; Wang, Y. X. J. Metal-Organic Framework-Based Nanomedicine Platforms for Drug Delivery and Molecular Imaging. *Small* **2015**, *11* (37), 4806–4822.
- (64) He, C.; Liu, D.; Lin, W. Nanomedicine Applications of Hybrid Nanomaterials Built from Metal-Ligand Coordination Bonds: Nanoscale Metal-Organic Frameworks and Nanoscale Coordination Polymers. *Chem. Rev.* **2015**, *115* (19), 11079–11108.
- (65) Dadfar, S. M.; Roemhild, K.; Drude, N. I.; von Stillfried, S.; Knüchel, R.; Kiessling, F.; Lammers, T. Iron Oxide Nanoparticles: Diagnostic, Therapeutic and Theranostic Applications. *Adv. Drug Deliv. Rev.* **2019**, *138*, 302–325.
- (66) Soetaert, F.; Korangath, P.; Serantes, D.; Fiering, S.; Ivkov, R. Cancer Therapy with Iron Oxide Nanoparticles: Agents of Thermal and Immune Therapies. *Adv. Drug Deliv. Rev.* **2020**, *163–164*, 65–83.
-

-
- (67) Kowalczyk, M.; Banach, M.; Rysz, J. Ferumoxytol: A New Era of Iron Deficiency Anemia Treatment for Patients with Chronic Kidney Disease. *J. Nephrol.* **2011**, *24* (6), 717–722.
- (68) Bhandari, S.; Pereira, D.; Chappell, H.; Drakesmith, H. Intravenous Irons: From Basic Science to Clinical Practice. *Pharmaceuticals* **2018**, *11* (3), 82.
- (69) Weissleder, R.; Stark, D.; Engelstad, B.; Bacon, B.; Compton, C.; White, D.; Jacobs, P.; Lewis, J. Superparamagnetic Iron Oxide: Pharmacokinetics and Toxicity. *Am. J. Roentgenol.* **1989**, *152* (1), 167–173.
- (70) Lee, N.; Yoo, D.; Ling, D.; Cho, M. H.; Hyeon, T.; Cheon, J. Iron Oxide Based Nanoparticles for Multimodal Imaging and Magnetoresponse Therapy. *Chem. Rev.* **2015**, *115* (19), 10637–10689.
- (71) Ouyang, B.; Poon, W.; Zhang, Y. N.; Lin, Z. P.; Kingston, B. R.; Tavares, A. J.; Zhang, Y.; Chen, J.; Valic, M. S.; Syed, A. M.; MacMillan, P.; Couture-Sénécal, J.; Zheng, G.; Chan, W. C. W. The Dose Threshold for Nanoparticle Tumour Delivery. *Nat. Mater.* **2020**, *19* (12), 1362–1371.
- (72) Sindhwani, S.; Syed, A. M.; Ngai, J.; Kingston, B. R.; Maiorino, L.; Rothschild, J.; MacMillan, P.; Zhang, Y.; Rajesh, N. U.; Hoang, T.; Wu, J. L. Y.; Wilhelm, S.; Zilman, A.; Gadde, S.; Sulaiman, A.; Ouyang, B.; Lin, Z.; Wang, L.; Egeblad, M.; Chan, W. C. W. The Entry of Nanoparticles into Solid Tumours. *Nat. Mater.* **2020**, *19* (5), 566–575.
- (73) Kingston, B. R.; Lin, Z. P.; Ouyang, B.; MacMillan, P.; Ngai, J.; Syed, A. M.; Sindhwani, S.; Chan, W. C. W. Specific Endothelial Cells Govern Nanoparticle Entry into Solid Tumors. *ACS Nano* **2021**, *15*, 14080–14094.
- (74) Harmsen, S.; Wall, M. A.; Huang, R.; Kircher, M. F. Cancer Imaging Using Surface-Enhanced Resonance Raman Scattering Nanoparticles. *Nat. Protoc.* **2017**, *12* (7), 1400–1414.
- (75) Wall, M. A.; Harmsen, S.; Pal, S.; Zhang, L.; Arianna, G.; Lombardi, J. R.; Drain, C. M.; Kircher, M. F. Surfactant-Free Shape Control of Gold Nanoparticles Enabled by Unified Theoretical Framework of Nanocrystal Synthesis. *Adv. Mater.* **2017**, *29* (21).
- (76) Varela-Moreira, A.; Shi, Y.; Fens, M. H. A. M.; Lammers, T.; Hennink, W. E.; Schiffelers, R. M. Clinical Application of Polymeric Micelles for the Treatment of Cancer. *Mater. Chem. Front.* **2017**, *1* (8), 1485–1501.
- (77) Cabral, H.; Kataoka, K. Progress of Drug-Loaded Polymeric Micelles into Clinical Studies. *J. Control. Release* **2014**, *190*, 465–476.
- (78) Irvine, D. J.; Dane, E. L. Enhancing Cancer Immunotherapy with Nanomedicine. *Nat. Rev. Immunol.* **2020**, *20* (5), 321–334.
- (79) McLaughlin, C. K.; Logie, J.; Shoichet, M. S. Core and Corona Modifications for the Design of Polymeric Micelle Drug-Delivery Systems. *Isr. J. Chem.* **2013**, *53* (9–10), 670–679.
- (80) Logie, J.; Ganesh, A. N.; Aman, A. M.; Al-awar, R. S.; Shoichet, M. S. Preclinical Evaluation of Taxane-Binding Peptide-Modified Polymeric Micelles Loaded with Docetaxel in an Orthotopic Breast Cancer Mouse Model. *Biomaterials* **2017**, *123*, 39–47.
- (81) Ulrich, S. Growing Prospects of Dynamic Covalent Chemistry in Delivery Applications. *Acc. Chem. Res.* **2019**, *52*, 510–519.
-

-
- (82) Klinker, K.; Schäfer, O.; Huesmann, D.; Bauer, T.; Capelôa, L.; Braun, L.; Stergiou, N.; Schinnerer, M.; Dirisala, A.; Miyata, K.; Osada, K.; Cabral, H.; Kataoka, K.; Barz, M. Secondary-Structure-Driven Self-Assembly of Reactive Polypept(o)Ides: Controlling Size, Shape, and Function of Core Cross-Linked Nanostructures. *Angew. Chemie Int. Ed.* **2017**, *56* (32), 9608–9613.
- (83) He, Z.; Wan, X.; Schulz, A.; Bludau, H.; Dobrovolskaia, M. A.; Stern, S. T.; Montgomery, S. A.; Yuan, H.; Li, Z.; Alakhova, D.; Sokolsky, M.; Darr, D. B.; Perou, C. M.; Jordan, R.; Luxenhofer, R.; Kabanov, A. V. A High Capacity Polymeric Micelle of Paclitaxel: Implication of High Dose Drug Therapy to Safety and in Vivo Anti-Cancer Activity. *Biomaterials* **2016**, *101*, 296–309.
- (84) Grüne, M.; Luxenhofer, R.; Iuga, D.; Brown, S. P.; Pöppler, A. C. 14N-1H HMQC Solid-State NMR as a Powerful Tool to Study Amorphous Formulations-an Exemplary Study of Paclitaxel Loaded Polymer Micelles. *J. Mater. Chem. B* **2020**, *8* (31), 6827–6836.
- (85) Cabral, H.; Matsumoto, Y.; Mizuno, K.; Chen, Q.; Murakami, M.; Kimura, M.; Terada, Y.; Kano, M. R.; Miyazono, K.; Uesaka, M.; Nishiyama, N.; Kataoka, K. Accumulation of Sub-100 Nm Polymeric Micelles in Poorly Permeable Tumours Depends on Size. *Nat. Nanotechnol.* **2011**, *6* (12), 815–823.
- (86) Weissig, V.; Whiteman, K. R.; Torchilin, V. P. Accumulation of Protein-Loaded Long-Circulating Micelles and Liposomes in Subcutaneous Lewis Lung Carcinoma in Mice. *Pharmaceutical Research*. 1998, 1552–1556.
- (87) Behzadi, S.; Serpooshan, V.; Tao, W.; Hamaly, M. A.; Alkawareek, M. Y.; Dreaden, E. C.; Brown, D.; Alkilany, A. M.; Farokhzad, O. C.; Mahmoudi, M. Cellular Uptake of Nanoparticles: Journey inside the Cell. *Chem. Soc. Rev.* **2017**, *46* (14), 4218–4244.
- (88) de Lázaro, I.; Mooney, D. J. Obstacles and Opportunities in a Forward Vision for Cancer Nanomedicine. *Nat. Mater.* **2021**, *20*, 1469–1479.
- (89) Anselmo, A. C.; Mitragotri, S. Nanoparticles in the Clinic. *Bioeng. Transl. Med.* **2016**, *1* (1), 10–29.
- (90) Ragelle, H.; Danhier, F.; Préat, V.; Langer, R.; Anderson, D. G. Nanoparticle-Based Drug Delivery Systems: A Commercial and Regulatory Outlook as the Field Matures. *Expert Opin. Drug Deliv.* **2017**, *14* (7), 851–864.
- (91) Duncan, R.; Gaspar, R. Nanomedicine(s) under the Microscope. *Mol. Pharm.* **2011**, *8* (6), 2101–2141.
- (92) Park, J. H.; Lee, H. K. Delivery Routes for COVID-19 Vaccines. *Vaccines* **2021**, *9* (5).
- (93) Ols, S.; Yang, L.; Thompson, E. A.; Pushparaj, P.; Tran, K.; Liang, F.; Lin, A.; Eriksson, B.; Karlsson Hedestam, G. B.; Wyatt, R. T.; Loré, K. Route of Vaccine Administration Alters Antigen Trafficking but Not Innate or Adaptive Immunity. *Cell Rep.* **2020**, *30* (12), 3964–3971.e7.
- (94) Zhang, L.; Wang, W.; Wang, S. Effect of Vaccine Administration Modality on Immunogenicity and Efficacy. *Expert Rev. Vaccines* **2015**, *14* (11), 1509–1523.
- (95) Youn, Y. S.; Bae, Y. H. Perspectives on the Past, Present, and Future of Cancer Nanomedicine. *Adv. Drug Deliv. Rev.* **2018**, *130*, 3–11.
- (96) Zhang, P.; Zhai, Y.; Cai, Y.; Zhao, Y.; Li, Y. Nanomedicine-Based Immunotherapy for the Treatment of Cancer Metastasis. *Adv. Mater.* **2019**, *31* (49), 1–18.
-

-
- (97) Shi, J.; Kantoff, P. W.; Wooster, R.; Farokhzad, O. C. Cancer Nanomedicine: Progress, Challenges and Opportunities. *Nat. Rev. Cancer* **2017**, *17* (1), 20–37.
- (98) Richtering, W.; Alberg, I.; Zentel, R. Nanoparticles in the Biological Context: Surface Morphology and Protein Corona Formation. *Small* **2020**, *16* (39), 1–8.
- (99) Docter, D.; Distler, U.; Storck, W.; Kuharev, J.; Wünsch, D.; Hahlbrock, A.; Knauer, S. K.; Tenzer, S.; Stauber, R. H. Quantitative Profiling of the Protein Coronas That Form around Nanoparticles. *Nat. Protoc.* **2014**, *9* (9), 2030–2044.
- (100) Alberg, I.; Kramer, S.; Leps, C.; Tenzer, S.; Zentel, R. Effect of Core-Crosslinking on Protein Corona Formation on Polymeric Micelles. *Macromol. Biosci.* **2021**, 2000414.
- (101) Owen, S. C.; Chan, D. P. Y.; Shoichet, M. S. Polymeric Micelle Stability. *Nano Today* **2012**, *7* (1), 53–65.
- (102) Hume, D. A. The Mononuclear Phagocyte System. *Curr. Opin. Immunol.* **2006**, *18* (1), 49–53.
- (103) Tsoi, K. M.; Macparland, S. A.; Ma, X. Z.; Spetzler, V. N.; Echeverri, J.; Ouyang, B.; Fadel, S. M.; Sykes, E. A.; Goldaracena, N.; Kathis, J. M.; Conneely, J. B.; Alman, B. A.; Selzner, M.; Ostrowski, M. A.; Adeyi, O. A.; Zilman, A.; McGilvray, I. D.; Chan, W. C. W. Mechanism of Hard-Nanomaterial Clearance by the Liver. *Nat. Mater.* **2016**, *15* (11), 1212–1221.
- (104) Chan, W. C. W. Nanomedicine 2.0. *Acc. Chem. Res.* **2017**, *50* (3), 627–632.
- (105) Fischer, H. C.; Liu, L.; Pang, K. S.; Chan, W. C. W. Pharmacokinetics of Nanoscale Quantum Dots: In Vivo Distribution, Sequestration, and Clearance in the Rat. *Adv. Funct. Mater.* **2006**, *16* (10), 1299–1305.
- (106) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. A Survey of Structure-Property Relationships of Surfaces That Resist the Adsorption of Protein. *Langmuir* **2001**, *17* (18), 5605–5620.
- (107) Klibanov, A. L.; Maruyama, K.; Torchilin, V. P.; Huang, L. Amphipathic Polyethyleneglycols Effectively Prolong the Circulation Time of Liposomes. *FEBS Lett.* **1990**, *268* (1), 235–237.
- (108) Klinker, K.; Barz, M. Polypept(o)ides: Hybrid Systems Based on Polypeptides and Polypeptoids. *Macromol. Rapid Commun.* **2015**, *36* (22), 1943–1957.
- (109) Bleher, S.; Buck, J.; Muhl, C.; Sieber, S.; Barnert, S.; Witzigmann, D.; Huwyler, J.; Barz, M.; Süss, R. Poly(Sarcosine) Surface Modification Imparts Stealth-Like Properties to Liposomes. *Small* **2019**, *15* (50), 1–10.
- (110) Logie, J.; Owen, S. C.; McLaughlin, C. K.; Shoichet, M. S. PEG-Graft Density Controls Polymeric Nanoparticle Micelle Stability. *Chem. Mater.* **2014**, *26* (9), 2847–2855.
- (111) Carstens, Myrra G; Rijcken, C. J. F. *Multifunctional Pharmaceutical Nanocarriers*; Torchilin, V., Ed.; Fundamental Biomedical Technologies; Springer New York: New York, NY, 2008; Vol. 4.
- (112) Lasic, D. D.; Martin, F. J. *Stealth Liposomes*; Boca Raton : CRC Press, 1995.
- (113) Barz, M.; Luxenhofer, R.; Zentel, R.; Vicent, M. J. Overcoming the PEG-Addiction: Well-Defined Alternatives to PEG, from Structure–Property Relationships to Better Defined Therapeutics. *Polym. Chem.* **2011**, *2* (9), 1900–1918.
- (114) Kwon, G. S.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. Biodistribution of
-

-
- Micelle-Forming Polymer-Drug Conjugates. *Pharm. Res.* **1993**, *10* (7), 970–974.
- (115) Kwon, G.; Suwa, S.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. Enhanced Tumor Accumulation and Prolonged Circulation Times of Micelle-Forming Poly (Ethylene Oxide-Aspartate) Block Copolymer-Adriamycin Conjugates. *J. Control. Release* **1994**, *29* (1–2), 17–23.
- (116) Du, B.; Yu, M.; Zheng, J. Transport and Interactions of Nanoparticles in the Kidneys. *Nat. Rev. Mater.* **2018**, *3* (10), 358–374.
- (117) Soo Choi, H.; Liu, W.; Misra, P.; Tanaka, E.; Zimmer, J. P.; Itty Ipe, B.; Bawendi, M. G.; Frangioni, J. V. Renal Clearance of Quantum Dots. *Nat. Biotechnol.* **2007**, *25* (10), 1165–1170.
- (118) Yamaoka, T.; Tabata, Y.; Ikada, Y. Body Distribution Profile of Polysaccharides after Intravenous Administration. *Drug Deliv.* **1993**, *1* (1), 75–82.
- (119) Yamaoka, T.; Tabata, Y.; Ikada, Y. Distribution and Tissue Uptake of Poly(Ethylene Glycol) with Different Molecular Weights after Intravenous Administration to Mice. *J. Pharm. Sci.* **1994**, *83* (4), 601–606.
- (120) Reske-Nielsen, E.; Bojsen-Møller, M.; Vetner, M.; Hansen, J. C. Polyvinylpyrrolidone-Storage Disease. *Acta Pathol. Microbiol. Scand. Sect. A Pathol.* **2009**, *84A* (5), 397–405.
- (121) Maeda, H. The 35th Anniversary of the Discovery of EPR Effect: A New Wave of Nanomedicines for Tumor-Targeted Drug Delivery—Personal Remarks and Future Prospects. *J. Pers. Med.* **2021**, *11* (3), 229.
- (122) Shi, Y.; van der Meel, R.; Chen, X.; Lammers, T. The EPR Effect and beyond: Strategies to Improve Tumor Targeting and Cancer Nanomedicine Treatment Efficacy. *Theranostics* **2020**, *10* (17), 7921–7924.
- (123) Maeda, H.; Matsumoto, T.; Konno, T.; Iwai, K.; Ueda, M. Tailor-Making of Protein Drugs by Polymer Conjugation for Tumor Targeting: A Brief Review on Smancs. *J. Protein Chem.* **1984**, *3* (2), 181–193.
- (124) Matsumura, Y.; Maeda, H. A New Concept for Macromolecular Therapeutics in Cancer Chemotherapy: Mechanism of Tumoritropic Accumulation of Proteins and the Antitumor Agent Smancs. *Cancer Res.* **1986**, *46* (8), 6387–6392.
- (125) Mochida, Y.; Cabral, H.; Kataoka, K. Polymeric Micelles for Targeted Tumor Therapy of Platinum Anticancer Drugs. *Expert Opin. Drug Deliv.* **2017**, *14* (12), 1423–1438.
- (126) Wilhelm, S.; Tavares, A. J.; Dai, Q.; Ohta, S.; Audet, J.; Dvorak, H. F.; Chan, W. C. W.; Chatterley, A.; Group, W. Analysis of Nanoparticle Delivery to Tumours. *Nat. Rev. Mater.* **2016**, *1* (1).
- (127) van der Meel, R.; Lammers, T.; Hennink, W. E. Cancer Nanomedicines: Oversold or Underappreciated? *Expert Opin. Drug Deliv.* **2017**, *14* (1), 1–5.
- (128) Lammers, T.; Kiessling, F.; Ashford, M.; Hennink, W.; Crommelin, D.; Strom, G. Cancer Nanomedicine: Is Targeting Our Target? *Nat. Rev. Mater.* **2016**, *1* (9), 1–2.
- (129) Meel, R. Van Der; Sulheim, E.; Shi, Y.; Kiessling, F.; Mulder, W. J. M. Smart Cancer Nanomedicine. *Nat. Nanotechnol.* **2019**, *14* (November), 1007–1017.
- (130) Nichols, J. W.; Bae, Y. H. EPR: Evidence and Fallacy. *J. Control. Release* **2014**, *190*, 451–464.
-

-
- (131) Sun, D.; Zhou, S.; Gao, W. What Went Wrong with Anticancer Nanomedicine Design and How to Make It Right. *ACS Nano* **2020**, *14* (10), 12281–12290.
- (132) Hare, J. I.; Lammers, T.; Ashford, M. B.; Puri, S.; Storm, G.; Barry, S. T. Challenges and Strategies in Anti-Cancer Nanomedicine Development: An Industry Perspective. *Adv. Drug Deliv. Rev.* **2017**, *108*, 25–38.
- (133) Lee, H.; Shields, A. F.; Siegel, B. A.; Miller, K. D.; Krop, I.; Ma, C. X.; Lorusso, P. M.; Munster, P. N.; Campbell, K.; Gaddy, D. F.; Leonard, S. C.; Geretti, E.; Blocker, S. J.; Kirpotin, D. B.; Moyo, V.; Wickham, T. J.; Hendriks, B. S. ⁶⁴Cu-MM-302 Positron Emission Tomography Quantifies Variability of Enhanced Permeability and Retention of Nanoparticles in Relation to Treatment Response in Patients with Metastatic Breast Cancer. *Clin. Cancer Res.* **2017**, *23* (15), 4190–4202.
- (134) Li, J.; Kataoka, K. Chemo-Physical Strategies to Advance the in Vivo Functionality of Targeted Nanomedicine: The Next Generation. *J. Am. Chem. Soc.* **2021**, *143* (2), 538–559.
- (135) Alkilany, A. M.; Zhu, L.; Weller, H.; Mews, A.; Parak, W. J.; Barz, M.; Feliu, N. Ligand Density on Nanoparticles: A Parameter with Critical Impact on Nanomedicine. *Adv. Drug Deliv. Rev.* **2019**, *143*, 22–36.
- (136) Kappel, C.; Seidl, C.; Medina-Montano, C.; Schinnerer, M.; Alberg, I.; Leps, C.; Sohl, J.; Hartmann, A.-K.; Fichter, M.; Kuske, M.; Schunke, J.; Kuhn, G.; Tubbe, I.; Paßlick, D.; Hobernik, D.; Bent, R.; Haas, K.; Montermann, E.; Walzer, K.; Diken, M.; Schmidt, M.; Zentel, R.; Nuhn, L.; Schild, H.; Tenzer, S.; Mailänder, V.; Barz, M.; Bros, M.; Grabbe, S. Density of Conjugated Antibody Determines the Extent of Fc Receptor Dependent Capture of Nanoparticles by Liver Sinusoidal Endothelial Cells. *ACS Nano* **2021**.
- (137) Darguzyte, M.; Holm, R.; Baier, J.; Drude, N.; Schultze, J.; Koynov, K.; Schwiertz, D.; Dadfar, S. M.; Lammers, T.; Barz, M.; Kiessling, F. Influence of Riboflavin Targeting on Tumor Accumulation and Internalization of Peptostar Based Drug Delivery Systems. *Bioconjug. Chem.* **2020**, *31* (12), 2691–2696.
- (138) Kunjachan, S.; Pola, R.; Gremse, F.; Theek, B.; Ehling, J.; Moeckel, D.; Hermanns-Sachweh, B.; Pechar, M.; Ulbrich, K.; Hennink, W. E.; Storm, G.; Lederle, W.; Kiessling, F.; Lammers, T. Passive versus Active Tumor Targeting Using RGD- and NGR-Modified Polymeric Nanomedicines. *Nano Lett.* **2014**, *14* (2), 972–981.
- (139) Poon, W.; Kingston, B. R.; Ouyang, B.; Ngo, W.; Chan, W. C. W. A Framework for Designing Delivery Systems. *Nat. Nanotechnol.* **2020**, *15* (10), 819–829.
- (140) Sofias, A. M.; Combes, F.; Koschmieder, S.; Storm, G.; Lammers, T. A Paradigm Shift in Cancer Nanomedicine: From Traditional Tumor Targeting to Leveraging the Immune System. *Drug Discov. Today* **2021**, *00* (00).
- (141) Matsumoto, Y.; Nichols, J. W.; Toh, K.; Nomoto, T.; Cabral, H.; Miura, Y.; Christie, R. J.; Yamada, N.; Ogura, T.; Kano, M. R.; Matsumura, Y.; Nishiyama, N.; Yamasoba, T.; Bae, Y. H.; Kataoka, K. Vascular Bursts Enhance Permeability of Tumour Blood Vessels and Improve Nanoparticle Delivery. *Nat. Nanotechnol.* **2016**, *11* (6), 533–538.
- (142) Biancacci, I.; Sun, Q.; Möckel, D.; Gremse, F.; Rosenhain, S.; Kiessling, F.; Bartneck, M.; Hu, Q.; Thewissen, M.; Storm, G.; Hennink, W. E.; Shi, Y.; Rijcken, C. J. F.; Lammers, T.; Sofias, A. M. Optical Imaging of the Whole-Body to Cellular Biodistribution of Clinical-Stage PEG-b-PHPMA-Based Core-Crosslinked
-

-
- Polymeric Micelles. *J. Control. Release* **2020**, *328*, 805–816.
- (143) Cheng, Y.; Song, S.; Wu, P.; Lyu, B.; Qin, M.; Sun, Y.; Sun, A.; Mu, L.; Xu, F.; Zhang, L.; Wang, J.; Zhang, Q. Tumor Associated Macrophages and TAMs-Based Anti-Tumor Nanomedicines. *Adv. Healthc. Mater.* **2021**, *10* (18), 1–21.
- (144) Mantovani, A.; Marchesi, F.; Malesci, A.; Laghi, L.; Allavena, P. Tumour-Associated Macrophages as Treatment Targets in Oncology. *Nat. Rev. Clin. Oncol.* **2017**, *14* (7), 399–416.
- (145) Greco, F.; Vicent, M. J. Combination Therapy: Opportunities and Challenges for Polymer–Drug Conjugates as Anticancer Nanomedicines. *Adv. Drug Deliv. Rev.* **2009**, *61* (13), 1203–1213.
- (146) Tyrrell, Z. L.; Shen, Y.; Radosz, M. Fabrication of Micellar Nanoparticles for Drug Delivery through the Self-Assembly of Block Copolymers. *Prog. Polym. Sci.* **2010**, *35* (9), 1128–1143.
- (147) Whitesides, G. M.; Grzybowski, B. Self-Assembly at All Scales. *Science* **2002**, *295* (5564), 2418–2421.
- (148) Bates, F. S. Polymer-Polymer Phase Behavior. *Science* **1991**, *251* (4996), 898–905.
- (149) Israelachvili, J. N.; Mitchell, D. J.; Ninham, B. W. Theory of Self-Assembly of Hydrocarbon Amphiphiles into Micelles and Bilayers. *J. Chem. Soc. Faraday Trans. 2 Mol. Chem. Phys.* **1976**, *72*, 1525–1568.
- (150) Adams, M. L.; Lavasanifar, A.; Kwon, G. S. Amphiphilic Block Copolymers for Drug Delivery. *J. Pharm. Sci.* **2003**, *92* (7), 1343–1355.
- (151) Huesmann, D.; Sevenich, A.; Weber, B.; Barz, M. A Head-to-Head Comparison of Poly(Sarcosine) and Poly(Ethylene Glycol) in Peptidic, Amphiphilic Block Copolymers. *Polym. (United Kingdom)* **2015**, *67*, 240–248.
- (152) Schaeffel, D.; Kreyes, A.; Zhao, Y.; Landfester, K.; Butt, H.-J.; Crespy, D.; Koynov, K. Molecular Exchange Kinetics of Diblock Copolymer Micelles Monitored by Fluorescence Correlation Spectroscopy. *ACS Macro Lett.* **2014**, *3* (5), 428–432.
- (153) Lübtow, M. M.; Hahn, L.; Haider, M. S.; Luxenhofer, R. Drug Specificity, Synergy and Antagonism in Ultrahigh Capacity Poly(2-Oxazoline)/Poly(2-Oxazine) Based Formulations. *J. Am. Chem. Soc.* **2017**, *139* (32), 10980–10983.
- (154) Hara, E.; Makino, A.; Kurihara, K.; Yamamoto, F.; Ozeki, E.; Kimura, S. Pharmacokinetic Change of Nanoparticulate Formulation Lactosome on Multiple Administrations. *Int. Immunopharmacol.* **2012**, *14* (3), 261–266.
- (155) Sevast'ianov, V. I.; Tseytlina, E. A. The Activation of the Complement System by Polymer Materials and Their Blood Compatibility. *J. Biomed. Mater. Res.* **1984**, *18* (9), 969–978.
- (156) Gabizon, A.; Szebeni, J. Complement Activation: A Potential Threat on the Safety of Poly(Ethylene Glycol)-Coated Nanomedicines. *ACS Nano* **2020**, *14* (7), 7682–7688.
- (157) Zheng, X.; Xie, J.; Zhang, X.; Sun, W.; Zhao, H.; Li, Y.; Wang, C. An Overview of Polymeric Nanomicelles in Clinical Trials and on the Market. *Chinese Chem. Lett.* **2021**, *32* (1), 243–257.
- (158) Hwang, D.; Ramsey, J. D.; Kabanov, A. V. Polymeric Micelles for the Delivery of Poorly Soluble Drugs: From Nanoformulation to Clinical Approval. *Adv. Drug Deliv. Rev.* **2020**, *156*, 80–118.
-

-
- (159) Cabral, H.; Miyata, K.; Osada, K.; Kataoka, K. Block Copolymer Micelles in Nanomedicine Applications. *Chem. Rev.* **2018**, *118* (14), 6844–6892.
- (160) Hamaguchi, T.; Matsumura, Y.; Suzuki, M.; Shimizu, K.; Goda, R.; Nakamura, I.; Nakatomi, I.; Yokoyama, M.; Kataoka, K.; Kakizoe, T. NK105, a Paclitaxel-Incorporating Micellar Nanoparticle Formulation, Can Extend in Vivo Antitumour Activity and Reduce the Neurotoxicity of Paclitaxel. *Br. J. Cancer* **2005**, *92* (7), 1240–1246.
- (161) Fujiwara, Y.; Mukai, H.; Saeki, T.; Ro, J.; Lin, Y. C.; Nagai, S. E.; Lee, K. S.; Watanabe, J.; Ohtani, S.; Kim, S. B.; Kuroi, K.; Tsugawa, K.; Tokuda, Y.; Iwata, H.; Park, Y. H.; Yang, Y.; Nambu, Y. A Multi-National, Randomised, Open-Label, Parallel, Phase III Non-Inferiority Study Comparing NK105 and Paclitaxel in Metastatic or Recurrent Breast Cancer Patients. *Br. J. Cancer* **2019**, *120* (5), 475–480.
- (162) Hamaguchi, T.; Tsuji, A.; Yamaguchi, K.; Takeda, K.; Uetake, H.; Esaki, T.; Amagai, K.; Sakai, D.; Baba, H.; Kimura, M.; Matsumura, Y.; Tsukamoto, T. A Phase II Study of NK012, a Polymeric Micelle Formulation of SN-38, in Unresectable, Metastatic or Recurrent Colorectal Cancer Patients. *Cancer Chemother. Pharmacol.* **2018**, *82* (6), 1021–1029.
- (163) Von Hoff, D. D.; Mita, M. M.; Ramanathan, R. K.; Weiss, G. J.; Mita, A. C.; Lorusso, P. M.; Burris, H. A.; Hart, L. L.; Low, S. C.; Parsons, D. M.; Zale, S. E.; Summa, J. M.; Youssoufian, H.; Sachdev, J. C. Phase I Study of PSMA-Targeted Docetaxel-Containing Nanoparticle BIND-014 in Patients with Advanced Solid Tumors. *Clin. Cancer Res.* **2016**, *22* (13), 3157–3163.
- (164) Miele, E.; Spinelli, G. P.; Miele, E.; Tomao, F.; Tomao, S. Albumin-Bound Formulation of Paclitaxel (Abraxane ABI-007) in the Treatment of Breast Cancer. *Int. J. Nanomedicine* **2009**, *4*, 99–105.
- (165) Gradishar, W. J.; Tjulandin, S.; Davidson, N.; Shaw, H.; Desai, N.; Bhar, P.; Hawkins, M.; O'Shaughnessy, J. Phase III Trial of Nanoparticle Albumin-Bound Paclitaxel Compared with Polyethylated Castor Oil-Based Paclitaxel in Women with Breast Cancer. *J. Clin. Oncol.* **2005**, *23* (31), 7794–7803.
- (166) Yardley, D. A. Nab-Paclitaxel Mechanisms of Action and Delivery. *J. Control. Release* **2013**, *170* (3), 365–372.
- (167) Socinski, M. A.; Bondarenko, I.; Karaseva, N. A.; Makhson, A. M.; Vynnychenko, I.; Okamoto, I.; Hon, J. K.; Hirsh, V.; Bhar, P.; Zhang, H.; Iglesias, J. L.; Renschler, M. F. Weekly Nab-Paclitaxel in Combination with Carboplatin versus Solvent-Based Paclitaxel plus Carboplatin as First-Line Therapy in Patients with Advanced Non-Small-Cell Lung Cancer: Final Results of a Phase III Trial. *J. Clin. Oncol.* **2012**, *30* (17), 2055–2062.
- (168) Lee, H.; Park, S.; Kang, J. E.; Lee, H. M.; Kim, S. A.; Rhie, S. J. Efficacy and Safety of Nanoparticle-Albumin-Bound Paclitaxel Compared with Solvent-Based Taxanes for Metastatic Breast Cancer: A Meta-Analysis. *Sci. Rep.* **2020**, *10* (1), 1–9.
- (169) Vergote, I.; Bergfeldt, K.; Franquet, A.; Lisyanskaya, A. S.; Bjermo, H.; Heldring, N.; Buyse, M.; Brize, A. A Randomized Phase III Trial in Patients with Recurrent Platinum Sensitive Ovarian Cancer Comparing Efficacy and Safety of Paclitaxel Micellar and Cremophor EL-Paclitaxel. *Gynecol. Oncol.* **2020**, *156* (2), 293–300.
- (170) Borgå, O.; Henriksson, R.; Bjermo, H.; Lilienberg, E.; Heldring, N.; Loman, N.
-

-
- Maximum Tolerated Dose and Pharmacokinetics of Paclitaxel Micellar in Patients with Recurrent Malignant Solid Tumours: A Dose-Escalation Study. *Adv. Ther.* **2019**, *36* (5), 1150–1163.
- (171) Sill, K. N.; Sullivan, B.; Carie, A.; Edward Semple, J. Synthesis and Characterization of Micelle-Forming PEG-Poly(Amino Acid) Copolymers with Iron-Hydroxamate Cross-Linkable Blocks for Encapsulation and Release of Hydrophobic Drugs. *Biomacromolecules* **2017**, *18* (6), 1874–1884.
- (172) Shi, Y.; Van Steenberg, M. J.; Teunissen, E. A.; Novo, L.; Gradmann, S.; Baldus, M.; Van Nostrum, C. F.; Hennink, W. E. II-II Stacking Increases the Stability and Loading Capacity of Thermosensitive Polymeric Micelles for Chemotherapeutic Drugs. *Biomacromolecules* **2013**, *14* (6), 1826–1837.
- (173) Wu, G.; Li, C.; Liu, X.; Lv, J.; Ding, Y.; Liu, Y.; Liu, Y.; Huang, F.; Shi, L.; An, Y.; Ma, R. Glucose-Responsive Complex Micelles for Self-Regulated Delivery of Insulin with Effective Protection of Insulin and Enhanced Hypoglycemic Activity in Vivo. *Colloids Surfaces B Biointerfaces* **2019**, *180* (May), 376–383.
- (174) Chen, J.; Yan, B.; Wang, X.; Huang, Q.; Thundat, T.; Zeng, H. Core Cross-Linked Double Hydrophilic Block Copolymer Micelles Based on Multiple Hydrogen-Bonding Interactions. *Polym. Chem.* **2017**, *8* (20), 3066–3073.
- (175) Lin, M.; Dai, Y.; Xia, F.; Zhang, X. Advances in Non-Covalent Crosslinked Polymer Micelles for Biomedical Applications. *Mater. Sci. Eng. C* **2021**, *119*, 111626.
- (176) Ke, X.; Ng, V. W. L.; Ono, R. J.; Chan, J. M. W.; Krishnamurthy, S.; Wang, Y.; Hedrick, J. L.; Yang, Y. Y. Role of Non-Covalent and Covalent Interactions in Cargo Loading Capacity and Stability of Polymeric Micelles. *J. Control. Release* **2014**, *193*, 9–26.
- (177) Hofmann, D.; Messerschmidt, C.; Bannwarth, M. B.; Landfester, K.; Mailänder, V. Drug Delivery without Nanoparticle Uptake: Delivery by a Kiss-and-Run Mechanism on the Cell Membrane. *Chem. Commun.* **2014**, *50* (11), 1369–1371.
- (178) Li, Y.; Xiao, K.; Zhu, W.; Deng, W.; Lam, K. S. Stimuli-Responsive Cross-Linked Micelles for on-Demand Drug Delivery against Cancers. *Adv. Drug Deliv. Rev.* **2014**, *66*, 58–73.
- (179) Fan, W.; Zhang, L.; Li, Y.; Wu, H. Recent Progress of Crosslinking Strategies for Polymeric Micelles with Enhanced Drug Delivery in Cancer Therapy. *Curr. Med. Chem.* **2019**, *26* (13), 2356–2376.
- (180) O'Reilly, R. K.; Hawker, C. J.; Wooley, K. L. Cross-Linked Block Copolymer Micelles: Functional Nanostructures of Great Potential and Versatility. *Chem. Soc. Rev.* **2006**, *35* (11), 1068–1083.
- (181) Thurmond, K. B.; Huang, H.; Clark, C. G.; Kowalewski, T.; Wooley, K. L. Shell Cross-Linked Polymer Micelles: Stabilized Assemblies with Great Versatility and Potential. *Colloids Surfaces B Biointerfaces* **1999**, *16* (1–4), 45–54.
- (182) Rolland, A.; O'Mullane, J.; Goddard, P.; Brookman, L.; Petrak, K. New Macromolecular Carriers for Drugs. I. Preparation and Characterization of Poly(Oxyethylene-b-isoprene-b-oxyethylene) Block Copolymer Aggregates. *J. Appl. Polym. Sci.* **1992**, *44* (7), 1195–1203.
- (183) Van Nostrum, C. F. Covalently Cross-Linked Amphiphilic Block Copolymer Micelles. *Soft Matter* **2011**, *7* (7), 3246–3259.
-

-
- (184) Rijcken, C. J.; Snel, C. J.; Schiffelers, R. M.; van Nostrum, C. F.; Hennink, W. E. Hydrolysable Core-Crosslinked Thermosensitive Polymeric Micelles: Synthesis, Characterisation and in Vivo Studies. *Biomaterials* **2007**, *28* (36), 5581–5593.
- (185) Hu, Q.; Rijcken, C. J.; Bansal, R.; Hennink, W. E.; Storm, G.; Prakash, J. Complete Regression of Breast Tumour with a Single Dose of Docetaxel-Entrapped Core-Cross-Linked Polymeric Micelles. *Biomaterials* **2015**, *53*, 370–378.
- (186) Hu, Q.; Rijcken, C. J. F.; van Gaal, E.; Brundel, P.; Kostkova, H.; Etrych, T.; Weber, B.; Barz, M.; Kiessling, F.; Prakash, J.; Storm, G.; Hennink, W. E.; Lammers, T. Tailoring the Physicochemical Properties of Core-Crosslinked Polymeric Micelles for Pharmaceutical Applications. *J. Control. Release* **2016**, *244*, 314–325.
- (187) Atrafi, F.; Dumez, H.; Mathijssen, R. H. J.; Menke van der Houven van Oordt, C. W.; Rijcken, C. J. F.; Hanssen, R.; Eskens, F. A. L. M.; Schöffski, P. A Phase I Dose-Escalation and Pharmacokinetic Study of a Micellar Nanoparticle with Entrapped Docetaxel (CPC634) in Patients with Advanced Solid Tumours. *J. Control. Release* **2020**, *325* (March), 191–197.
- (188) Atrafi, F.; van Eerden, R. A. G.; van Hylckama Vlieg, M. A. M.; Oomen-de Hoop, E.; de Bruijn, P.; Lolkema, M. P.; Moelker, A.; Rijcken, C. J.; Hanssen, R.; Sparreboom, A.; Eskens, F. A. L. M.; Mathijssen, R. H. J.; Koolen, S. L. W. Intratumoral Comparison of Nanoparticle Entrapped Docetaxel (CPC634) with Conventional Docetaxel in Patients with Solid Tumors. *Clin. Cancer Res.* **2020**, *26* (14), 3537–3545.
- (189) Atrafi, F.; van Eerden, R. A. G.; Koolen, S. L. W.; de Bruijn, P.; Rijcken, C. J. F.; Hanssen, R.; Eskens, F. A. L. M.; Lolkema, M. P.; Oomen-de Hoop, E.; Damman, J.; Mathijssen, R. H. J. Docetaxel Skin Exposure and Micronucleation Contributes to Skin Toxicity Caused by CPC634. *Cancers (Basel)*. **2021**, *13* (15), 3741.
- (190) Sevier, C. S.; Kaiser, C. A. Formation and Transfer of Disulphide Bonds in Living Cells. *Nat. Rev. Mol. Cell Biol.* **2002**, *3* (11), 836–847.
- (191) Poole, L. B. The Basics of Thiols and Cysteines in Redox Biology and Chemistry. *Free Radic. Biol. Med.* **2015**, *80*, 148–157.
- (192) Li, L.; Li, D.; Zhang, M.; He, J.; Liu, J.; Ni, P. One-Pot Synthesis of PH/Redox Responsive Polymeric Prodrug and Fabrication of Shell Cross-Linked Prodrug Micelles for Antitumor Drug Transportation. *Bioconjug. Chem.* **2018**, *29* (8), 2806–2817.
- (193) Huesmann, D.; Schäfer, O.; Braun, L.; Klinker, K.; Reuter, T.; Barz, M. Exploring New Activating Groups for Reactive Cysteine NCAs. *Tetrahedron Lett.* **2016**, *57* (10), 1138–1142.
- (194) Schäfer, O.; Barz, M. Of Thiols and Disulfides: Methods for Chemoselective Formation of Asymmetric Disulfides in Synthetic Peptides and Polymers. *Chem. - A Eur. J.* **2018**, *24* (47), 12131–12142.
- (195) Schäfer, O.; Huesmann, D.; Muhl, C.; Barz, M. Rethinking Cysteine Protective Groups: S -Alkylsulfonyl- l -Cysteines for Chemoselective Disulfide Formation. *Chem. - A Eur. J.* **2016**, *22* (50), 18085–18091.
- (196) Huesmann, D.; Klinker, K.; Barz, M. Orthogonally Reactive Amino Acids and End Groups in NCA Polymerization. *Polym. Chem.* **2016**, *8* (6), 957–971.
- (197) Schäfer, O.; Huesmann, D.; Barz, M. Poly(S -Ethylsulfonyl- l -Cysteines) for
-

-
- Chemoselective Disulfide Formation. *Macromolecules* **2016**, *49* (21), 8146–8153.
- (198) Barz, M. Complexity and Simplification in the Development of Nanomedicines. *Nanomedicine* **2015**, *10* (20), 3093–3097.
- (199) Leroux, J. C. Drug Delivery: Too Much Complexity, Not Enough Reproducibility? *Angew. Chemie - Int. Ed.* **2017**, 2–4.
- (200) Waldman, A. D.; Fritz, J. M.; Lenardo, M. J. A Guide to Cancer Immunotherapy: From T Cell Basic Science to Clinical Practice. *Nat. Rev. Immunol.* **2020**, *20* (11), 651–668.
- (201) Bourgeois-Daigneault, M. C.; Roy, D. G.; Aitken, A. S.; El Sayes, N.; Martin, N. T.; Varette, O.; Falls, T.; St-Germain, L. E.; Pelin, A.; Lichty, B. D.; Stojdl, D. F.; Ungerechts, G.; Diallo, J. S.; Bell, J. C. Neoadjuvant Oncolytic Virotherapy before Surgery Sensitizes Triple-Negative Breast Cancer to Immune Checkpoint Therapy. *Sci. Transl. Med.* **2018**, *10* (422), 1–12.
- (202) Ho, D. Artificial Intelligence in Cancer Therapy. *Science* **2020**, *367* (6481), 982–983.
- (203) Birke, A.; Huesmann, D.; Kelsch, A.; Weilbacher, M.; Xie, J.; Bros, M.; Bopp, T.; Becker, C.; Landfester, K.; Barz, M. Polypeptoid-Block-Polypeptide Copolymers: Synthesis, Characterization, and Application of Amphiphilic Block Copolypept(o)ides in Drug Formulations and Miniemulsion Techniques. *Biomacromolecules* **2014**, *15* (2), 548–557.
- (204) Birke, A.; Ling, J.; Barz, M. Polysarcosine-Containing Copolymers: Synthesis, Characterization, Self-Assembly, and Applications. *Prog. Polym. Sci.* **2018**, *81*, 163–208.
- (205) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K. Peptoids: A Modular Approach to Drug Discovery. *Proc. Natl. Acad. Sci.* **1992**, *89* (20), 9367–9371.
- (206) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. Efficient Method for the Preparation of Peptoids [Oligo(N-Substituted Glycines)] by Submonomer Solid-Phase Synthesis. *J. Am. Chem. Soc.* **1992**, *114* (26), 10646–10647.
- (207) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, P.; Bradley, E. K.; Truong, K. T. V.; Dill, K. A.; Cohen, F. E.; Zuckermann, R. N. Sequence-Specific Polypeptoids: A Diverse Family of Heteropolymers with Stable Secondary Structure. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95* (8), 4303–4308.
- (208) Secker, C.; Brosnan, S. M.; Luxenhofer, R.; Schlaad, H. Poly(α -Peptoid)s Revisited: Synthesis, Properties, and Use as Biomaterial. *Macromol. Biosci.* **2015**, *15* (7), 881–891.
- (209) Gangloff, N.; Ulbricht, J.; Lorson, T.; Schlaad, H.; Luxenhofer, R. Peptoids and Polypeptoids at the Frontier of Supra- and Macromolecular Engineering. *Chem. Rev.* **2016**, *116* (4), 1753–1802.
- (210) Sanborn, T. J.; Wu, C. W.; Zuckermann, R. N.; Barron, A. E. Extreme Stability of Helices Formed by Water-Soluble Poly-N-Substituted Glycines (Polypeptoids) with α -Chiral Side Chains. *Biopolymers* **2002**, *63* (1), 12–20.
- (211) Mannige, R. V.; Haxton, T. K.; Proulx, C.; Robertson, E. J.; Battigelli, A.; Butterfoss, G. L.; Zuckermann, R. N.; Whitelam, S. Peptoid Nanosheets Exhibit a New Secondary-Structure Motif. *Nature* **2015**, *526* (7573), 415–420.
- (212) Weber, B.; Birke, A.; Fischer, K.; Schmidt, M.; Barz, M. Solution Properties of
-

- Polysarcosine: From Absolute and Relative Molar Mass Determinations to Complement Activation. *Macromolecules* **2018**, *51* (7), 2653–2661.
- (213) Lau, K. H. A.; Ren, C.; Sileika, T. S.; Park, S. H.; Szleifer, I.; Messersmith, P. B. Surface-Grafted Polysarcosine as a Peptoid Antifouling Polymer Brush. *Langmuir* **2012**, *28* (46), 16099–16107.
- (214) Astbury, W. T.; Dalglish, C. E.; Darmon, S. E.; Sutherland, G. B. B. M. Studies of the Structure of Synthetic Polypeptides. *Nature* **1948**, *162* (4120), 596–600.
- (215) Brosnan, M. E.; Brosnan, J. T. The Role of Dietary Creatine. *Amino Acids* **2016**, *48* (8), 1785–1791.
- (216) Snow, R. J.; Murphy, R. M. Creatine and the Creatine Transporter: A Review. *Mol. Cell. Biochem.* **2001**, *224* (1–2), 169–181.
- (217) Horner, W. H.; Mackenzie, C. G. The Biological Formation of Sarcosine. *J. Biol. Chem.* **1950**, *187* (1), 15–22.
- (218) Ogawa, H.; Gomi, T.; Takusagawa, F.; Fujioka, M. Structure, Function and Physiological Role of Glycine N-Methyltransferase. *Int. J. Biochem. Cell Biol.* **1998**, *30* (1), 13–26.
- (219) Porter, D. H.; Cook, R. J.; Wagner, C. Enzymatic Properties of Dimethylglycine Dehydrogenase and Sarcosine Dehydrogenase from Rat Liver. *Arch. Biochem. Biophys.* **1985**, *243* (2), 396–407.
- (220) Glorieux, F. H.; Scriver, C. R.; Delvin, E.; Mohyuddin, F. Transport and Metabolism of Sarcosine in Hypersarcosinemic and Normal Phenotypes. *J. Clin. Invest.* **1971**, *50* (11), 2313–2322.
- (221) Katchalski, E. Poly- α -Amino Acids. In *Advances in Protein Chemistry*; **1951**; 123–185.
- (222) Kricheldorf, H. R. Polypeptides and 100 Years of Chemistry of α -Amino Acid N-Carboxyanhydrides. *Angew. Chemie - Int. Ed.* **2006**, *45* (35), 5752–5784.
- (223) Hanby, W. E.; Waley, S. G.; Watson, J. Synthetic Polypeptides. Part I. *J. Chem. Soc.* **1950**, 3009.
- (224) Ballard, D. G. H.; Bamford, C. H. Studies in Polymerization. X. 'The Chain-Effect'. *Proc. R. Soc. London. Ser. A. Math. Phys. Sci.* **1956**, *236* (1206), 384–396.
- (225) Kricheldorf, H. R.; Schilling, G. ¹³C NMR Sequence Analysis, 15. Copolymerization of Alanine-NCA with Other α -Amino Acid NCAs. *J. Macromol. Sci.-Chem.* **1978**, *179* (11), 1175–1191.
- (226) Katchalski, E.; Sela, M. Synthesis and Chemical Properties of Poly- α -Amino Acids. *Adv. Protein Chem.* **1958**, *13* (C), 243–492.
- (227) Katchalski, E.; Berger, A.; Bichowsky-Slomnicki, L.; Kurtz, J. Antibiotically Active Amino-Acid Copolymers Related to Gramicidin S. *Nature* **1955**, *176* (4472), 118–119.
- (228) Kidchob, T.; Kimura, S.; Imanishi, Y. Preparation, Structure and Release Profile of Polypeptide Microcapsules. *J. Control. Release* **1996**, *40* (3), 285–291.
- (229) Kidchob, T.; Kimura, S.; Imanishi, Y. PH-Responsive Release from Polypeptide Microcapsules. *J. Appl. Polym. Sci.* **1997**, *63* (4), 453–458.
- (230) Kidchob, T. Thermoresponsive Release from Poly(Glu(OMe))-Block-Poly(Sar) Microcapsules with Surface-Grafting of Poly(N-Isopropylacrylamide). *J. Control.*
-

-
- Release* **1998**, *50* (1–3), 205–214.
- (231) Kidchob, T.; Kimura, S.; Imanishi, Y. Amphiphilic Poly(Ala)-b-Poly(Sar) Microspheres Loaded with Hydrophobic Drug. *J. Control. Release* **1998**, *51* (2–3), 241–248.
- (232) Vayaboury, W.; Giani, O.; Cottet, H.; Deratani, A.; Schué, F. Living Polymerization of α -Amino Acid N-Carboxyanhydrides (NCA) upon Decreasing the Reaction Temperature. *Macromol. Rapid Commun.* **2004**, *25* (13), 1221–1224.
- (233) Aliferis, T.; Iatrou, H.; Hadjichristidis, N. Living Polypeptides. *Biomacromolecules* **2004**, *5* (5), 1653–1656.
- (234) Gangloff, N.; Fetsch, C.; Luxenhofer, R. Polypeptoids by Living Ring-Opening Polymerization of N-Substituted N-Carboxyanhydrides from Solid Supports. *Macromol. Rapid Commun.* **2013**, *34* (12), 997–1001.
- (235) Bailey, J. A New Peptide Synthesis. *Nature* **1949**, *164* (4177), 889–889.
- (236) Merrifield, R. B. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **1963**, *85* (14), 2149–2154.
- (237) Li, Y. Recombinant Production of Antimicrobial Peptides in Escherichia Coli: A Review. *Protein Expr. Purif.* **2011**, *80* (2), 260–267.
- (238) Kawakami, T.; Murakami, H.; Suga, H. Ribosomal Synthesis of Polypeptoids and Peptoid-Peptide Hybrids. *J. Am. Chem. Soc.* **2008**, *130* (50), 16861–16863.
- (239) Leuchs, H. Ueber Die Glycin-Carbonsäure. *Berichte der Dtsch. Chem. Gesellschaft* **1906**, *39* (1), 857–861.
- (240) Leuchs, H.; Manasse, W. Über Die Isomerie Der Carbäthoxyl-Glycyl Glycinester. *Berichte der Dtsch. Chem. Gesellschaft* **1907**, *40* (3), 3235–3249.
- (241) Leuchs, H.; Geiger, W. Über Die Anhydride von α -Amino-N-Carbonsäuren Und Die von α -Aminosäuren. *Berichte der Dtsch. Chem. Gesellschaft* **1908**, *41* (2), 1721–1726.
- (242) Staudinger, H. Über Polymerisation. *Berichte der Dtsch. Chem. Gesellschaft (A B Ser.)* **1920**, *53* (6), 1073–1085.
- (243) Wessely, F. Untersuchungen Über α -Amino-N-Carbonsäureanhydride. I. *Hoppe-Seyler's Zeitschrift für Physiol. Chemie* **1925**, *146* (1–3), 72–90.
- (244) Go, Y.; Tani, H. Über Die Hochkondensierten Produkte Aus Einigen Aminosäuren. *Bull. Chem. Soc. Jpn.* **1939**, *14* (11), 510–516.
- (245) Sigmund, F.; Wessely, F. Untersuchungen Über α -Amino-N-Carbonsäureanhydride. II. *Hoppe-Seyler's Zeitschrift für Physiol. Chemie* **1926**, *157* (1–3), 91–105.
- (246) Wessely, F.; Sigmund, F. Untersuchungen Über α -Amino-N-Carbonsäureanhydride. III. (Zur Kenntnis Höhermolekularer Verbindungen.). *Hoppe-Seyler's Zeitschrift für Physiol. Chemie* **1926**, *159* (1–4), 102–119.
- (247) Woodward, R. B.; Schramm, C. H. Synthesis of Protein Analogs. *J. Am. Chem. Soc.* **1947**, *69* (6), 1551–1552.
- (248) Waley, S. G.; Watson, J. The Kinetics of the Polymerization of Sarcosine Carbonic Anhydride. *Proc. R. Soc. London. Ser. A. Math. Phys. Sci.* **1949**, *199* (1059), 499–517.
- (249) Fessler, J. H.; Ogston, A. G. Studies of the Sedimentation, Diffusion and Viscosity of Some Sarcosine Polymers in Aqueous Solution. *Trans. Faraday Soc.* **1951**, *47*,
-

- 667.
- (250) Ballard, D. G.; Bamford, C. H. Kinetics of the Formation of Polypeptides from N-Carboxy- α -Amino-Acid Anhydrides. *Nature* **1953**, *172* (4385), 907–908.
- (251) Fuchs, F. Über N-Carbonsäure-Anhydride. *Chem. Ber.* **1922**, *55B*, 2943.
- (252) Farthing, A. C.; Reynolds, R. J. W. Anhydro-N-Carboxy-DL- β -Phenylalanine. *Nature* **1950**, *165* (4199), 647–647.
- (253) Oya, M.; Katakai, R.; Nakai, H.; Iwakura, Y. A Novel Synthesis of N -Carboxy- α -Amino Acid Anhydride. *Chem. Lett.* **1973**, *2* (11), 1143–1144.
- (254) Daly, W. H.; Poché, D. The Preparation of N-Carboxyanhydrides of α -Amino Acids Using Bis(Trichloromethyl)Carbonate. *Tetrahedron Lett.* **1988**, *29* (46), 5859–5862.
- (255) Schäfer, O.; Schollmeyer, D.; Birke, A.; Holm, R.; Johann, K.; Muhl, C.; Seidl, C.; Weber, B.; Barz, M. Investigation of α -Amino Acid N-Carboxyanhydrides by X-Ray Diffraction for Controlled Ring-Opening Polymerization. *Tetrahedron Lett.* **2019**, *60* (3), 272–275.
- (256) Fischer, K. Neues Verfahren Zur Maßanalytischen Bestimmung Des Wassergehaltes von Flüssigkeiten Und Festen Körpern. *Angew. Chemie* **1935**, *48* (26), 394–396.
- (257) Semple, J. E.; Sullivan, B.; Sill, K. N. Large-Scale Synthesis of α -Amino Acid- N -Carboxyanhydrides. *Synth. Commun.* **2017**, *47* (1), 53–61.
- (258) Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Sakellariou, G. Synthesis of Well-Defined Polypeptide-Based Materials via the Ring-Opening Polymerization of α -Amino Acid N -Carboxyanhydrides. *Chem. Rev.* **2009**, *109* (11), 5528–5578.
- (259) Liu, J.; Ling, J. DFT Study on Amine-Mediated Ring-Opening Mechanism of α -Amino Acid N -Carboxyanhydride and N-Substituted Glycine N -Carboxyanhydride: Secondary Amine versus Primary Amine. *J. Phys. Chem. A* **2015**, *119* (27), 7070–7074.
- (260) Webster, O. W. Living Polymerization Methods. *Science* **1991**, *251* (4996), 887–893.
- (261) Deming, T. J. Facile Synthesis of Block Copolypeptides of Defined Architecture. *Nature* **1997**, *390* (6658), 386–389.
- (262) Deming, T. J. Cobalt and Iron Initiators for the Controlled Polymerization of α -Amino Acid-N-Carboxyanhydrides. *Macromolecules* **1999**, *32* (13), 4500–4502.
- (263) Deming, T. J. Synthesis of Side-Chain Modified Polypeptides. *Chem. Rev.* **2016**, *116* (3), 786–808.
- (264) Detwiler, R. E.; Schlirf, A. E.; Kramer, J. R. Rethinking Transition Metal Catalyzed N-Carboxyanhydride Polymerization: Polymerization of Pro and AcOPro N-Carboxyanhydrides. *J. Am. Chem. Soc.* **2021**, *143* (30), 11482–11489.
- (265) Conejos-Sánchez, I.; Duro-Castano, A.; Birke, A.; Barz, M.; Vicent, M. J. A Controlled and Versatile NCA Polymerization Method for the Synthesis of Polypeptides. *Polym. Chem.* **2013**, *4* (11), 3182.
- (266) Dimitrov, I.; Schlaad, H. Synthesis of Nearly Monodisperse Polystyrene–Polypeptide Block Copolymers via Polymerisation of N-Carboxyanhydrides. *Chem. Commun.* **2003**, No. 23, 2944–2945.
- (267) Habraken, G. J. M. M.; Peeters, M.; Dietz, C. H. J. T. J. T.; Koning, C. E.; Heise, A. How Controlled and Versatile Is N-Carboxy Anhydride (NCA) Polymerization at 0

-
- °C? Effect of Temperature on Homo-, Block- and Graft (Co)Polymerization. *Polym. Chem.* **2010**, *1* (4), 514–524.
- (268) Rasines Mazo, A.; Allison-Logan, S.; Karimi, F.; Chan, N. J. A.; Qiu, W.; Duan, W.; O'Brien-Simpson, N. M.; Qiao, G. G. Ring Opening Polymerization of α -Amino Acids: Advances in Synthesis, Architecture and Applications of Polypeptides and Their Hybrids. *Chem. Soc. Rev.* **2020**, *49* (14), 4737–4834.
- (269) Zhao, W.; Lv, Y.; Li, J.; Feng, Z.; Ni, Y.; Hadjichristidis, N. Fast and Selective Organocatalytic Ring-Opening Polymerization by Fluorinated Alcohol without a Cocatalyst. *Nat. Commun.* **2019**, *10* (1), 3590.
- (270) Xia, Y.; Song, Z.; Tan, Z.; Xue, T.; Wei, S.; Zhu, L.; Yang, Y.; Fu, H.; Jiang, Y.; Lin, Y.; Lu, Y.; Ferguson, A. L.; Cheng, J. Accelerated Polymerization of N-Carboxyanhydrides Catalyzed by Crown Ether. *Nat. Commun.* **2021**, *12* (1).
- (271) Wang, X.; Song, Z.; Tan, Z.; Zhu, L.; Xue, T.; Lv, S.; Fu, Z.; Zheng, X.; Ren, J.; Cheng, J. Facile Synthesis of Helical Multiblock Copolypeptides: Minimal Side Reactions with Accelerated Polymerization of N-Carboxyanhydrides. *ACS Macro Lett.* **2019**, 1517–1521.
- (272) Heatley, F.; Bovey, F. A. Polymer Nuclear Magnetic Resonance Spectroscopy. XIV. The Nuclear Magnetic Resonance Spectrum of Poly (Isopropyl Acrylate). *Macromolecules* **1968**, *1* (4), 303–304.
- (273) Edison, J. R.; Spencer, R. K.; Butterfoss, G. L.; Hudson, B. C.; Hochbaum, A. I.; Paravastu, A. K.; Zuckermann, R. N.; Whitelam, S. Conformations of Peptoids in Nanosheets Result from the Interplay of Backbone Energetics and Intermolecular Interactions. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (22), 5647–5651.
- (274) Sisido, M.; Imanishi, Y.; Higashimura, T. Monte Carlo Calculation on Trans/Cis-Polysarcosine. *Macromolecules* **1976**, *9* (3), 389–395.
- (275) Ostuni, E.; Chapman, R. G.; Liang, M. N.; Meluleni, G.; Pier, G.; Ingber, D. E.; Whitesides, G. M. Self-Assembled Monolayers That Resist the Adsorption of Proteins and the Adhesion of Bacterial and Mammalian Cells. *Langmuir* **2001**, *17* (20), 6336–6343.
- (276) Schneider, M.; Fetsch, C.; Amin, I.; Jordan, R.; Luxenhofer, R. Polypeptoid Brushes by Surface-Initiated Polymerization of N-Substituted Glycine N - Carboxyanhydrides. *Langmuir* **2013**, *29* (23), 6983–6988.
- (277) Schneider, M.; Tang, Z.; Richter, M.; Marschelke, C.; Förster, P.; Wegener, E.; Amin, I.; Zimmermann, H.; Scharnweber, D.; Braun, H. G.; Luxenhofer, R.; Jordan, R. Patterned Polypeptoid Brushes. *Macromol. Biosci.* **2016**, *16* (1), 75–81.
- (278) Settanni, G.; Schäfer, T.; Muhl, C.; Barz, M.; Schmid, F. Poly-Sarcosine and Poly(Ethylene-Glycol) Interactions with Proteins Investigated Using Molecular Dynamics Simulations. *Comput. Struct. Biotechnol. J.* **2018**, *16*, 543–550.
- (279) Wheeler, A. W.; Henderson, D. C.; Garman, A. J.; Moran, D. M. Suppression of Murine IgE Responses with Amino Acid Polymer/Allergen Conjugates. *Int. Arch. Allergy Immunol.* **1985**, *76* (4), 361–368.
- (280) Cook, R. M.; Henderson, D. C.; Wheeler, A. W.; Moran, D. M. Suppression of Murine IgE Responses with Amino Acid Polymer/Allergen Conjugates. *Int. Arch. Allergy Immunol.* **1986**, *80* (4), 355–360.
- (281) Viricel, W.; Fournet, G.; Beaumel, S.; Perrial, E.; Papot, S.; Dumontet, C.; Joseph,
-

- B. Monodisperse Polysarcosine-Based Highly-Loaded Antibody-Drug Conjugates. *Chem. Sci.* **2019**, *10* (14), 4048–4053.
- (282) Hou, Y.; Lu, H. Protein PEPylation: A New Paradigm of Protein-Polymer Conjugation. *Bioconjug. Chem.* **2019**, *30* (6), 1604–1616.
- (283) Wang, H.; Hou, Y.; Hu, Y.; Dou, J.; Shen, Y.; Wang, Y.; Lu, H. Enzyme-Activatable Interferon-Poly(α -Amino Acid) Conjugates for Tumor Microenvironment Potentiation. *Biomacromolecules* **2019**, *20* (8), 3000–3008.
- (284) Hu, Y.; Hou, Y.; Wang, H.; Lu, H. Polysarcosine as an Alternative to PEG for Therapeutic Protein Conjugation. *Bioconjug. Chem.* **2018**, *29* (7), 2232–2238.
- (285) Son, K.; Ueda, M.; Taguchi, K.; Maruyama, T.; Takeoka, S.; Ito, Y. Evasion of the Accelerated Blood Clearance Phenomenon by Polysarcosine Coating of Liposomes. *J. Control. Release* **2020**, *322* (March), 209–216.
- (286) Nogueira, S. S.; Schlegel, A.; Maxeiner, K.; Weber, B.; Barz, M.; Schroer, M. A.; Blanchet, C. E.; Svergun, D. I.; Ramishetti, S.; Peer, D.; Langguth, P.; Sahin, U.; Haas, H. Polysarcosine-Functionalized Lipid Nanoparticles for Therapeutic mRNA Delivery. *ACS Appl. Nano Mater.* **2020**, *3* (11), 10634–10645.
- (287) Klinker, K.; Holm, R.; Heller, P.; Barz, M. Evaluating Chemical Ligation Techniques for the Synthesis of Block Copolypeptides, Polypeptoids and Block Copolypept(o)ides: A Comparative Study. *Polym. Chem.* **2015**, *6* (25), 4612–4623.
- (288) Heller, P.; Mohr, N.; Birke, A.; Weber, B.; Reske-Kunz, A.; Bros, M.; Barz, M. Directed Interactions of Block Copolypept(o) Ides with Mannose-Binding Receptors: Peptomicelles Targeted to Cells of the Innate Immune System. *Macromol. Biosci.* **2015**, *15* (1), 63–73.
- (289) Johann, K.; Bohn, T.; Shahneh, F.; Luther, N.; Birke, A.; Jaurich, H.; Helm, M.; Klein, M.; Raker, V. K.; Bopp, T.; Barz, M.; Becker, C. Therapeutic Melanoma Inhibition by Local Micelle-Mediated Cyclic Nucleotide Repression. *Nat. Commun.* **2021**, *12* (1), 5981.
- (290) Steinborn, B.; Hirschle, P.; Höhn, M.; Bauer, T.; Barz, M.; Wuttke, S.; Wagner, E.; Lächelt, U. Core-Shell Functionalized Zirconium-Pemetrexed Coordination Nanoparticles as Carriers with a High Drug Content. *Adv. Ther.* **2019**, *2* (11), 1900120.
- (291) Yoo, J.; Birke, A.; Kim, J.; Jang, Y.; Song, S. Y.; Ryu, S.; Kim, B.-S.; Kim, B.-G.; Barz, M.; Char, K. Cooperative Catechol-Functionalized Polypept(o)Ide Brushes and Ag Nanoparticles for Combination of Protein Resistance and Antimicrobial Activity on Metal Oxide Surfaces. *Biomacromolecules* **2018**, *19* (5), 1602–1613.
- (292) Tanisaka, H.; Kizaka-Kondoh, S.; Makino, A.; Tanaka, S.; Hiraoka, M.; Kimura, S. Near-Infrared Fluorescent Labeled Peptosome for Application to Cancer Imaging. *Bioconjug. Chem.* **2008**, *19* (1), 109–117.
- (293) Weber, B.; Kappel, C.; Scherer, M.; Helm, M.; Bros, M.; Grabbe, S.; Barz, M. PeptoSomes for Vaccination: Combining Antigen and Adjuvant in Polypept(o)Ide-Based Polymersomes. *Macromol. Biosci.* **2017**, *17* (10), 1–6.
- (294) Heller, P.; Weber, B.; Birke, A.; Barz, M. Synthesis and Sequential Deprotection of Triblock Copolypept(o)Ides Using Orthogonal Protective Group Chemistry. *Macromol. Rapid Commun.* **2015**, *36* (1), 38–44.
- (295) Heller, P.; Hobernik, D.; Lächelt, U.; Schinnerer, M.; Weber, B.; Schmidt, M.;

-
- Wagner, E.; Bros, M.; Barz, M. Combining Reactive Triblock Copolymers with Functional Cross-Linkers: A Versatile Pathway to Disulfide Stabilized-Polyplex Libraries and Their Application as PDNA Vaccines. *J. Control. Release* **2017**, *258* (March), 146–160.
- (296) Ren, J. M.; McKenzie, T. G.; Fu, Q.; Wong, E. H. H.; Xu, J.; An, Z.; Shanmugam, S.; Davis, T. P.; Boyer, C.; Qiao, G. G. Star Polymers. *Chem. Rev.* **2016**, *116* (12), 6743–6836.
- (297) Holm, R.; Weber, B.; Heller, P.; Klinker, K.; Westmeier, D.; Docter, D.; Stauber, R. H.; Barz, M. Synthesis and Characterization of Stimuli-Responsive Star-Like Polypept(o)ides: Introducing Biodegradable PeptoStars. *Macromol. Biosci.* **2017**, *17* (6), 1–14.
- (298) Holm, R.; Douverne, M.; Weber, B.; Bauer, T.; Best, A.; Ahlers, P.; Koynov, K.; Besenius, P.; Barz, M. Impact of Branching on the Solution Behavior and Serum Stability of Starlike Block Copolymers. *Biomacromolecules* **2019**, *20* (1), 375–388.
- (299) Holm, R.; Schwiertz, D.; Weber, B.; Schultze, J.; Kuhn, J.; Koynov, K.; Lächelt, U.; Barz, M. Multifunctional Cationic PeptoStars as siRNA Carrier: Influence of Architecture and Histidine Modification on Knockdown Potential. *Macromol. Biosci.* **2020**, *20* (1), 1–17.
- (300) Hörtz, C.; Birke, A.; Kaps, L.; Decker, S.; Wächtersbach, E.; Fischer, K.; Schuppan, D.; Barz, M.; Schmidt, M. Cylindrical Brush Polymers with Polysarcosine Side Chains: A Novel Biocompatible Carrier for Biomedical Applications. *Macromolecules* **2015**, *48* (7), 2074–2086.
- (301) Stéen, E. J. L.; Jørgensen, J. T.; Johann, K.; Nørregaard, K.; Sohr, B.; Svatunek, D.; Birke, A.; Shalgunov, V.; Edem, P. E.; Rossin, R.; Seidl, C.; Schmid, F.; Robillard, M. S.; Kristensen, J. L.; Mikula, H.; Barz, M.; Kjær, A.; Herth, M. M. Trans-Cyclooctene-Functionalized PeptoBrushes with Improved Reaction Kinetics of the Tetrazine Ligation for Pretargeted Nuclear Imaging. *ACS Nano* **2020**, *14* (1), 568–584.
- (302) Longmire, M.; Choyke, P. L.; Kobayashi, H. Clearance Properties of Nano-Sized Particles and Molecules as Imaging Agents: Considerations and Caveats. *Nanomedicine* **2008**, *3* (5), 703–717.
- (303) Johann, K.; Svatunek, D.; Seidl, C.; Rizzelli, S.; Bauer, T. A.; Braun, L.; Koynov, K.; Mikula, H.; Barz, M. Tetrazine- and Trans-Cyclooctene-Functionalised Polypept(o)ides for Fast Bioorthogonal Tetrazine Ligation. *Polym. Chem.* **2020**, *11* (27).
- (304) Barz, M.; Huesmann, D.; Schäfer, O.; Reuter, T.; Birke, A.; Heller, P. Thiol-Protected Amino Acid Derivatives and Uses Thereof, **2014**, EP2942348A1.
- (305) Muhl, C.; Schäfer, O.; Bauer, T.; Räder, H.-J.; Barz, M. Poly(S-Ethylsulfonyl-L-Homocysteine): An α -Helical Polypeptide for Chemoselective Disulfide Formation. *Macromolecules* **2018**, *51* (20), 8188–8196.
- (306) Schäfer, O.; Klinker, K.; Braun, L.; Huesmann, D.; Schultze, J.; Koynov, K.; Barz, M. Combining Orthogonal Reactive Groups in Block Copolymers for Functional Nanoparticle Synthesis in a Single Step. *ACS Macro Lett.* **2017**, *6* (10), 1140–1145.
-