Triblock polypept(o)ides for siRNA delivery: unique polymeric designs for versatile carrier systems

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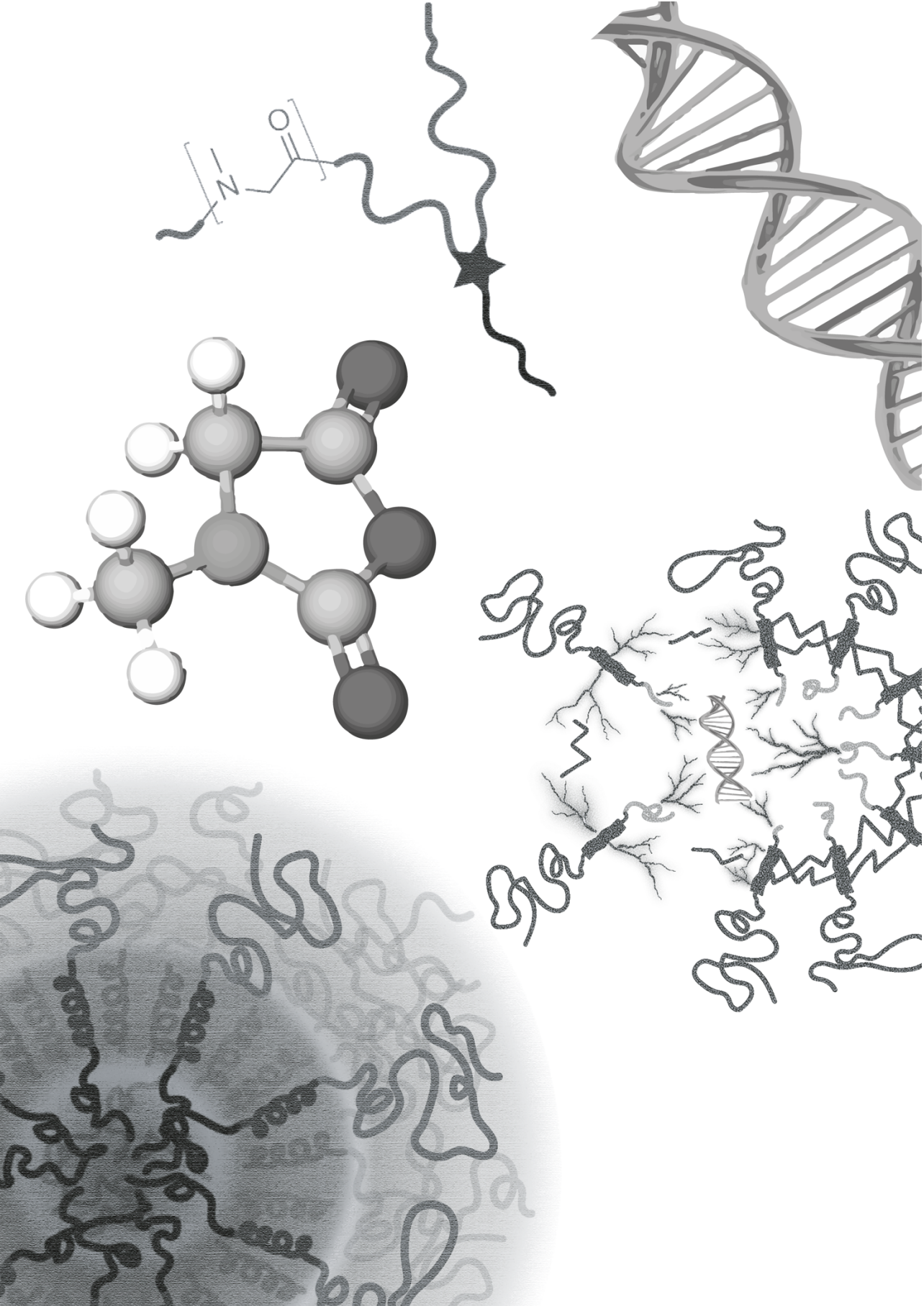
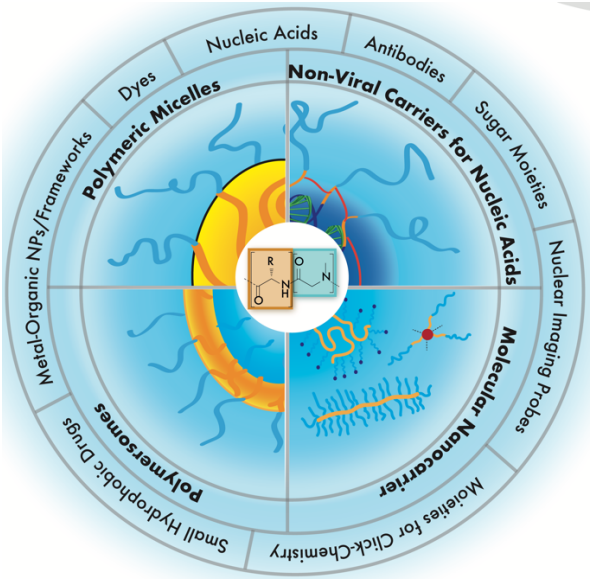


CHAPTER

Introduction Part I - Polypept(o)ides

Origins, Synthesis and Applications



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Polypept(o)ides – Origins, Synthesis and Applications

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Abstract

Polypept(o)ides combine the stealth-like properties of polypeptoids such as polysarcosine (poly(*N*-methyl glycine)) with the multifunctionality and intrinsic stimuli-responsiveness of synthetic polypeptides. This class of copolymers can be synthesized by controlled living ring-opening polymerization of the corresponding α-amino acid *N*-carboxyanhydrides (NCAs) and *N*-substituted glycine *N*-carboxyanhydrides (NNCAs). When the polymerization is performed under clean conditions, the resulting copolymers are characterized by high end-group fidelity and Poisson-like molecular weight distributions with dispersities below 1.2. While polysarcosine might be able to tackle most of the current concerns of poly(ethylene glycol) (PEG), e.g., acute immune responses, the polypeptide part can provide a plethora of reactivity or functionality, allowing to tailor the polymer for specific tasks. In this review, we provide an overview on the origins of NCA polymerization and polypept(o)ides and provide a detailed overview on the last decade of research focusing on synthesis, characterization, and application. Arguably the biggest applicational progress for polypept(o)ides has been made in nanomedicine. Here, the remarkable combination of functionality, biocompatibility and a high degree of synthetic control has led to established protocols for the certified production of polypept(o)ides, which will enable the rapid clinical translation for the years to come.

Introduction

Polypept(o)ides have been introduced by Birke *et al.* in 2014 as hybrid materials combining the intrinsic functionality and stimuli-responsiveness of polypeptides with polypeptoids, e.g., polysarcosine (pSar) for solubility and steric shielding.[1–3] 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.[4,5] The term was later expanded by Zuckerman and co-workers, referring to polypeptoids for large and peptoids for short sequences. In general, polypeptoids can be conceived as structural isomers of polypeptides.[3,6] Unlike polypeptides, polypeptoids generally lack the acidic hydrogen atom at the amide nitrogen and are thus exclusive hydrogen bond acceptors that do not form secondary structures unless specific substituents are introduced in the side chain.[7–10] The highly water-soluble pSar, poly(*N*-methyl glycine), is among the most intensively studied polypeptoids.[11–13] 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.[14–16] Sarcosine can be synthesized from glycine *via* the enzyme glycine-*N*-methyl transferase and degraded by sarcosine dehydrogenase.[17–19] Therefore, polypept(o)ides can be synthetic polymers entirely based on endogenous amino acids.[1–3] While polypept(o)ides have been already applied to multiple applications, including surface modifications[12,20–22] and surfactants,[23,24] they are mostly applied as materials for diagnosis and therapy as nanomedicines. In the past decades, nanomedicine has evolved to a well-accepted tool for drug and gene delivery or for imaging probes to visualize certain disease pathologies or disease-related biomarkers.[25–27] Several classes of therapeutic drug delivery systems have been developed and applied to multiple diseases including bacterial and viral infections, inflammation, and cancer. Especially over the last couple of years, polypept(o)ides have seen a steadily increasing attention as a new class of functional materials for the design of nanomedicines as indicated by an increase in publications and active development programs in pharmaceutical industry (**Figure 1**).[28] The process has led to the establishment of facilities for the GMP production of such

materials, and the recent launch of the excipient Apisolex® for solubility enhancement of poorly soluble drugs by The Lubrizol Corporation.[29,30]

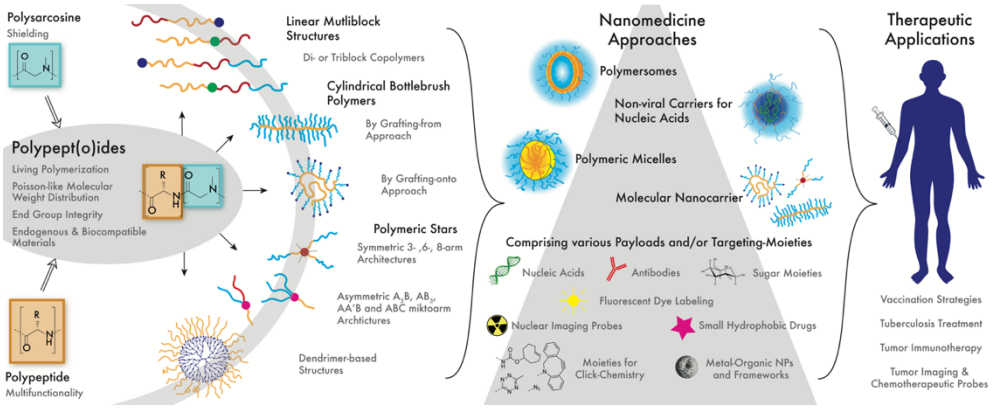


Figure 1. General chemical composition of polypept(o)ides, overview of polymeric architectures and resulting nanomedical delivery platforms.

The established synthetic methodologies provide access to various well-defined polymeric architectures leading toward functional approaches, especially in the field of nanomedicine. This review aims to elucidate the origins of polypept(o)ides, the current synthetic methods based on NCA polymerization, present a systematic overview on the application of polypept(o)ides and provide an outlook on future applications of these hybrid materials.

Polypeptides

Polypeptides, natural or synthetic, depict a class of polymers comprising α-amino acid repetitive units. Beside other strategies, the synthesis of polypeptides can also be performed by NCA polymerization, particularly whenever a precise sequence control is not warranted but high molecular weights intended. We would like to refer the interested reader to other excellent reviews on this subject.[31–35]

Polypeptoids

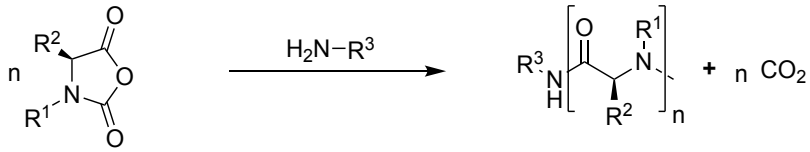
Polypeptoids are defined as *N*-substituted polyglycines, that are connected by amide bonds in the main chain.[4–6] This class of materials has seen increasing attention over the last decades, and polypeptoids have been subjected by excellent reviews.[36–38] The synthesis of polypeptoids can take place by solid phase methods as well as by ring-opening polymerization (ROP) of the corresponding NNCAs. Among these polypeptoids, pSar is particularly interesting. It 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.[11,39–41] 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 $l_{k,\,\mathrm{pSar}}$ = 1.5 nm and $l_{k,\,\mathrm{PEG}}$ = 1.1 nm.[11] PSar matches the requirements for protein resistant surfaces summarized by the Whitesides' rules in 2001.[42] 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.[42,43] These results were later confirmed by Messersmith and co-workers reporting excellent resistance of pSar-grafted TiO_2 surfaces towards non-specific adhesion of proteins or any attachment of mammalian or bacterial cells.[12] Additionally, Jordan/Luxenhofer and co-workers investigated the resistance of inorganic surfaces to biofouling after modification with polypeptoid brushes.[20,22] 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.[44] Moreover, both pSar and PEG do not elicit activation of the complement cascade, but acetylation of the amine end-group remains significant.[11,24] 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 mononuclear phagocyte system (MPS) recognition are desired.[3,11] The search for alternatives to PEG was specifically boosted by the detailed study of potentially PEG-related side-effects and increased number of anti-PEG antibodies after vaccination with lipid nanoparticle-based SARS CoV-2 vaccines.[45,46]

Polysarcosine in Nanomedicine

The qualification of pSar as an alternative material in medical and medicinal applications has been explored over the past decades and is now rising as a new star. 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.[47,48] More recently, pSar attracted increasing attention as an alternative material to PEG and has been investigated for shielding of antibody-drug-conjugates,[49,50] proteins,[51] liposomes,[24,52] inorganic nanoparticles,[53] and lipid nanoparticles (LNPs).[54,55] In 2020, Son *et al.* prepared PEG and pSar functionalized liposomes and compared the immune response after intravenous administration to rats.[52] As a result, significantly higher levels of IgM and IgG antibodies were found for the PEGylated liposomes. Hereafter, the second administration revealed the accelerated blood clearance (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.[54] In summary, pSar has emerged as a biocompatible polymeric material suitable for medicinal applications. Since our review focuses on polypept(o)ides, thus combinations of polysarcosine with polypeptides, we refer the interested reader to the review by Birke *et al.* for combinations of pSar with other polymers.[3]

Polypept(o)ides

Synthetic polypept(o)ides can be conveniently prepared by living amine-initiated ROP of NCAs/NNCAs.[56–58] Despite early attempts by Bailey *et al.*,[59] 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.[60–62] A general scheme for the amine-initiated polymerization of NCAs or NNCAs ($R^1 \neq H$) is given in **Scheme 1**.



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

Polypept(o)ide Architectures

Depending on the desired application, polypept(o)ides can be designed with block-wise primary sequences, linear or branched architectures and Poisson-like molecular weight distribution.[2,3] The various architectures of polypept(o)ides are summarized in **Figure 2**. Besides block-wise construction of copolypept(o)ides by sequential polymerization of NCAs and NNCAs, in 2015 Klinker *et al.* firstly demonstrated their preparation by chemical ligation techniques utilizing functional initiators and complementary end-group modifications for the ligation of different homopolymers.[63] The extension from di- to triblock copolypept(o)ides by sequential polymerization was introduced by Heller *et al.* in 2015, with ε-*tert*-butyloxycarbonyl-L-lysine (Lys(Boc))-, ε-trifluoroacetamide-L-lysine (Lys(TFA))- and sarcosine-NNCA.[64] Just recently, the synthesis of triblock copolypept(o)ides could also be demonstrated utilizing a bifunctional initiator approach, which was introduced earlier to construct diblock structures, further enabling the incorporation of functional groups between the blocks.[65,66] Reaching out to more complex architectures than linear polymers, in 2015, Hörtz *et al.* presented the synthesis of molecular polymer brushes based on polypept(o)ides which emerged from a grafting-from approach utilizing poly(*N*-(6-aminohexyl)methacrylamide) (PAHMA) and pLys backbones for NCA/NNCA polymerization.[67] Moreover, polymer brush architectures from a grafting-onto approach have been described by Stéen *et al.* in 2020.[68] Investigating the broad variety of small molecular structures with multiple amine functions as initiators, access was found to polymeric star architectures. In 2017, 3- and 6-arm star-like polypept(o)ides have been introduced by Holm *et al.,* followed by A_3B and AB_3-type miktoarm analogues by Schwiertz *et al.* in 2020.[69–71] Just recently, the access to AA'B- and ABC-type miktoarm architectures has been demonstrated by Capelôa *et*

al., introducing a lysine end group to a pSar chain, comprising two amine functions with orthogonal protective groups.[72]

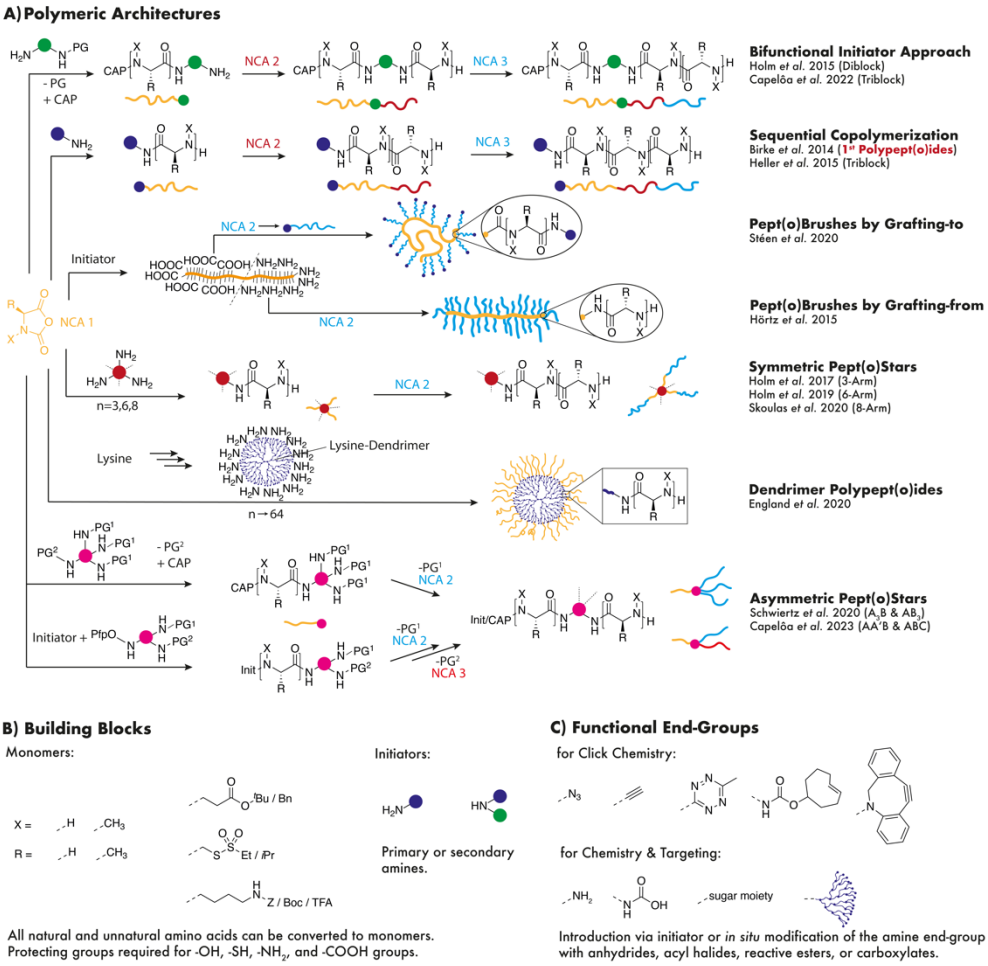


Figure 2. A) Summary of synthetic strategies to access various polypept(o)ide architectures by amine-initiated ROP of NCAs/NNCAs, including B) an overview of common amino acid-based building blocks and initiators as well as C) introduced functional end-groups. Seminal publications of these synthetic approaches to yield well-defined polymeric architectures are assigned.

In 2020, Skoulas et al. firstly demonstrated polypept(o)ide-based 8-arm star like architectures.[73] Utilizing lysine dendrimeric structures, the Ashford lab was able to

create star architectures with up to 32 arms as well as telodendrimeric polypept(o)ides in 2020 and 2022, respectively.[74,75] As a consequence of providing access to functional materials of defined polymeric architecture and narrow molecular weight distribution by NCA polymerization, polypept(o)ides have attracted increasing attention.[2,3] The latest developments for medical applications will be discussed in the paragraph *Polypept(o)ides in Nanomedicine*.

Origins of Polypept(o)ides and (N)NCA Polymerization

The term polypept(o)ides was coined by Birke *et al.* in 2014 referring to the new class of polymeric materials combining polypeptides with polypeptoids.[1,2] Early examples of polypeptide/polypeptoid copolymers, however, date back until the 1950s, to the origins of NCA and NNCA polymerization, when studying fundamental properties and reaction kinetics was the major focus of research.[35,76] Besides block copolypept(o)ides, early on, also copolypept(o)ides have been synthesized and studied, aiming to understand the structure and function of the synthetic polypeptides and their natural analogs.[77–79] Considering medical applications, Kimura and co-workers investigated polypept(o)ides in the 1990s.[80–84] 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, including release upon different triggers like pH or temperature and loading of hydrophobic drugs. The first detailed proof for the controlled polymerization of polypept(o)ides with high molecular weights was provided by Birke *et al*. in 2014. In this work, applications in drug formulations or as surfactants in emulsion polymerization were described.[1] The first NCA synthesis was discovered by Hermann Leuchs in 1906, and NCAs are thus also called Leuchs' anhydrides.[35,85] A summary of important milestones on the origin of NCAs and polypept(o)ides is displayed with a timeline in Error! Reference source not found..

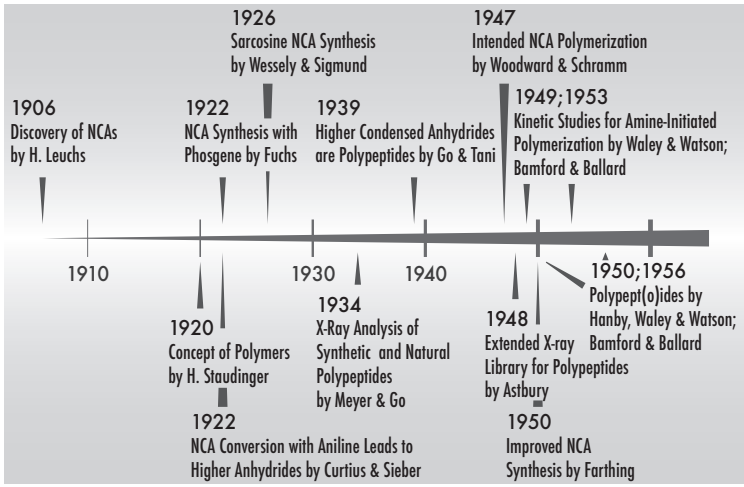
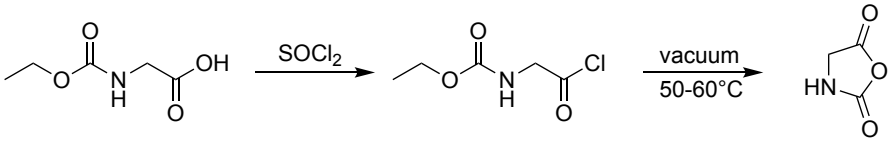


Figure 3. Origins of NCA & NCCA synthesis and polymerization leading towards the intended synthesis of polypept(o)ides. Important milestones for discovering NCAs and understanding NCA polymerization for the synthesis of polypept(o)ides.

In the initial publication, Leuchs obtained glycine NCA upon heating of *N*-ethoxycarbonyl glycine chloride (**Scheme 2**).[85] Moreover, Leuchs carefully described the release of CO_2 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.[86,87] 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.[35,88] Early reports on NCAs thus mainly focused on the analysis of the degradation products of NCAs, e.g., diketopiperazines and hydantoins, and referred to so-called higher condensed anhydrides for products with higher molecular weights.[89–91] Sigmund and Wessely described the first synthesis of sarcosine NCA in 1926.[92] 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. These findings were later revised by Meyer and Go in 1934, detecting no differences between the higher condensed anhydrides derived from NCA

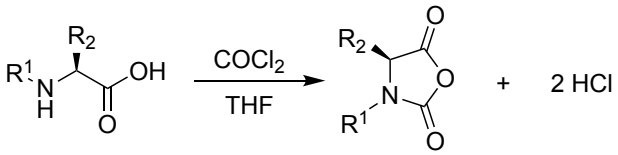
polymerization compared to other polypeptides in X-Ray analysis, establishing the connection between NCAs and polypeptides.[90,93] In 1947, Woodward and Schramm claimed the first intended NCA polymerization using traces of water for the initiation of the reaction in benzene-solutions yielding polypeptides as synthetic analogs of proteins.[94] In the following, various polypeptides have been synthesized from this methodology and studied for their physicochemical properties.[13,76,78] The kinetics of the NCA polymerization were first examined by Waley and Watson, revealing a first-order reaction for the polymerization of sarcosine NCA in nitrobenzene.[95–97] Earliest examples for polypept(o)ide copolymers can be found from Hanby, Waley, and Watson, who briefly described the synthesis of pSar-*block*-poly(DL-phenylalanine) in 1950, yet, comprehensive study design and characterization of the copolymer was provided only by Bamford and Ballard in 1956.[98,99]



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

To generate NCAs and NNCAs, Fuchs suggested the direct reaction of amino acids with phosgene, which was refined and improved by Farthing (**Scheme 3**).[100,101] 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*.[102,103] Despite a large variety of routes to (N)NCAs, the Fuchs-Farthing method is the preferred synthetic route to (N)NCA monomers, allowing for larger scales, high yields and sufficient monomer purity after sublimation and/or repetitive recrystallization.[3,104] Nevertheless, alternative approaches aiming for high monomer purity have been presented more recently. Laconde *et al.* reported on the synthesis of NCAs and NNCAs using Boc-protected amino acids and *n*-propanephosphonic acid anhydride (T3P) yielding numerous products with high melting points.[105] Toshiyuki *et al.* reported on the photo-on-demand synthesis of NCAs by light-induced reaction of amino-acids with oxygen and chloroform, thus

representing an *in situ* phosgenation approach.[106] Of note, the purity of all reagents, i.e., monomer, solvent, and initiator, is of major significance for successful NCA polymerization. Beyond characterization of NCAs by nuclear magnetic resonance (NMR) spectroscopy, special attention should be devoted to determination of melting points,[104] Karl-Fischer-Titration to determine residual water content in monomers and solvents, and ion chromatography to detect inorganic contaminations, e.g., nucleophilic chloride ions, to limit the occurrence of side reactions during the NCA polymerization.[107–109]



Scheme 3. NCA synthesis according to the Fuchs-Farthing method.

NCA Polymerization

From a mechanistic understanding, two competing reaction pathways exist in parallel, namely, the activated monomer mechanism (AMM) and the normal amine mechanism (NAM). In the NAM, the amine initiator solely acts as a nucleophile attacking at C-5, while deprotonation of the NCA takes place within the AMM. For more details on mechanistic pathways and potential side reactions we would like to refer the interested reader to detailed reviews by Birke *et al.* and Hadjichristidis and co-workers.[3,110] Of note, living amine-initiated polymerization of NCAs is only possible for the NAM pathway, whereby Poisson-like molecular weight distributions can be obtained, when the initiation is faster than the propagation reaction.[2,110] Since NNCAs 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.[2,35,110] In addition and comparable to other living polymerization techniques, also NCA/NNCA polymerization is highly sensitive to impurities since these may promote the AMM pathway, catalyze side reactions, and initiate or terminate the chain growth.[104,110,111] In 1997, Deming reported on the living NCA polymerization by using zero-valent nickel

amido-amidate complexes initiating and mediating the chain growth reaction.[112] Herein, homo- and block copolypeptides of poly(γ-benzyl-L-glutamate) (pGlu(OBn)) and pLys(Z) could be synthesized with high molecular weights and narrow dispersity ($Đ$ < 1.2). Deming further expanded the concept to cobalt and iron complexes, and synthesized a library of functional polypeptides.[34,113] However, polymerization of NNCAs was not substantiated until recently, since initiation required an acidic proton for β-hydride elimination. The Kramer group thus improved the nickel and cobalt initiators allowing for polymerization of proline NCA.[114] Nevertheless, elaborate synthesis and potentially remaining traces of toxic heavy metal ions may hamper this technique.[2] In 2004, the groups of Schué and Hadjichristidis reported that reaction temperature, sufficient removal of CO_2, and the purity of the components, are the key parameters for the living amine-initiated polymerization of NCAs, which from our point of view can already ensure the controlled living polymerization of various NCAs until degree of polymerization of 100-200.[56,57] In the following, several groups contributed to expanding the living amine-initiated NCA polymerization leading to a robust and well-established type of polymerization.[1,58,115–117]

With access to conditions for amine-initiated NCA polymerization to possess living nature, featuring active end-group integrity, sequential polymerization of different NCAs/NNCAs was enabled with the absence of homopolymer-forming side reactions and maintaining Poisson-like molecular weight distribution with low dispersity. Exemplarily, consistent and controlled chain extensions to yield pSar-*b*-poly(γ-tert-butyl-L-glutamate) (pSar-*b*-pGlu(O*t*Bu)) or pSar-*b*-poly(*S*-ethylsulfonyl-D,L-cysteine)-*b*-pGlu(OBn) (pSar-*b*-p(DL)Cys(SO_2Et)-*b*-pGlu(OBn)) block copolymers were nicely visualized by Yoo *et al.* and Bauer *et al.*, respectively, utilizing size exclusion chromatography (SEC) presenting clean shifts of the elugrams towards lower elution volumes upon block addition (**Figure 4 A,B**).[118,119] Moreover, active end-group fidelity paired with the toleration of functional initiators, allows for installation of diverse functional groups at one or both ends of the synthesized polymer, as already indicated in **Figure 2**. Klinker *et al.,* for example, demonstrated quantitative end-group integrity of pSar polymers by, among others, utilizing a dibenzocyclooctyne (DBCO)-functionalized initiator or *S*-benzyl thiosuccinic acid for end-group attachment,

confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) (**Figure 4 C**).[63]

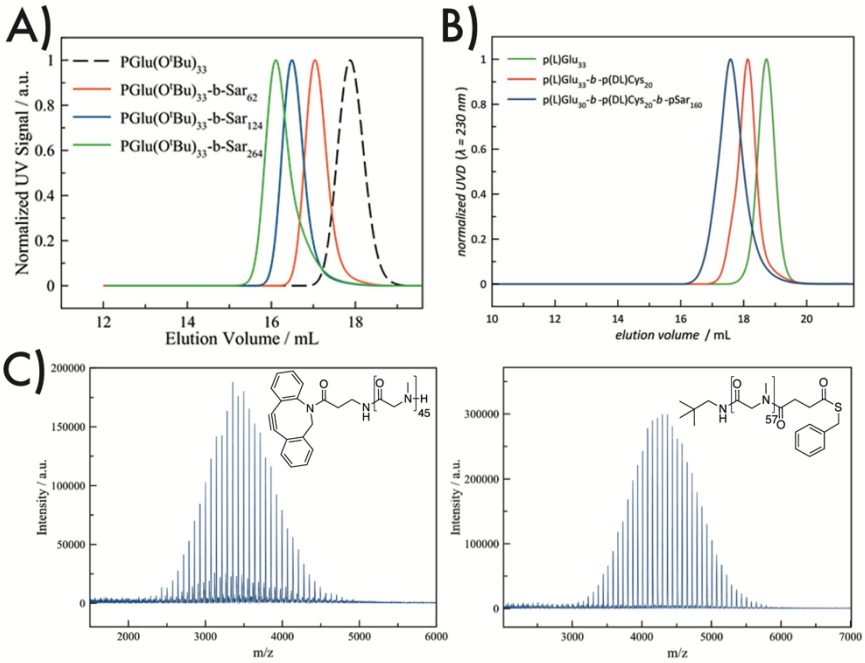


Figure 4. Polymer analysis demonstrates clean NCA polymerization with complete block initiation and end-group fidelity. GPC elugrams in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) of A) pSar-*b*-pGlu(O*t*Bu) diblock copolymers with varying chain lengths and pGlu(O*t*Bu) precursor (Reprinted with permission from Yoo *et al.*[118] © 2018 American Chemical Society), and B) pSar-*b*-p(DL)Cys(SO_2Et)-*b*-pGlu(OBn) triblock copolymers and respective precursors, (Reprinted from Bauer *et al.*[119] with permission). C) MALDI-TOF MS analysis of end-group modified pSar polymers as proof of end-group integrity (Reproduced from Klinker *et al.*[63] with permission from the Royal Society of Chemistry).

At present, the slow propagation rates of NCA/NNCA ROP remain a limitation. Current research thus aims to accelerate the reaction rates of polymerization to achieve chain lengths beyond 1000.[31] 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.[120] Moreover, Xia *et al.* demonstrated accelerated polymerization in DCM upon addition

of crown ether as a catalyst.[121] With a view towards biomedical applications, large molecular weights are often not necessarily an advantage or simply not needed, since nanosized structures can be obtained by solution self-assembly. Nevertheless, fast and robust mechanisms facilitating large-scale production will aid translation of polypept(o)ides, and organocatalysis represents a promising tool for chemists. For extended information on current developments in NCA polymerization techniques we would like to refer the interested reader to reviews by Song *et al.* and Rasines Mazo *et al*.[31,32]

Certified Production of Polypept(o)ides

Over the last years, more than 10 published patents have protected various polypept(o)ides as well as their use in various pharmaceutical applications, e.g. as mRNA delivery systems, drug excipients or core cross-linked polymeric micelles.[122–131] Nevertheless, progress of polypept(o)ide-based therapeutics from early R&D programs to clinical phases and eventually marketed products relies inevitably on the certified production of the materials to be used as regulatory ready precursors, excipients or drug substances (DS) in nanomedicine-based drug products (DP). This implies a comprehensive approach from initial technology transfer, process and analytical development in feasibility programs, to process scale-up, and final transfer of protocols for Good Manufacturing Practice (GMP) implementation and GMP-compliant batches production. In this industrialization and manufacturing process development following the International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use should be careful considered in order to secure approval from regulatory authorities. Of particular relevance are the Quality Guidelines, so-called ICH-Q. In this respect, progress made in the recent years in the validation of analytical methods for the characterization of macromolecules has enabled Drug Developers and Manufacturers to stablish the adequate Control Strategy for Chemistry, Manufacturing and Control (CMC) development of these materials following ICH-Q2 “Analytical validation” Guidelines. This has allowed to identify critical process parameters (CPPs) and to stablish in-

process controls (IPCs) for a robust and reproducible manufacturing process of polypeptides, polypeptoids, and polypept(o)ides.

In order to guarantee target acceptance criteria is met during the process, a normalized operational range (NOR) of the CPPs is usually stablished. This is defined and adjusted by previous proven operational range (POR) studies where parameters such as concentration, temperature, reaction time, purification and drying, hold times within the process operation units and the stability of the product & intermediates during these are stressed to identify the CPPs. Critical Quality Attributes (CQAs) of polypept(o)ides must be defined at the beginning of the process and will depend on the particular manufacturing, dosage, indication, administration route and regulatory classification (excipient, raw material or key intermediate for further manufacturing and DS). Fishbone or Ishikawa diagrams are a useful and illustrative approach to outline all parameters affecting the CQAs (**Figure 5**).[132] These representations summarize the steps in the manufacturing process, highlighting possible quality control issues and defining resources and action plans based on the ICH-Q9 “Quality Risk Management” and Q11 “Development and Manufacture of DS” guidelines. Basic CQAs for polypept(o)ides have been established and are identity, purity, Mw distribution & polydispersity, enantiomeric excess in case of the introduction of chiral monomers, ratio of monomers in case of multiblock copolymers, counterion content for polyelectrolytes, impurities profile (whether there are raw materials, process or degradation related products or solvents) and bioburden.

Although, the overall number of companies being currently able to provide with GMP compliant supply of polypept(o)ide-based delivery systems is limited, these materials are available in certified grade and can support drug development innovators in their clinical development journey from preclinical/clinical trials to commercialization.

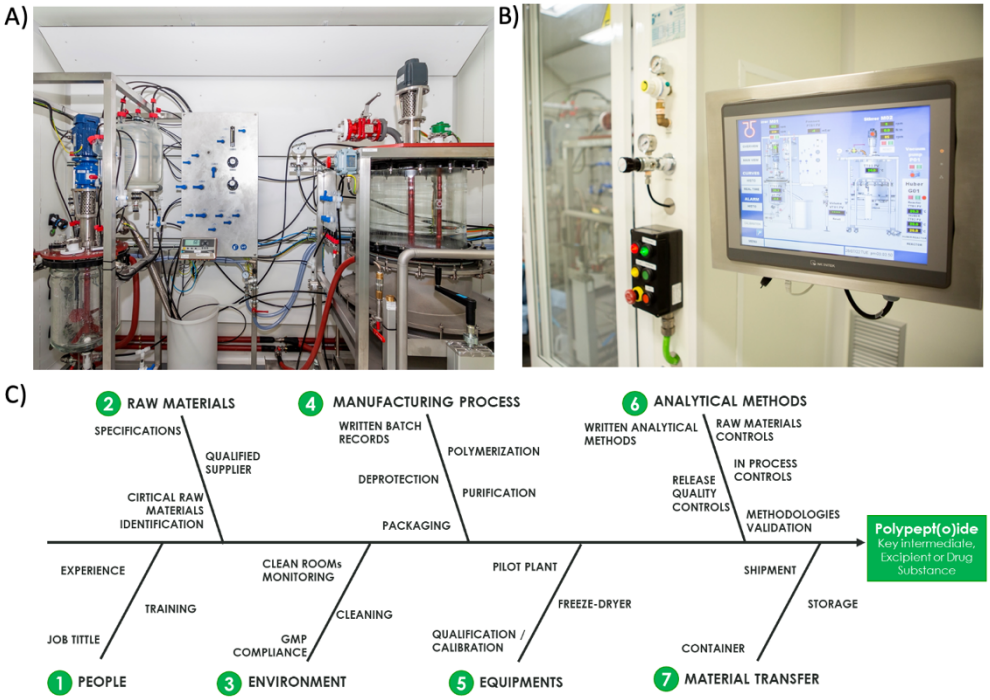


Figure 5. A+B) GMP facility for the certified production of polypeptides, polypeptoids and polypept(o)ides, C) Fishbone or Ishikawa diagram for the identification of internal and external factors affecting CQAs based on ICH Q9 “Quality Risk Management” and Q11 “Development and Manufacture of drug substances”. Adapted to the polypept(o)ide manufacturing process from Glodek *et al*.[132]

Nanomedicine

Since most of the applications of polypept(o)ides are in the area of nanomedicine, we feel that a brief introduction in the related needs is valuable for readers. The term 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, are drugs themselves, and improve or enable *in vivo* or *ex vivo* disease diagnosis.[133–139] 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.[140–142] 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.[143–145] 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.[141,146,147] The nanocarrier design follows the structural demands of the API while referring to the intended application.[148–151] 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 LNPs.[152–155] Nanomedicine thus opened the therapeutic window for RNA-based therapies substantially.[156–158] *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.[138,159,160] Despite being highly potent in the mode of action, many small molecule APIs are hydrophobic and require excipients and high dilutions for administration.[144,161–163] 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.[160,164,165] Nevertheless, anaphylactic reactions, hypersensitivity, hemolysis, and peripheral neuropathy are common side effects attributed to these excipients,[166,167] which restricts the maximum tolerated dose (MTD), while prolonged administration times limit patient compliance.[166,167] In this case, nanomedicine aims to provide solubility and a selective distribution profile to APIs to reduce off-target toxicity and improve therapeutic success.[136,168,169]

Following the ambitions of nanomedicine shown in **Figure 6**, 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.[151,170–173] In consecutive *in vivo* studies a sustained drug release was observed.[171] 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.[171] Jatzkewitz clearly described the

potential of polymer-drug conjugates to alter the pharmacokinetics of APIs and should be recognized for his ground breaking work.[170,171] The idea for polymer-drug conjugates was later refined and conceptualized by Helmut Ringsdorf considering solubility, drug conjugation, and targeting.[151,174]

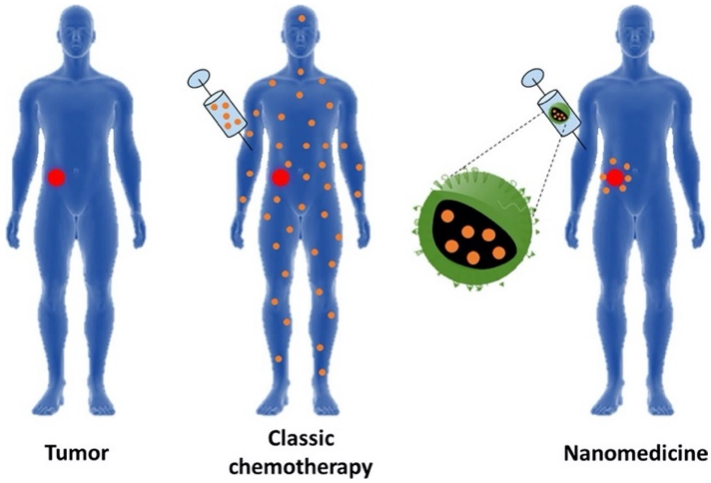


Figure 6. Idealized perspective on chemotherapy by nanomedicine. Reprinted from Gonzalez-Valdivieso *et al.*[168], © 2021, with permission from Elsevier.

Beyond polymer-drug conjugates many different nanocarriers have been designed and evaluated in (pre-)clinical investigations.[137,144,145,173,175,176] A schematic overview is shown in **Figure 7**.[26,175] 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 as well as RNA/DNA.[175]

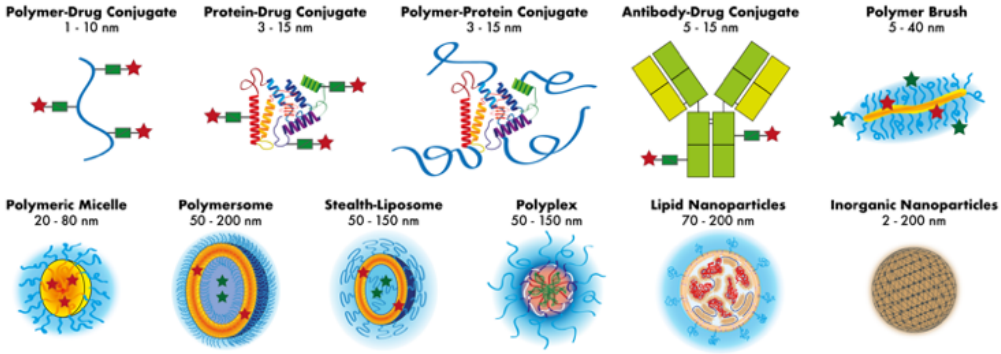


Figure 7. Schematic overview of the most common drug delivery systems in nanomedicine with approximate hydrodynamic diameters. Small molecule drugs are represented by red (hydrophobic) and green (hydrophilic) stars, RNA/DNA, drug linkers by dark green rectangles.

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.[148,173,177] In particular, self-assembled nanoparticles are characterized by their core-shell architecture and can be readily formed from amphiphilic lipids or polymers.[178–180] 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.[180–182] 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.[173,183,184] While PEG is still the most frequently used polymer, the polypeptoid pSar reduces the immunogenicity even further. For therapeutic inventions using hydrophobic small molecule APIs, polymeric micelles (PMs) are among the most promising carrier types.[182,185–187] Herein, the core compartment allows for drug loading and can be designed and adjusted for the conjugation of (pro-)drugs featuring stimuli-responsive release.[188–193] 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.[145,194–197] In contrast, polyplexes and LNPs 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.[21,154,157,173,198] Besides lipid- and polymer-based nanocarriers, inorganic nanoparticles, colloids, and metal-organic frameworks have been established for drug delivery and diagnosis.[26,199–202]

Physiological Barriers for Nanomedicine

On the journey to the target site, nanomaterials face several barriers and obstacles for successful drug delivery.[133,203,204] In general, the majority of nanocarriers are applied by parenteral administration routes.[205–207] Activation of the adaptive immune system typically follows intramuscular injection for addressing transport into the draining lymph node.[208–210] In contrast, for cancer therapy, nanomedicines are mainly administered by intravenous injection aiming to target metastasis as well as the primary tumor site.[211–213] 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.[214–217] Stabilization and shielding strategies are thus required to prevent unspecific complement activation, opsonization, and recognition by the MPS.[133,181,217,218] The MPS consists of bone marrow progenitors, monocytes and tissue macrophages located in organs such as the liver and spleen.[218,219] 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 8**).[219,220]

Strategies to avoid rapid clearance comprise the decoration of nanocarriers with hydrophilic polymers, such as PEG, polyoxazolines (POx) or pSar, which follow the Whitesides' rules for protein resistant materials and reduce MPS recognition, phagocytosis, and clearance from the circulation.[2,24,42,221,222] Mechanistically, the enhanced repulsive forces among the hydrated polymer strands form an impermeable coating preventing van der Waals, electrostatic and hydrophobic interaction with proteins.[181,223] 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).[183,224,225] Herein, the molecular weight of the hydrophilic polymer significantly influences the shielding efficiency.[223] 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 °C)), and PEG_{2k}(DP = 50)-lipid (lipid: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; DSPE) was selected considering circulation time and lipid metabolism.[145] For block copolymer micelles, Kwon *et al.* reported that PEG_{12k} (DP = 270) significantly reduced the nanoparticle clearance compared to shielding by PEG_{5k} (DP = 120), resulting in a 5-fold increase of nanoparticles in circulation after 4 h post-injection.[226,227] These results underline that polymer chain lengths between 50 and 300 are particularly interesting.

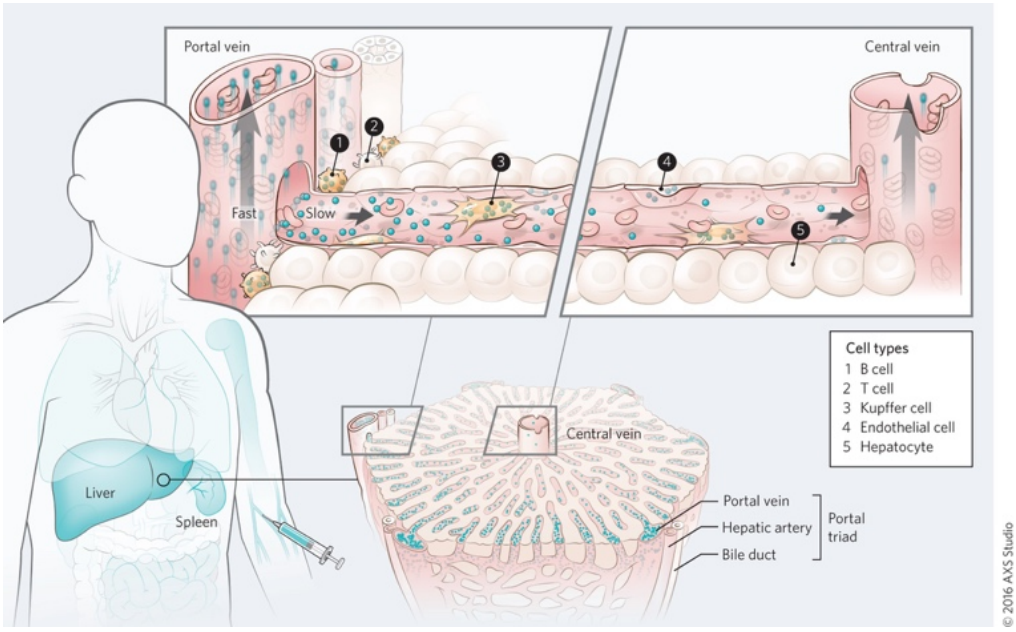


Figure 8. Intravenously administered nanoparticles encounter non-specific interaction with the MPS. The intensity of the turquoise color refers to the nanoparticle uptake within each organ. The reduced flow rates in the liver sinusoid facilitates nanoparticle uptake by the residing immune cells, e.g., Kupffer cells. Figure reprinted from Tsoi *et al.* with permission.[219]

Beyond the surface properties, 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.[149,151,182] Contrariwise, glomerular filtration in the kidneys defines the lower size limit for nanoparticles that aim for long blood circulation.[133,228] 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.[229–231] 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.[170,225,232] Having a biodegradable stealth-like polymer would therefore be highly advantageous.

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.[159,233,234] 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.[235] After detailed elucidating studies in tumor-bearing mice using ^{51}Cr-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.[236] 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.[173,236,237] Moreover, insufficient lymphatic drainage reduces nanoparticle clearance from the tumor retaining the accumulated particles.[236] Consequently, if unspecific excretion and interaction with the MPS can be prevented, passive accumulation of the nanocarriers can be achieved.[162,173,238] This passive accumulation correlates *vice versa* with circulation time of nanoparticles, which in turn underlines the importance of stealth-like polymers in nanomedicine.

Nevertheless, the EPR effect has been critically discussed, and cannot be seen as a general concept for all tumor types.[159,239–242] As such, the EPR effect was reported to be more prominent in murine (xenograft) tumor models compared to human patients.[243,244] 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.[245,246]

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.[204,247–249] Herein, Chan and co-workers employed PEGylated gold nanoparticles to investigate the exact mechanism accounting for nanoparticle entry into tumor tissue.[250,251] 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.[250] Moreover, a specific type of endothelial cells, nanoparticle transport endothelial cells (N-TECs), was identified as a gatekeeper for the transport process.[251] On the other hand, Biancacci *et al.* combined optical whole-animal imaging by micro-computed tomography-fluorescence tomography (μCT-FLT) with immunohistochemistry for a detailed biodistribution analysis of dye-labeled core cross-linked polymeric micelles.[252] 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.[252] This underlines the potential of nanomedicine for therapies combining chemotherapy and immune modulation.[248,252–255]

Polypept(o)ides in Nanomedicine

Since the term polypept(o)ides was coined in 2014 and first reviewed by Klinker *et al*. in 2015, several polypept(o)ide-based polymer and nanoparticle architectures and assembled constructs have been established and investigated for therapeutic applications.[2] In these systems, the hydrophilic pSar provides the desired solubility and prevents recognition of the structures by the MPS, while the polypeptide section can be selected and tuned for drug or gene delivery.[2] As polypept(o)ides are easily accessible by the mild chemical conditions of the NCA/NNCA 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.

Polymeric Micelles

In their initial publication from 2014, Birke *et al.* reported that for pSar-*b*-pGlu(OBn) and pSar-*b*-pLys(Z) of elevated molecular weights, the succession of the block copolymerization (block sequence) is not relevant for the properties of the final block copolymers, and polymers with precise control over molecular weight and narrow dispersity (Đ < 1.2) can be obtained in all cases.[1] The amphiphilic copolymers were then applied for stabilization of organic colloids and for encapsulation of small molecules like paclitaxel[258] or the adenylate cyclase (cAMP) inhibitor MDL-12.330A into PeptoMicelles.[1,259] Just recently, Johann *et al.* demonstrated the potential of the cAMP inhibitor-loaded PMs, formed by dual-asymmetric centrifugation **(Figure 9A)**. Herein, MDL-12.330A **(Figure 9B)** is stabilized in the micelle core by π-π-interactions with the aromatic benzyl groups.[1,260] Peritumoral injection of loaded PMs presented dose-depended suppression of cAMP levels within the melanoma tissue, leading to reduced tumor growth **(Figure 9C)**. Importance of immune infiltration could be demonstrated by non-effective treatment of adaptive immune cell-depleted mice (DEREG). Combinatorial treatment then achieved full tumor rejection and long-term immunity **(Figure 9D)** in a B16F10 melanoma model, which underlines both, the remarkable potency of this approach and the often underestimated potential of small molecules for tumor immune therapy. Furthermore, mild synthetic conditions of the applied sequential NCA ROP enabled the use of a mannose-containing initiator, leading to mannose-decorated micelles. Uptake into DC2.4 and bone marrow-derived dendritic cells (BMDCs) was increased and discovered to be mannose-receptor depended, indicating an active targeting approach.[257]

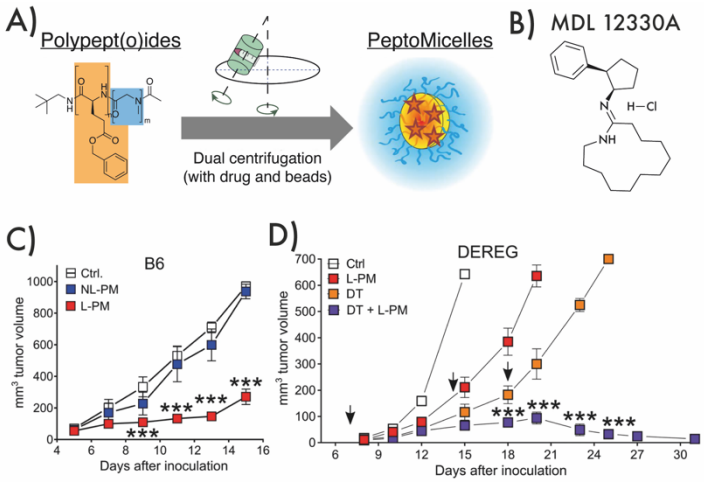


Figure 9: A) Illustration of micelle formulation and drug loading by dual-asymmetric centrifugation. B) Chemical structure of MDL-12.330A. C) Inhibition of B16 melanoma growth by peritumorally injected MDL-loaded (red, n=6)/non-loaded (blue, n=5) micelles in C57BL/6 mice (as mean from three independent experiments). D) Similar to C) with DEREG mice and additional treatment with diphtheria toxin (DT) at day 7, 14 an 18 (arrows) (as mean from two independent experiments). Reprinted with permission from Johann et *al.*[260], © 2021, Springer Nature.

Besides an application in tumor-immune therapy pSar-*b*-pGlu(OBn) polymeric micelles have been employed to encapsulate pretomanid derivatives for tuberculosis therapy. The PeptoMicelles loaded with antibiotics showed remarkable stability in water, blood plasma, and lung surfactant as demonstrated by fluorescence cross-correlation spectroscopy and displayed prolonged circulation times of several days in zebrafish larvae and mice.[261] The most efficacious PeptoMicelle formulation eradicated the bacteria at non-toxic doses in the zebrafish larvae infection model and significantly reduced bacterial burden and inflammatory responses in the lungs and spleens of infected mice, which underlines the potential of core cross-linking of micelles by non-covalent π-π-interactions.[261]

The combination of pSar with functional polyglutamic acid derivatives further led to micellar structures enabling simultaneous encapsulation and cross-linking by metal-based drugs. Herein, cisplatin was integrated into a polypept(o)ide-based delivery system resembling NC-6004 that was developed in the Kataoka lab using PEG-*b*-pGlu(ONa).[238,262,263] As reported recently by Siemer *et al.*, masking cisplatin in

polymeric micelles of pSar-*b*-pGlu(ONa) resulted in a well-tolerated nanomedicine that enabled an alternative endocytic cell-uptake mechanism which mediated effective toxicity for LRRC8A-downregulated tumor cells circumventing cisplatin resistance.[264] Beyond platinum-based metal drugs, light-sensitive ruthenium(II) complexes granted fabrication of photocleavable PMs, demonstrating enhanced circulation times *in ovo* and releasing the drug upon irradiation as an external stimulus.[265] To reversibly conjugate ruthenium(II) complexes the polyglutamic acid block was modified with aromatic nitrile linkers that also directed self-assembly into either spherical or worm-like micelles. In addition to the stabilization of organic colloids and integration of metal complexes, polypept(o)ides based on pSar-*b*-pGlu(ONa) have also been applied for the shielding of metal-organic frameworks (MOFs), metal-oxide surfaces.[118,200,266]

With the purpose of making core cross-linked polypept(o)ide micelles biodegradable and responsive to intracellular glutathione levels, Huesmann *et al.* introduced reactive *S*-alkylsulfonyl protecting groups for thiol-containing amino acids such as cysteine and homocysteine.[267–270] The *S*-ethyl- and *S*-isopropylsulfonyl protective group combines stability towards amines (hard nucleophiles) and provides a high reactivity towards thiols (soft nucleophiles), which enables their use in NCA/NNCA polymerization followed by self-assembly in water and rapid bioreversible core cross-linking by chemo-selective disulfide formation.[191] In combination with functional di- or olgio thiols containing cross-linkers, nanoparticle morphology and core- and surface functionality could be controlled in a single step.[271] Access to a wide range of payloads from hydrophobic drugs such as paclitaxel to hydrophilic small interfering RNA (siRNA) was thus created.[191,271] Of note, the secondary structure formation of the polypeptide segment governs the nanoparticle morphology and can be selected and tuned to define the desired shape.[191,272]

The synthesis of multifunctional nanoparticles is commonly a complex endeavor that complicates the clinical translation of effective nanomedicines. To tackle this issue, Bauer *et al.* recently presented a microfluidic setup based on the aforementioned thiol-reactive pSar-*b*-pCys(SO_2Et) polypept(o)ides and a combination of slit interdigital micromixers and tangential flow filtration.[273] The setup enabled the

synthesis of core cross-linked polymeric micelles (CCPMs) in a continuous flow process that combined the commonly separated steps of micelle formation, core cross-linking, functionalization and purification into a single process. The precisely controlled microfluidic self-assembly process allowed the production of 350-700 mg of spherical CCPMs/h (D_h = 35 nm) with low polydispersity values (PDI < 0.1) while the online tangential flow filtration removed impurities (unimer ≤ 0.5%). In the given setup, the CCPMs displayed an ideal screening tool, as drug conjugation can be performed in an independent step. As a proof-of-concept, paclitaxel was conjugated to the micellar core via pH-sensitive hydrazone bonds. These paclitaxel-loaded CCPMs showed the desired pH-responsive release profile, stable drug encapsulation and an improved toxicity profile compared to Abraxane, and therapeutic efficiency in the B16F1-xenotransplanted zebrafish model.[273]

Just recently Bauer *et al.* transferred the concept of CCPMs and presented these structures as basis for encapsulation of superparamagnetic iron oxide nanoparticles (SPIONs) and their potential for macrophage activation.[274] Colloidal SPION nanoparticles were encapsulated by co-self-assembly with pSar-*b*-pCys(SO_2Et) diblock polypept(o)ides and subsequently treated with dihydro lipoic acid, simultaneously stabilizing the PM by disulfide core cross-linking and the cargo by surface interaction of the carboxylic acid with the inorganic nanoparticle surface **(Figure 10A)**. Bioreversible cross-linking was nicely visualized by treatment with intracellular glutathione levels (10 mM) causing the precipitation of iron oxide/phosphate, whereas minor effects were observed at extracellular reductive conditions (10 µM). Interestingly, treatment with SPION-loaded and non-loaded CCPMs on a co-culture of primary murine bone marrow-derived macrophages (BMDMs) and Lewis lung carcinoma cells (LLCs) resulted in a significantly higher accumulation of SPION-loaded micelles in BMDMs, whereas non-loaded micelles preferably ended up in LLCs. **(Figure 10B)**. On the cellular level, efficient iron delivery by CCPMs into BMDMs induced pro-inflammatory response by upregulating cytokines and characteristic surface proteins as summarized in **Figure 10C.** Identical effects could be observed in human macrophages **(Figure 10D**), pointing out the therapeutic potential of such systems. Finally, the effect was confirmed *in vivo*.

Intratracheal administration of SPION-loaded micelles to wild-type C57Bl/6N mice lead to elevated iron levels in lung macrophages and increased pro-inflammatory cytokines and surface markers **(Figure 10E)**. The immunomodulatory properties were even able to outperform commercial iron sources like Feraheme, and therefore display a promising approach as adjuvant in cancer therapy calling for combination with other therapies.

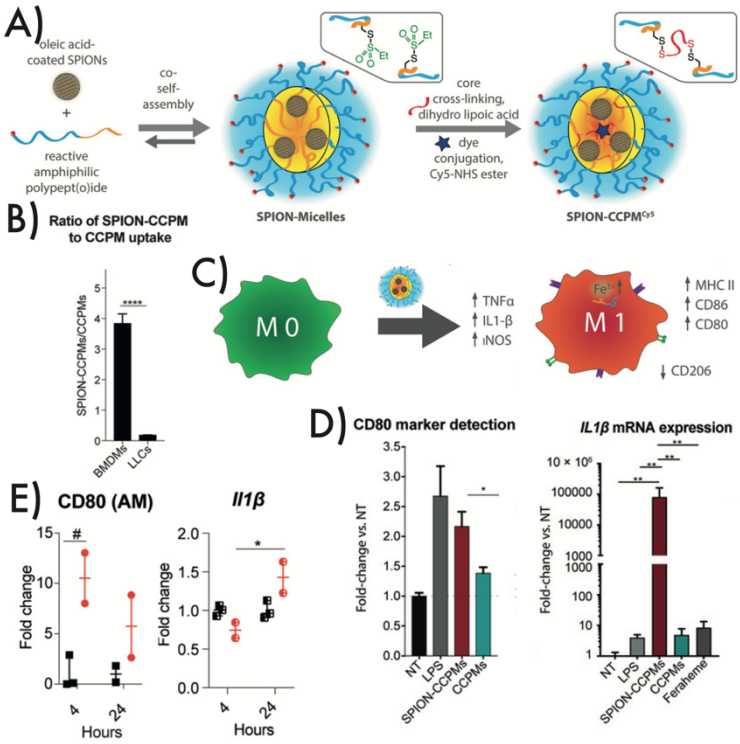


Figure 10: A) Illustration of the synthesis of SPION-loaded CCPMs by co-self-assembly and subsequent cross-linking. B) In co-cultures of LLCs and BMDMs, SPION-CCPMs show significantly higher uptake in BMDMs compared to LLCs. Inverse effect for CCPMs without iron, as analyzed by FACS 24 h after incubation. C) Illustrative summary of SPION-CCPM-induced inflammatory response of macrophages. D) *In vitro* upregulation of inflammatory surface marker and cytokine levels as measured by protein and mRNA expression in human macrophages (compared to non-treated condition or corrected to *Rpl19*, respectively; as mean from three independent experiments). E) *In vivo* evaluation, 4 and 24 h after intratracheal administration of SPION-CCPMs to C57Bl/6 mice (red), compared to PBS treatment (black); represented by increased cell-surface marker CD80 in alveolar macrophages (left) and mRNA expression of pro-inflammatory cytokines Il1β in lung tissue (right). Reprinted from Bauer *et al.*[274], © 2021, with permission from Wiley-VCH GmbH.

Polymersomes

Besides PMs as self-assembled structures from amphiphilic block copolymers, polymersomes, so-called Peptosomes, have been established from polymers with higher ratio of the hydrophobic segment by Tanisaka *et al.* using pSar-*b*-pGlu(OMe).[275] By representing one of the first applicational approaches of polypept(o)ides, near-infrared fluorescent labeled Peptosomes were used for cancer imaging in SUIT2/EF-luc xenograft tumor bearing-mice upon tail vein injection. Retention in the bloodstream were comparable to PEGylated liposomes and pronounced accumulation was detected in tumor tissues.[275] Contrary to classical micellar constructs, Peptosomes further allow for encapsulation of hydrophobic and/or hydrophilic cargo due to their vesicular structure. Later, Weber *et al.* utilized pSar-*b*-pGlu(OBn) to prepare Peptosomes encapsulating the antigen (SIINFEKL) and the adjuvant (CpG) at the same time, aiming for a vaccination approach. The authors demonstrated the activation of BMDCs, characterized by enhanced expression of activation markers and cytokine excretion. Additionally, antigen specific T-cell proliferation was observed, indicating processing of the loaded antigen by BMDCs.[276]

Non-Viral Delivery Systems for Nucleic Acids

Due to the remarkable success of mRNA vaccines against SARS-COV2 infections, lipid- and polymer-based delivery systems for nucleic acids have received widespread attention in pharmaceutical industry and research.[152,277–279] The strong anionic character of RNA or DNA requires cationic counterparts to form a complex by electrostatic interaction. Therefore, various lipid- or polymer-based delivery systems have been developed and tested, culminating in the final mRNA lipid vaccines BNT162b2 "Comirnaty" (BioNTech/Pfizer) and mRNA-1273 "Spikevax" (Moderna). While during the broad application of such vaccines initial concerns regarding the immunogenicity of PEGylated lipids have been raised, substitutes for PEG have been investigated and in particular pSar has shown an improved immunological profile.[23,24,54,123]

Besides lipid-based structures, polymer-based non-viral delivery systems for nucleic acids have been developed utilizing polypept(o)ides.[21,64,198,280] In the first publication from 2014, Heller *et al.* reported on the synthesis and evaluation of pSar-*b*-pLys diblock structures to complex plasmid DNA (pDNA) into so-called PeptoPlexes. Transfection ability was demonstrated *in vitro* by dose-depended eGFP expression of HEK293T cells without toxic side-effects.[281] Further development of the polymeric architecture created triblock copolymers by insertion of a second pLys block with orthogonal protective moieties, namely TFA and Boc. Thus, sequential deprotection gave access to partial and selective functionalization of the complexing segment.[280] In this respect, the significance of the block ionomer microstructure on the formation and transfection efficiency was investigated based on random or block-wise alteration of the cationic block with pH-responsive imidazole groups. Supported by simulation studies, block structures were most effective in protecting pDNA from degradation and lead to highest transfection efficiencies, outperforming the randomly modified copolymers.[198]

Following these sequentially deprotectable triblock structures, Heller *et al.* further reported on disulfide-mediated stabilization by cross-linking structures, introduced by succinimidyl 3-(2-pyridyldithio)propionate (SPDP), a concept originated within the Kataoka group's research.[198,282] Introducing this functional group enabled bioreversible cross-linking by multifunctional cross-linkers, e.g., virus derived endosomolytic peptides, containing two or more thiols for bioreversible covalent stabilization of polyplexes (**Figure 11A)**. A few examples of cross-linker structures are displayed in **Figure 11B**. Dynamic light scattering (DLS) experiments in human blood plasma as model for an *in vivo* environment revealed prevention of aggregate formation upon applying the cross-linking step **(Figure 11C)**, whereas non-cross-linked PeptoPlexes showed the same significant aggregation caused by interaction with plasma proteins like LNPs or lipoplexes. By building up a library of PeptoPlexes introducing different cross-linker molecules, triethylenetetramine dicysteine (cTETAc) was identified as candidate with the best balance of stability, transfection and toxicity, and therefore applied for further application as pDNA vaccine formulation for dendritic cell-induced T cell stimulation **(Figure 11D)**. After transfection of DC2.4 cells with

OVA-encoding pDNA by the PeptoPlex approach, cells were co-cultured with OVA-responsive $CD8^+$ T cells to evaluate stimulation. While polypept(o)ide material itself did not induce any detectable immune response, OVA-pDNA transfected cells induced strong T cell proliferation, even after pre-incubation with native mouse serum to mimic *in vivo* conditions. Stability, non-toxicity, pDNA transfection efficiency, specific immune activation by the cargo and no influence after serum incubation display the PeptoPlexes' potential as pDNA vaccine approach.[280]

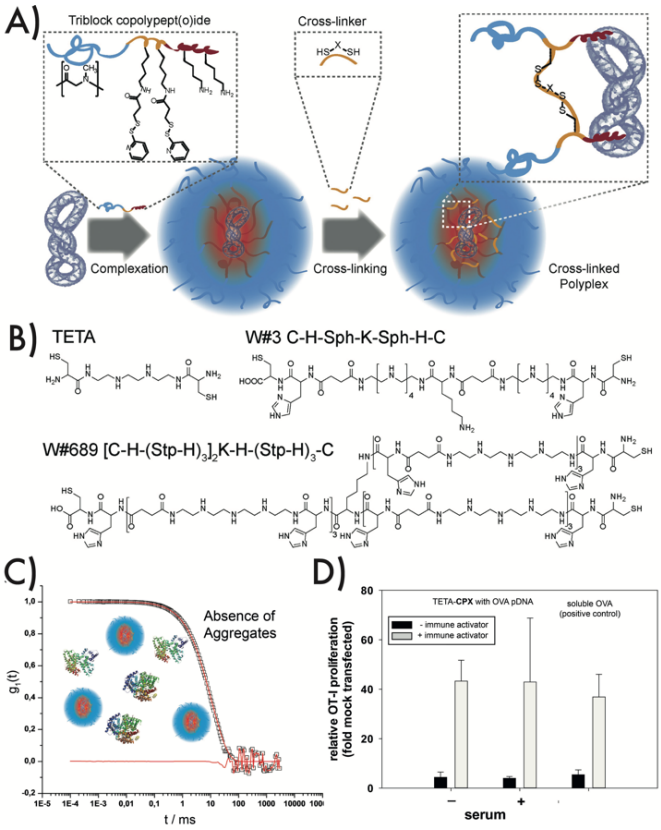


Figure 11: A) Illustration of PeptoPlex formation by triblock copolypept(o)ides and pDNA with subsequent cross-linking step. B) Exemplary selection of evaluated disulfide cross-linker structures. C) DLS in human blood plasma of cross-linked PeptoPlexes. D) OT-I T cell proliferation upon co-culturing with transfected DC2.4 cells. DC2.4 cells were transfected with OVA encoding pDNA complexed by TETA-cross-linked PeptoPlexes or soluble OVA protein, either stimulated with immune activator LPS or left untreated prior to transfection. PeptoPlexes were partially incubated with native mouse serum 1:1 (v/v) (n=3). Reprinted from Heller *et al.*[280], © 2017, with permission from Elsevier.

Following a similar strategy, Capelôa *et al.* implemented siRNA as cargo, by encapsulation into Polyion Complex Micelles (PICMs) comprising pSar-*b*-pCys(SO_2Et)-*b*-pLys triblock copolymers. Thus, introduction of a cross-linkable block-structure from previously mentioned micellar approaches was combined with the complexing abilities of PeptoPlexes.[66] This approach avoids the post-polymerization modification used by Heller *et al.* and allows for the direct chemo-selective formation of asymmetric disulfides stabilizing the nucleic acid complexing vehicle.

Molecular Nanocarriers: PeptoStars

Beyond self-assembled structures, also molecular nanocarriers have been prepared from polypept(o)ides.[67,283] Compared to their self-assembled counterparts, molecular nanocarriers are chemically synthesized and defined nanoparticles, whereby size and stability can be precisely controlled by chemical synthesis. In particular, carrier systems with small hydrodynamic diameters, high stability or defined architecture can easily be prepared by these techniques.[2,177,284] Since small nanoparticles just above the renal filtration limit (> 10 nm) have shown superior passive or active tumor accumulation and penetration as explained above, star-like polymers seem to be ideal candidates for drug delivery to solid tumors, even though drug content per particle will be limited.[237,285,286]

In 2017, Holm *et al.* introduced PeptoStars with Poisson-like molecular weight distribution and small hydrodynamic diameters.[69,71,283,287] Primarily, a library of 3-arm structured polymeric star architectures was presented. The core-first method was used, starting from trifunctional initiators with or without GSH-responsive cleavable moieties. PSar-stars as well as core-shell structured 3-arm star architectures comprising a pLys core exhibited hydrodynamic diameters of 4 to 10 nm as determined by DLS and visualized by atomic force microscopy (AFM). Degradation studies cleaving off single arms by elevated GSH levels revealed homogenous and uniform arms, confirming a controlled synthetic approach.[69] In a follow-up study, riboflavin targeting moieties were utilized to enhance cellular internalization *in vitro* as well as *ex* and *in vivo.* Non-targeted and targeted 3-arm PeptoStars displayed an identical safety, circulation and biodistribution profile in balb/c tumor-bearing mice

upon intravenous administration, indicating successful shielding by the pSar corona. Moreover, riboflavin-conjugated structures presented higher cell-internalization within the tumor tissue demonstrating the effects of ligand-modification on passive and active tumor targeting.[286] Further, 6-arm star architectures based on pSar-*b*-pLys arms have been utilized as siRNA carrier system, presenting slightly increased hydrodynamic diameters of up to ~20 nm but still unimolecular appearance, even upon loading with siRNA.[283] However, the impact of branching revealed its importance when the peptidic core-material switched into hydrophobic, displayed by Holm *et al.* with pSar-*b*-pGlu(O*t*Bu) arm structures. Interestingly, supramolecular assembly in aqueous solution and human citrated plasma was observed for 3-arm but not for 6-arm structures. Further, fluorescence correlation spectroscopy (FCS) in human blood plasma revealed an effect on the shielding efficiency, since significant protein adsorption was observed for the 3-arm PeptoStars yet not for the 6-arm analogon.[71] Following research by Schwiertz *et al.* and Capelôa *et al.* was focused on miktoarm star architectures, while consequences on the structure-activity relationship for biomedical applications are the scope of current investigations.[70,72]
Skoulas *et al.* investigated 8-arm core-shell star structures based on a poly(propyleneimine) dendrimer initiator and pSar-*b*-pGlu(OBn) arms **(Figure 12A)**. The tendency of aggregation could also be observed by high hydrodynamic diameters in DLS and shapes of elongated particles in TEM images, even for 8-arm PeptoStars. Most likely, this can be attributed to the strong hydrophobic character of pGlu(OBn) and additional aggregation driving forces like π-π-stacking and secondary structure formation. Nevertheless, increasing the sarcosine shell size by higher pSar chain lengths counteracted to this effect resulting in smaller particles **(Figure 12B)**. The authors evaluated the potential of this system to tackle the challenging task of overcoming mucus and epithelial barrier to enable non-invasive administration. As *ex vivo* model, FITC-labeled 8-arm PeptoStars presented ability to penetrate through a polycarbonate membrane. An artificial mucus layer indeed lowered the speed but couldn't hinder membrane penetration **(Figure 12C)**. Finally, transepithelial permeability and thus therapeutic potential of this approach was evaluated by rat jejunal mucusae mounted in Franz diffusion cells and apical-side addition of the

polypept(o)ide stars. The apparent permeability coefficient (P_{app}), displayed in **Figure 12D**, revealed dose-dependent barrier crossing, comparable to FITC-labeled dextran (FD-40), but still almost a magnitude lower than positive controls like ketoprofen, a small molecule well-known for its high oral bioavailability. Histological investigations confirmed material safety by not detecting tissue damage upon 2 h incubation. Overall, regardless of the underlying mechanism, the ability of penetrating and crossing mucus and epithelial barrier could be demonstrated.[73] Just recently, the researchers extended their 8-arm star-design by presenting core-shell star-polymers with pSar-pLys arms in both sequences as well as randomly structured. The authors demonstrated the ability of DNA-complexation by the polycationic structure as well as the ability of mucus penetration. Interestingly, the authors detected a strong structural dependency of the star-polymers on trypsin-mediated degradation. Increased levels of free lysine amino acid were detected after 48 h with a pLys outer layer in comparison to architectures with pLys inner layer, demonstrating the shielding pSar-effect. Lowest levels, however, were detected at the randomly structured stars.[288]

In the aim of increasing arm quantity of star-architectures, England *et al.* used lysine dendrimers as multifunctional initiators for sarcosine-NNCA ROP.[289] With generation-five dendrimers 32-arm star polypept(o)ides were accessible. By increasing pSar chain lengths (28, 56 and 100) the hydrodynamic diameter grew from 10 nm to 18 nm, indicating unimolecular star polymers. Prolonged circulation half-lives of up to 88 h have been observed, correlating with increased pSar chain length. Further, conjugation of the topoisomerase inhibitor SN-38 revealed great potential as anticancer treatment upon systemic injection of tumor-bearing mice (subcutaneous colorectal SW620 xenografted tumor model) by significant reduction of total tumor volume without loss of body weight. Further, Yu *et al.* just recently synthesized lysine based dendrimeric structures of different generations, to which a linear pSar was introduced yielding polypept(o)ide telodendrimers. Subsequent conjugation of the hydrophobic drug fulvestrant generated amphiphilic polymers and enabled the formation of micelles with high drug loading and tunable size.[75]

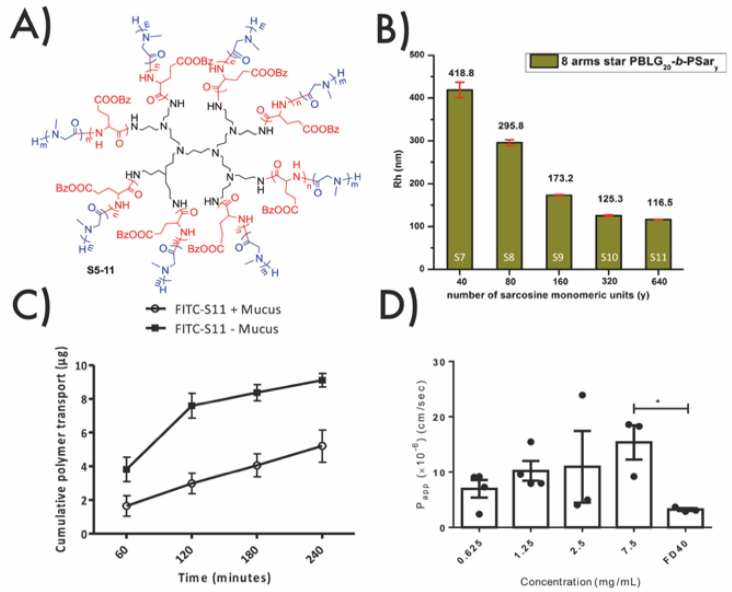


Figure 12: A) Chemical structure of investigated 8-arm polypept(o)ide star architectures. B) Correlation of hydrodynamic radii of polymeric stars with increasing chain length of polysarcosine (S7 - S11) as evaluated by DLS in water at 5 g·L^{-1}. C) Passage of FITC-labeled S11 star-polymers through a polycarbonate membrane in the presence (circles) and absence (squares) of artificial mucus correlated to the time. D) P_{app} of FITC-labeled S11 at different concentrations and FD40 (2.5 g·L^{-1}) across isolated rat jejunal mucosae. Reprinted from Skoulas *et al.*[73] with permission, © 2020 American Chemical Society.

Molecular Nanocarriers: PeptoBrushes

Besides star-shaped polymers, research has also been conducted on the synthesis of cylindrical bottlebrush polypept(o)ides (PeptoBrushes). Here, grafting-onto as well as grafting-from strategies have been established.

Stéen *et al.* designed PeptoBrushes based on pGlu backbones with *trans*-cyclooctene (TCO) functionalization at the side chains (approx. 7.5% to 30%) and pSar polymers grafted onto residual pGlu functions **(Figure 13A**) to enable pre-targeted imaging and therapy by *in vivo* click chemistry. More precisely, the *trans*-cyclooctene tetrazine (Tz) ligation *via* inverse electron demand Diels-Alder ligation was used.[68] The highest degree of TCO-functionalization (PeptoBrush 1) encountered an enhancement of the reaction rate constants by two orders of magnitude compared to a simple water-

soluble TCO derivative, as displayed in **Figure 13B**. Supported by simulation studies as well as a positive correlation between lipophilicity of the Tz derivative and the rate constant, the authors describe a specific function since the microphase separation of the TCO-modified amino acids undergo local clustering, which leads to an enhanced reactivity of TCOs by creating binding sides for lipophilic Tz-frameworks. Concurrently, the PeptoBrush demonstrated structural integrity in human plasma without appearance of aggregated products and further enabled an increased TCO-moiety half-life, outlining pSar-mediated shielding and protection. For pre-targeted imaging and therapy, the TCO-PeptoBrush is administered first, whereby the small size (≈ 10 nm) slightly above the value for renal filtration ensures optimal tumor tissue penetration.[228,290] When either tumor accumulation was highest (therapy) or blood levels were lowest (diagnosis), since non-accumulated PeptoBrushes were already excreted, a Tz-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 Tz and TCO. The seminal pre-targeting approach thus decoupled the tumor accumulation of the radiolabeled probe from the imaging step leading to enhanced contrast and reduced radiation exposure. The designed PeptoBrush was examined as excellent primary targeting agent, presenting passive tumor accumulation values of up to 5.1 ± 0.3 %ID/g (22 h p.i.) upon intravenous injection of a [^{111}In]-labeled probe into CT26 tumor-bearing mice (mouse colorectal cancer), detected by single-photon emission computed tomography (SPECT) (**Figure 13C**). Under optimized conditions for pre-targeted imaging the TCO-PeptoBrush enabled adequate image contrast of the tumor already 2 h after administration of the radiolabeled Tz (**Figure 13D**). Particularly at early timepoints the pre-targeting strategy outperforms conventional imaging by resulting in higher image contrasts. Future developments will be directed to reduce the amount of residual PeptoBrush circulating in the blood by masking or clearing agents between the two steps to further increase the tumor-to-background ratio in imaging and directed effect of radionucleotide therapy.

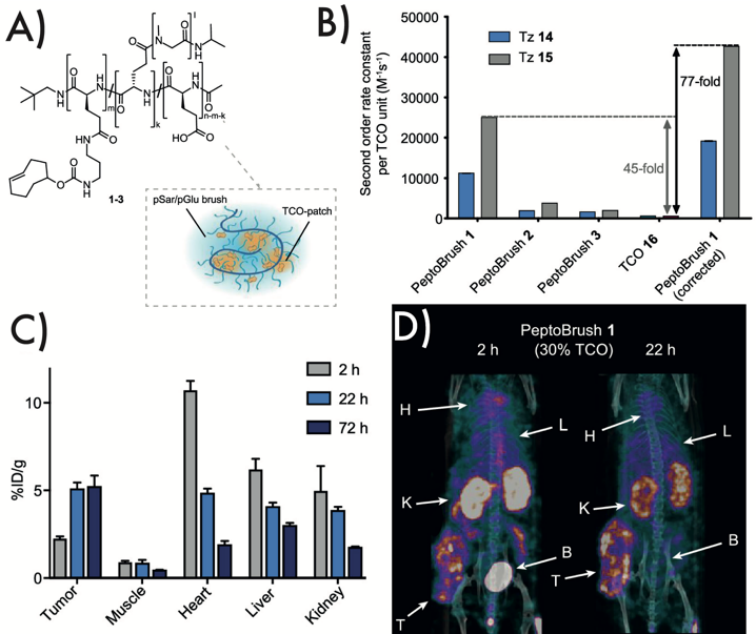


Figure 13: A) Chemical and illustrative structure of TCO-modified PeptoBrushes synthesized by grafting-onto approach. B) Reaction kinetics for the Tz ligation of different PeptoBrushes with fluorogenic "turn-on" Tz 14 (HELIOS 347Me) and Tz 15 (HELIOS 388Me) compared to water-soluble TCO-derivative 16 (n=5). "Corrected" data were related to single TCO-units. C) SPECT-image derived mean *in vivo* uptake values of [^{111}In]-modified PeptoBrush in tissues (n=4) for evaluation of primary targeting abilities. D) Representative SPECT/CT images of pre-targeted imaging with PeptoBrush1 under optimized condition 2 h and 22 h p.i. H=heart, L=liver, K=Kidney, B=bladder, T=Tumor. Reprinted from Steen *et al.*[68], © 2019, with permission from American Chemical Society.

Of note, the PeptoBrush-assisted TCO/Tz click reaction is among the fastest bio-orthogonal ligation techniques, directing towards new drug release strategies (click-to-release) for tissue-selective drug release by functional nanomedicines. Interestingly, polypept(o)ide-based micelles could not provide the unique combination of enhanced stability and higher TCO reactivity, but have been investigated with respect to microstructure variation by Johann *et al.*.[291] The authors utilized the TCO/Tz click reaction for a chemical ligation approach to form pSar-*b*-pGlu(OBn) from homopolymers synthesized with respective functional initiators. Moreover, the synthesis of an end group Tz-functionalized diblock copolypept(o)ide enabled the

formation of Tz-decorated PMs as well as organic colloids, displaying interesting candidates as masking or clearing agents in pre-targeted imaging or therapy strategies. Further, synthetic strategies have been developed for [^{11}C]-labeling of Tz-derivatives and thus the modified PeptoBrushes. Thereby, PeptoBrush's *in vivo* fate upon injection was simplified to follow by the utility of positron emission tomography (PET).[68]

Besides the grafting-onto approach, which leads most of the time to spherical or slightly elongated polymer nanoparticles, the grafting-from approach has been realized first by Hörtz *et al.* in 2015.[67] Here, cylindrical brush polymers were synthesized by utilizing PAHMA and pLys as backbones and multi-amine structures to initiate ROP of sarcosine-NNCA to generate the grafted pSar side chains. Moreover, the synthesis of core-shell brush-architectures has been described with PAHMA as backbone by sequentially polymerizing Lys(TFA)-NCA and sarcosine-NNCA, and thus creating brushes with polypept(o)idic side chains. Upon TFA-deprotection, siRNA complexation was achieved by the polycationic core, enabling moderate knockdown efficiencies of ApoB100 mRNA in AML-12 hepatocytes in low-protein medium. However, reduced efficiency was observed in high-protein medium, correlating with reduced cellular uptake, likely caused by protein interaction of the secondary amine of pSar side chains.

This synthetic methodology was further improved and applied to pLys backbones with higher molecular weights with the vision to develop molecular vaccines based on cylindrical bottle brush polymers. The desired molecular vaccines shall enable the directed delivery of defined amounts of antigen and adjuvant to induce either antigen specific immunity or immune tolerance. Therefore, antibodies have been applied to provide specificity for specific immune cell types, e.g., dendritic cells or macrophages. On the way to develop such a molecular vaccine, Kappel *et al.* recently used PeptoBrushes based on a pLys backbone with a dense pSar corona generated by grafting-from approach **(Figure 14A)** to demonstrate the significance of an average number of antibodies for active targeting of nanoparticles to dendritic cells *in vivo*.[292,293] Therefore, fluorescently labeled PeptoBrushes with grafted $pSar_{100}$ side chains were precisely engineered to bear on average either 2, 6, or 12 antibodies

(anti-DEC205) per nanoparticle (R_h ≈ 23 nm), as pictured in **Figure 14B**. Interestingly, circulation half-life evaluation revealed 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 **(Figure 14C)**. Conversely, in biodistribution experiments, low amounts of anti-DEC205 were efficient for targeting the cells of the lymphoid organs bypassing liver accumulation, while high amounts of anti-DEC205 forced accumulation in liver and spleen **(Figure 14D)**. Representing a platform to effectively and selectively accumulate in lymphoid organs, the research on these PeptoBrushes displays a suitable foundation for future development of systemic cancer vaccination strategies.[292]

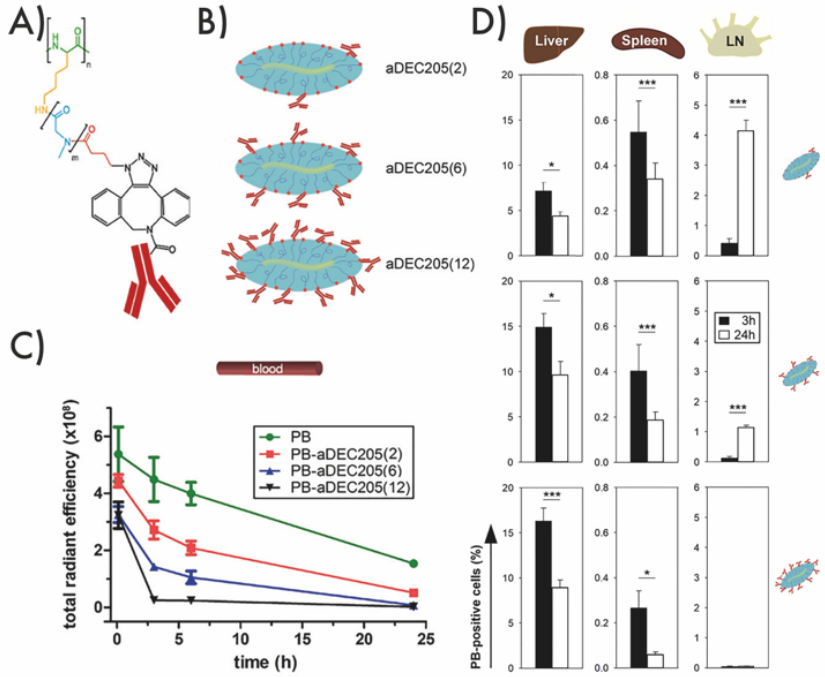


Figure 14: A) Chemical and B) illustrative structure of PeptoBrushes by grafting-from approach, decorated with variable amounts of aDEC205. On average 2, 6, and 12 antibodies were conjugated per PeptoBrush and analyzed for biodistribution and circulation time. C) Estimation of antibody-density-related liver uptake upon systemic injection of CW800-labeled PeptoBrushes by fluorescence intensities in blood at different timepoints (n=3). D) Flow cytometric *ex vivo* quantification of systemically administered PeptoBrushes in liver, spleen, and lymph nodes (LN), 3 h and 24 h p.i. (n=6-8, obtained from 2-3 experiments). Reprinted with permission from Kappel *et al.*[292]© 2021 American Chemical Society.

Other Applications

Despite their major application as delivery platform in the field of nanomedicine, polypept(o)ides have already reached out into other applicational fields to deploy their potential as multifunctional material class. Considering the implication of pSar's stealth-mediating effect on surfaces in nanomedical approaches, most evident applicational extension is the control of macroscopic surface properties. Especially for biomedical devices, nonspecific protein adsorption, cell attachment and inflammatory reactions as biological responses as well as bacterial infections display major hurdles for their utility.[294,295] The design of antifouling and antibacterial surface coatings thus enables improved performances of these devices. Messersmith and co-workers therefore created pSar 10- and 20-mer coatings for titanium(IV) oxide (TiO_2) surfaces utilizing a catechol (DOPA)-Lys pentapeptide anchor for surface attachment. PSar coatings effectively inhibited fibrinogen adsorption and resisted to attachment of mammalian or bacterial cells for several weeks.[12] Later, the authors utilized sequence-specific zwitterionic peptoid oligomer variants with similar surface attachment moieties to investigate their structure-property relationship.[296] Starting from pGlu(O*t*Bu)-*b*-pSar diblock polymers, Yoo *et al.* highly increased the catechol amount per polymer by polymer-analogous functionalization of pGlu side chains.[118] Therefore, catechol moieties additionally could function as silver(I) ion reductant at the TiO_2 surface to induce the formation of interfacial silver nanoparticles as adjuvant antimicrobial agent.

For an almost contrary purpose, Yu and co-workers utilized polypept(o)ide-based hydrogels as tissue engineering scaffold platform, enabling the internal cultivation of peripheral blood-derived mesenchymal stem cells (PBMSCs) as treatment for osteochondral defects.[297] The hydrogel comprised a mixture of an α-helical hyperbranched polypeptide p(2-(2-(2-methoxyethoxy) ethoxy)ethyl Glu-*co*-Cys) by copolymerization of respective NCAs, a four arm pSar with maleimide end groups and the integrin-binding peptide CRGD (cysteine-arginine-glycine-aspartic acid). The authors demonstrated excellent promotion of PBMSC-mediated chondrogenesis within the hydrogel and subsequent promising osteochondral repair results in New Zealand white rabbits upon transplantation. Interestingly, pSar-based hydrogels

outperformed PEG and methacryloyl gelatin analogues in this regard and further presented significantly reduced intra-articular immune response. Superior immunomodulatory performance was additionally confirmed by the least foreign body reaction and most macrophage polarization towards M2 phenotypes upon subcutaneous implantation into C57/BL6 mice.

Conclusions

During the last decade, polypept(o)ides have evolved as auspicious class of materials entirely based on endogenous amino acids. Having provided a detailed overview on the origins and the state-of-the-art polypept(o)ide research for synthesis strategies, properties, and applications, we conclude that this unique class of synthetic polymers combines high biocompatibility with a high degree of functionality. Polypept(o)ides can be derived by controlled living polymerization techniques providing convenient access to multiple polymer architectures. To date, most applications of polypept(o)ides are related to nanomedicine, namely the delivery of small molecules and/or nucleic acids, molecular imaging approaches, and immune therapies. Complex tasks calling for functional materials. Several key requirements in these areas can be achieved by the combination of polypeptides and the polypeptoid pSar, including the translatability and certified production of polypept(o)ides. Therefore, the next years to come may provide further insight on the behavior of such materials in patients. But even nowadays, achievements in polypept(o)ide-related developments have created access to intrinsically highly functional material, by the controlled, straightforward, and reproducible construction of complex polymeric architectures.

Author Contributions

Tobias A. Bauer & Leon Capelôa: Conceptualization, Visualization, Writing, Reviewing - Original Draft. Joachim van Guyse, Aroa Duro-Castano & Vicent J. Nebot: Writing - Review & Editing. Matthias Barz: Supervision, Project administration, Writing - Review & Editing, Funding acquisition.

Conflicts of interest

M.B. and T.A.B. are named as a co-inventor in the patent application Nanoparticles comprising iron oxide particles embedded in polymeric micelles, EP2021017. M.B. is named as a co-inventor in the following patents and patent applications Polypept(o)id-based Graft Copolymers for In Vivo Imaging by Tetrazine Transcyclooctene Click Chemistry, WO patent 2020002459A1 & Thiol-protected amino acid derivatives and uses thereof, WO patent 2015169908. A. D. C. and V. J. N. are employes of Curapath (former PTS, Valencia, Spain). M. B. is member of the scientific advisory board of Curapath.

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References

[1] A. Birke, D. Huesmann, A. Kelsch, M. Weilbächer, J. Xie, M. Bros, T. Bopp, C. Becker, K. Landfester, M. Barz, *Biomacromolecules* **2014**, *15*, 548.
[2] K. Klinker, M. Barz, *Macromol. Rapid Commun.* **2015**, *36*, 1943.
[3] A. Birke, J. Ling, M. Barz, *Prog. Polym. Sci.* **2018**, *81*, 163.
[4] R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, *Proc. Natl. Acad. Sci.* **1992**, *89*, 9367.
[5] R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* **1992**, *114*, 10646.
[6] K. Kirshenbaum, A. E. Barron, R. A. Goldsmith, P. Armand, E. K. Bradley, K. T. V. Truong, K. A. Dill, F. E. Cohen, R. N. Zuckermann, *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 4303.
[7] C. Secker, S. M. Brosnan, R. Luxenhofer, H. Schlaad, *Macromol. Biosci.* **2015**, *15*, 881.
[8] N. Gangloff, J. Ulbricht, T. Lorson, H. Schlaad, R. Luxenhofer, *Chem. Rev.* **2016**, *116*, 1753.
[9] T. J. Sanborn, C. W. Wu, R. N. Zuckermann, A. E. Barron, *Biopolymers* **2002**, *63*, 12.
[10] R. V. Mannige, T. K. Haxton, C. Proulx, E. J. Robertson, A. Battigelli, G. L. Butterfoss, R. N. Zuckermann, S. Whitelam, *Nature* **2015**, *526*, 415.

[11] B. Weber, A. Birke, K. Fischer, M. Schmidt, M. Barz, *Macromolecules* **2018**, *51*, 2653.
[12] K. H. A. Lau, C. Ren, T. S. Sileika, S. H. Park, I. Szleifer, P. B. Messersmith, *Langmuir* **2012**, *28*, 16099.
[13] W. T. Astbury, C. E. Dalgliesh, S. E. Darmon, G. B. B. M. Sutherland, *Nature* **1948**, *162*, 596.
[14] M. E. Brosnan, J. T. Brosnan, *Amino Acids* **2016**, *48*, 1785.
[15] R. J. Snow, R. M. Murphy, *Mol. Cell. Biochem.* **2001**, *224*, 169.
[16] W. H. Horner, C. G. Mackenzie, *J. Biol. Chem.* **1950**, *187*, 15.
[17] H. Ogawa, T. Gomi, F. Takusagawa, M. Fujioka, *Int. J. Biochem. Cell Biol.* **1998**, *30*, 13.
[18] D. H. Porter, R. J. Cook, C. Wagner, *Arch. Biochem. Biophys.* **1985**, *243*, 396.
[19] F. H. Glorieux, C. R. Scriver, E. Delvin, F. Mohyuddin, *J. Clin. Invest.* **1971**, *50*, 2313.
[20] M. Schneider, Z. Tang, M. Richter, C. Marschelke, P. Förster, E. Wegener, I. Amin, H. Zimmermann, D. Scharnweber, H. G. Braun, R. Luxenhofer, R. Jordan, *Macromol. Biosci.* **2016**, *16*, 75.
[21] P. Heller, A. Birke, D. Huesmann, B. Weber, K. Fischer, A. Reske-Kunz, M. Bros, M. Barz, *Macromol. Biosci.* **2014**, *14*, 1380.
[22] M. Schneider, C. Fetsch, I. Amin, R. Jordan, R. Luxenhofer, *Langmuir* **2013**, *29*, 6983.
[23] B. Weber, C. Seidl, D. Schwiertz, M. Scherer, S. Bleher, R. Süss, M. Barz, *Polymers (Basel).* **2016**, *8*, 427.
[24] S. Bleher, J. Buck, C. Muhl, S. Sieber, S. Barnert, D. Witzigmann, J. Huwyler, M. Barz, R. Süss, *Small* **2019**, *15*, 1.
[25] A. I. S. van den Berg, C. O. Yun, R. M. Schiffelers, W. E. Hennink, *J. Control. Release* **2021**, *331*, 121.
[26] M. J. Mitchell, M. M. Billingsley, R. M. Haley, M. E. Wechsler, N. A. Peppas, R. Langer, *Nat. Rev. Drug Discov.* **2021**, *20*, 101.
[27] S. Y. Ong, C. Zhang, X. Dong, S. Q. Yao, *Angew. Chemie - Int. Ed.* **2021**, *60*, 17797.
[28] H. Haas, "PNI Tea Time Webinar Series: Polysarcosine-Functionalized Lipid Nanoparticles for Therapeutic mRNA Delivery," can be found under https://www.youtube.com/watch?v=DgX8KoURBs4, **n.d.**
[29] The Lubrizol Corporation, "Apisolex Launch," can be found under https://www.lubrizol.com/-/media/Lubrizol/Health/Campaigns/Apisolex-Brochure---Injectable-Solubility-Enhancement.pdf, **2022**.
[30] C. A. Challener, *Pharm. Technol.* **2022**, *46*, 20.
[31] A. Rasines Mazo, S. Allison-Logan, F. Karimi, N. J. A. Chan, W. Qiu, W. Duan, N. M. O'Brien-Simpson, G. G. Qiao, *Chem. Soc. Rev.* **2020**, *49*, 4737.
[32] Z. Song, Z. Tan, J. Cheng, *Macromolecules* **2019**, *52*, 8521.
[33] D. Huesmann, K. Klinker, M. Barz, *Polym. Chem.* **2016**, *8*, 957.
[34] T. J. Deming, *Chem. Rev.* **2016**, *116*, 786.
[35] H. R. Kricheldorf, *Angew. Chemie - Int. Ed.* **2006**, *45*, 5752.
[36] J. Sun, R. N. Zuckermann, *ACS Nano* **2013**, *7*, 4715.
[37] B. A. Chan, S. Xuan, A. Li, J. M. Simpson, G. L. Sternhagen, T. Yu, O. A.

Darvish, N. Jiang, D. Zhang, *Biopolymers* **2018**, *109*, 1.
[38] R. Luxenhofer, C. Fetsch, A. Grossmann, *J. Polym. Sci. Part A Polym. Chem.* **2013**, *51*, 2731.
[39] F. Heatley, F. A. Bovey, *Macromolecules* **1968**, *1*, 303.
[40] J. R. Edison, R. K. Spencer, G. L. Butterfoss, B. C. Hudson, A. I. Hochbaum, A. K. Paravastu, R. N. Zuckermann, S. Whitelam, *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 5647.
[41] M. Sisido, Y. Imanishi, T. Higashimura, *Macromolecules* **1976**, *9*, 389.
[42] E. Ostuni, R. G. Chapman, R. E. Holmlin, S. Takayama, G. M. Whitesides, *Langmuir* **2001**, *17*, 5605.
[43] E. Ostuni, R. G. Chapman, M. N. Liang, G. Meluleni, G. Pier, D. E. Ingber, G. M. Whitesides, *Langmuir* **2001**, *17*, 6336.
[44] G. Settanni, T. Schäfer, C. Muhl, M. Barz, F. Schmid, *Comput. Struct. Biotechnol. J.* **2018**, *16*, 543.
[45] B. M. Chen, T. L. Cheng, S. R. Roffler, *ACS Nano* **2021**, DOI 10.1021/acsnano.1c05922.
[46] Y. Ju, W. S. Lee, E. H. Pilkington, H. G. Kelly, S. Li, K. J. Selva, K. M. Wragg, K. Subbarao, T. H. O. Nguyen, L. C. Rowntree, L. F. Allen, K. Bond, D. A. Williamson, N. P. Truong, M. Plebanski, K. Kedzierska, S. Mahanty, A. W. Chung, F. Caruso, A. K. Wheatley, J. A. Juno, S. J. Kent, *ACS Nano* **2022**, *16*, 11769.
[47] A. W. Wheeler, D. C. Henderson, A. J. Garman, D. M. Moran, *Int. Arch. Allergy Immunol.* **1985**, *76*, 361.
[48] R. M. Cook, D. C. Henderson, A. W. Wheeler, D. M. Moran, *Int. Arch. Allergy Immunol.* **1986**, *80*, 355.
[49] W. Viricel, G. Fournet, S. Beaumel, E. Perrial, S. Papot, C. Dumontet, B. Joseph, *Chem. Sci.* **2019**, *10*, 4048.
[50] L. Conilh, G. Fournet, E. Fourmaux, A. Murcia, E. L. Matera, B. Joseph, C. Dumontet, W. Viricel, *Pharmaceuticals* **2021**, *14*, 1.
[51] Y. Hu, Y. Hou, H. Wang, H. Lu, *Bioconjug. Chem.* **2018**, *29*, 2232.
[52] K. Son, M. Ueda, K. Taguchi, T. Maruyama, S. Takeoka, Y. Ito, *J. Control. Release* **2020**, *322*, 209.
[53] Y. Chen, Z. Xu, D. Zhu, X. Tao, Y. Gao, H. Zhu, Z. Mao, J. Ling, *J. Colloid Interface Sci.* **2016**, *483*, 201.
[54] S. S. Nogueira, A. Schlegel, K. Maxeiner, B. Weber, M. Barz, M. A. Schroer, C. E. Blanchet, D. I. Svergun, S. Ramishetti, D. Peer, P. Langguth, U. Sahin, H. Haas, *ACS Appl. Nano Mater.* **2020**, *3*, 10634.
[55] D. Bi, D. M. Unthan, L. Hu, J. Bussmann, K. Remaut, M. Barz, H. Zhang, *J. Control. Release* **2023**, *356*, 1.
[56] W. Vayaboury, O. Giani, H. Cottet, A. Deratani, F. Schué, *Macromol. Rapid Commun.* **2004**, *25*, 1221.
[57] T. Aliferis, H. Iatrou, N. Hadjichristidis, *Biomacromolecules* **2004**, *5*, 1653.
[58] N. Gangloff, C. Fetsch, R. Luxenhofer, *Macromol. Rapid Commun.* **2013**, *34*, 997.
[59] J. Bailey, *Nature* **1949**, *164*, 889.
[60] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149.

[61] Y. Li, *Protein Expr. Purif.* **2011**, *80*, 260.
[62] T. Kawakami, H. Murakami, H. Suga, *J. Am. Chem. Soc.* **2008**, *130*, 16861.
[63] K. Klinker, R. Holm, P. Heller, M. Barz, *Polym. Chem.* **2015**, *6*, 4612.
[64] P. Heller, B. Weber, A. Birke, M. Barz, *Macromol. Rapid Commun.* **2015**, *36*, 38.
[65] R. Holm, K. Klinker, B. Weber, M. Barz, *Macromol. Rapid Commun.* **2015**, *36*, 2083.
[66] L. Capelôa, M. Yazdi, H. Zhang, X. Chen, Y. Nie, E. Wagner, U. Lächelt, M. Barz, *Macromol. Rapid Commun.* **2022**, 2100698.
[67] C. Hörtz, A. Birke, L. Kaps, S. Decker, E. Wächtersbach, K. Fischer, D. Schuppan, M. Barz, M. Schmidt, *Macromolecules* **2015**, *48*, 2074.
[68] E. J. L. Stéen, J. T. Jørgensen, K. Johann, K. Nørregaard, B. Sohr, D. Svatunek, A. Birke, V. Shalgunov, P. E. Edem, R. Rossin, C. Seidl, F. Schmid, M. S. Robillard, J. L. Kristensen, H. Mikula, M. Barz, A. Kjær, M. M. Herth, *ACS Nano* **2020**, *14*, 568.
[69] R. Holm, B. Weber, P. Heller, K. Klinker, D. Westmeier, D. Docter, R. H. Stauber, M. Barz, *Macromol. Biosci.* **2017**, *17*, 1.
[70] D. Schwiertz, R. Holm, M. Barz, *Polym. J.* **2020**, *52*, 119.
[71] R. Holm, M. Douverne, B. Weber, T. Bauer, A. Best, P. Ahlers, K. Koynov, P. Besenius, M. Barz, *Biomacromolecules* **2019**, *20*, 375.
[72] L. Capelôa, D. Schwiertz, M. Barz, *Eur. Polym. J.* **2023**, *187*, 111896.
[73] D. Skoulas, V. Stuettgen, R. Gaul, S. A. Cryan, D. J. Brayden, A. Heise, *Biomacromolecules* **2020**, *21*, 2455.
[74] R. M. England, J. I. Moss, A. Gunnarsson, J. S. Parker, M. B. Ashford, *Biomacromolecules* **2020**, *21*, 3332.
[75] Q. Yu, R. M. England, A. Gunnarsson, R. Luxenhofer, K. Treacher, M. B. Ashford, *Macromolecules* **2022**, 55, 2, 401-412.
[76] E. Katchalski, in *Adv. Protein Chem.*, **1951**, pp. 123–185.
[77] H. R. Kricheldorf, G. Schilling, *J. Macromol. Sci.-Chem.* **1978**, *179*, 1175.
[78] E. Katchalski, M. Sela, *Adv. Protein Chem.* **1958**, *13*, 243.
[79] E. Katchalski, A. Berger, L. Bichowsky-Slomnicki, J. Kurtz, *Nature* **1955**, *176*, 118.
[80] T. Kidchob, S. Kimura, Y. Imanishi, *J. Control. Release* **1996**, *40*, 285.
[81] T. Kidchob, S. Kimura, Y. Imanishi, *J. Appl. Polym. Sci.* **1997**, *63*, 453.
[82] T. Kidchob, *J. Control. Release* **1998**, *50*, 205.
[83] T. Kidchob, S. Kimura, Y. Imanishi, *J. Control. Release* **1998**, *51*, 241.
[84] S. Kimura, T. Kidchob, Y. Imanishi, *Polym. Adv. Technol.* **2001**, *12*, 85.
[85] H. Leuchs, *Berichte der Dtsch. Chem. Gesellschaft* **1906**, *39*, 857.
[86] H. Leuchs, W. Manasse, *Berichte der Dtsch. Chem. Gesellschaft* **1907**, *40*, 3235.
[87] H. Leuchs, W. Geiger, *Berichte der Dtsch. Chem. Gesellschaft* **1908**, *41*, 1721.
[88] H. Staudinger, *Berichte der Dtsch. Chem. Gesellschaft (A B Ser.* **1920**, *53*, 1073.
[89] F. Wessely, *Hoppe-Seyler´s Zeitschrift für Physiol. Chemie* **1925**, *146*, 72.
[90] Y. Go, H. Tani, *Bull. Chem. Soc. Jpn.* **1939**, *14*, 510.
[91] F. Sigmund., F. Wessely, *Hoppe-Seyler´s Zeitschrift für Physiol. Chemie* **1926**,

157, 91.
[92] F. Wessely, F. Sigmund, *Hoppe-Seyler´s Zeitschrift für Physiol. Chemie* **1926**, *159*, 102.
[93] K. H. Meyer, Y. Go, *Helv. Chim. Acta* **1934**, *17*, 1081.
[94] R. B. Woodward, C. H. Schramm, *J. Am. Chem. Soc.* **1947**, *69*, 1551.
[95] S. G. Waley, J. Watson, *Proc. R. Soc. London. Ser. A. Math. Phys. Sci.* **1949**, *199*, 499.
[96] J. H. Fessler, A. G. Ogston, *Trans. Faraday Soc.* **1951**, *47*, 667.
[97] D. G. Ballard, C. H. Bamford, *Nature* **1953**, *172*, 907.
[98] W. E. Hanby, S. G. Waley, J. Watson, *J. Chem. Soc.* **1950**, 3009.
[99] D. G. H. Ballard, C. H. Bamford, *Proc. R. Soc. London. Ser. A. Math. Phys. Sci.* **1956**, *236*, 384.
[100] F. Fuchs, *Chem. Ber.* **1922**, *55B*, 2943.
[101] A. C. Farthing, R. J. W. Reynolds, *Nature* **1950**, *165*, 647.
[102] M. Oya, R. Katakai, H. Nakai, Y. Iwakura, *Chem. Lett.* **1973**, *2*, 1143.
[103] W. H. Daly, D. Poché, *Tetrahedron Lett.* **1988**, *29*, 5859.
[104] O. Schäfer, D. Schollmeyer, A. Birke, R. Holm, K. Johann, C. Muhl, C. Seidl, B. Weber, M. Barz, *Tetrahedron Lett.* **2019**, *60*, 272.
[105] G. Laconde, M. Amblard, J. Martinez, *Org. Lett.* **2021**, *23*, 6412.
[106] T. Sugimoto, T. Kuwahara, F. Liang, H. Wang, A. Tsuda, *ACS Omega* **2022**, *7*, 39250.
[107] K. Fischer, *Angew. Chemie* **1935**, *48*, 394.
[108] J. E. Semple, B. Sullivan, K. N. Sill, *Synth. Commun.* **2017**, *47*, 53.
[109] Z.-Y. Tian, Z. Zhang, S. Wang, H. Lu, *Nat. Commun.* **2021**, *12*, 5810.
[110] N. Hadjichristidis, H. Iatrou, M. Pitsikalis, G. Sakellariou, *Chem. Rev.* **2009**, *109*, 5528.
[111] O. W. Webster, *Science.* **1991**, *251*, 887.
[112] T. J. Deming, *Nature* **1997**, *390*, 386.
[113] T. J. Deming, *Macromolecules* **1999**, *32*, 4500.
[114] R. E. Detwiler, A. E. Schlirf, J. R. Kramer, *J. Am. Chem. Soc.* **2021**, *143*, 11482.
[115] I. Conejos-Sánchez, A. Duro-Castano, A. Birke, M. Barz, M. J. Vicent, *Polym. Chem.* **2013**, *4*, 3182.
[116] I. Dimitrov, H. Schlaad, *Chem. Commun.* **2003**, 2944.
[117] G. J. M. Habraken, M. Peeters, C. H. J. T. Dietz, C. E. Koning, A. Heise, *Polym. Chem.* **2010**, *1*, 514.
[118] J. Yoo, A. Birke, J. Kim, Y. Jang, S. Y. Song, S. Ryu, B.-S. Kim, B.-G. Kim, M. Barz, K. Char, *Biomacromolecules* **2018**, *19*, 1602.
[119] T. A. Bauer, C. Muhl, D. Schollmeyer, M. Barz, *Macromol. Rapid Commun.* **2021**, *42*, 2000470.
[120] W. Zhao, Y. Lv, J. Li, Z. Feng, Y. Ni, N. Hadjichristidis, *Nat. Commun.* **2019**, *10*, 3590.
[121] Y. Xia, Z. Song, Z. Tan, T. Xue, S. Wei, L. Zhu, Y. Yang, H. Fu, Y. Jiang, Y. Lin, Y. Lu, A. L. Ferguson, J. Cheng, *Nat. Commun.* **2021**, *12*, DOI 10.1038/s41467-020-20724-w.
[122] J. Sun, J. Ling, J. Cen, Y. Miao, *Amphiphilic Polymer Nanomicelle Containing*

Polydopamine Amino Acid-Chelated Ferric Ions and Application Thereof, **2019**, WO2019218960-A1.
[123] M. Barz, B. Weber, H. Haas, P. Heller, S. Nogueira, A. Schlegel, *RNA Particles Comprising Polysarcosine*, **2018**, WO2020069718A1.
[124] S.-A. Cryam, A. Heise, D. Walsh, J. O'DWYER, F. O'brien, G. Duffy, *Star Polypeptides*, **2019**, WO2020128089-A1.
[125] V. Moreno Manzano, E. Giraldo Reboloso, R. Requejo Aguilar, A. Alastrue Agudo, M. J. Vicent Docon, A. Arminan De Benito, J. J. Arroyo-Crespo, V. J. Nebot Carda, O. Zagorodko, S. Dordevic, *Polymeric Conjugates and Uses Thereof*, **2020**, WO2020193802-A1.
[126] G. Battaglia, A. D. Castano, *Production of Nanoparticles and Microparticles*, **2020**, EP3962456-A1.
[127] H. Haas, S. Nogueira, A. Schlegel, *Rna Particles Comprising Polysarcosine*, **2021**, WO2021191265-A1.
[128] H. Zhang, X. Meng, *Preparation Method and Application of Antibody Drug Conjugate*, **2022**, WO2022228493-A1.
[129] M. J. Vicent Docon, A. D. Castano, V. J. Nebot, *Cross-Linked Star-Shaped Self-Assembled Polypeptides and Its Use as Carriers in Biomedical Applications*, **2016**, US10155050B2.
[130] L. Herrera Muñoz, I. Dolz Pérez, C. Felip Leon, V. J. Nebot Carda, M. J. Vicent Docón, *Star-Shaped PAsp-Oligoamine Derivatives*, **2023**, WO2023002012.
[131] F. L. Carles, I. Dolz Perez, S. Esteban Perez, L. Herrera Munoz, V. J. Nebot Carda, *Non-Covalent Shielding Polymers*, **2023**, WO2023002014.
[132] M. Glodek, S. Liebowitz, R. McCarthy, G. McNally, C. Oksanen, T. Schultz, M. Sundararajan, R. Vorkapich, K. Vukovinsky, C. Watts, G. Millili, *Pharm. Eng.* **2006**, *26*, 1.
[133] N. Bertrand, J. C. Leroux, *J. Control. Release* **2012**, *161*, 152.
[134] R. Duncan, *J. Control. Release* **2014**, *190*, 371.
[135] M. F. Kircher, *Nanomedicine* **2017**, *12*, 171.
[136] M. Barz, R. Luxenhofer, M. Schillmeier, *Nanomedicine* **2015**, *10*, 3089.
[137] S. Sindhwani, W. C. W. Chan, *J. Intern. Med.* **2021**, *290*, 486.
[138] A. G. Arranja, V. Pathak, T. Lammers, Y. Shi, *Pharmacol. Res.* **2017**, *115*, 87.
[139] R. Duncan, *Curr. Opin. Biotechnol.* **2011**, *22*, 492.
[140] M. Vert, Y. Doi, K.-H. Hellwich, M. Hess, P. Hodge, P. Kubisa, M. Rinaudo, F. Schué, *Pure Appl. Chem.* **2012**, *84*, 377.
[141] S. Bisso, J. C. Leroux, *Int. J. Pharm.* **2020**, *578*, DOI 10.1016/j.ijpharm.2020.119098.
[142] S. Soares, J. Sousa, A. Pais, C. Vitorino, *Front. Chem.* **2018**, *6*, 1.
[143] T. Lammers, M. Ferrari, *Nano Today* **2020**, *31*, 0.
[144] W. W. Ma, M. Hidalgo, *Clin. Cancer Res.* **2013**, *19*, 5572.
[145] Y. Barenholz, *J. Control. Release* **2012**, *160*, 117.
[146] E. Markovsky, H. Baabur-Cohen, A. Eldar-Boock, L. Omer, G. Tiram, S. Ferber, P. Ofek, D. Polyak, A. Scomparin, R. Satchi-Fainaro, *J. Control. Release* **2012**, *161*, 446.
[147] M. Hashida, *Adv. Drug Deliv. Rev.* **2020**, *157*, 71.
[148] P. Mi, K. Miyata, K. Kataoka, H. Cabral, *Adv. Ther.* **2021**, *4*, 2000159.

[149] E. Blanco, H. Shen, M. Ferrari, *Nat. Biotechnol.* **2015**, *33*, 941.
[150] S. Quader, K. Kataoka, *Mol. Ther.* **2017**, *25*, 1501.
[151] R. A. Petros, J. M. DeSimone, *Nat. Rev. Drug Discov.* **2010**, *9*, 615.
[152] F. P. Polack, S. J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. Pérez Marc, E. D. Moreira, C. Zerbini, R. Bailey, K. A. Swanson, S. Roychoudhury, K. Koury, P. Li, W. V. Kalina, D. Cooper, R. W. Frenck, L. L. Hammitt, Ö. Türeci, H. Nell, A. Schaefer, S. Ünal, D. B. Tresnan, S. Mather, P. R. Dormitzer, U. Şahin, K. U. Jansen, W. C. Gruber, *N. Engl. J. Med.* **2020**, *383*, 2603.
[153] L. Schoenmaker, D. Witzigmann, J. A. Kulkarni, R. Verbeke, G. Kersten, W. Jiskoot, D. J. A. Crommelin, *Int. J. Pharm.* **2021**, *601*, 120586.
[154] C. Delehedde, L. Even, P. Midoux, C. Pichon, F. Perche, *Pharmaceutics* **2021**, *13*, 945.
[155] L. R. Baden, H. M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert, S. A. Spector, N. Rouphael, C. B. Creech, J. McGettigan, S. Khetan, N. Segall, J. Solis, A. Brosz, C. Fierro, H. Schwartz, K. Neuzil, L. Corey, P. Gilbert, H. Janes, D. Follmann, M. Marovich, J. Mascola, L. Polakowski, J. Ledgerwood, B. S. Graham, H. Bennett, R. Pajon, C. Knightly, B. Leav, W. Deng, H. Zhou, S. Han, M. Ivarsson, J. Miller, T. Zaks, *N. Engl. J. Med.* **2021**, *384*, 403.
[156] H. Cabral, S. Uchida, F. Perche, C. Pichon, *Mol. Pharm.* **2020**, *17*, 3654.
[157] I. Gómez-Aguado, J. Rodríguez-Castejón, M. Vicente-Pascual, A. Rodríguez-Gascón, M. Á. Solinís, A. Del Pozo-Rodríguez, *Nanomaterials* **2020**, *10*, DOI 10.3390/nano10020364.
[158] N. Chaudhary, D. Weissman, K. A. Whitehead, *Nat. Rev. Drug Discov.* **2021**, *20, 817-838.*
[159] T. Lammers, F. Kiessling, W. E. Hennink, G. Storm, *J. Control. Release* **2012**, *161*, 175.
[160] R. A. G. Van Eerden, R. H. J. Mathijssen, S. L. W. Koolen, *Int. J. Nanomedicine* **2020**, *15*, 8151.
[161] K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Deliv. Rev.* **2001**, *47*, 113.
[162] K. S. Ho, M. S. Shoichet, *Curr. Opin. Chem. Eng.* **2013**, *2*, 53.
[163] T. J. Anchordoquy, Y. Barenholz, D. Boraschi, M. Chorny, P. Decuzzi, M. A. Dobrovolskaia, Z. S. Farhangrazi, D. Farrell, A. Gabizon, H. Ghandehari, B. Godin, N. M. La-Beck, J. Ljubimova, S. M. Moghimi, L. Pagliaro, J. H. Park, D. Peer, E. Ruoslahti, N. J. Serkova, D. Simberg, *ACS Nano* **2017**, *11*, 12.
[164] P. Ma, R. J. Mumper, *J. Nanomed. Nanotechnol.* **2013**, *4*, 1000164.
[165] A. Montero, F. Fossella, G. Hortobagyi, V. Valero, *Lancet Oncol.* **2005**, *6*, 229.
[166] H. Gelderblom, J. Verweij, K. Nooter, A. Sparreboom, *Eur. J. Cancer* **2001**, *37*, 1590.
[167] A. Sparreboom, O. Van Teilingen, W. J. Nooijen, J. H. Beijnen, *Cancer Res.* **1996**, *56*, 2112.
[168] J. Gonzalez-Valdivieso, A. Girotti, J. Schneider, F. J. Arias, *Int. J. Pharm.* **2021**, *599*, 120438.
[169] M. Talelli, C. J. F. Rijcken, W. E. Hennink, T. Lammers, *Curr. Opin. Solid State Mater. Sci.* **2012**, *16*, 302.

[170] R. Luxenhofer, *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* **2020**, *75*, 303.
[171] H. Jatzkewitz, *Zeitschrift fur Naturforsch. - Sect. B J. Chem. Sci.* **1955**, *10*, 27.
[172] H. Jatzkewitz, *Hoppe Seyler's Zeitschrift für Physiol. Chemie* **1954**, *297*, 149.
[173] R. Duncan, *Nat. Rev. Drug Discov.* **2003**, *2*, 347.
[174] H. Ringsdorf, *J Polym Sci Polym Symp* **1975**, *153*, 135.
[175] M. Talelli, M. Barz, C. J. F. F. Rijcken, F. Kiessling, W. E. Hennink, T. Lammers, *Nano Today* **2015**, *10*, 93.
[176] A. C. Anselmo, S. Mitragotri, *Bioeng. Transl. Med.* **2019**, *4*, 1.
[177] M. Müllner, *Macromol. Chem. Phys.* **2016**, *217*, 2209.
[178] B. K. Johnson, R. K. Prud'homme, *Phys. Rev. Lett.* **2003**, *91*, 1.
[179] Y. Mai, A. Eisenberg, *Chem. Soc. Rev.* **2012**, *41*, 5969.
[180] T. M. Allen, P. R. Cullis, *Adv. Drug Deliv. Rev.* **2013**, *65*, 36.
[181] A. Vonarbourg, C. Passirani, P. Saulnier, J. P. Benoit, *Biomaterials* **2006**, *27*, 4356.
[182] H. Cabral, K. Miyata, K. Osada, K. Kataoka, *Chem. Rev.* **2018**, *118*, 6844.
[183] F. F. Davis, *Adv. Drug Deliv. Rev.* **2002**, *54*, 457.
[184] M. L. Graham, *Adv. Drug Deliv. Rev.* **2003**, *55*, 1293.
[185] A. Varela-Moreira, Y. Shi, M. H. A. M. Fens, T. Lammers, W. E. Hennink, R. M. Schiffelers, *Mater. Chem. Front.* **2017**, *1*, 1485.
[186] H. Cabral, K. Kataoka, *J. Control. Release* **2014**, *190*, 465.
[187] D. J. Irvine, E. L. Dane, *Nat. Rev. Immunol.* **2020**, *20*, 321.
[188] C. K. McLaughlin, J. Logie, M. S. Shoichet, *Isr. J. Chem.* **2013**, *53*, 670.
[189] J. Logie, A. N. Ganesh, A. M. Aman, R. S. Al-awar, M. S. Shoichet, *Biomaterials* **2017**, *123*, 39.
[190] S. Ulrich, *Acc. Chem. Res.* **2019**, *52*, 510.
[191] K. Klinker, O. Schäfer, D. Huesmann, T. Bauer, L. Capelôa, L. Braun, N. Stergiou, M. Schinnerer, A. Dirisala, K. Miyata, K. Osada, H. Cabral, K. Kataoka, M. Barz, *Angew. Chemie Int. Ed.* **2017**, *56*, 9608.
[192] Z. He, X. Wan, A. Schulz, H. Bludau, M. A. Dobrovolskaia, S. T. Stern, S. A. Montgomery, H. Yuan, Z. Li, D. Alakhova, M. Sokolsky, D. B. Darr, C. M. Perou, R. Jordan, R. Luxenhofer, A. V. Kabanov, *Biomaterials* **2016**, *101*, 296.
[193] M. Grüne, R. Luxenhofer, D. Iuga, S. P. Brown, A. C. Pöppler, *J. Mater. Chem. B* **2020**, *8*, 6827.
[194] A. D. Bangham, R. W. Horne, *J. Mol. Biol.* **1964**, *8*, 660.
[195] D. E. Discher, A. Eisenberg, *Science.* **2002**, *297*, 967.
[196] D. E. Discher, F. Ahmed, *Annu. Rev. Biomed. Eng.* **2006**, *8*, 323.
[197] R. Bleul, R. Thiermann, M. Maskos, *Macromolecules* **2015**, *48*, 7396.
[198] P. Heller, J. Zhou, B. Weber, D. Hobernik, M. Bros, F. Schmid, M. Barz, *Small* **2017**, *13*, 1603694.
[199] S. J. Soenen, W. J. Parak, J. Rejman, B. Manshian, *Chem. Rev.* **2015**, *115*, 2109.
[200] A. Zimpel, N. Al Danaf, B. Steinborn, J. Kuhn, M. Höhn, T. Bauer, P. Hirschle, W. Schrimpf, H. Engelke, E. Wagner, M. Barz, D. C. Lamb, U. Lächelt, S. Wuttke, *ACS Nano* **2019**, *13*, 4, 3884-3895.
[201] W. Cai, C. C. Chu, G. Liu, Y. X. J. Wáng, *Small* **2015**, *11*, 4806.
[202] C. He, D. Liu, W. Lin, *Chem. Rev.* **2015**, *115*, 11079.

[203] S. Behzadi, V. Serpooshan, W. Tao, M. A. Hamaly, M. Y. Alkawareek, E. C. Dreaden, D. Brown, A. M. Alkilany, O. C. Farokhzad, M. Mahmoudi, *Chem. Soc. Rev.* **2017**, *46*, 4218.
[204] I. de Lázaro, D. J. Mooney, *Nat. Mater.* **2021**, *20*, 1469.
[205] A. C. Anselmo, S. Mitragotri, *Bioeng. Transl. Med.* **2016**, *1*, 10.
[206] H. Ragelle, F. Danhier, V. Préat, R. Langer, D. G. Anderson, *Expert Opin. Drug Deliv.* **2017**, *14*, 851.
[207] R. Duncan, R. Gaspar, *Mol. Pharm.* **2011**, *8*, 2101.
[208] J. H. Park, H. K. Lee, *Vaccines* **2021**, *9*, 5, 524.
[209] S. Ols, L. Yang, E. A. Thompson, P. Pushparaj, K. Tran, F. Liang, A. Lin, B. Eriksson, G. B. Karlsson Hedestam, R. T. Wyatt, K. Loré, *Cell Rep.* **2020**, *30*, 3964.
[210] L. Zhang, W. Wang, S. Wang, *Expert Rev. Vaccines* **2015**, *14*, 1509.
[211] Y. S. Youn, Y. H. Bae, *Adv. Drug Deliv. Rev.* **2018**, *130*, 3.
[212] P. Zhang, Y. Zhai, Y. Cai, Y. Zhao, Y. Li, *Adv. Mater.* **2019**, *31*, 1.
[213] J. Shi, P. W. Kantoff, R. Wooster, O. C. Farokhzad, *Nat. Rev. Cancer* **2017**, *17*, 20.
[214] W. Richtering, I. Alberg, R. Zentel, *Small* **2020**, *16*, 1.
[215] D. Docter, U. Distler, W. Storck, J. Kuharev, D. Wünsch, A. Hahlbrock, S. K. Knauer, S. Tenzer, R. H. Stauber, *Nat. Protoc.* **2014**, *9*, 2030.
[216] I. Alberg, S. Kramer, C. Leps, S. Tenzer, R. Zentel, *Macromol. Biosci.* **2021**, *21*, 2000414.
[217] S. C. Owen, D. P. Y. Chan, M. S. Shoichet, *Nano Today* **2012**, *7*, 53.
[218] D. A. Hume, *Curr. Opin. Immunol.* **2006**, *18*, 49.
[219] K. M. Tsoi, S. A. Macparland, X. Z. Ma, V. N. Spetzler, J. Echeverri, B. Ouyang, S. M. Fadel, E. A. Sykes, N. Goldaracena, J. M. Kaths, J. B. Conneely, B. A. Alman, M. Selzner, M. A. Ostrowski, O. A. Adeyi, A. Zilman, I. D. McGilvray, W. C. W. Chan, *Nat. Mater.* **2016**, *15*, 1212.
[220] W. C. W. Chan, *Acc. Chem. Res.* **2017**, *50*, 627.
[221] A. L. Klibanov, K. Maruyama, V. P. Torchilin, L. Huang, *FEBS Lett.* **1990**, *268*, 235.
[222] J. Logie, S. C. Owen, C. K. McLaughlin, M. S. Shoichet, *Chem. Mater.* **2014**, *26*, 2847.
[223] C. J. F. Carstens, Myrra G; Rijcken, *Multifunctional Pharmaceutical Nanocarriers*, Springer New York, New York, NY, **2008**.
[224] D. D. Lasic, F. J. Martin, *Stealth Liposomes*, Boca Raton : CRC Press, **1995**.
[225] M. Barz, R. Luxenhofer, R. Zentel, M. J. Vicent, *Polym. Chem.* **2011**, *2*, 1900.
[226] G. S. Kwon, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, *Pharm. Res.* **1993**, *10*, 970.
[227] G. Kwon, S. Suwa, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, *J. Control. Release* **1994**, *29*, 17.
[228] B. Du, M. Yu, J. Zheng, *Nat. Rev. Mater.* **2018**, *3*, 358.
[229] H. Soo Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. Itty Ipe, M. G. Bawendi, J. V. Frangioni, *Nat. Biotechnol.* **2007**, *25*, 1165.
[230] T. Yamaoka, Y. Tabata, Y. Ikada, *Drug Deliv.* **1993**, *1*, 75.
[231] T. Yamaoka, Y. Tabata, Y. Ikada, *J. Pharm. Sci.* **1994**, *83*, 601.

[232] E. Reske-Nielsen, M. Bojsen-Møller, M. Vetner, J. C. Hansen, *Acta Pathol. Microbiol. Scand. Sect. A Pathol.* **2009**, *84A*, 397.
[233] H. Maeda, *J. Pers. Med.* **2021**, *11*, 229.
[234] Y. Shi, R. van der Meel, X. Chen, T. Lammers, *Theranostics* **2020**, *10*, 7921.
[235] H. Maeda, T. Matsumoto, T. Konno, K. Iwai, M. Ueda, *J. Protein Chem.* **1984**, *3*, 181.
[236] Y. Matsumura, H. Maeda, *Cancer Res.* **1986**, *46*, 6387.
[237] H. Cabral, Y. Matsumoto, K. Mizuno, Q. Chen, M. Murakami, M. Kimura, Y. Terada, M. R. Kano, K. Miyazono, M. Uesaka, N. Nishiyama, K. Kataoka, *Nat. Nanotechnol.* **2011**, *6*, 815.
[238] Y. Mochida, H. Cabral, K. Kataoka, *Expert Opin. Drug Deliv.* **2017**, *14*, 1423.
[239] S. Wilhelm, A. J. Tavares, Q. Dai, S. Ohta, J. Audet, H. F. Dvorak, W. C. W. Chan, A. Chatterley, W. Group, *Nat. Rev. Mater.* **2016**, *1*.
[240] R. van der Meel, T. Lammers, W. E. Hennink, *Expert Opin. Drug Deliv.* **2017**, *14*, 1.
[241] T. Lammers, F. Kiessling, M. Ashford, W. Hennink, D. Crommelin, G. Strom, *Nat. Rev. Mater.* **2016**, *1*, 1.
[242] R. Van Der Meel, E. Sulheim, Y. Shi, F. Kiessling, W. J. M. Mulder, *Nat. Nanotechnol.* **2019**, *14*, 1007.
[243] J. W. Nichols, Y. H. Bae, *J. Control. Release* **2014**, *190*, 451.
[244] D. Sun, S. Zhou, W. Gao, *ACS Nano* **2020**, *14*, 12281.
[245] J. I. Hare, T. Lammers, M. B. Ashford, S. Puri, G. Storm, S. T. Barry, *Adv. Drug Deliv. Rev.* **2017**, *108*, 25.
[246] H. Lee, A. F. Shields, B. A. Siegel, K. D. Miller, I. Krop, C. X. Ma, P. M. Lorusso, P. N. Munster, K. Campbell, D. F. Gaddy, S. C. Leonard, E. Geretti, S. J. Blocker, D. B. Kirpotin, V. Moyo, T. J. Wickham, B. S. Hendriks, *Clin. Cancer Res.* **2017**, *23*, 4190.
[247] W. Poon, B. R. Kingston, B. Ouyang, W. Ngo, W. C. W. Chan, *Nat. Nanotechnol.* **2020**, *15*, 819.
[248] A. M. Sofias, F. Combes, S. Koschmieder, G. Storm, T. Lammers, *Drug Discov. Today* **2021**, 26, 6, 1482-1489.
[249] Y. Matsumoto, J. W. Nichols, K. Toh, T. Nomoto, H. Cabral, Y. Miura, R. J. Christie, N. Yamada, T. Ogura, M. R. Kano, Y. Matsumura, N. Nishiyama, T. Yamasoba, Y. H. Bae, K. Kataoka, *Nat. Nanotechnol.* **2016**, *11*, 533.
[250] S. Sindhwani, A. M. Syed, J. Ngai, B. R. Kingston, L. Maiorino, J. Rothschild, P. MacMillan, Y. Zhang, N. U. Rajesh, T. Hoang, J. L. Y. Wu, S. Wilhelm, A. Zilman, S. Gadde, A. Sulaiman, B. Ouyang, Z. Lin, L. Wang, M. Egeblad, W. C. W. Chan, *Nat. Mater.* **2020**, *19*, 566.
[251] B. R. Kingston, Z. P. Lin, B. Ouyang, P. MacMillan, J. Ngai, A. M. Syed, S. Sindhwani, W. C. W. Chan, *ACS Nano* **2021**, *15*, 14080.
[252] I. Biancacci, Q. Sun, D. Möckel, F. Gremse, S. Rosenhain, F. Kiessling, M. Bartneck, Q. Hu, M. Thewissen, G. Storm, W. E. Hennink, Y. Shi, C. J. F. Rijcken, T. Lammers, A. M. Sofias, *J. Control. Release* **2020**, *328*, 805.
[253] Y. Cheng, S. Song, P. Wu, B. Lyu, M. Qin, Y. Sun, A. Sun, L. Mu, F. Xu, L. Zhang, J. Wang, Q. Zhang, *Adv. Healthc. Mater.* **2021**, *10*, 1.
[254] A. Mantovani, F. Marchesi, A. Malesci, L. Laghi, P. Allavena, *Nat. Rev. Clin.*

Oncol. **2017**, *14*, 399.
[255] F. Greco, M. J. Vicent, *Adv. Drug Deliv. Rev.* **2009**, *61*, 1203.
[256] O. Schäfer, M. Barz, *Chem. - A Eur. J.* **2018**, *24*, 12131.
[257] P. Heller, N. Mohr, A. Birke, B. Weber, A. Reske-Kunz, M. Bros, M. Barz, *Macromol. Biosci.* **2015**, *15*, 63.
[258] C. Lebleu, L. Rodrigues, J. M. Guigner, A. Brûlet, E. Garanger, S. Lecommandoux, *Langmuir* **2019**, *35*, 13364.
[259] R. Seifert, G. H. Lushington, T. C. Mou, A. Gille, S. R. Sprang, *Trends Pharmacol. Sci.* **2012**, *33*, 64.
[260] K. Johann, T. Bohn, F. Shahneh, N. Luther, A. Birke, H. Jaurich, M. Helm, M. Klein, V. K. Raker, T. Bopp, M. Barz, C. Becker, *Nat. Commun.* **2021**, *12*, 5981.
[261] N. J. K. Dal, G. Schäfer, A. M. Thompson, S. Schmitt, N. Redinger, N. Alonso-Rodriguez, K. Johann, J. Ojong, J. Wohlmann, A. Best, K. Koynov, R. Zentel, U. E. Schaible, G. Griffiths, M. Barz, F. Fenaroli, *J. Control. Release* **2023**, *354*, 851.
[262] N. Nishiyama, S. Okazaki, H. Cabral, M. Miyamoto, Y. Kato, Y. Sugiyama, K. Nishio, Y. Matsumura, K. Kataoka, *Cancer Res.* **2003**, *63*, 8977.
[263] V. Subbiah, J. E. Grilley-Olson, A. Combest, N. Sharma, R. H. Tran, I. Bobe, A. Osada, K. Takahashi, J. Balkissoon, A. Camp, A. Masada, D. J. Reitsma, L. Bazhenova, *Clin. Cancer Res.* **2017**, 1.
[264] S. Siemer, T. A. Bauer, P. Scholz, C. Breder, F. Fenaroli, G. Harms, D. Dietrich, J. Dietrich, C. Rosenauer, M. Barz, S. Becker, S. Strieth, C. Reinhardt, T. Fauth, J. Hagemann, R. H. Stauber, *ACS Nano* **2021**, *15*, 18541.
[265] T. A. Bauer, J. Eckrich, N. Wiesmann, F. Kuczelinis, W. Sun, X. Zeng, B. Weber, S. Wu, N. H. Bings, S. Strieth, M. Barz, *J. Mater. Chem. B* **2021**, *9*, 8211.
[266] B. Steinborn, P. Hirschle, M. Höhn, T. Bauer, M. Barz, S. Wuttke, E. Wagner, U. Lächelt, *Adv. Ther.* **2019**, *2*, 1900120.
[267] D. Huesmann, O. Schäfer, L. Braun, K. Klinker, T. Reuter, M. Barz, *Tetrahedron Lett.* **2016**, *57*, 1138.
[268] O. Schäfer, D. Huesmann, M. Barz, *Macromolecules* **2016**, *49*, 8146.
[269] O. Schäfer, D. Huesmann, C. Muhl, M. Barz, *Chem. - A Eur. J.* **2016**, *22*, 18085.
[270] C. Muhl, O. Schäfer, T. Bauer, H.-J. Räder, M. Barz, *Macromolecules* **2018**, *51*, 8188.
[271] O. Schäfer, K. Klinker, L. Braun, D. Huesmann, J. Schultze, K. Koynov, M. Barz, *ACS Macro Lett.* **2017**, *6*, 1140.
[272] T. A. Bauer, J. Imschweiler, C. Muhl, B. Weber, M. Barz, *Biomacromolecules* **2021**, *22*, 2171.
[273] T. A. Bauer, J. Schramm, F. Fenaroli, S. Siemer, C. I. Seidl, C. Rosenauer, R. Bleul, R. Stauber, K. Koynov, M. Maskos, M. Barz, *Adv. Mater.* **2023**, *7*, 2210704.
[274] T. A. Bauer, N. K. Horvat, O. Marques, S. Chocarro, C. Mertens, S. Colucci, S. Schmitt, L. M. Carrella, S. Morsbach, K. Koynov, F. Fenaroli, P. Blümler, M. Jung, R. Sotillo, M. W. Hentze, M. U. Muckenthaler, M. Barz, *Adv. Healthc.*

Mater. **2021**, *10*, 2100385.
[275] H. Tanisaka, S. Kizaka-Kondoh, A. Makino, S. Tanaka, M. Hiraoka, S. Kimura, *Bioconjug. Chem.* **2008**, *19*, 109.
[276] B. Weber, C. Kappel, M. Scherer, M. Helm, M. Bros, S. Grabbe, M. Barz, *Macromol. Biosci.* **2017**, *17*, 1.
[277] U. Sahin, K. Karikó, Ö. Türeci, *Nat. Rev. Drug Discov.* **2014**, *13*, 759.
[278] A. J. Barbier, A. Y. Jiang, P. Zhang, R. Wooster, D. G. Anderson, *Nat. Biotechnol.* **2022**, *40*, 840.
[279] R. Verbeke, I. Lentacker, S. C. De Smedt, H. Dewitte, *J. Control. Release* **2021**, *333*, 511.
[280] P. Heller, D. Hobernik, U. Lächelt, M. Schinnerer, B. Weber, M. Schmidt, E. Wagner, M. Bros, M. Barz, *J. Control. Release* **2017**, *258*, 146.
[281] P. Heller, B. Weber, A. A. Birke, M. Barz, *Macromol. Rapid Commun.* **2015**, *36*, 38.
[282] K. Miyata, Y. Kakizawa, N. Nishiyama, A. Harada, Y. Yamasaki, H. Koyama, K. Kataoka, *J. Am. Chem. Soc.* **2004**, *126*, 2355.
[283] R. Holm, D. Schwiertz, B. Weber, J. Schultze, J. Kuhn, K. Koynov, U. Lächelt, M. Barz, *Macromol. Biosci.* **2020**, *20*, 1.
[284] J. M. Ren, T. G. McKenzie, Q. Fu, E. H. H. Wong, J. Xu, Z. An, S. Shanmugam, T. P. Davis, C. Boyer, G. G. Qiao, *Chem. Rev.* **2016**, *116*, 6743.
[285] S. Kunjachan, R. Pola, F. Gremse, B. Theek, J. Ehling, D. Moeckel, B. Hermanns-Sachweh, M. Pechar, K. Ulbrich, W. E. Hennink, G. Storm, W. Lederle, F. Kiessling, T. Lammers, *Nano Lett.* **2014**, *14*, 972.
[286] M. Darguzyte, R. Holm, J. Baier, N. Drude, J. Schultze, K. Koynov, D. Schwiertz, S. M. Dadfar, T. Lammers, M. Barz, F. Kiessling, *Bioconjug. Chem.* **2020**, *31*, 2691.
[287] M. Darguzyte, R. Holm, J. Baier, N. Drude, J. Schultze, K. Koynov, D. Schwiertz, S. M. Dadfar, T. Lammers, M. Barz, F. Kiessling, *Bioconjug. Chem.* **2020**, *31*, 2691.
[288] D. Skoulas, S. Fattah, D. Wang, S. A. Cryan, A. Heise, *Macromol. Biosci.* **2022**, *22*, 1.
[289] R. M. England, J. I. Moss, A. Gunnarsson, J. S. Parker, M. B. Ashford, *Biomacromolecules* **2020**, *21*, 3332.
[290] M. Longmire, P. L. Choyke, H. Kobayashi, *Nanomedicine* **2008**, *3*, 703.
[291] K. Johann, D. Svatunek, C. Seidl, S. Rizzelli, T. A. Bauer, L. Braun, K. Koynov, H. Mikula, M. Barz, *Polym. Chem.* **2020**, *11*, 4396.
[292] C. Kappel, C. Seidl, C. Medina-Montano, M. Schinnerer, I. Alberg, C. Leps, J. Sohl, A.-K. Hartmann, M. Fichter, M. Kuske, J. Schunke, G. Kuhn, I. Tubbe, D. Paßlick, D. Hobernik, R. Bent, K. Haas, E. Montermann, K. Walzer, M. Diken, M. Schmidt, R. Zentel, L. Nuhn, H. Schild, S. Tenzer, V. Mailänder, M. Barz, M. Bros, S. Grabbe, *ACS Nano* **2021**, *15*, 15191.
[293] A. M. Alkilany, L. Zhu, H. Weller, A. Mews, W. J. Parak, M. Barz, N. Feliu, *Adv. Drug Deliv. Rev.* **2019**, *143*, 22.
[294] C. J. Wilson, R. E. Clegg, D. I. Leavesley, M. J. Pearcy, *Tissue Eng.* **2005**, *11*, 1.
[295] M. Ribeiro, F. J. Monteiro, M. P. Ferraz, *Biomatter* **2012**, *2*, 176.

[296] K. H. A. Lau, T. S. Sileika, S. H. Park, A. M. L. Sousa, P. Burch, I. Szleifer, P. B. Messersmith, *Adv. Mater. Interfaces* **2015**, *2*, 1.
[297] M. Yang, Z. C. Zhang, F. Z. Yuan, R. H. Deng, X. Yan, F. B. Mao, Y. R. Chen, H. Lu, J. K. Yu, *Bioact. Mater.* **2023**, *19*, 678.