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

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General Discussion

Summary

This thesis aimed to investigate core cross-linked polymeric micelles (CCPMs) and expand their potential for the delivery of hydrophobic drugs and co-factors. Applying polypept(o)ides as the polymeric platform technology, the fundamental implications of secondary structure formation on ring-opening *N*-carboxyanhydride (NCA) polymerization and self-assembly were examined and optimized. CCPMs with functional core architectures serving external or disease-related stimuli were developed. To establish robust CCPM production, overcome drug resistance mechanisms, and explore therapeutic agents for immunomodulation, polymer science was combined with organic and inorganic chemistry.

A general introduction about nanomedicine and polypept(o)ides was given in **chapter 1**. Herein, the rationale for the use of nanocarriers to tune the pharmacokinetic profile of active pharmaceutical ingredients (API) was explained. Relevant carrier systems were presented, and the significance of surface shielding to prevent non-specific uptake by the mononuclear phagocyte system (MPS) was outlined. The enhanced permeability and retention (EPR) effect was introduced and critically discussed as a mechanism for passive drug targeting, and future directives were disclosed. Current developments on polymeric micelles and CCPMs were reviewed. In addition, polypept(o)ides were explored as a novel class of functional polymers entirely based on endogenous amino acids. The mechanism of the ring-opening NCA polymerization was explained, and current trends in NCA polymerization were identified. Ultimately, the early and most recent developments on the biomedical application of polypept(o)ides were outlined.

In **chapter 2**, racemic *S*-ethylsulfonyl-DL-cysteine was investigated to improve the ring-opening polymerization of polypeptides such as polycysteine that form strong anti-parallel ß-sheets during polymerization. The thiol-reactive *S*-ethylsulfonyl-DL-cysteine NCA was synthesized and polymerized in analogy to enantiopure *S*-ethylsulfonyl-L-cysteine. For the racemic NCA, complete monomer conversion and increased chain lengths up to X_n = 102 corresponding to molecular weights of 20.0 kDa could be obtained. Moreover, kinetic investigations revealed higher rate constants (40% on average) even though the reaction kinetics still followed the Avrami model indicating low solubility. Indeed, only a reduced tendency but not entirely resolved ß-sheets were detected by infrared (IR)

spectroscopy. Nevertheless, the full monomer conversion of *S*-ethylsulfonyl-DL-cysteine NCA granted the synthesis of a triblock copolymer by sequential monomer addition otherwise inaccessible to the enantiopure amino acid unless purification steps were introduced.

The influence of the secondary structure on the self-assembly of thiol-reactive copolymers was examined in **chapter 3**. Therefore, a library of copolymers of enantiopure polysarcosine-*block*-poly(*S*-ethylsulfonyl-L-cysteine) (pSar-*b*-p(L)Cys(SO_2Et)), racemic pSar-*b*-p(DL)Cys(SO_2Et), and pSar-*b*-poly(*S*-ethylsulfonyl-L-homocysteine) (pSar-*b*-p(L)Hcy(SO_2Et)) was synthesized. The secondary structure formation of the polypeptides was analyzed by IR and circular dichroism spectroscopy, whereby an α-helix was detected for pSar-*b*-pHcy(SO_2Et), anti-parallel ß-sheets for pSar-*b*-p(L)Cys(SO_2Et), and disrupted ß-sheets for racemic pSar-*b*-p(DL)Cys(SO_2Et). During self-assembly induced by solvent switch, anti-parallel ß-sheets showed the strongest tendency for self-assembly followed by the α-helical copolymer and ultimately the disrupted ß-sheets, as quantified by analysis *via* dynamic light scattering (DLS). These findings translated to the morphology of the assemblies, leading to worm-like, entirely spherical, or slightly elongated structures, as analyzed by atomic force microscopy (AFM) or transmission electron microscopy (TEM). Secondary structure formation of thiol-reactive copolymers was demonstrated to be an elegant tool for adjusting the features of nanomedicines.

In **chapter 4**, the cross-linking density was assessed as a parameter to fine-tune the stability of CCPMs based on pSar-*b*-pCys(SO_2Et)$_n$. To define structure-activity relationships, the length of the cross-linkable p(L)Cys(SO_2Et)$_n$ block was selected as X_n = 17 or 30, and mono-, bi-, or trifunctional thiol-reagents were synthesized and applied to address the *S*-ethylsulfonyl group for quenching or cross-linking reactions. Asymmetrical flow field-flow fractionation (AF4) and fluorescence correlation spectroscopy (FCS) were applied as the screening techniques. Analysis by AF4 in human blood plasma revealed a clear connection between the cross-linking density and the particle stability. Large fractions of aggregates were found for non-cross-linked particles and still for CCPMs from bifunctional cross-linkers and X_n = 17, yet not when the trifunctional peptide cross-linker was applied. Nevertheless, no significant differences for the circulation half-life or biodistribution could be detected after intravenous administration to mice, whereby the circulation half-lives of 11.3 - 19.1 h indicated sufficient stability for drug delivery *via* passive targeting mechanisms.

Envisioning large-scale production of CCPMs, a continuous flow process, and a strategy for decoupled drug conjugation was presented in **chapter 5**. Self-assembly and core cross-linking of pSar-*b*-p(L)Cys(SO_2Et) with dihydrolipoic acid hydrazide were adjusted and transferred to the production *via* slit-interdigital micromixers. The process parameters were optimized, yielding a robust procedure for up to 650 mg CCPMs/h without numbering up. CCPMs were further purified by online tangential flow filtration reducing the concentration of unconjugated polymer to below the limit of detection (≤ 0.5%). The CCPMs could then be loaded with paclitaxel-levulinic acid in a separate step (PTX@CCPMs), allowing for stimuli-responsive drug release at endo-lysosomal pH values. When tested in cell culture and xenograft B16F1 zebrafish larvae models, PTX@CCPMs showed comparable performance yet reduced toxicity compared to state-of-the-art treatment with nanoparticle albumin-bound paclitaxel. The disclosed strategy may ease CCPM production, allowing to screen for combination therapies.

In **chapter 6**, polypept(o)ides were combined with photo-responsive ruthenium(II) complexes as metal-based APIs offering to use light as an external trigger for drug release from CCPMs. Polypept(o)ides based on pSar-*b*-poly(glutamic acid) (pSar-*b*-pGlu) were synthesized and modified with aromatic nitrile moieties for coordination of bis(bipyridine)ruthenium(II) ($[Ru(bpy)_2]^{2+}$) or bis(biquinoline)ruthenium(II) ($[Ru(biq)_2]^{2+}$) complexes. Depending on the flexibility or hydrophobicity of the linker, either spherical or worm-like micelles were obtained. The progress of the cross-linking reaction could be monitored *via* the color change originating from the ligand exchange reaction. Cross-linking did not affect the size distribution yet resulted in stable CCPMs according to GPC in hexafluoroisopropanol (HFIP) and DLS in human blood plasma. For drug release, CCPMs containing $[Ru(bpy)_2]^{2+}$ could be cleaved within 300 s, whereas only insufficient light-induced solvolysis was observed for $[Ru(biq)_2]^{2+}$ referring to low quantum yields and the dense micellar core. The photocleavable CCPMs were assessed in cell culture and the *in ovo* model confirming high biocompatibility and prolonged vascular circulation providing the basis for future investigations incorporating metal-based APIs with high cytotoxicity and fast-cleavage profiles.

Chapter 7 was focused on assessing precision medicine to account for the heterogeneous response of head and neck cancer patients to therapeutic regimens. Therefore, a cisplatin-resistant head and neck cancer cell line was established and sequenced. Drug uptake *via* the ion channel LRRC8A was identified as the molecular pathway for cisplatin resistance. The clinical significance of LRRC8A

as a biomarker was examined among a cohort of 500 head and neck cancer patients with data from The Cancer Genome Atlas. For patients under cisplatin treatment, low levels of LRRC8A correlated with lower overall survival. To bypass drug uptake by LRRC8A, cisplatin was conjugated to polypept(o)ides of pSar-*b*-pGlu(ONa). The chloride ligands of cisplatin were exchanged by the carboxylate groups in the side chain of glutamic acid, leading to small spherical polymeric micelles (NP_{Cis}, $D_h \approx 28$ nm) with high biocompatibility. NP_{Cis} did not induce complement activation, colloidal stability was confirmed by DLS in human blood plasma, and the circulation half-life of approx. 4 h in zebrafish larvae indicated effective stabilization providing the basis for passive tumor targeting. In cell culture, NP_{Cis} successfully reversed cisplatin resistance by circumventing the LRRC8A channel, which confirmed the significance of combining nanomedicine and molecular pathways for patient stratification.

The therapeutic potential of the co-factor iron upon specific delivery to macrophages was investigated in **chapter 8**. Iron oxide nanoparticles (SPIONs) were embedded in CCPMs based on pSar-*b*-p(L)Cys(SO_2Et). The building blocks were combined by cross-linking with dihydrolipoic acid aimed at chemoselective disulfide bond formation and coordination to the SPION surface *via* the carboxylate group. The resulting SPION-CCPMs showed colloidal stability in human blood plasma, and glutathione-responsive particle degradation. In co-cultures of primary murine macrophages and Lewis lung carcinoma cells SPION-CCPMs specifically sequestered in macrophages in an iron-related feedback-loop manner. The sustained iron release of SPION-CCPMs induced a strong inflammatory phenotype in both murine and human macrophages. Significantly elevated levels of inflammatory surface markers, e.g., cluster of differentiation 86 (CD86), and cytokines such as tumor necrosis factor α (TNFα) and interleukin 1ß (IL1ß) were thus detected. The observed sterile inflammation of macrophages was further confirmed *in vivo* after intratracheal administration of SPION-CCPMs to mice. Based on the design-to-release concept, SPION-CCPMs could be introduced as a promising adjuvant to overcome pathological immune tolerance and activate macrophages in the tumor microenvironment.

Discussion and Outlook

Throughout this thesis, CCPMs have been investigated as drug delivery systems, and tailored strategies have been applied to combine external or disease-related stimuli for controlled drug release. Established APIs such as paclitaxel and cisplatin, as well as novel therapeutics and co-factors, e.g., ruthenium(II) complexes and iron, were successfully implemented to CCPMs. The toolbox of polypept(o)ides as functional copolymers for straightforward synthesis of complex polymeric architectures was therefore explored and expanded.[1–3]

Considering the influence of the secondary structure formation on the synthesis of polypeptides *via* NCA polymerization, the focus of **chapter 2** was to improve the synthesis of thiol-reactive polypeptides.[4] The *S*-ethylsulfonyl-protecting group was introduced by Huesmann *et al.* in 2016 and remains intact during NCA polymerization with hard amine nucleophiles but can be addressed by soft nucleophile thiols in post-polymerization modification reactions for chemo-selective disulfide bond formation.[5] Despite this outstanding feature, the polymerization of cysteine NCA is severely hampered by the formation of strong anti-parallel ß-sheets during the polymerization.[6–8] Consequently, only low monomer conversion can be achieved albeit with long reaction times. Moreover, for chain lengths above $X_n = 20$, multimodal molecular weight distributions are obtained by HFIP-GPC, complicating reliable analysis.[8–10] On the other hand, the polymerization of *S*-ethylsulfonyl-L-homocysteine NCA leads to a favorable α-helix promoting the polymerization, but the synthesis of the protected amino acid requires laborious and time-consuming purification by preparative high-performance liquid chromatography compromising larger-scale production.[11–14] To disrupt the interfering hydrogen bonds, the addition of chaotropic thiourea was suggested, however did not contribute to improving the polymerization of *S*-ethylsulfonyl cysteine NCA.[15,16] As outlined, the use of racemic *S*-ethylsulfonyl-DL-cysteine successfully improved the polymerization. Full monomer conversion and polymers with molecular weights up to 20 kDa and well-defined dispersity could be achieved. Even though the reaction kinetics still followed the Avrami model of a physically hindered polymerization, the rate constant was on average 40 % faster compared to the enantiopure L-cysteine analog.[8,17–19] Nevertheless, the polymerization of α-helical *S*-ethylsulfonyl-L-homocysteine NCA was 5-fold faster, which underlines the critical influence of the secondary structure on the polymerization. For these reasons, mostly α-helical polypeptides have been

investigated throughout the literature, and recent developments on accelerated NCA polymerization using organocatalysts do not cover ß-sheet forming polypeptides.[7,12,14,20–23] Since more complex polymeric architectures, such as triblock copolymers, could be conveniently realized with racemic *S*-ethylsulfonyl-DL-cysteine, this chapter provides a valuable contribution to the field of NCA polymerization. Future applications of the thiol-reactive protecting group may thus be facilitated by the easier synthesis and handling of p(DL)Cys(SO_2Et), supporting the design of cross-linkable materials and drug delivery systems.

Building up on the results of chapter 2, the influence of secondary structure formation on self-assembly of thiol-reactive block copolymers was investigated in **chapter 3**.[16] As reported by Klinker *et al.*, anti-parallel ß-sheet formation can be used to direct self-assembly of pSar-*b*-p(L)Cys(SO_2Et), leading to worm-like micelles unless the secondary structure was suppressed by the addition of chaotropic thiourea during self-assembly.[9] In a similar manner, ß-sheets have been exploited previously as a driving force for hydrogelation.[24] To expand the application of thiol-reactive copolymers, the influence of the three secondary structure motifs was elucidated by direct comparison of enantiopure pSar-*b*-p(L)Cys(SO_2Et), racemic pSar-*b*-p(DL)Cys(SO_2Et), and α-helical pSar-*b*-p(L)Hcy(SO_2Et). Indeed, albeit similar primary structure and thus similar hydrophobicity of the polypeptide block, secondary structure formation was confirmed as the major driving force for self-assembly. In particular the comparison of racemic pSar-*b*-p(DL)Cys(SO_2Et) and enantiopure pSar-*b*-p(L)Cys(SO_2Et) revealed that aggregation during the solvent switch starts at significantly lower water content for intact anti-parallel ß-sheets. Regarding the nanoparticle morphology, interestingly the racemic pSar-*b*-p(DL)Cys(SO_2Et) induced the formation of less uniform spherical to slightly elongated structures indicating a residual directing character of the racemic ß-sheets.[25,26] In contrast, solely spherical morphologies were detected for CCPMs based on α-helical pSar-*b*-p(L)Hcy(SO_2Et) for copolymers containing up to 27 wt.% of pHcy(SO_2Et). These findings relate to reports by the Kataoka group for the bundled assembly of poly(ethylene glycol)-*b*-pGlu helices for NC-6004 containing cisplatin conjugated to the pGlu block.[27,28] The detailed understanding of the relation between secondary structure, self-assembly and particle morphology of the thiol-reactive copolypept(o)ides may support future studies elucidating the effect of the shape on the performance of the core cross-linked drug delivery system with a soft nanoparticle surface.[29–32]

To further develop CCPMs from the established copolypept(o)ides of enantiopure pSar-*b*-p(L)Cys(SO_2Et), the influence of the core cross-linking itself on nanoparticle stability was analyzed in **chapter 4**. The detailed study was motivated by initial findings that CCPMs cross-linked with lipoic acid derivatives induced aggregation during analysis by AF4 in human blood plasma, despite being considered stable according to analysis by HFIP-GPC, DLS in human blood plasma, and circulation half-life in zebrafish larvae.[33–35] The analysis of nanoparticles by AF4 in human blood plasma was previously realized by Alberg *et al.* to investigate the protein corona formation for soft nanoparticles of low density.[36] In fact, a negligible protein corona was found for CCPMs (CPC634) and pSar-shielded peptobrushes, which was attributed to the steric shielding by the dense hydrophilic polymer shell. These results are of significance for the clinical investigation of CPC634 (CCPMs containing covalently encapsulated docetaxel) since otherwise patient-specific protein corona formation could impact the performance of the nanomedicine.[36–40] For non-cross-linked PMs, however, clear signs of interaction with plasma proteins were observed.[41] Since pSar was confirmed as protein resistant material, the results described in chapter 4 thus indicated that the core cross-linking for $X_{n,\,pCys(SO2Et)} = 17$ and bifunctional cross-linkers did not sufficiently stabilize the micellar structure to prevent interaction with blood plasma components.[36,42–44] By increasing the number of cross-linking net points, the particle stability could be precisely tuned. In particular, the designed trifunctional peptide cross-linker yielded a lower tendency for aggregate formation. Besides more efficient cross-linking referring to the Carothers equation, the peptide containing cysteine mimics symmetrical disulfide bonds, and self-immolative cleavage by shuffling of disulfide bonds is unlikely since only 11-membered rings can be formed.[45,46] Conversely, 5-membered dithiolane rings can be released in the case of lipoic acid.[45] Surprisingly, the results of the AF4 analysis were not reflected by FCS analysis in human blood plasma, despite being a sensitive tool to elucidate nanoparticle stability in complex media.[47,48] Moreover, intravenous administration to mice did not reveal statistically significant differences for the circulation half-life and biodistribution among the different particle groups. Variations in the sensitivity of the detection modes may account for the observed disparities. As such, light scattering is highly sensitive to larger structures ($I \sim r^6$) overinterpreting aggregates.[49,50] A deeper understanding of the core architecture using NMR spectroscopy or neutron scattering may thus be required to assess the microstructural differences in the micellar core.[51] *Vice*

versa, the non-significant differences observed in the *in vivo* experiment advocate for reducing complexity when designing functional cross-linkers.[52]

Envisioning the larger-scale production of drug-loaded CCPMs, a continuous flow process for self-assembly, cross-linking, and purification, as well as a strategy for decoupled drug conjugation was presented in **chapter 5**. Bottlenecks for CCPM production were identified as the cross-linking and, more severely, the nanoparticle purification. Microfluidics are considered the state-of-the-art technique for liposome and lipid nanoparticle production.[53–56] Micro-structured mixers allowing for precise control of solvent mixing were thus adapted and applied as the central device defining self-assembly and core cross-linking.[57,58] Furthermore, the tedious manual spin-filtration process was substituted by the online tangential flow filtration. In combination, the designed continuous flow process enabled the robust production of purified CCPMs with significantly higher throughput compared to the laboratory procedure. Additional scale-up can be readily performed by numbering-up circumventing classical scale-up tasks of re-defining the optimum parameters. Polypept(o)ides of pSar-*b*-p(L)Cys(SO_2Et) were selected as the functional material accounting for their fast and chemoselective disulfide bond formation.[5,9,59–61] The starting material was complemented by dihydrolipoic acid hydrazide and paclitaxel-levulinic acid, enabling hydrazone bond formation, adding the pH-value as an additional trigger for drug release.[16,62–64] The dual stimuli-responsive system was designed aiming for complete drug release, since slow and deficient clearance was observed during the clinical investigation of NK105 (PMs containing paclitaxel stabilized by π-π interactions) and CPC634 (CCPMs with conjugated docetaxel for gradual release at pH 7.4).[38,39,65–67] In the presented concept, CCPM synthesis and purification were first completed before drug conjugation was performed in a second step. Despite mediocre conjugation efficiency for the relatively large molecule paclitaxel, this approach can be expanded to other APIs allowing for rapid screening of combination therapies featured by nanomedicine.[68–70]

Implementing light as an external trigger for drug release, polypept(o)ides were synthesized and modified for stimuli-responsive conjugation of ruthenium(II) complexes in **chapter 6**.[71] Besides monoclonal antibodies and classical taxane or anthracycline small-molecule drugs, metal-based chemotherapeutics are frequently used in the first-line treatment for many types of cancer.[72–78] Ruthenium complexes have been investigated as an alternative to conventional cisplatin, combining the ability to induce cytotoxicity by DNA cross-links with a

rich photochemistry prone to light-induced ligand exchange reactions and singlet oxygen production.[79–82] In combination with nanomedicine, the hydrophobic ruthenium complexes can be encapsulated in carrier systems providing passive tumor targeting while being activated only after irradiation which provides additional spatial resolution for drug release.[83,84] To serve as the functional material, polypept(o)ides of pSar-*b*-pGlu were synthesized, and the carboxyl side chain of pGlu was modified with linkers containing aromatic nitrile moieties.[71] The coordination of the nitrile-nitrogen atom to the central ruthenium ion was previously investigated and established as a light-responsive dynamic covalent bond for polypyridyl ruthenium(II) complexes.[84–87] Aiming to improve the grafting efficiency of the post-polymerization modification reaction, two linkers were applied to differentiate the connection to pGlu *via* amide or ester bond. Interestingly, the variations induced alterations in the morphology of the PMs. Modification with the shorter and more hydrophobic amine resulted in worm-like PMs, whereas solely spherical morphologies were obtained for the ester linkage with a longer and more flexible alkyl chain. Despite flexibility and hydrophobicity, also differences in hydrogen bond and secondary structure formation could account for the structural transitions.[7,88,89] Cross-linking with the ruthenium(II) complexes did not influence the morphologies, and similar conjugation efficiencies were obtained. Referring to the biological application, high biocompatibility and particle stability were found for the photocleavable CCPMs. Nevertheless, in cell culture and the *in ovo* model, the intended cytotoxicity upon photoactivation was mainly attributed to the irradiation itself. In particular, for $[Ru(biq)_2]^{2+}$, the low quantum yield of the ligand exchange reaction combined with the dense micellar core reduced the toxicity of the conjugated complex.[87,90] Since combining nanomedicine and photoactivated chemotherapy represents a promising concept for therapy of certain types of cancer, e.g., head and neck cancer, future studies will be focused on the design of metal complexes with high cytotoxicity and fast release profile.[91]

In **chapter 7**, the mechanisms for resistance to cisplatin therapy were analyzed and correlated to the survival of head and neck cancer patients. Nanomedicine featured by polypept(o)ides was then applied to provide an additional drug uptake pathway *via* endocytosis and overcome drug resistance. Cisplatin resistant head and neck cancer cells (Fadu) were established by prolonged treatment with sub-toxic drug concentrations mimicking the induced selection process upon chemotherapy.[92,93] By RNA sequencing transcriptomics, among other transporter

genes, genetic alterations in the expression of the ion channel LRRC8A were identified as the most prominent variation between resistant and wild-type Fadu cells. Low expression levels of LRRC8A impaired cisplatin uptake and were even maintained when cells were cultured in the absence of cisplatin, accounting for stable genetic modification. Besides reduced expression of VRAC channels required for cisplatin uptake, other mechanisms for drug resistance have been described in the literature.[76,93–95] In particular, elevated levels of glutathione, methionine, and other cysteine-rich proteins were identified to detoxify cisplatin, rescuing cancer cells from chemotherapy.[94,96,97] Nevertheless, for head and neck cancer patients under cisplatin therapy, decreased levels of LRRC8A could be correlated with reduced overall survival, giving a rationale and a biomarker for personalized nanomedicine.[98,99] Polypept(o)ides of pSar-*b*-pGlu(ONa) were thus synthesized, and cisplatin was conjugated to the carboxyl side chain of pGlu(ONa) yielding NP_{Cis}. During nanoparticle synthesis, the exchange of the chloride ligands by the carboxyl groups converted the hydrophilic charged pGlu(ONa) block to an uncharged and hydrophobic polymer, inducing self-assembly. Hence, NP_{Cis} resemble NC-6004 based on PEG-*b*-pGlu(ONa), which is currently under clinical investigation (phase III) for the treatment of pancreatic cancer.[28,100,101] Of note, the preparation NP_{Cis} based on polypept(o)ides by the mild NCA polymerization offers block copolymer synthesis by sequential polymerization, and access to functional end-groups for further modification with targeting moieties to enhance or specify cellular uptake.[3,102,103] Concerning the core structure, for NC-6004, predominantly bundled assembly of cisplatin-containing α-helices was reported from small-angle X-ray scattering, however, a certain degree of cross-linking cannot generally be excluded.[27,101,104] Compared to the free cisplatin, nanoparticles allow for uptake *via* endocytic pathways bypassing LRRC8A. In cell culture, NP_{Cis} thus significantly reduced the viability of cisplatin-resistant Fadu cells. The presented approach demonstrates the potential of nanomedicine when combined with genomic analysis of drug resistance mechanisms. Future studies will continue elucidating relevant resistance pathways to target therapeutic failures and relapse. In addition, the obtained results will be further validated in cell culture and in *in vivo* disease models.

Empowering the co-factor iron as a therapeutic for immunomodulation, SPION-CCPMs were designed for sustained release and specific delivery of iron to macrophages, as introduced in **chapter 8**.[34] The essential co-factor iron is involved in numerous cellular processes in the human body, and impaired iron

homeostasis is linked to multiple pathologies and diseases.[105–107] Despite that potential, iron oxide nanoparticles were initially intended as contrast agents for magnetic resonance imaging, and drug delivery systems were designed for effective shielding of the delicate cargo.[108–110] In spite, the majority of the administered iron was metabolized, and Ferumoxytol (SPIONs encapsulated in a matrix of cross-linked carboxymethyl dextran) was instead approved for the treatment of iron deficiency anemia.[110–112] Referring to the therapeutic potential of iron, Zanganeh *et al.* reported on reduced tumor growth upon local administration of Ferumoxytol in 2016.[113] Moreover, Thielmann *et al.* correlated increased iron levels in tumor-associated macrophages with higher overall survival of patients with lung adenocarcinoma.[114] The therapeutic effects were attributed to the activation of tumor-associated macrophages leading to a stimulated anti-tumor immune response.[113–116] To specifically enable sustained and stimuli-responsive release and exploit the potential of iron as a stimulating agent for immunotherapy, SPION-CCPMs were designed based on polypept(o)ides of pSar-*b*-p(L)Cys(SO_2Et). Herein, SPIONs were encapsulated in a matrix of polycysteine that was cross-linked with dihydrolipoic acid. Since lipoic acid can anchor to the iron oxide nanoparticle surface *via* the carboxyl group, all building blocks were connected while being sensitive to redox-responsive release.[117–119] In the co-culture of macrophages and cancer cells, interestingly, a feedback mechanism leads to enhanced uptake of SPION-CCPMs in macrophages, whereas non-iron-loaded CCPMs were predominantly accumulated in the cancer cells. The selective distribution may thus direct to cell-specific therapies applying stimulating agents to macrophages and cytotoxic APIs to cancer cells. In addition, the detailed mechanisms of the sterile inflammation induced by SPION-CCPMs remain to be elucidated. The described activation of primary murine and human macrophages by SPION-CCPMs extended the effects induced by other iron sources, suggesting an influence of the factual co-delivery of cysteine and iron. Replacing SPIONs with iron sulfide nanoparticles or iron-containing metal-organic frameworks may thus direct to novel classes of therapeutics to defeat pathologic immune tolerance mechanisms.

Taken together, polypept(o)ides and CCPMs were explored as functional materials to improve the therapeutic potential of APIs. The developed understanding of the relationship between secondary structure formation on polymerization and self-assembly will support exploring thiol-reactive polypept(o)ides in nanomedicine and for the design of advanced materials. Next-

generation nanomedicines that aim to refine the potential of novel APIs and drug combinations embrace a complex subset of tasks. The presented design concepts of stimuli-responsive CCPMs may thus add to produce adaptive drug carriers by scalable and reproducible techniques.

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