Max-Planck-Institut für Kolloid- und Grenzflächenforschung

EXPLOITING SELF-ORGANIZATION AND FUNCTIONALITY OF PEPTIDES FOR POLYMER SCIENCE

Habilitationsschrift

zur Erlangung des akademischen Grades
doctor rerum naturalium habilitatus
(*Dr. rer. nat. habil.*)

in den Wissenschaftsdisziplinen Makromolekulare Chemie und Kolloidchemie

eingereicht an der
Mathematisch-Naturwissenschaftlichen Fakultät
der Universität Potsdam

von

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Potsdam, im März 2008

Meinen Eltern
&
Andrea

„Exakte Wissenschaft ist der wahre Reichtum der Welt“

C. R. Darwin

Zusammenfassung

Die Kontrolle von Wechselwirkungen in synthetischen Polymersystemen mit vergleichbarer Präzision wie in Polypeptiden und Proteinen hätte einen dramatischen Einfluss auf die Möglichkeiten in den Polymer- und Materialwissenschaften. Um dies zu erreichen, werden im Rahmen dieser Arbeit Eigenschaften von Oligopeptiden mit definierter Monomersequenz ausgenutzt. Die Integration dieser monodispersen Biosegmente in synthetische Polymere erlaubt z. B. den Aufbau von Peptid-block-Polymer Copolymeren. In solchen sogenannten Peptid-Polymer-Konjugaten sind die Funktionalitäten, die Sekundärwechselwirkungen und die biologische Aktivität des Peptidsegments präzise programmierbar. In den vergangen vier Jahren konnte demonstriert werden, wie in Biokonjugatsystemen die Mikrostrukturbildung gesteuert werden kann, wie definierte Wechselwirkungen in diesen Systemen programmiert und ausgenutzt werden können und wie Grenzflächen zwischen anorganischen und organischen Komponenten in Faserkompositmaterialien kontrolliert werden können. Desweiteren konnten Peptid-Polymer-Konjugate verwendet werden, um für biomedizinische Anwendungen DNS gezielt zu komprimieren oder Zelladhäsion auf Oberflächen zu steuern.

Abstract

Controlling interactions in synthetic polymers as precisely as in proteins would have a strong impact on polymer science. Advanced structural and functional control can lead to rational design of, integrated nano- and microstructures. To achieve this, properties of monomer sequence defined oligopeptides were exploited. Through their incorporation as monodisperse segments into synthetic polymers we learned in recent four years how to program the structure formation of polymers, to adjust and exploit interactions in such polymers, to control inorganic-organic interfaces in fiber composites and induce structure in Biomacromolecules like DNA for biomedical applications.

1 Introduction and Potential Scope of Peptide-polymer Conjugates

1.1 Generation of structure and function (block copolymers versus proteins)

Amphiphilic block copolymers combine polymer segments with different properties. Presumably they are the most widely examined model system for the study of self-assembly and organization to larger scale structures with controlled structural features on the nanometer length scale.[1-6] These studies have clearly identified the precise control of (dynamic) nano- and microstructures in synthetic polymer materials as one key-factor to achieve advanced functional control.[7-13] However, comparing the structural and functional diversity that exists in biology to the functional diversity accessible in synthetic polymer science, it is evident that the capabilities of polymer chemists are still limited. As illustrated in Figure 1, common amphiphilic AB-block copolymers provide the possibility to generate only a limited set of structures in solution. Depending on the block length ratio and the Flory-Huggins interaction parameter χ of the polymer system, polymer micelles, worm-like micellar structures or vesicles ("polymersomes") can be obtained.[1, 3, 14-19] Even though other, kinetically controlled types of block copolymer aggregates have been described, such as toroidal or tubular assemblies,[20-27] the control over nano- and sub-nanoscale structures is still rudimentary as compared to structural biology. Distinct chain-folding events and hierarchically assembly processes – two features abundant in peptides – indicate that polymer science is still full of opportunities.

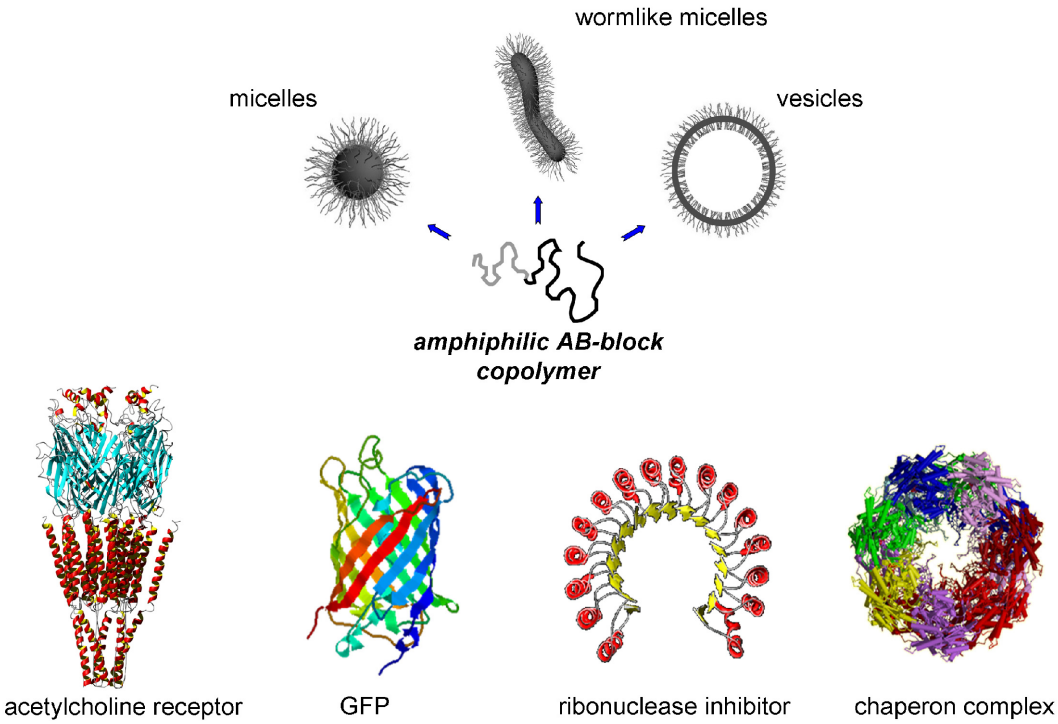


Figure 1. Structural diversity in synthetic and biological macromolecules: The comparison of block copolymers *vs.* proteins shows the limited set of common structures of amphiphilic AB-block copolymers in solution (top) and a selection of proteins (bottom) (assemblies are not drawn to scale for clarity and structures are adapted from ref [19] and from PDB sources).

The concepts present in biological systems demonstrate that an enormous structural variability can be accessed by rather simple and practicable means. Commonly biology combines a limited set of simple building blocks, using a restricted number of connectivities to form biomacromolecules. Nucleic acid (deoxyribonucleic acid (DNA)) for example, has only four different, linearly connected monomers and peptides or proteins combine only 20 simple building blocks (native α-amino acids) in linear chains (*cf.* Figure 2, right).

In contrast to this, polymer chemists have progressively increased the complexity of synthetic polymers in order to access structurally more diverse aggregates. For example, AB-block copolymers have been evolved to ABC-systems (see Figure 2 left) or linear architectures to branched, star or graft systems.[13, 28-35] However, this development is contrary to the conservative concept of biology that - instead of increasing the architectural complexity - increases the information content encoded in a linear polymer chain.

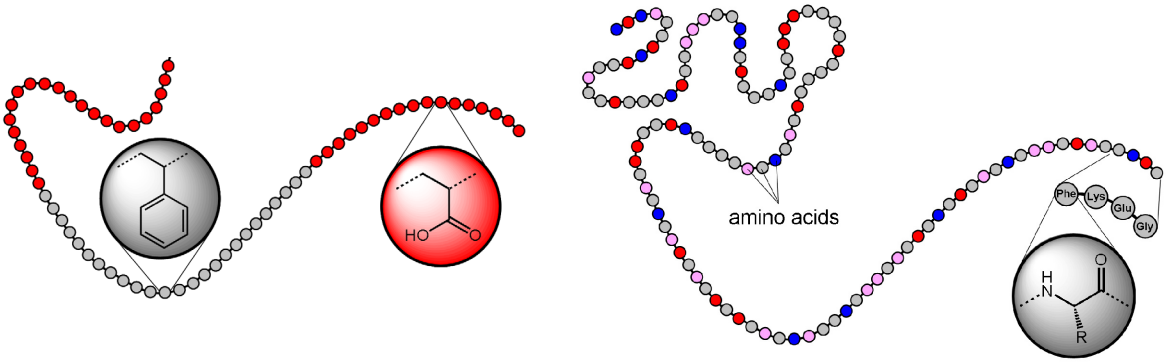


Figure 2. Schematic illustration of the construction of an ABA-block copolymer with "block wise" defined chain properties (left) and a polypeptide where chain properties are defined with "monomer resolution" (right) (color code: blue (polar, basic), red (polar, acidic), pink (polar, uncharged) and gray (non-polar, hydrophobic).

Polypeptides are indeed an excellent example, since they are monodisperse macromolecules that exhibit a defined monomer sequence and possess neither a chemical nor a molecular weight distribution.[36, 37] This enables the precise control of secondary interactions[8] along the peptide chain, allowing the sequence specific constitution of H-bridges, hydrophobic (entropic)-, coulombic-, and dipolar interactions. Such secondary interactions are rather soft, reversible and often highly localized point contacts. They are the basis to encode distinct supramolecular organization processes within a monomer sequence.[38] The complex translation process of the interaction code (linear chemical code) into a structure, and as a result of this the expression of a distinct function, is generally referred to as "protein folding".[39] Figure 3 (a) schematically shows that the folding of proteins proceeds formally on three distinguishable structure levels: (*i.*) the generation of simple secondary structure elements (*e. g.* α-helix, β-sheet and γ-turn), which are (*ii.*) organized into a 3D tertiary

structure and (*iii.*) the assembly of tertiary structures that leads to the quaternary structure as an assembly of multiple peptide chains. The latter usually describes the biologically active form of a protein, *e. g.* an enzyme possessing catalytic activity. In fact, folding processes relying on hierarchical levels are structurally more diverse than linear self-assembly processes, which make evolutionary adaptation feasible.

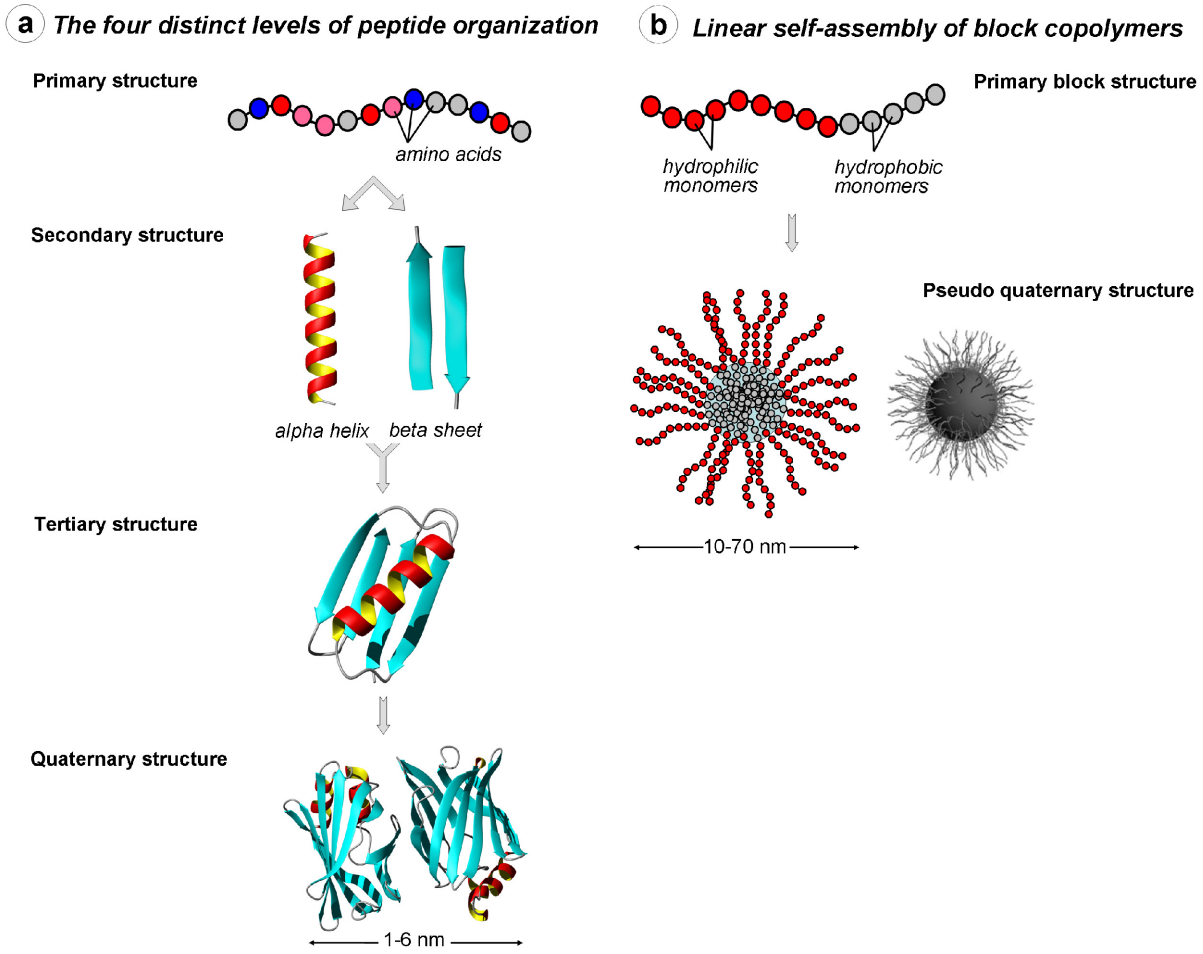


Figure 3. Schematic illustration of the structure formation processes in polypeptides (A) and amphiphilic AB-block copolymers (B). While the block copolymer forms micellar aggregates with a simple core-shell fine structure,

the peptide organization leads to distinct nano structures with a precise hierarchical inner structure (**Primary structure** - the amino acid sequence of the peptide chains; **Secondary structure** - locally defined sub-structures in a single protein molecule; **Tertiary structure** - spatial arrangement of the secondary structures in a 3D-structure of a single protein molecule (example shown B1 domain of the *streptococcal* protein G; PDB:1GB1[40]) and **Quaternary structure** - complex of several polypeptide chains (example shows adipocyte lipid-binding protein; PDB:1A2D[41])).

It is interesting that usually in synthetic superstructures, for example assemblies of block copolymers, the relative importance of the different types of interactions encode each for one separate level of supramolecular organization.[34, 42] However, the interaction code in proteins is programmed with "monomer resolution", realizing a degree of specificity that allows structure formation on a much smaller length scale, which makes nanostructures with a distinct inner sub-nanometer fine structure accessible (Figure 3, A). In these systems suitable interactions cannot longer be provided by single groups or blocks, instead so called "*fit interactions*" play a dominant role.[42-44] Fit interactions are collective interactions between the surfaces of substructure modules, describing the perfect fit with respect to geometry, electron density, amphiphilicity, and polarizability. Such interactions must be sufficiently weak, directed and short-range in nature. Hence, enable the system to dynamically explore the potential energy surface. This highlights the differences between biomacromolecules and "common" block copolymers, since the latter usually exhibit elementary block-block interactions based on *e. g.* selective solubility or polarity differences, only (Figure 3, B)).[1]

1.2 Aim of research and outline of the thesis

The simple, molecular concepts present in structural biology have inspired us to investigate in this present thesis measures to bridge the gap between "common" block copolymers and proteins. While each of these classes certainly has their own special characteristics and limitations, a combination of both protein and synthetic polymer elements will prospectively result in synergistic properties far beyond the single components and indeed may overcome several of their limitations.[45]

As mentioned before, secondary interactions are encoded precisely along the chain within the monomer sequence of a polypeptide. These interactions are used to control the organization processes (chain folding and assembly). The resulting structures can be seen as complex scaffolds to position chemical functionalities within precise geometries, for instance to generate a catalytically active pocket in an enzyme. Evaluating the basic concepts behind those functional protein structures elucidates four fundamental properties:

i. Precisely defined interactions along the polymer chain
ii. Programmable formation of hierarchical structures with defined sub-structure
iii. Positioning of chemical functionalities to generate functions
iv. Capability to actively interact with biological systems (bioactivity)

These fundamental concepts are ubiquitous in high molecular weight proteins. However, inherently they are already present in oligopeptides, if with reduced complexity and specificity. It is this analogy which belongs to the fascinating properties of the class of polypeptides and makes them highly interesting for bioinspired material sciences. In recent

years insight was gained into how protein properties can be exploited for polymer science, making use of the integration of short peptide segments into the existing polymer world (*cf.* Figure 4). Sequence-defined oligopeptides with up to 20 amino acids in length were emphasized, because their sequence-property relationships are still fairly simple and often rationally predictable. The synthesis of these segments proceeds via chemical means on fully automated synthesizer platforms in up to multi-gram scales. Moreover, peptides possess a stable amide backbone and are rather inert against hydrolysis. This might make them more suitable for material science applications compared to other highly interesting bioorganic macromolecules such as oligonucleotides or oligosaccharides. For the choice of all those functional oligomers, biological building blocks are favorable, as they have already shown their extraordinary performance. The main advantage of polymer chemistry, however is not to be restricted to natural availability. Thus, there is ground to believe that systems known from nature could be extended to high temperatures, non-aqueous media and artificial functions.

The precise integration of oligopeptides (or proteins) into well-defined synthetic polymers results in hybrid macromolecules (*e. g.* peptide-*block*-polymer copolymers) that are usually referred to as "*peptide-polymer conjugates*", but also terms like "*bio-hybrids*" or "*macromolecular chimera*"[46] can be found in the literature.[12, 47] This interesting class of macromolecules has the potential to *i.* tune the interaction capabilities of the monodisperse segment precisely, and to enhance both *ii.* the structural and *iii.* the functional space available for polymer assemblies, which might allow the rational design of hierarchically ordered (nano)-structures (*cf.* Figure 4).[10] Furthermore, peptides have the potential to interact with biological systems. Hence the resulting materials might be capable of communicating with biosystems at the interface, making *iv.* bioactive assemblies and materials attainable (*cf.* Figure 4).[48]

The present thesis is outlined along these four fundamental properties of proteins, summarizing our efforts to provide insight into how effectively these concepts can be transferred to synthetic polymer systems.

Before discussing the scope of peptide-polymer conjugates, the synthetic issues have to be addressed. Chapter 2 describes the evaluation of existing polymer synthesis techniques and conjugation strategies and summarizes how these are adapted to provide a synthesis platform to peptide-polymer conjugates.[49] Ideally, the developed routes should envelope a wide range of different synthetic polymers with adjustable molecular weights and low polydispersity indices. Moreover, suggested routes should be independent of both type and molecular weight of synthetic polymer block, as well as of the peptide primary structure. This requires strategies that are highly regio (sequence) selective and compatible to the multifunctional character of peptides.

Beside the development of effective tools to synthesize peptide-polymer conjugates, it has been the scope of the present thesis to transfer concepts from peptide science to polymer science. For that, efforts were made to evolve the oligopeptide segment toward a completely synthetic polymer platform, while preserving both monomer sequence control and the monodisperse character (Chapter 2.2). Beyond the integration of non-natural α-amino acids with synthetic side chain moieties, a library of fully synthetic, non-amino acid monomers should be established, which provides a high synthetic fidelity.[50] To address this issue, polymer synthesis techniques, which give monomer sequence control beyond techniques of established living/controlled polymerization methodologies, are required. These are solid-phase supported synthesis techniques by forced-step chain-growth mechanisms performed in a robotized fashion on fully automated synthesizers. The resulting AB-block copolymers combine one monodisperse "*peptidomimetic*" segment with a synthetic block and might allow to envision sophisticated self-assembly or even folding, similarly to peptide-polymer conjugates but realized on a alternative synthetic basis.

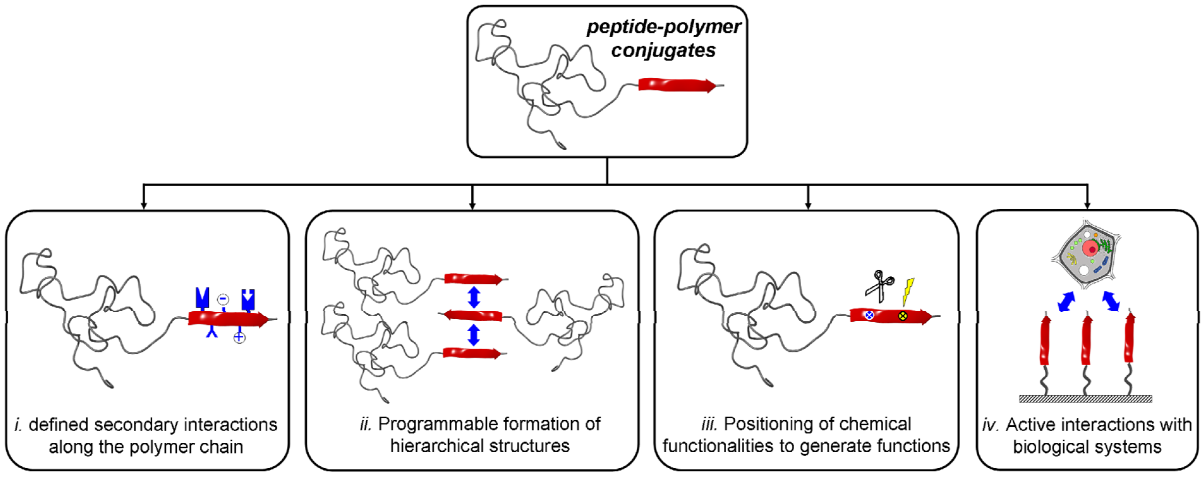


Figure 4. Transfer of the four basic concepts of proteins into the synthetic polymer world by utilizing peptide-polymer conjugates as tools.

Definition of secondary interactions: Chapter 3.1 describes our activities in those approaches to understand and better control interactions in block copolymer systems. Peptide-polymer conjugates or analogous pseudopeptide systems possess a monodisperse (pseudo)peptide segment that can be fine-tuned, enabling – probably for the first time – the precise adjustment of the interaction capabilities of such block copolymers. This makes these macromolecules interesting to study for instance *i.* the complexation of biomacromolecules (*e.g.* DNA or proteins) for biomedical delivery applications;[50, 51] *ii.* the interactions with inorganic or organic crystal surfaces;[52, 53] and *iii.* the complex formation with pharmacologically active compounds (*e.g. cis*-Platinum).[54]

Programming the structure formation: Keeping in mind the enormous structural precision of proteins, the most important aspect of this present thesis is to investigate the self-assembly behavior of peptide-polymer conjugates.[12, 55] Particularly the formation of well-defined micro- and nanostructures with hierarchical organization levels is of interest. In such conjugates, the formation of structure dominantly relies on the directed self-assembly, driven by specific, programmable interactions between peptide-segments. Chapter 3.2 describes our activities and approaches to better controlled polymer superstructures. Given the fact that complex protein functions originate from a defined structure, it is to be expected that advanced control of (nano)structures in synthetic systems might generate functions that are beyond those of the bulk material. Additionally, strategies should be developed to further organize the generated (nano)structures into more complex assemblies with several levels of hierarchical order. Particularly the interplay with inorganic materials was investigated as a way to provide access routes to hierarchical composites.[56]

Positioning of chemical functionalities: The positioning of a highly defined chemical moiety *e. g.* at a specific sequence position in the monodisperse peptide segment of a conjugate, opens the possibilities to establish advanced functions in macromolecules. Such, functions should be designable in an exact manner and beyond those of common polymers. Chapter 3.3 provides two examples. It is shown on one hand how interfacial positioning of a functional peptide domain within a classical block copolymer micelle could be achieved and on the other hand how the positioning of a digestible chemical moiety in a polymer bioconjugate could lead to a responsive two-stage delivery system for DNA.

Generation of bioactivity: Polypeptides are considered the language of biology, transferring signals in complex biological systems. Interestingly, these signaling units are frequently rather small peptide entities with less than 30 amino acids. Thus it appears to be straightforward to integrate peptide signaling domains into synthetic polymers, leading to peptide-polymer conjugates that have a biological meaning. In Chapter 3.4 our efforts are summarized to generate for instance thermo responsive polymers that mediate specific cell adhesion on gold surfaces.[57] Moreover, a single step process was introduced, allowing to spin sub-micrometer polymer fibers with peptide labels at the fiber surface.[58]

2 Strategies to Access Polymer-peptide Conjugates

The combination of polymers of natural and synthetic origin is an appealing strategy to prepare hybrid macromolecules with synergistic properties, beyond both constituents. Generically, such hybrid macromolecules are referred to as "polymer bioconjugates" and in the specific case of integrated peptides, the term "peptide-polymer conjugate" is most frequently used. In the presented chapter, emphasis was laid on the development of broad access routes toward such conjugates. The limitations of existing synthesis routes, which have been practically exclusively focused on poly(ethylene oxide) (PEO) as synthetic polymer block, and which usually yield small amounts of bioconjugates, have been overcome. In order to provide bioconjugates for materials science applications, the introduction of a wide range of organo-soluble synthetic polymers with diverse functionalities and functions has been addressed and multi-gram synthesis scales were realized, without the usually required chromatographic purification steps. Moreover, the platform of monomer sequence controlled peptides was extended to monodisperse poly(amides). For that, the library of natural α-amino acids was not only enlarged by non-natural α-amino acids, but also complemented with synthetic building blocks like for instance functional monomers well-known in polycondensation chemistry.

The research field of bioconjugate synthesis originally gained motivation from pharmaceutical science, where the conjugation of peptides or proteins with PEO have been a central topic for a few decades.[59-62] The covalent attachment of PEO to therapeutically active proteins (a process commonly referred to as PEGylation) leads to improved stability by preventing aggregation, and reduces both immunogenicity and toxicity of the compounds. This frequently increases blood clearance half-life times and *in vivo* bioavailability of the drug.[61, 63, 64]

The field of protein PEGylation grew tremendously within the last decades.[62, 64, 65] Due to traditional barriers between the scientific fields, these important developments have been nearly unnoticed by polymer science, even if the advantages of polymer chemistry are evident, not to be restricted to PEO and capable of extending the nature of the synthetic polymer block to (multi)functional macromolecules. However, with the recent explosion of the fields of nanotechnology and biotechnology, peptide-polymer conjugates have gained increased attention in materials sciences.[10, 66-70] Consequently, the construction of hybrids of biological and synthetic macromolecules became in the last few years an important trend.[12, 46, 71, 72]

Polymer bioconjugates can be generally defined as synthetic macromolecules covalently linked to biological moieties. The latter can be any molecule, which can be found in living organisms such as, lipids, nucleic acids, peptides or carbohydrates. However, this thesis focuses on the platform of peptides or pseudopeptides (monomer sequence defined polyamides), due to the fact that this class of bioorganic macromolecules is multifunctional and has the important potential to exhibit biological activity.

2.1 Synthesis of peptides

Monodisperse polypeptides with a defined amino acid sequence can be accessed via three major approaches: *i.* solid-phase supported synthesis, *ii.* chemical ligation and *iii.* genetic engineering. All these routes allow monomer sequence-control and the integration of non-natural amino acids as selective loci for bioconjugation.

Modern synthetic methods based on sequential solid-phase supported peptide synthesis (SPPS) were pioneered by Merrifield in 1963.[73] These chemical synthesis approaches provide access to essentially any peptide shorter than about 40 residues. As a purely chemical route it offers the advantage for polymer chemistry, not to be restricted to natural α-amino acids. This enables one to extend nature to artificial building blocks, useful for high temperatures and non-aqueous media. Even if the synthesis platform is restricted to oligopeptides that hardly reach the size of the smallest proteins known (about 6 kDa),[74] numerous oligopeptides are described, showing distinct functions and even biological activity. The developments in SPPS have overcome a number of difficulties related to the selectivity of chemical reactions, the insolubility of protected peptides, the optimization of the applied polymer supports concerning accessibility, diffusion properties and non-specific interactions with the peptide as well as the difficulty to drive the step-wise reaction to quantitative conversion. SPPS has greatly facilitated the synthesis of peptides and has made them widely available through automated procedures. Moreover, the sequence-specific incorporation of non-natural amino acids by chemical synthesis is virtually unlimited. Versatile modifications of peptides can be easily achieved, ranging from single position mutants where one amino acid is substituted to the synthesis of pseudopeptides comprising non-peptidic backbones and chemistry.[50, 75] Table 1 summarizes the amino acid derivatives and the functional end-caps that have been utilized within this thesis to synthesize peptide derivatives. These include artificial adhesive groups to "glue" peptides to metal/metal oxide surfaces, photo switchable moieties changing the polarity on photo response, but also diverse initiator or chain transfer groups for the "grafting from" polymerization strategies as well as orthogonally addressable functionalities for click ligation.

To overcome the size limitations of SPPS, efficient strategies have been developed to couple synthetic peptide segments together.[74, 76] These segment coupling strategies are commonly referred to as ligation. The combination of SPPS and enzymatic or chemo-selective ligation now permits construction of entirely synthetic proteins as large as 25 kDa.[74] Moreover, recombinant fragments produced by genetically modified bacterial hosts can be attached to chemically synthesized peptides, utilizing selective fragment coupling reactions.[77-79] An elegant strategy referred to as "native chemical ligation" allows the selective coupling of peptide fragments under natural conditions to form a native peptide linkage.[80]

High-molecular weight polypeptides or proteins comprising native and a progressively increasing variety of non-natural amino acids[81] can also be accessed via recombinant DNA

methods (i. e. genetic engineering) that uses bacterial expression of corresponding artificial genes.[82] These genes can be used to express proteins in bacteria, yeast, plants, insect cells, mammalian cells, or even goats, enabling the generation of reasonable quantities of proteins. The effort of designing artificial genes, modifying host organisms and in particular the isolation of the expressed proteins from the host, limits the application of this technique to peptides with comparatively high-molecular weight much larger than 10 kDa. However, accuracy of the peptide synthesis by the biosynthetic machinery is unsurpassed, and modern biotechnology makes large-scale production possible.

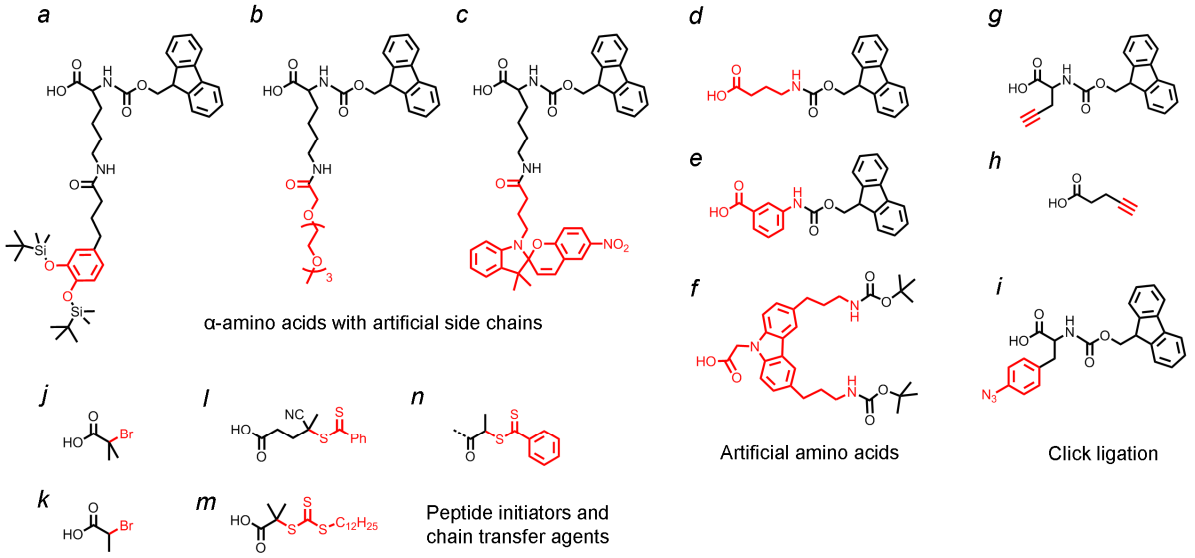
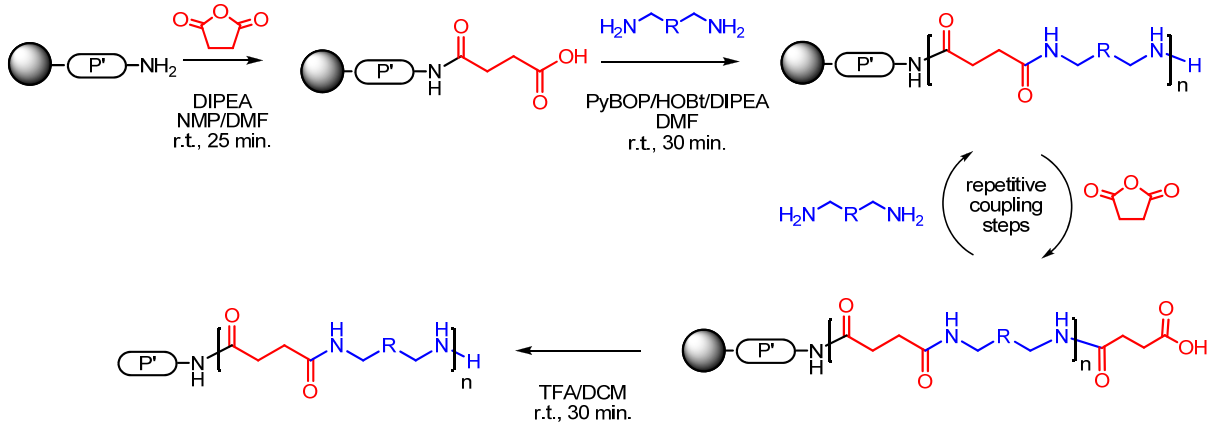


Table 1. Non-natural and artificial monomers for the solid-phase supported synthesis of derived peptides and peptides derivatives (α-amino acids with artificial side chain moieties (a-c), artificial amino acids (d-f and h, j), chemical moieties for the Click ligation (g-h) and residues for the synthesis of peptide ATRP initiators (j-k) or peptide macro chain transfer agents (l-n)).

2.2 Expanding the library of α-amino acids to fully synthetic building blocks

Despite the enormous progress made in living/controlled polymerization techniques to access well-defined macromolecules, the polymerization mechanisms utilized are still statistical processes, yielding polymers with inherent molecular weight distributions. So far none of the established polymerization methodologies enable the synthesis of linear polymers as monodisperse systems with defined monomer sequences. Considering the enormous structural and functional advances of peptides compared to common polymers, the establishment of monomer sequence control in synthetic polymer science is one of the major rewarding challenges. The combination of the existing technology and chemistry of solid-phase supported peptide synthesis with established step-growth polycondensation or polyaddition reactions a route to monodisperse polyamides was established.

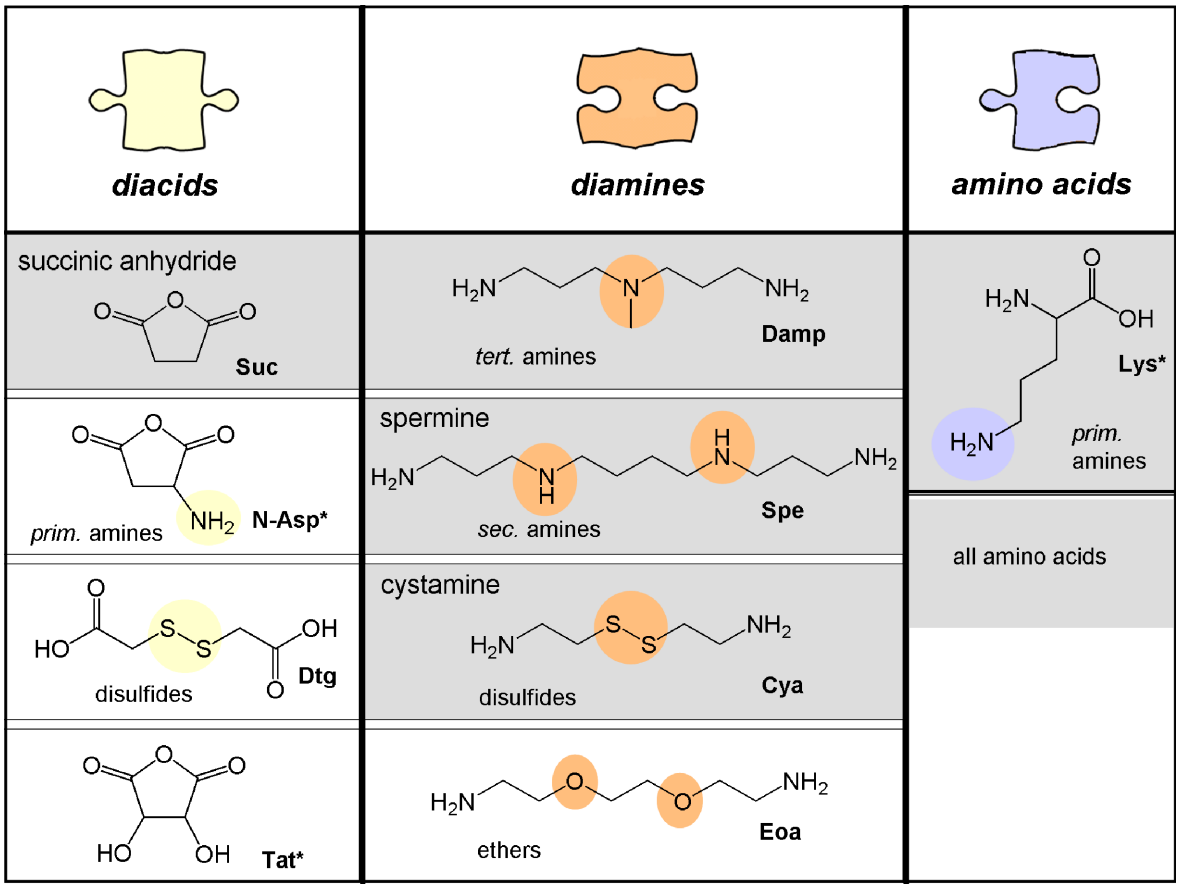
Focus was devoted on the synthesis of linear poly(amidoamines) (PAA). As cationic *pseudopeptides* they combine peptide linkages (amide connectivities) with backbone amino groups. This class of multifunctional polymers already received considerable attention as polydisperse, ill-defined analogues[83, 84] due to their excellent biocompatibility,[85,86] low cyto- and hemotoxicity.[87, 88] The key-advantage of PAAs compared to peptides is, however, the absence of an inherent immunogenicity.[89] It is noteworthy, that peptides are simply "too well known" for the immune system. Depending on the sequence of a peptide an immune cascade can be triggered, resulting in a response of the immune system and hence limiting their biomedical applicability. In contrast PAAs have proven to be suitable for diverse medical applications *e. g.* in the fields of gene delivery or tissue-engineering.[83, 90, 91]



Scheme 1. Sequential synthesis of monodisperse PAA segments. The solid-phase supported synthesis proceeds analogues to SPPS and can be performed in a fully automated manner, on common peptide synthesizer platforms. The utilization of preloaded resins allows the synthesis of peptide-*block*-PAA and the PEO-*block*-PAA conjugates (P' = peptide or PEO, respectively).

In order to establish a synthesis route to monomer sequence-defined, monodisperse PAA segments a solid-phase supported synthesis strategy was applied (Scheme 1).[92] In contrast to peptide synthesis, where N-protected α-amino acid derivatives are sequentially coupled to a functionalized support, the PAA segment is constituted by alternating condensation of functional diacids and functional diamines, respectively. The sequential synthesis could be performed in an automated manner on a common peptide synthesizer platform, resulting in PAAs with defined monomer sequence and absence of a molecular weight distribution. A library of different dicarboxylate and diamine building blocks was evaluated and is summarized in Table 2, several monomers are suitable for fully or semi-automated synthesis of PAAs. Among these there are also naturally occurring building blocks like for instance spermine, spermidine, tartaric acid or aspartic acid (α-δ-connected). It was an important task to preserve the compatibility of the PAA synthesis protocols to those of SPPS. This limits the possible chemistry, however, allows the straightforward synthesis of peptide-*block*-PAA and PEO-*block*-PAA conjugates in up to multi-gram scales.

Table 2. Library of building blocks for supported synthesis of monodisperse PAA segments.



*) selective protecting groups are required; color code: fully automated synthesis (gray) and semi-automated synthesis (white).

Prospectively, the synthesis of sequence defined oligo- and polymers can be readily expanded to various building blocks with diverse secondary functionalities. Completely non-natural polymer classes might be developed, which combine novel units capable of specific molecular recognition with new monomer alphabets to fine-tune secondary interactions along the linear polymer chains. Moreover, the sequence-defined integration of chiral compounds might lead to advanced structural control such as stereo selective folding or aggregation.

2.3 Bioconjugation strategies

The following paragraph describes synthetic strategies for synthesizing well-defined polymer-peptide bioconjugates.[49] More detailed information about established strategies may be found in the following reviews.[59, 61, 62, 93]

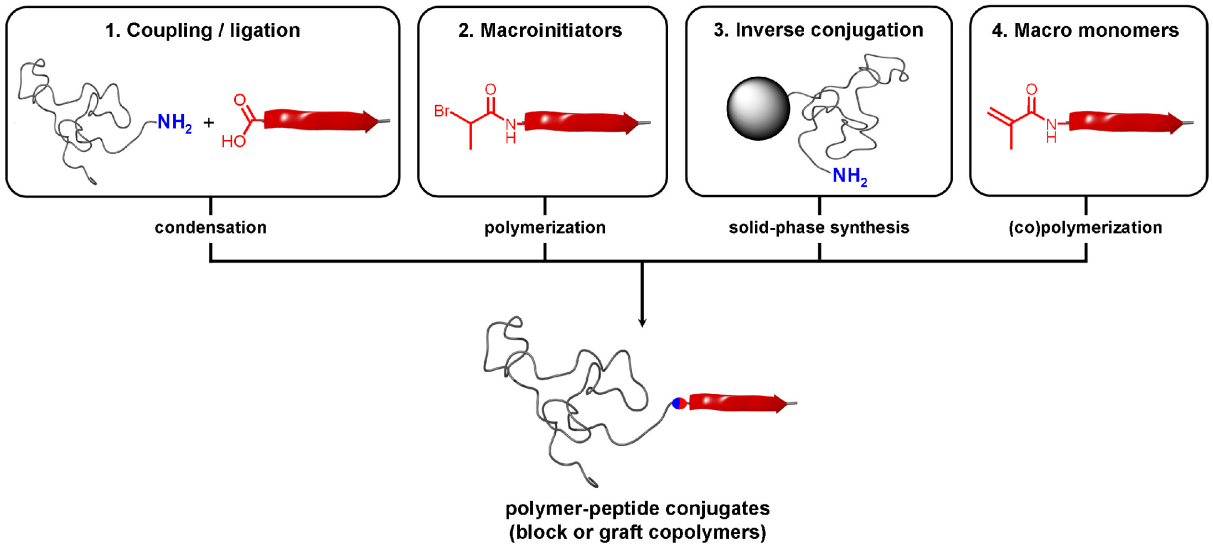


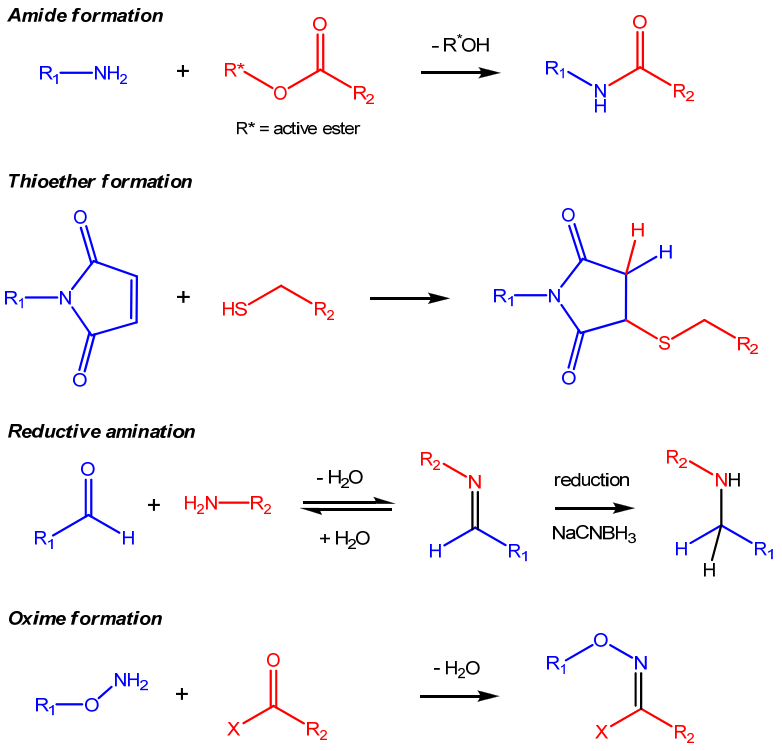
Figure 5. Schematic illustration of the principal synthesis strategies to polymer-peptide conjugates.

Figure 5 summaries the different strategies for the preparation of peptide-polymer conjugates. Typically the routes can be classified into four main approaches: *i.* the coupling strategies, where peptide segments are coupled to preformed polymer blocks using one or multiple reactive sites, *ii.* the polymerization strategies, where the synthetic polymer block is grown *in situ* from the peptide segment ("grafting from approach"), and *iii.* the inverse conjugation strategy, where the peptide is sequentially assembled on a preformed synthetic polymer. The three different strategies can be performed either homogeneously, using solution phase chemistry, or in a solid-phase supported manner. To complete the picture, *iv.* the macromonomer approaches have to be mentioned, too. These rely on the polymerization of short peptide segments that possess a polymerizable functionality and lead to comb structures, which are certainly interesting, but of less importance in this thesis.

2.3.1 Bioconjugation via coupling

2.3.1.1 Standard coupling chemistry

The coupling approach is probably the most common route, applied to introduce synthetic polymers to sequence defined peptides or proteins. This approach involves the attachment of a synthetic polymer that exhibits a well-defined end-functionality to a complementary functionality of a peptide. The most straightforward route relies on the formation of an amide bond (Scheme 2). Frequently this exploits the reaction of an active ester *e. g.* a *N*-hydroxysuccinimide ester derivatized polymer with nucleophiles on the peptide or protein, such as ε-amino group of lysine or the N-terminal amine group. Particularly, the sequence specific introduction of PEO is a rather developed field. A variety of chemo-selective handles are described that can be introduced at a specific position in a peptide sequence, allowing selective conjugation of end-functionalized PEO-chains. These conjugation procedures are commonly performed homogeneously in solution and the most frequently used linker chemistries are summarized in Scheme 2.

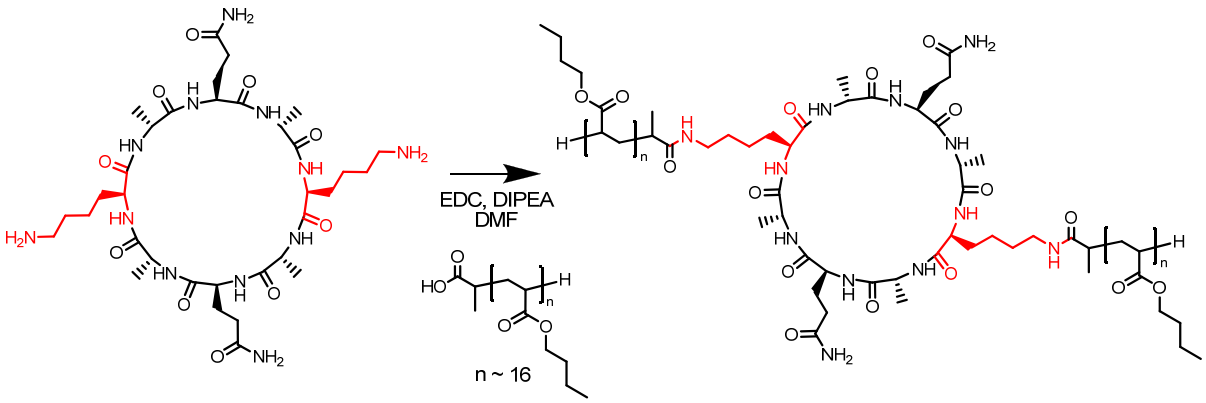


Scheme 2. Commonly used ligation chemistry to couple an end-functionalized polymer to a selective sequence position of a peptide segment.

Aside the conjugation of PEO, other water soluble polymers with biomedical relevance have been linked to peptides, using solution-phase coupling approaches. For instance, the work of Hoffman *et al.* (PNIPAM), Haddleton *et al.* (poly(oligo(ethylene glycol) methacrylates), and Müller *et al.* (poly(*N*-methacryloxy-succinimide)) should be highlighted as interesting approaches, leading to bioconjugates with block- or graft-architectures.[94-100]

2.3.1.2 Homogeneous conjugation in solution

As discussed above, modern applications of peptide-polymer conjugates go far beyond the field of PEGylation and therefore require a wider palette of synthetic polymers. In this thesis we extended the coupling approach to poly((meth)acrylates)) as organo-soluble polymers that result in a new platform of peptide-polymer conjugates.[101, 102] To demonstrate this approach, an α-carboxyl-functionalized poly(*n*-butyl acrylate) (P*n*BA) was synthesized via controlled radical polymerization techniques and coupled in solution to a (D-*alt*-L)-cyclopeptide ("*Ghadiri cycle*") by using standard activation chemistry (Scheme 3).[101] The site-selective coupling was achieved by limiting the anchor functionalities in the cyclopeptide to two lysine residues, positioned at opposite sides of the ring (cyclo(L-Gln-D-Ala-L-Lys-D-Ala-L-Gln-D-Ala-L-Lys-D-Ala). An excess of the P*n*BA was necessary to drive the coupling to quantitative conversion, leading to the P*n*BA-cyclopeptide-P*n*BA conjugate. Usually, the solution-phase coupling is severely hampered, because it requires extensive purification procedures *e. g.* preparative HPLC-purification in order to separate the conjugate from the excess of unreacted P*n*BA. This limits the applicability of the approach. However, in this particular example, the self-assembly properties of the product could be exploited to easily separate the conjugate from the excess of P*n*BA, making a rapid multi-gram scale synthesis possible.[101]



Scheme 3. Synthesis of the conjugate from poly(*n*-butyl acrylate) and cyclic (D-*alt*-L)-α-octapeptide. Adapted from Reference [101].

2.3.1.3 Orthogonal coupling via "Click" chemistry

In collaboration with the group of J.-F. Lutz (IAP, Potsdam) the copper catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes was investigated as tool for the synthesis of peptide-polymer conjugates. The "click" cycloaddition azide/alkyne might be the most frequently utilized example of "click" chemistry[103-105] and has proven to be a robust, regioselective and highly efficient tool.[106, 107] The copper catalyzed Huisgen cycloadditions can be performed at room temperature in various solvents (including water) and in the presence of numerous other functional groups.[104, 105] This latter feature makes this reaction particularly attractive for sequence selective modification of (multi)functional peptides. The

alkyne moiety can be easily introduced either to the N-terminus of supported peptides by capping the terminal amine group with pentinoic acid or to any specific sequence position by using the Fmoc-propargylglycine as amino acid derivative in the SPPS (*cf.* Table 1). For the introduction of the azide compound into a peptide segment, the Fmoc-(*para*-azido phenylalanine) is available, too (*cf.* Table 1).[108, 109] If carefully performed, both approaches lead to highly pure products, which do not require further purification. It was shown, that ATRP offers a continent approach to the complementary azide functionalized polymer, making well-defined polymers with ω-functionality accessible.[110] This methodology was used to synthesize bioconjugates, with potentials for biomedical applicability, by coupling peptides such as RGD[108] (peptide that allows cell adhesion) or TAT[109] (peptide that facilitates cell membrane crossing and nucleus localization) to azide functionalized polymers.

2.3.1.4 Solid-phase supported conjugation

Oligopeptides that have been prepared by SPPS offer the possibility to directly take advantage of protecting group strategies for side-selective conjugation onto resin supported peptides. This is a clear advantage in comparison to the solution-phase coupling procedures, which have been discussed above. Since the SPPS requires anyway the coupling of side chain protected α-amino acid derivatives, the introduction of a functional amino acid with selectively cleavable side-chain protecting group is possible. For this, amino acids such as lysine (ε-amine), cysteine (thiol) or glutamic acid (carboxylate) are available with protecting groups, which can be cleaved in an orthogonal manner to the SPPS reaction steps, to the cleavage of commonly used side-chain protecting groups and to the liberation of the oligopeptide from the support. The generated anchor functionality can be used to introduce a synthetic polymer. This on support conjugation route can take advantage of solid-phase supported chemistry, such as ease of purification and high conversion by large excesses of the polymer compound. However, the coupling of an end-functionalized polymer to a supported peptide is a reaction that takes place in a polystyrene micro-gel (solid-phase support) and hence it proceeds usually diffusion limited. This gets progressively more evident the higher the molecular weight of the polymer is. The strong decrease of the overall coupling rates is consistent with the early work of Mutter *et al.* (coupling of α-methoxy-ω-carboxy-PEO) and can be explained by mainly three different factors: *i.* the reduction of the reactivity of the polymer end-group, *ii.* by the decrease of diffusion rate of the polymer in the micro-gel and potentially by *iii.* an increased incompatibility of the polymer with the solid support.[111 112]

However, the on-support coupling strategy that uses SPPS techniques has strong advantages, particularly if the product is rather sensitive or difficult to handle *e. g.* because of a high aggregation tendency. This was demonstrated by the quantitative conjugation of a carboxyl end-functionalized P*n*BA ($M_n \sim 2000$, $M_w/M_n = 1.16$) to a peptide aggregator domain ((threonine-valine)$_5$).[113] Even if the aggregation tendency of the (TV)$_5$-domain was

temporarily disturbed by the incorporation of structure breaking defects into the peptide sequence ("Switch-ester" segments[114-116]), a purification of this conjugate *e. g.* by HPLC methods would have been highly challenging and both time and materials consuming.

2.3.2 Direct polymerization from a predefined peptide

The controlled growth of a synthetic polymer from a defined position in a preassembled peptide is conceptually the most straightforward approach for synthesizing polymer-peptide bioconjugates.[49, 117] In such approaches, the peptides have to be chemically modified with a low molecular weight initiator moiety and subsequently used as macroinitiators for a polymerization of the synthetic monomer. This strategy overcomes the limitations of the coupling methods, since the polymer chain grows from the peptide and hence it is not restricted in molecular weight. Moreover, such an approach involves only low molecular weight reactants and therefore generally leads to clean products. However, the utilization of a peptide based macroinitiator is not trivial because the polymerization processes, used for preparing the synthetic polymer block should be unaffected by diverse functional groups, which might be present in the multifunctional peptide system. So far, radical polymerization is the only process, which has been evidenced to be broadly tolerant to the presence of biological segments. The methodologies of controlled radical polymerization (CRP) such as atom transfer radical polymerization (ATRP), nitroxide mediated polymerization (NMP) and reversible addition-fragmentation transfer polymerization (RAFT) seem to be most suited for the preparation of polymer-peptide bioconjugate.[118, 119] Indeed, these polymerization techniques combine the advantages of standard free radical polymerizations (*e. g.* experimental simplicity, tolerance towards functional groups) and anionic polymerizations (*i. e.* controlled character). Moreover, CRP methods allow the controlled polymerization of diverse monomers, giving access to a variety of polymers with defined molecular weights with low polydispersity indexes.

Nitroxide mediated polymerization (NMP) is historically the first widespread CRP method.[120-123] Wooley and coworkers investigated two alkoxyamine-functionalized oligopeptides (i. e. TAT peptide and the antimicrobial peptide tritrpticin) as macroinitiators for NMP.[124, 125] Their general strategy includes a two step procedure to introduce the alkoxyamine moiety to the N-terminal amino group of a supported peptide. Subsequently, the supported peptide macroinitiator was used directly to initiate a solid-phase supported NMP. Two different triblock copolymers were synthesized: TAT-*block*-poly(acrylic acid)-*block*-poly(methyl acrylate) and tritrpticin-*block*-poly(acrylic acid)-*block*-poly(styrene).[124, 125] After polymerization, the polymer bioconjugates were liberated from the support by using standard procedures. These first results suggested that NMP is a very promising tool for bioconjugation. However, no details were presented concerning the degree of control of the CRP reaction, and only limited characterization data were accessible due to the complexity of the conjugates. So far, this interesting aspect has not been studied by other authors.

2.3.2.1 Grafting from peptides via ATRP

In our efforts to develop a simple and straightforward access platform to peptide-polymer conjugates, atom transfer radical polymerization (ATRP) was investigated. ATRP is the most studied and applied method of CRP.[126, 127] This is probably due to both its experimental simplicity and the commercial availability of most ATRP initiators and catalysts. In ATRP, the growth of the polymer is controlled by a transition metal complex (usually copper- or ruthenium-based), which establishes a redox equilibrium between active macroradicals and dormant species end-capped by a halogen atom. This straightforward mechanism provides control over the polymerization of a wide variety of functional monomers.[128]

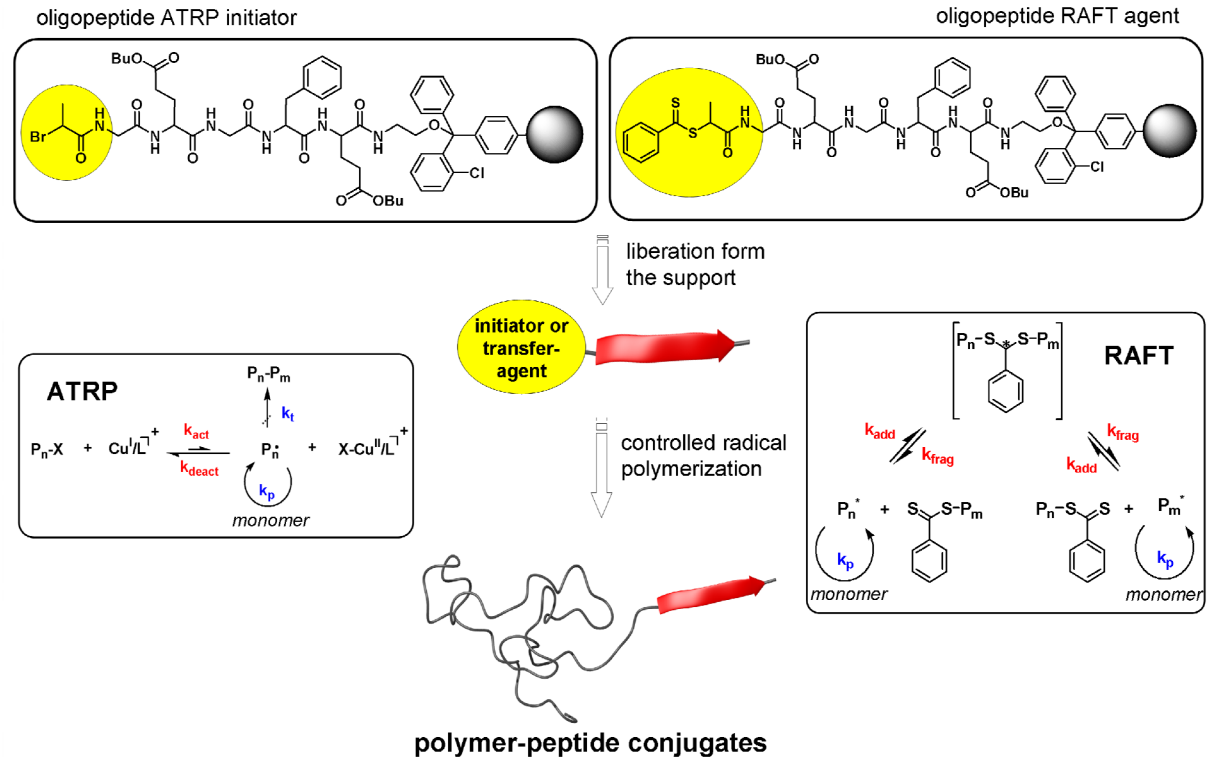


Figure 6. Schematic illustration of the "grafting from" approach relying on (*i.*) the sequence specific introduction of an initiator or a chain-transfer moiety into the peptide by solid-phase supported means, (*ii.*) the liberation of the peptide macroinitiator or the peptide chain transfer agent from the support and (*iii.*) homogeneous solution-phase polymerization of a synthetic monomer by ATRP or RAFT to grow the polymer chain from the peptide.

We investigated the homogenous solution-phase ATRP of butyl acrylate (*n*BuA) in the presence of an oligopeptide macroinitiator.[129] As summarized in Figure 6 (left) the peptide macroinitiator was synthesized via standard SPPS methodologies. The N-terminus of the supported peptide was utilized to introduce a common ATRP initiator moiety (α-bromopropionate). After liberation of the fully protected peptide-macroinitiator from the support, the solution-phase polymerization of *n*BuA was performed. The use of unsupported

peptides offers advantages over the solid-phase supported strategy of Wooley *et al.* For instance, it was shown for "grafting from" polymerizations from either silica supports or polystyrene microgel particles that the deactivation step of ATRP as the most delicate step for optimal control is diffusion-limited.[130-132] Such drawback is circumvented in homogeneous solution-phase polymerizations. The solution ATRP of *n*BuA in the presence of a model peptide macroinitiator resulted in well-defined peptide-polymer conjugates with low polydispersity index ($M_w/M_n = 1.19$) and predictable molecular weight of the synthetic polymer block.[129] Although interactions between the copper-catalyst and the oligopeptide were evident, it was demonstrated that they were not critical in terms of synthesis control.

Following the initial reports,[129, 133] other research groups described the ATRP construction of oligopeptide-polymer bio-hybrids using either the solid-phase or the solution-phase macroinitiator strategy.[125, 134-136] Moreover, not only oligopeptides, but also proteins were successfully conjugated to polymers by using the "grafting from" strategy via *in situ* ATRP.[137-140] This route seems to be promising and straightforward for preparing polypeptide-polymer conjugates. However, *in situ* ATRP approaches require transition metal catalysts, which may in many cases interact with the peptide or the protein. Despite these possible drawbacks, the ATRP "grafting from" method is, by many aspects, probably more practical than conventional coupling approaches.

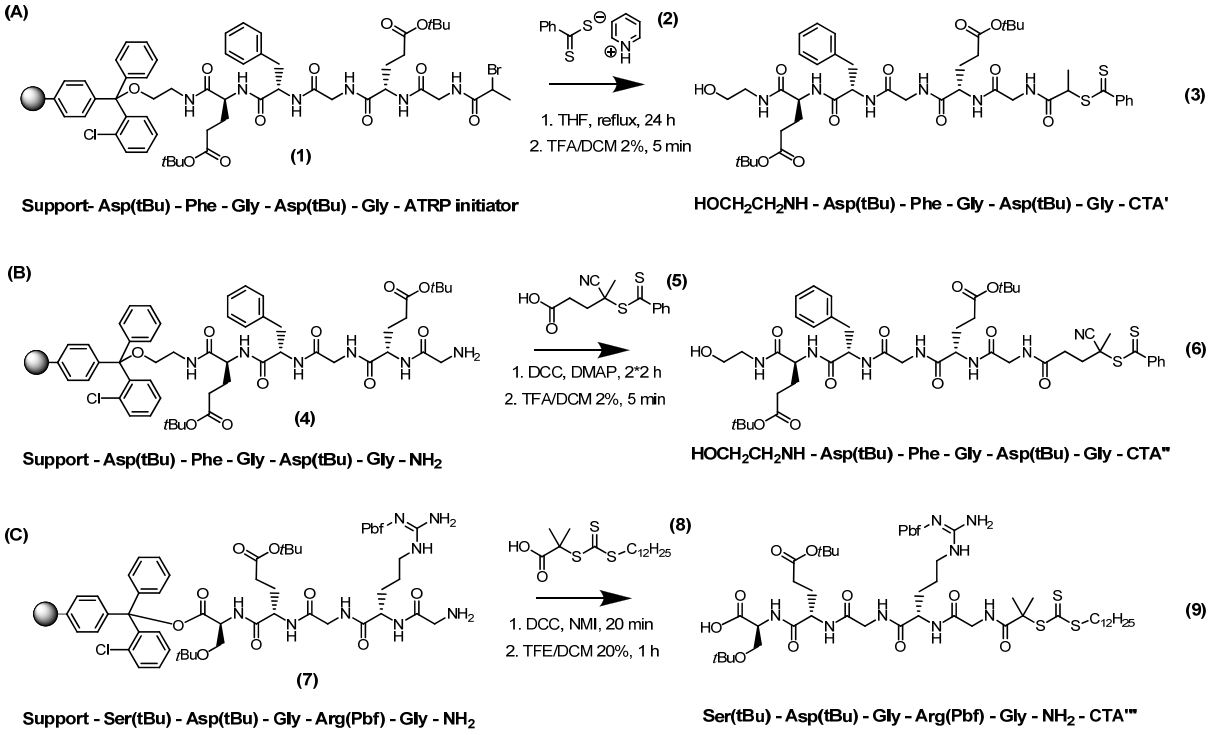
2.3.2.2 Grafting from peptides via RAFT

Analyzing the advantages but also the method immanent difficulties of ATRP we started the investigation of the reversible addition fragmentation transfer polymerization (RAFT) as promising tool to access peptide-polymer conjugates. The RAFT radical polymerization technique has been established as a versatile CRP tool.[141-147] Particularly, the tolerance toward many functional groups (relevant exceptions are indeed nucleophilic groups such as amines or thiols), the absence of metal catalysts and the close relation of the RAFT process to conventional free radical polymerizations are advantageous for the synthesis of bioconjugates.

McCormick *et al.* followed by Perrier *et al.* introduced the first amide-based RAFT chain transfer agents (CTAs). These low molecular weight CTAs gave efficient control in the polymerization of DMA, styrene, methyl acrylates.[148] Thus, the studies demonstrated that amidic CTAs do not suffer from slow primary fragmentation steps or inefficient re-initiation.

Taking this promising observation into account, we expanded the RAFT polymerization technique for the first time to the synthesis of well-defined peptide-polymer conjugates.[117, 149] This enables the straightforward synthesis of polymers with multifunctional and potentially bio-functional peptide segments. The peptide CTAs were synthesized via solid-phase supported synthesis strategies, making the usually required chromatographic purification step of the CTA obsolete (*cf.* Figure 6 (right)). Particularly this strategy is of advantage if large

and/or multifunctional peptide CTAs are synthesized, because a chromatographic purification step would proceed strongly dependent on the amino acid sequence and get progressively more difficult with peptide length. Two different synthesis pathways have been established, giving *i.* peptide-dithiobenzoate CTAs[149] and *ii.* peptide-trithiocarbonate CTAs[57] in almost quantitative yields (cf. Scheme 4). The dithiobenzoate CTAs have been accepted as generally applicable RAFT CTAs, mediating effectively the polymerization of a broad variety of functional monomers. Peptide-dithiobenzoate CTAs can be accessed by clean functionality transformation of a resin-bound oligopeptide ATRP macroinitiator[129] into an oligopeptide chain transfer agent (Scheme 4, route a).[149] The alternative approach that involves the coupling of 4-cyano-4-((thiobenzoyl)sulfanyl)pentane carboxylic acid (Scheme 4 (5)) to the N-terminal amine group of a supported peptide yielded the macro RAFT agent in about 76% purity. This direct approach suffers from the formation of about 24% thioamide side product as a result of the nucleophilic attack of the peptide amine on the dithioester. Kinetic investigations, in which *n*BA was polymerized homogeneously in solution, using oligopeptide CTAs (Scheme 4, compound 3 and 6), revealed an efficient control of the polymerization processes. Peptide-polymer conjugates with polydispersity indices of about 1.1 and controllable molecular weights were obtained.[149]



Scheme 4. Synthetic routes to oligopeptide chain transfer agents (3, 6 and 9) either via functionality transformation of a peptide ATRP-macroinitiator into a peptide macro-CTA (route A (1→3)) or by coupling of carboxylate functionalized CTAs (5 or 8) to the N-terminus of a supported decapeptide (route B (4→6) and route C (7→9)); (decapeptide ATRP-macroinitiator attached to a pS-(2-Cl-trityl) resin as solid support (1), supported decapeptide (4 and 7) and peptide-based CTAs after liberation from the support (2, 6 and 9)).

Despite all these advantages, and the potentials of the RAFT methodology to synthesize bioconjugates, the synthesis protocol for the introduction of the dithiobenzoate CTA cannot be automated and hence remains relatively time-consuming. Therefore, a convenient and fully automated one-step approach was developed to synthesize peptide CTAs, while strongly reducing the synthetic efforts and the costs.[57] The strategy does not rely on standard dithioester-based CTAs, but on trithiocarbonates (*cf.* Scheme 4 route C). The latter have been recently shown to be very efficient CTAs for controlling the polymerization of various monomers (*e. g.* St,[150] MMA,[142] *n*BA,[151] or NIPAM[152]) and moreover exhibit a higher tolerance against nucleophiles than the dithiobenzoate analoges.[152, 153] Additionally, while the synthesis of RAFT CTAs often requires multi-step reactions and chromatographic purifications, the S-1-dodecyl-S'-(R,R'-dimethyl-R''-acetic acid) trithiocarbonate (Scheme 4 (8)) is readily accessible from commodity compounds (acetone, chloroform, carbon disulfide and 1-dodecanethiol) in a one-pot reaction. The ease of synthesis, the absence of further purification steps and the cost-effective large scale accessibility (100 g scales) makes this CTA an appealing candidate for the design of peptide-based CTAs. The S-1-dodecyl-S'-(R,R'-dimethyl-R''-acetic acid) was treated like a Fmoc amino acid derivative and was coupled to the N-terminus of a supported peptide in a fully automated manner. Substitution side products like *e. g.* dithiourea (R-SC(S)NH-R') were practically negligible, as they were limited to ~0.3%, indicating the high tolerance of the trithiocarbonate functionality toward nucleophiles. After liberation of the peptide-CTA (Scheme 4 (9)), the polymerization of *n*BA was effectively mediated, leading to peptide-polymer conjugates with adjustable molecular weight and low polydispersity index (M_w/M_n ~1.1). To demonstrate the relevance and potentials of this particular approach the peptide-CTA with trithiocarbonate functionality was used to mediate the polymerization of *N*-isopropylacrylamide (NIPAM) and oligo(ethylene glycol) acrylate (OEGA). Well-defined peptide-polymer conjugates were obtained with adjustable molecular weights and rather low polydispersity indices, which are both interesting polymers for biomedical or pharmaceutical applications.

Moreover, the peptide macro-CTR strategy is not limited to AB-block structures. It can be applied further toward the synthesis of more complex peptide-polymer conjugates.[154] This was shown by the synthesis of a functional, amphiphilic ABC-triblock copolymer, comprising a central decapeptide segment that links a PEO-block with a P*n*BA-block. The required PEO-peptide macro chain transfer agent was easily accessible via solid-phase supported techniques. The macro-CTA was cleanly liberated from the support and used to mediate the RAFT polymerization of *n*BA in solution, providing well-defined ABC-triblock copolymers.

2.3.3 Inverse bioconjugation approach

In contrast to the conjugation strategies described above, which are means to couple or graft synthetic polymers to or from peptides, the inverse bioconjugation strategy relies on the stepwise synthesis of the bio-segment on a preformed synthetic polymer. This strategy points back to 1965, where Kozhevnikova *et al.* described the use of synthetic polymers as soluble supports for liquid-phase synthesis of oligopeptides.[155] However, the soluble support was cleaved and sacrificed after finishing the sequential synthesis of the peptide.

An elegant approach to PEO-peptide conjugates was pioneered by the research groups of Mutter and Bayer.[111, 156-158] For that a common PS-resin was preloaded with functionalized PEO, leading to the nowadays commercialized PAP-resin (PEO-attached PS-resin).[159] It is noteworthy that the synthesis of the PAP-resin combines solid-phase supported polymerization of ethylene oxide by means of anionic polymerization and subsequent transformation of the ω-hydroxyl group of the PEO-grafts into ω-amino functionalities.[158] However, while the α-functionality of the PEO-block is connected to the PS-resin by a benzyl-ether linker (*cf.* Figure 7), the ω-amino group can be used to assemble a peptide segment sequentially with automated SPPS.[159] The resulting PEO-peptide conjugate exhibits a permanent amide connectivity between the PEO and the peptide segment, but an acid cleavable benzyl-ether linker between the PS-backbone and the PEO. Hence, the complete PEO-peptide conjugate can be liberated after finishing the peptide synthesis. Using the PAP-resin strategy provides well-defined PEO-peptide conjugates, where the PEO-block is attached at the C-terminal side of the peptide-segment.

It was demonstrated also in our work that the PAP-resin can be used to synthesize highly pure PEO-peptide conjugates, comprising rather complex, bulky diamino acids and aggregator domains (Figure 13 a).[160] The PEO-grafts of the resin facilitate the peptide synthesis of difficult sequences due to both an enhanced swelling behavior and depletion effects by the PEO in the PS-microgel. As a consequence micro aggregation or adsorption of the peptide onto the hydrophobic PS-backbone is reduced.[111, 158, 159]

However, one drawback of the PAP-resin results from the rigorous acidic conditions that are required for cleavage of the conjugate (99% TFA, up to 1% bromotrimethyl-silane, up to 6 h).[159] Even if the PEO-block surprisingly survives these harsh acidic conditions without fragmentation, the convenient PAP route is not suitable for conjugates composed of more sensitive non-natural amino acids, or peptide derivatives such as switch-ester segments and pseudopeptides.[50, 102, 114, 115] To overcome these limitations we developed in collaboration with Rapp *et al.* a Wang linker based PAP-resin. By using the hydroxymethylphenoxy instead of the benzyl alcohol linker (Figure 7) to attach the functional PEO to the PS-backbone the ether connectivity between the PEO and the PS resin is activated.[50] This allows the liberation of the conjugate under gentle conditions as only 2-5 vol.% of TFA in dichloromethane has to be used and quantitative cleavage occurs within about 10-45 min.

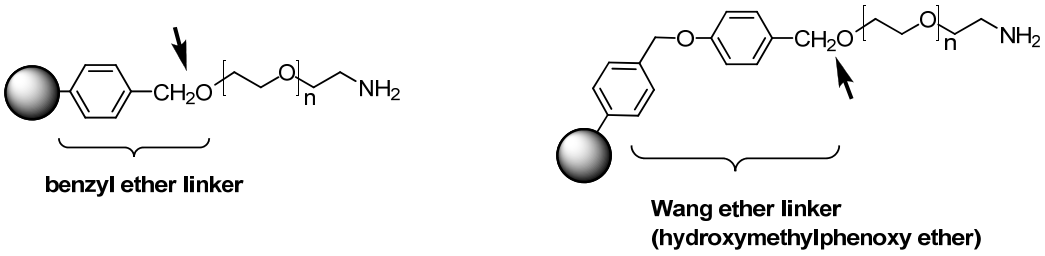


Figure 7. PAP-resin compared to PAP-Wang-resin (PEO-attached PS-resins, cleavage point is marked with an arrow).

3 Implementing Protein Properties into Synthetic Polymer Systems

As discussed in the introduction, natural peptides and proteins cover not only a wide range of molecular and material properties, but also realize diverse functions. It is interesting to note, that the fundamental properties of proteins are in fact inherently present in oligopeptides. The present work discusses the efforts undertaken to transfer the fundamental properties of proteins into polymer science by integrating oligopeptides (and their synthetic analogues *i.e.* pseudopeptides) into synthetic polymers. In this chapter, the results are summarized, showing how effective peptide functions can be transferred to synthetic polymer materials.

3.1 Precisely defined secondary interactions along the polymer chain

The capability to define the monomer sequence in a monodisperse (pseudo)peptide segment, makes it possible to fine-tune polymer properties with "monomer resolution". Different monomeric building blocks can be selected from a library of natural and non-natural α-amino acids as well as from their fully synthetic analogues. These monomers are incorporated at a specific sequence position, locally defining the properties and the functionalities of each position in the polymer chain. In contrast to common polydisperse polymers this leads to sharply expressed sequence-property relationships, because the overall property is defined by one discrete species and does not result from the convolution of a set of polymer molecules.

The following chapter provides a selection of examples, discussing the potentials of polymers with precisely adjustable secondary interactions in biomedicine and materials sciences.

3.1.1 Polymer-peptide conjugates with distinct interactions to DNA

Even if the solid-phase supported synthesis of peptides is nowadays routine in many laboratories and operating expenses have been strongly reduced in the last five years, peptide synthesis is still rather time consuming and expensive, as compared to other polymerization procedures. However, the monodisperse definition of the products, combined with high reproducibility makes these systems promising candidates for applications in the biomedical or pharmacological field. Indeed, legislation is claiming more and more defined products for registration, so that synthetic concepts for highly defined structures are mandatory.

We have demonstrated the potentials of peptide-polymer conjugates, by designing block copolymer carriers for DNA transport, which actively pack and unpack the cargo. As a requirement for the effective transport of DNA for gene delivery applications, a high molecular weight drug has to be strongly condensed (double stranded plasmid DNA (*ds*DNA) with *e. g.* 5.000 base pairs has an average molecular weight of $M \sim 3.3 \times 10^6$ Da). This packing of *ds*DNA should, however, be reversible, because unpacking is required at the location of action (*i. e.* in the target cell, where transfection should take place). Nature solves the problem of DNA condensation in the cell nucleus by sophisticated packing tools. The overall process is up to now not fully understood. However, in a simplified way a set of globular proteins referred to as Histones are utilized. These have defined cationic surface charges, which interact with the negatively charged phosphate groups along the DNA-backbone. The interactions are soft, reversible and moreover, highly regulated by diverse enzymes (acetylases, kinases, and methylases). From the point of view of a physical chemist, a strongly regulated polyion complex formation takes place, which guides the rigid DNA double helix construct (~60-80 nm persistence length) into coils and supercoils as illustrated in Figure 8 a.

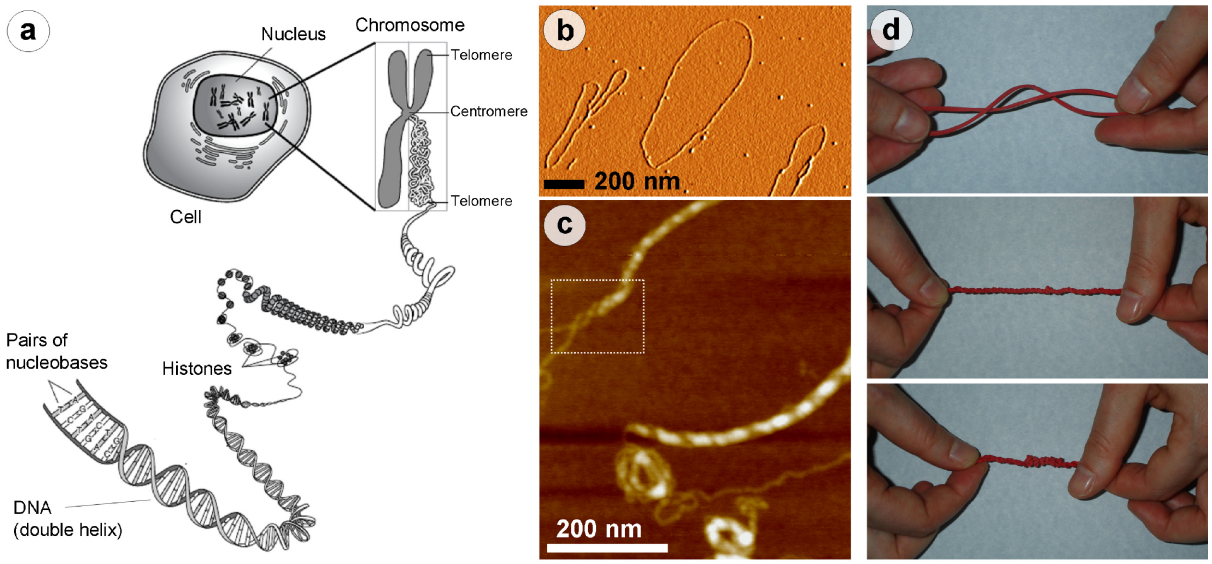


Figure 8. a) Biological packing of DNA via supercoiling to condense DNA in the cell nucleus. The packing is induced and regulated by distinct DNA-histone interactions. ***b, c)*** Controlling the compression of plasmid *ds*DNA by well-defined contacts of the DNA with different PEO-PAA conjugates ((b) Expanded DNA loops using PEO-*t*PAA with tertiary amines and (c) compact supercoiled DNA using a PEO-*sp*PAA with a mixture of secondary and primary amines (the ongoing process of DNA supercoiling can be seen by the entanglement of two DNA double strands (marked box)). ***d)*** schematic illustration of DNA supercoiling with the help of a rubber band model. (Image (a) was adapted from: http://www.accessexcellence.org).

The condensation of plasmid *ds*DNA for gene delivery appears to be state of the art.[161-166] However, if the literature that covers this topic is carefully analyzed, it becomes evident that the polymers utilized for transfection and gene delivery were optimized empirically. The most frequently applied polymers are branched poly(ethylene imine) and linear poly(L-Lysine), often with multimodal, ill-defined molecular weight and chemical distributions.[167] To illustrate the need for further developments in this field, SuperFect® as a commercially available transfection agent should be discussed briefly.[168] SuperFect® is based on Starburst®, a poly(amidoamine) dendrimer.

Even though it was described that Starburst® interacts with DNA, the perfectly defined dendrimers have to be treated by statistical hydrolysis to obtain SuperFect® with an increased activity. Certainly SuperFect® is more active, but it is molecularly ill-defined. This makes it difficult or even impossible to learn which molecular parameters are important for the different steps in the transfection process (*i.* DNA condensation; *ii.* translocation via the cell membrane and *iii.* delivery of DNA into the cell nucleus).

As the peptide segment in poly(ethylene oxid)-*block*-peptide conjugates is monodisperse, the cationic character can be programmed by positioning *e. g.* lysines, histidines, or arginines in the sequence. Considering the inherent risk of immunogenicity and/or toxicity of such conjugates, we have developed a biocompatible platform of cationic pseudopeptides on the basis of linear poly(amidoamine)s (PAAs).[50, 51] This polymer class combines amides with main chain amine groups and synthesis proceeds via sequential monomer assembly in a supported manner. A library of functional monomers is available (Table 2) that allows fine-tuning of the monodisperse PAA segments (*cf.* Section 2.2).

To gain further insight into the interaction of cationic polymers and *ds*DNA, a set of three different poly(ethylene oxide-*block*-amidoamine) conjugates (PEO-*block*-PAA) was synthesized. The polymers have PAA-segments with systematically altered monomer sequences, while keeping the PEO-block constant. The choice of building blocks used to assemble the PAA-segments allows for the precise positioning of amine functionalities with various base strengths. Thus the cationic character was tuned by introducing tertiary, secondary and primary amine groups at specific sequence positions. The polymeric carriers exhibit sharply defined sequence-property relationships, which enable precise understanding of how the polymer structure influences the interaction capabilities of the carrier with DNA. To ensure reproducible formation of *ds*DNA-polymer ion complexes (polyplexes) a microfluidic device was used referred to as a microfluidizer.[169] This consists of a Y-shaped channel system that allows non-turbulent mixing of solutions of DNA and carrier polymer through diffusion at a defined interface.

Easily comparable polyplexes were obtained and the complexation as well as the compaction behavior depending on the used carrier was investigated. Figure 8 (b-c) shows a strong influence of the PAA-segments on the polyplex structures. In contrast to the usually applied branched poly(ethyleneimine)s, which result in ill-defined, globular, multi-plasmid polyplexes, the PEO-*block*-PAA conjugates lead dominantly to uniform, single-plasmid complexes (Figure 8 b-c). Their structure strongly depends on the cationic character, which is encoded by the number and type of cationic functionalities within the PAA-segment. This allows controlling the polyplex structure. Extended ring-like polyplexes could be observed, if carriers with tertiary amines have been used (Figure 8 b, PEO-*t*PAA), whereas strongly compacted polyplexes are formed for carriers with combined secondary and primary amine groups have been applied (Figure 8 c, PEO-*sp*PAA). The latter exhibited a well-defined mode of compaction, as “supercoiling” of the DNA cargo could be observed. PEO-*sp*PAA was certainly the most promising candidate for further biological testing, because it not only realizes highly condensed polyplexes, which are favorable for transport and cellular uptake, but also provides excellent stabilization against enzymatic DNA-degradation, as was shown by DNase assays.

Taking into account the architecture of the carrier polymers and the resulting polyplex structures, a model has been proposed that explains the complexation and condensation properties of the carriers. In this model the cationic PAA-segment binds to the *ds*DNA, screening the negative charges of the DNA backbone, while the PEO-block stabilizes the polyplex, preventing cross-linking and producing a "stealth" aggregate. This leads to stable single-plasmid complexes and even stabilizes the DNA structure itself, as was demonstrated by intercalation fluorescence assays.

Such a soft, stabilizing compression mode is of great importance for *in vitro* and *in vivo* applications, and cooperative unpacking of the cargo DNA at the desired location of action might be possible. Thus the progress in fundamental understanding of these processes might even contribute to novel strategies of drug delivery systems with increased effectiveness.

The scope of the (pseudo)peptide-polymer platform is indeed much broader as applications are not restricted to the field of pharmacological and biomedical sciences. In fact high precision block copolymers that combine an inert synthetic polymer block with a highly adaptable, multifunctional segment might be useful for modern materials sciences, which certainly require selective or even specific interactions. Surface or structure specific gluing, selective absorption of organic, inorganic or biological compounds from mixtures or selective compatibilization to laminate incompatible compounds are of importance for future applications. To address these issues, however, the monomer library for the solid-phase supported synthesis of PAAs has to be diversified, and establishing directed screening assays comparable to those existing for peptides would certainly be advantageous.

3.1.2 PEO-peptide conjugates as crystal growth modifier

In the following paragraph two examples form Cölfen and co-workers (MPI-KGF) will be discussed, which provide only a small glance on the possibilities that arise when specific interactions are purposefully employed in materials sciences. Modern material strategies demand progressively specific interactions and applications go far beyond the classical fields of pharmacological drug delivery or targeting. For instance highly effective, biological or bioinspired additives in washing powders are requested as scale inhibitors to prevent calcium carbonate precipitation, or cosmetics should be "delivered" to the keratin structures of the hair, but not to the collagen of the skin.

Compounds that change or control the growth of crystals are usually referred to as crystal growth modifiers. These are of enormous interest for academic research, as they might reveal some secrets related to the biomineralization processes, which result in multiplicity of materials *e. g.* crustacean carapaces or mollusk shells and bone or teeth tissues in vertebrates. Cölfen *et al.* pioneered the field of double hydrophilic block copolymers for the use as synthetic crystal growth modifiers.[170, 171] Combining a water soluble, non-functional polymer

block with a functional one, permits the definition of interactions with ions and crystal surfaces feasible. Depending on the nature of the functional block, crystallization processes can be influenced and guided. It appears straightforward that the utilization of poly(ethylene oxid)-*block*-peptide conjugates provide interesting systems because the functionalities of the peptide segment can be precisely programmed. Moreover, the monodisperse character of the peptide should lead to rather uniform processes, since *e. g.* adsorption phenomena of polymers on surfaces are certainly depending on the length and multi-valence of the polymer.

Besides the investigation of PEO-peptide conjugates with more classical sequences, relying on multimers of glutamic acid or aspartic acid (*e.g.* Asp_5, Asp_{12} or Glu_{15}) and its systematic variation of the functional group density (*e.g.* $(Asp\text{-}Gly)_6$, $(Asp\text{-}Asp\text{-}Gly)_4$ or $(Asp\text{-}Gly\text{-}Gly)_4$) a PEO-peptide conjugate with a zwitterionic adhesion motif was studied.[172] The crystallization of calcium carbonate was the subject of a study and interestingly, mesoporous crystalline materials with pore sizes consistently smaller than 50 nm were generated by using a the zwitterionic $(Arg\text{-}Gly\text{-}Asp)_5$-PEO conjugate. This extends the accessible pore sizes via ionic crystallization from several hundred nm by latex templating down to the mesopore-range. It has been suggested that the repetitive RGD motif is responsible for two functions necessary to control the crystallization process. On the one hand the generation of amorphous $CaCO_3$ precursors (ACC), stabilized due to a crystallization inhibition effect and on the other hand the formation of polymer aggregates, acting as soft templates. It appears to be noteworthy that these soft templates are probably preserved during the crystallization process. This allows the formation of porous crystalline materials in analogy to established sol-gel chemistry routes for mesoporous metal oxides. Such processes utilize metal alkoxide precursors and proceed *via* deformable amorphous precursor phases. Analogues to this, the crystallization of aggregated amorphous $CaCO_3$ containing the conjugate templates, leads to calcite superstructures with enclosed polymer domains. Upon calcination, a transformation into materials with rhombohedral lattice-shaped pores can be observed. Interestingly, the PEO-$(RGD)_5$ conjugate plays multiple roles in the formation process of the inorganic structures. This is typical of biological mineralization processes, showing that complex multifunctionality of biopolymers can be mimicked. However, systematic variations of the peptide sequence have to be studied, and combinatorial screening processes *e. g.* phage display assays might be very useful tools to provide more insight into the sequence-function relationships, directing such complex processes as biomineralization.

To demonstrate the versatility of this approach, the modification of the crystal growth of an organic compound was investigated, too. The crystallization of DL-alanine as model system in the presence of a PEO-peptide conjugate as a growth modifier was studied. A triade of the repeat Glu-Glu-Ser (PEO-*block*-$(EES)_3$) was chosen as peptide sequence, providing solubility and binding capabilities to alanine. A defined crystallization generates hollow DL-alanine tubes with quadratic cross-sections. The process relies on a dissolution–recrystallization mechanism, where amorphous precursor nanoparticles are formed and aggregates into rod-

like structures. While the shell of these rods crystallizes, the core remains amorphous and is sacrificed to finally provide hollow tubes. As mentioned in the previous example, the PEO-peptide conjugate is involved in the generation of amorphous DL-alanine precursor nanoparticles, and moreover it facilitates the subsequent mesoscopic transformation toward crystalline hollow tubes. Interestingly, a similar mechanism is discussed for the formation of biological silica hollow fibers.[173] The marine organism *Acanthoecaceae* builds hollow silica fibers by the aggregation of amorphous precursor phases into rods and the sacrification of the core, while compaction the outer shell.

The results show that bioconjugates can be applied as specific stabilizers and compatibilizers with inherent chirality. These are useful for instance to modify and define the growth of inorganic crystals which give complex crystal morphologies. Even more interestingly organic crystalline compounds could be shaped in analogy. Prospectively, this have impact on pharmacological sciences, because crystallization of organic drugs is frequently required to prevent or reduce the rate of drug decomposition as well as to modulate the redissolution kinetics of a pharmacologically active compound. Moreover, chirality of the conjugates might even enable chiral selection of one enantiomer from a racemic mixture, which certainly is demanded for purification purposes.[174]

3.1.3 PEO-peptide conjugates to complex and mediate drugs

The peptide segment of PEO-peptide conjugates is not limited only to tune and modulate interactions, rather the pendent side chain functionalities of the peptide segment are interesting itself to be applied performing further chemistry. The library of natural and non-natural α-amino acids provides a multitude of functional amino acids with various side chain functionalities, interesting for metal complexation as well as basic or acidic catalysis. In collaboration with Werner and Dünne (HNO Marburg, Germany) PEO-peptide conjugates were used to complex a cytostatic drug. It is a common principle in pharmacological research to complex, particularly highly toxic, insoluble or fragile drugs with polymeric carriers to enhance pharmacological indices.[59] Complexation frequently leads to an increase of bioavailability of the drug accompanied by a reduction of toxicity or other side effects, which are beneficial for patients. In this study the complexation of *cis*-Platinum (CDDP)[175, 176] as one of the most effective chemotherapeutics used for the treatment of squamous cell cancer was addressed.[54] CDDP is a highly cytotoxic drug, and several negative side effects like rapid blood clearance and occurrence of necrosis and nephrotoxicity have been described. These side effects clearly limit the clinical use of intravenous administration of CDDP and even prevent subcutaneous administration of the drug. PEO-peptide conjugates, exhibiting multiple cationic side chain functionalities in the peptide block were used to effectively complex CDDP. Lysine was proven to complex CDDP effectively, dramatically reducing the amount of free drug and thus the toxic side effects. However, drug complex formation was apparently

only reducing the non-specific toxicity of CDDP, because pharmacological activity was still preserved. PEO-*block*-Lys_{35} and PEO-*block*-$(Gly_3Lys_7)_4$ were tested as carriers, showing rather similar results. However, it is an established fact that the toxicity of multivalent, cationic polymers increases with the number of continuous cationic repeats.[177, 178] Thus, it should be expected that the PEO-*block*-$(Lys_2Gly_3)_{10}$ carrier possesses reduced toxicity, compared to the PEO-*block*-Lys_{35}. Analysis of polymer drug complexes by analytical ultracentrifugation (AUC) and dynamic light scattering indicated that these are molecular carriers, rather than colloidal ones. AUC results further suggested the absence of non-conjugated CDDP. Moreover, agglomeration could not be observed even after one year-storage at 4 °C, demonstrating an extraordinary long term stability of the dissolved CDDP-complexes. The effective reduction of toxicity enables one to perform interstitial administration (injection into the narrow spaces between the tissues), without obvious side effects like local necrosis or severe inflammatory responses. In the study the interstitial administration of a solution of CDDP-complexes was used to mimic the physiological pathway of lymph fluids. This allows lymphogenic absorption and transport of the CDDP-carrier complexes, making lymphatic chemotherapy of metastazing squamous cell carcinoma possible and avoiding systemic distribution of the drug.

In the past chapter, our work was summarized, demonstrating the advantages that arise from the integration of monodisperse segments into the existing world of polymer science. By precisely adjusting functionalities and fine-tuning interaction capabilities of (pseudo)peptide segments, several applications can prospectively be addressed in the fields of biomedical and material sciences. The most important characteristics of peptide-polymer conjugates remain the precise definition and ease of variability of the peptide segments. This allows for the correlation of chemical structure with properties of the macromolecules, providing potent tools, which can contribute to the deeper understanding of the interactions between polymers and complex systems, such as for example biological systems.

3.2 Programming structure formation in synthetic polymer systems

Proteins and polypeptides are certainly the most important and most versatile platform of bioorganic macromolecules. Massive research efforts progressively elucidate the fundamental understanding of the dependencies between primary amino acid sequence and the formation of both protein structure and protein functions. This elegance perfection of controlling (nano)structures in macromolecules inspired us as polymer chemists and raised the question if the fundamental concepts could also be applied to organize synthetic polymers. In this chapter the biological concept of peptide-guided structure formation has been introduced for the organization of synthetic polymers.[12] To address this, different peptide-based organizer units were synthesized and incorporated into synthetic polymers. These organizers drive structure formation by directed peptide-peptide interactions and hence control microstructure formation in the conjugate systems.[55]

3.2.1 The β-sheet secondary structure as organization motif

The β-sheet secondary structure might be one of the most suitable organization motifs for peptide-guided assembly of synthetic polymers. This is due to intense investigations, contributing to the understanding of this basic secondary structure element of proteins.[179-185] Interesting tape structures with structural and functional anisotropy can be generated by directed self-assembly of oligopeptides in a β-sheet motif (Figure 9c).[180] Such structures are indeed beyond those available through common amphiphilic block copolymers. The assemblies are stabilized by directed hydrogen bonding between β-strands as well as by secondary contributions resulting from side chain interactions (i.e. entropic, hydrophobic, ionic or aromatic effects). Frequently, the β-sheet tape propagates in an autocatalytic manner and could in principle lead to structures with infinite lengths. Moreover, several distinct hierarchical levels of structures exist, as summarized in Figure 9. These levels span structures from nanometers to several microns, as β-strands can assemble to β-sheets, which can further stack to double sheets, called ribbons (Figure 9, b → c → d). Additional aggregation can lead to fibrils and fibers with a well-defined inner structure, and, depending on the peptide sequence, a length of up to several micrometers (Figure 9, e → f).

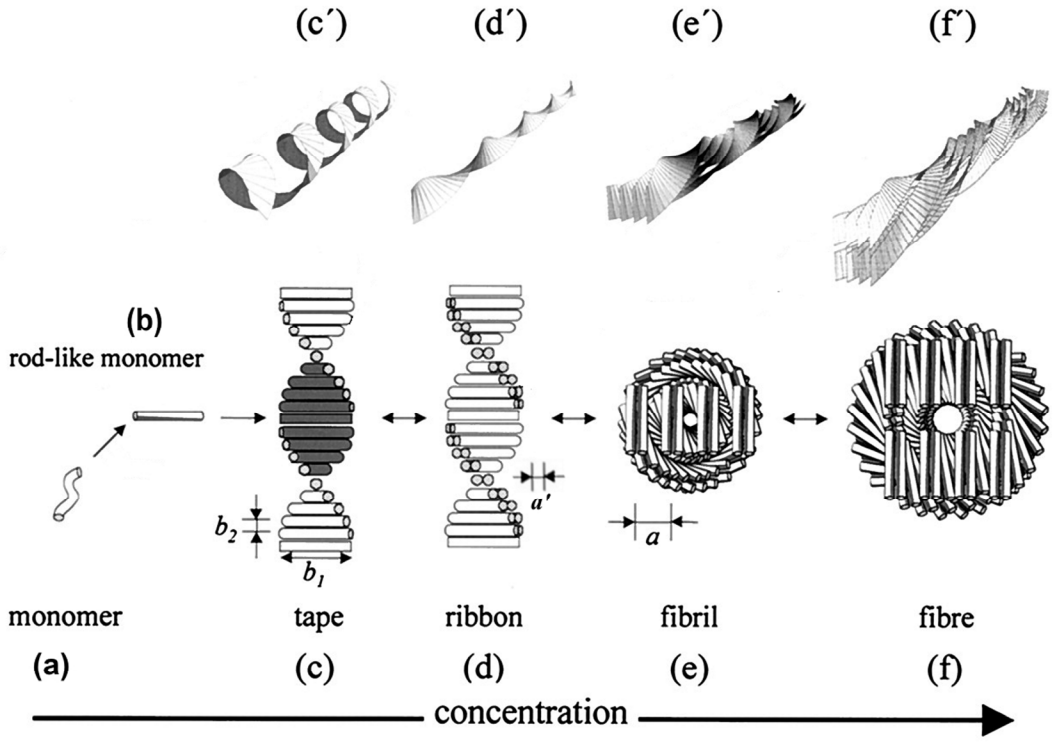


Figure 9. Schematic illustration of the hierarchical self-assembly of β-sheet forming peptides. Local arrangements (c–f) and the corresponding global equilibrium conformations (c'–f'): Peptide unimer, statistical segment configuration (a); peptide unimer, β-strand configuration (b); peptide aligned to form helical β-sheet-tapes (c, c'); twisted ribbons (d, d'); fibrils ($e_{\text{front view}}$, e'); and fiber ($f_{\text{front view}}$, f'). Geometrical dimensions: interstrand spacing in a β-sheet (b_2 = 0.47 nm); tape width, equal to the length of a β-strand ($b_1 = n_{\text{amino acids}} \times 0.35$ nm); β-sheet-β-sheet spacing in a ribbon (a' = 0.9–1.2 nm); inter ribbon distance in the fibril (a = 1.6–2.4 nm). Reprinted with permission from Ref.[180], copyright (2001) National Academy of Sciences USA.

The structural characteristics of the open β-sheet motif appear highly attractive because anisometric tape, fibrillar or fiber-like nanostructures can be accessed. Fibrils or fibers are important structural elements in native materials. Diverse properties like anisotropic strength,[186, 187] structural stability,[188] precise spacing of chemical functionalities,[189-191] and directed transport[186, 188] are generated by naturally occurring fiber-like structures.[189, 192] In materials sciences, nanofibers are of high interest[193] because of their potential applicability as high-strength components in composite materials,[194] nanowires,[195] medical fibers,[196] or actuators.[197, 198] Therefore, preparation of polymer nanofibers by peptide-guided routes is currently a field of high interest.[199] Particularly, because the obtained nanofibers or filaments possess the advantage of well-defined functional faces, compared to other nanofibers like carbon nanotubes or common electrospin fibers.

3.2.2 Design of peptide based organizers

There is a general understanding of the rules determining the relationship of sequence and structure for the three different secondary structure motifs (α-helix, β-sheet and turns). Particularly so far the rules for the α-helix folding are better developed. Nevertheless, in materials sciences more progress was made in the field of oligopeptide self-assembly for β-sheet fibers. This is foremostly driven by the enormous medical interest on Amyloid-like β-sheet assemblies, related to diverse diseases (*e. g.* Alzheimer's disease, Parkinson's disease and type II (adult onset) diabetes.[200-204] Despite the sequential diversity of the different proteins that are involved in pathological fibrillogenesis, the rules for β-sheet forming oligopeptides are well established, and diverse model peptides have been described, which assemble to β-sheet structures in water or organic media.[183, 199]

In our work we focus on a well-studied aggregator domain that exhibits high β-sheet propensities.[205] The primary structure of the peptide strands comprises multiple repeats of a diade of alternating threonine and valine residues $[(Thr\text{-}Val)_x]$.[206] The sequence matches the hydrophobic–hydrophilic repeat pattern of the β-strand[207] and both threonine and valine have high β-sheet propensities.[208] Among other factors, the latter is due to the sterically demanding side chain moieties that contribute to the extended β-strand conformation. For linear peptides it was shown that 5–6 repeats of a TV diade are required to form stable β-sheets in water.[205]

Figure 10 summarizes the different types of peptide based organizers, which have been studied in this thesis. Besides the most simple linear peptide organizers (Figure 10 a), more complex peptides such as template preorganized systems and cyclic systems inspired by the work of Kelly *et al.* and Ghadiri *et al.* respectively, have been investigated.[185, 209]

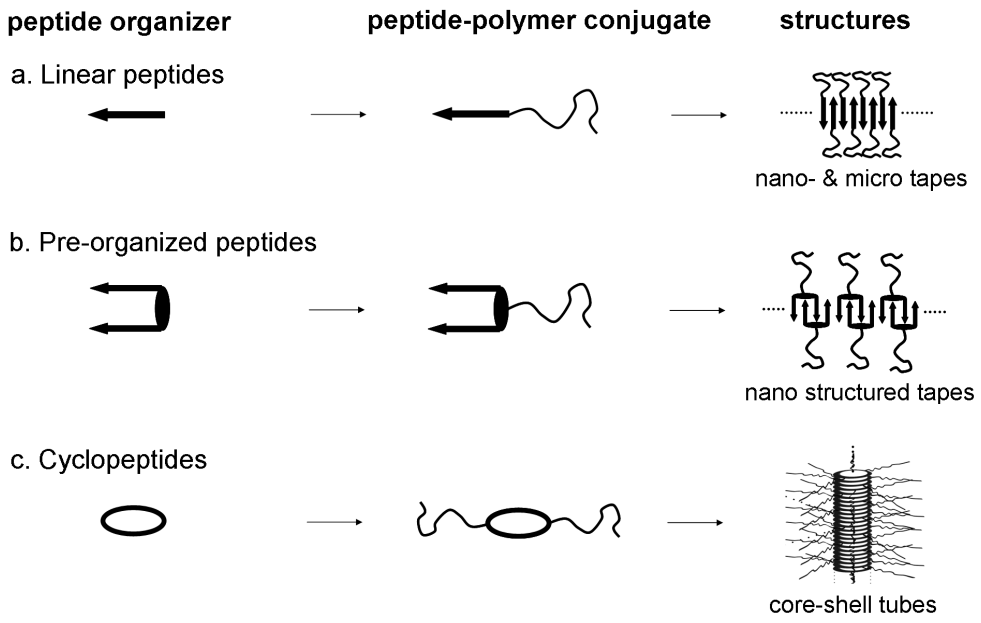


Figure 10. Schematic presentation of the peptide-guided organization via self-assembly of peptide-polymer conjugates. The peptide organizer dominates the assembly process, controlling the nano- and microstructure formation of the synthetic polymer block.

3.2.3 Peptide-guided organization in water

Lynn *et al.* pioneered the route of peptide-guided organization by investigating a conjugate of PEO and the Aβ10-30 fragment of the amyloid sequence associated with *e. g.* Alzheimer's disease.[210, 211] The conjugate assembled into short, soluble amyloid fibrils in water. Although the work aimed to prevent the lateral aggregation of the amyloid fibrils in order to investigate the fibrillogenesis at dispersed structures in aqueous solution, the concept of peptide-guided assembly of polymers was already visible. They described the association of the Aβ10-30 fragment into β-sheet structures, resulting in well-defined fibrillar aggregates.

3.2.3.1 Linear peptide organizers as aggregator domains in peptide-polymer conjugates

To obtain extended and robust nanofibers, which are more stable to mechanical stress and thus, more interesting for materials sciences, peptides with a high tendency to form stable aggregates are required. Unfortunately the solid-phase supported peptide synthesis as well as the analysis and handling of those peptides remain challenging, because irreversible aggregation takes place, frequently already on the solid support.[212, 213] This inherent problem was overcome by the incorporation of temporary structure breaking defect-segments into the peptide backbone (Figure 11 and Figure 12).[116, 212, 214, 215] The defects are denoted as “switch” segments,[214] they disrupt the amidic backbone, disturb the function of the peptide and hence suppress the aggregation tendency of a $[Val\text{-}Thr]_x$ aggregator domain. However the native peptide backbone can be re-established by a selective rearrangement in the switch segments, restoring the native peptide function. We have demonstrated that the switch segment is a highly useful tool for the peptide-guided microstructure formation of synthetic polymers. It not only facilitates the synthesis of the peptide aggregator domains but most importantly it allows – probably for the first time – to control the aggregation kinetics by defining the rate of switching via the pH-value (Figure 11).[102, 113]

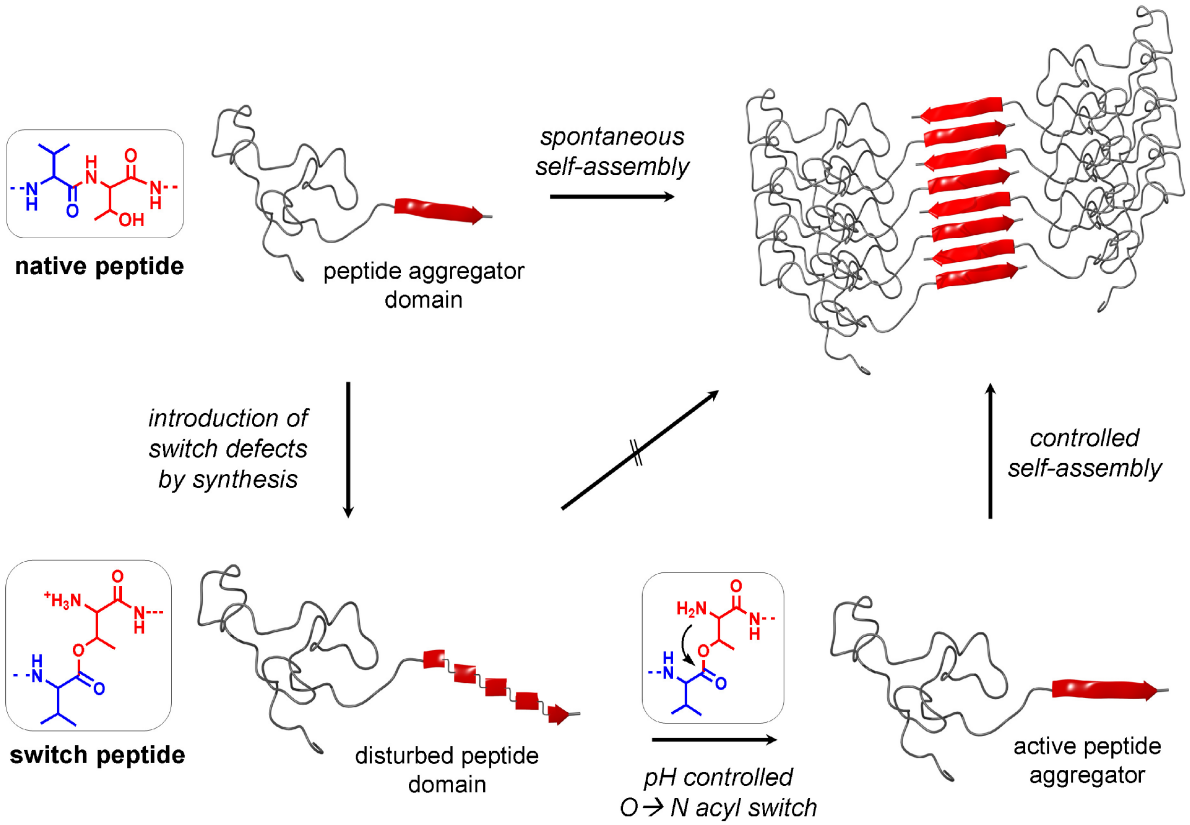


Figure 11. Peptide-guided organization of peptide-polymer conjugates. The self-assembly process is controlled by the organization of peptide domains, leading to β-sheet microstructures (comparison of the direct organization of the undisturbed conjugates (top route) *versus* the detour via disturbed switch peptide systems that allow a controlled induction of the aggregation tendency of the peptide aggregator (bottom route)).

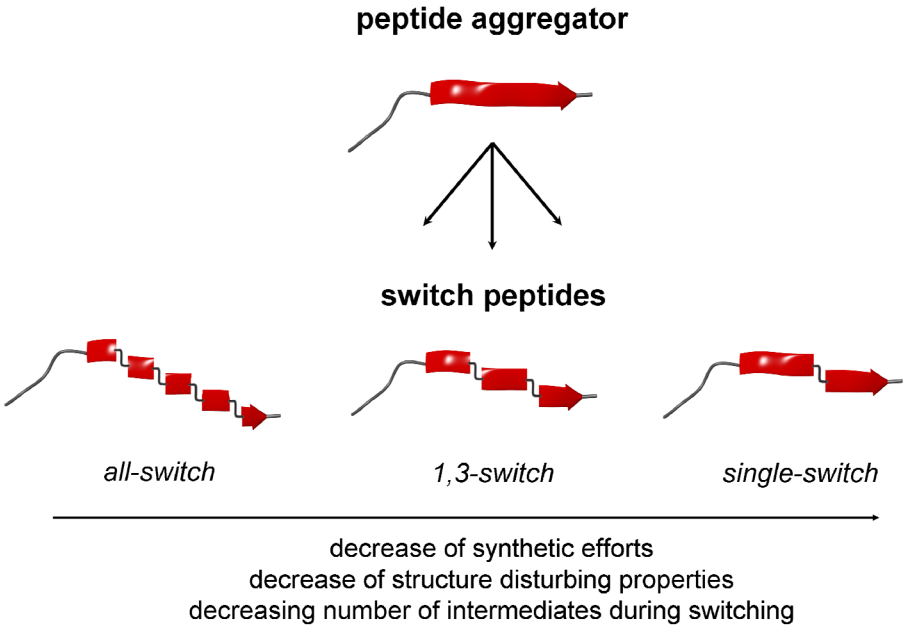


Figure 12. Evolution of the switch segments proceeded from the highly disturbed *all*-switch systems with demanding synthesis, to the easily accessible *single*-switch system that shows a binary off/on-transition.

PEO was conjugated to a peptide segment, consisting of five repeats of a threonine-valine diade ((Thr-Val)$_5$). The aggregation tendency of this domain was completely suppressed by incorporating four switch segments (Figure 12, *all*-switch).[102] The resulting PEO-peptide conjugates were easy to handle, readily soluble and neither peptide organization nor microstructure formation could be observed. The rearrangement in the switch segments triggers the assembly process by switching on the aggregation properties of the peptide segment. Due to a very slow generation of the peptide aggregators (switching over ~7 days), assembly of the conjugates occurs in a highly-controlled manner, leading to well-defined, tape structures, which are up to several millimeters in length, have a width of about 2 μm and a height of ~50 nm.[102] The inner structure of the macrotapes was investigated by polarized IR-microscopy at an oriented bundle of macrotapes, revealing evidence of a hierarchical sub-structure of stacked β-sheet tapes. The macroscopic dimensions of the uniform structures might indicate a growth mechanism via a nucleation growth process. This supposed mechanism could allow the realization of a chain growth assembly and with this the transfer of concepts known from polymer chemistry such as “grafting tapes from surfaces” or “block-wise co-assembly”.

3.2.3.2 Preorganized peptides as aggregator domains in peptide-polymer conjugates

The β-sheet formation tendency of a peptide-polymer conjugate can be strongly enhanced in order to minimize the relative amount of peptide, which certainly is the expensive constituent of bioconjugates.[160] This issue was addressed by tethering two short pentapeptides (*dm*Gly-(Val-Thr)$_2$, where *dm*Gly is *N*,*N*-dimethylglycine) to a suitable organic template (Figure 13). The latter was designed to preorganize the peptide strands in the appropriate geometry (spacing) of a β-sheet, enhancing the aggregation tendency due to entropic reasons. Moreover such a system avoids the synthesis of long peptide strands with difficult sequences, too.[160]

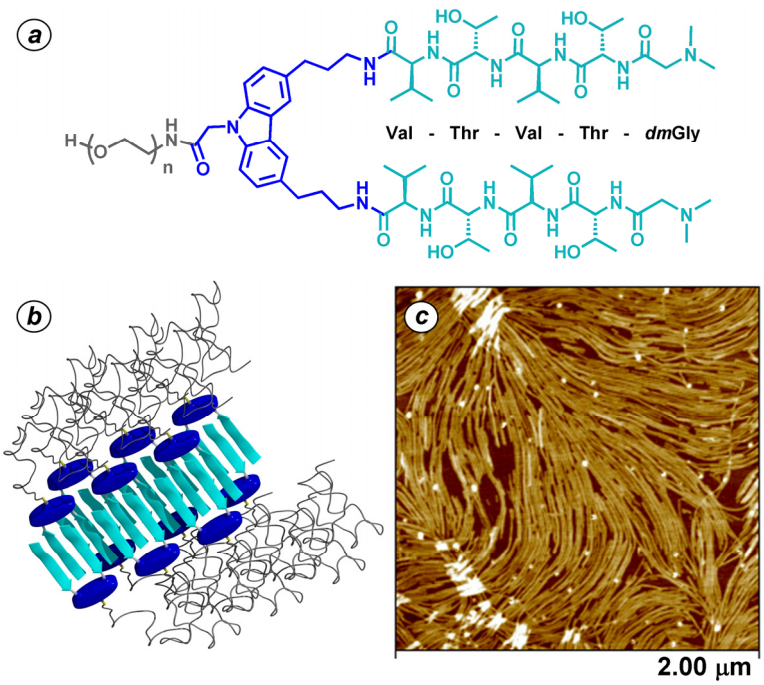


Figure 13. Peptide-guided organization of PEO-peptide conjugates with template pre-organized pentapeptides to direct the assembly. (a) Molecular structure, (b) idealized model describing the self-assembled nanotapes with peptide double β-sheet core (turquoise) and PEO shell (grey) and (c) AFM image showing the morphology of nano-structured tapes (tapping mode, height).

As shown in Figure 13, two pentapeptides were attached to a template based on carbazole. The PEO-peptide conjugate was synthesized in a straightforward manner by an inverse conjugation strategy and subsequent self-assembly results in the formation of defined β-sheet structures. A controlled de-aggregation/re-aggregation approach was required to obtain nanostructured tapes in water. These exhibit a uniform height of 1.4 nm, a width of 14 nm, and a maximum length of about two microns. Evidence could be found that the fibers consist of two antiparallel β-sheets, which assemble to a double β-sheet. Such motifs are commonly

observed in protein structures and are referred to as ribbons (Figure 13 b and Figure 9 d). The apparent stiffness of the PEO-peptide nanofibers suggests the presence of a LC-phase in the phase diagram.[160] This was verified by small angle neutron scattering (SANS), showing the strong tendency of the nanofibers to form bundles. Moreover, the analysis of the SANS data has led to a deeper insight into the substructure of the stiff nanofibers in solution, as it showed evidence for an extended core-shell nanostructure.[216]

The obtained double tapes are not only interesting structural elements due to their anisometry and high persistence length, but also because they possess functionalizable β-sheet faces.[217] These can be decorated with a variety of different side chain functionalities from natural and non-natural α-amino acids (Table 2). The versatility of this functional scaffold is illustrated in Figure 14, summarizing functional nanotapes that have been realized in this thesis. The variability, combined with precision of positioning functional moieties makes these supramolecular tapes potentially useful as catalysts (lipase mimics), scaffolds for nanowires or even as rails for directed transport.

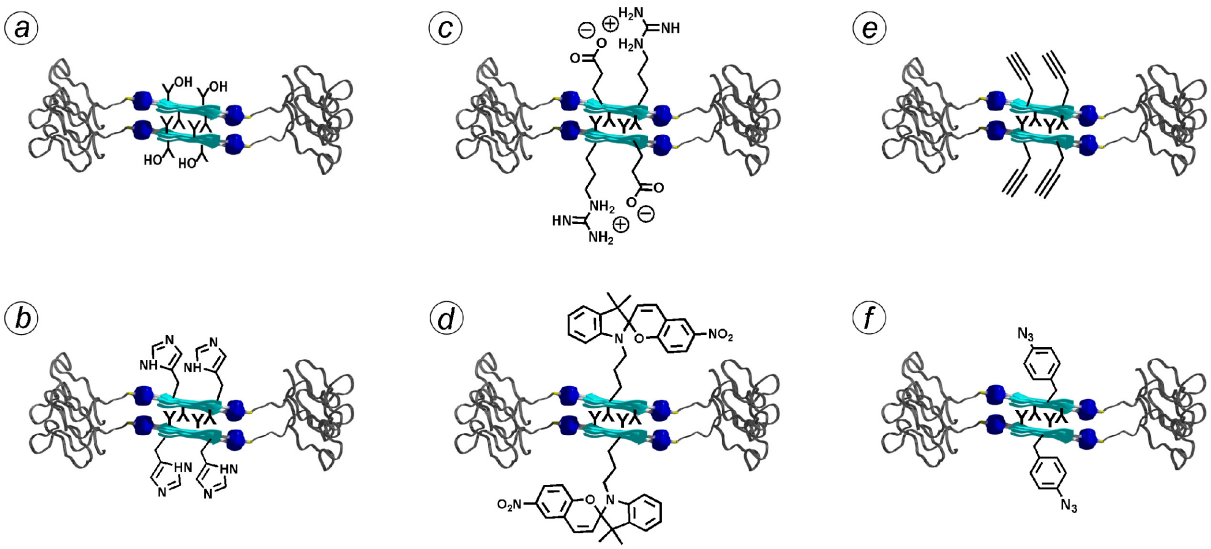


Figure 14. PEO-peptide nanotapes as functional scaffolds. Precise positioning of side chain moieties of threonine (a), histidine (b), arginine-glutamic acid (c) and the presentation of spiro pyranes (d), alkynes (e) or azides (f) on flat β-sheet faces of the core-shell nanostructures (front views).

3.2.3.3 Cyclopeptides as organizer units

Cyclopeptides composed of an even number of α-amino acids with alternating chirality and rather small ring sizes show an interesting self-assembly behavior to tubular ring stacks (Figure 15 a).[218] We have demonstrated that (D-*alt*-L)-cyclopeptides are highly interesting organizer units.[101] The conjugation of two P*n*BA blocks to a cyclic (D-*alt*-L)-α-octapeptide leads to a coil-ring-coil conjugate with preserved self-organization tendency of the (D-*alt*-L)-cyclopeptide. Thus the conjugates assembled into structures with a tubular peptide core and a polymer shell that wrapped around the hollow tube (Figure 15 b,c).[101] Moreover, network formation could be observed, which is probably due to the lateral interaction of the polymer-peptide tubes (Figure 15 c).

The resulting peptide-polymer nanotubes possess a functionalizable exterior and a hollow interior that potentially allows passive transport of substrates based on size exclusion modes.[219] However, for recognition or active transport, it is essential to functionalize the interior of the tubes. Hence our research currently focuses on the design of cyclopeptides, where non-natural amino acids provide orthogonal intra- and extraannular functionalities.

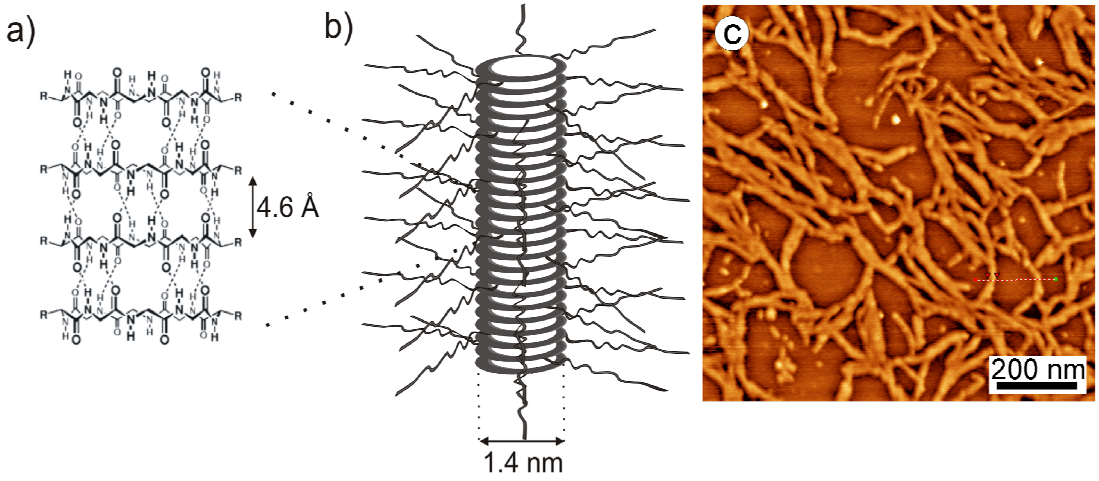


Figure 15. Formation of nanotubes by self-assembly of coil-ring-coil conjugates from P*n*BA and (D-*alt*-L)-cyclopeptides: Idealized assembly motif of unfunctionalized cyclopeptides (a), of the conjugates (b) and AFM micrograph (tapping mode, height) of the tube structures (c).

3.2.4 Peptide-guided organization in organic solvents

The transfer of the concept of peptide-guided organization from aqueous to organic media allows the incorporation and the organization of organo-soluble polymers, covering a wider spectrum of mechanical properties, functionalities and functions. The use of synthetic polymers, for instance, would not only allow the integration of conductivity (*e. g.* oligo(thiophenes) or poly(ferrocenes)) but also the realization of responsiveness to external stimuli such as thermo-responsive property transitions, photo crosslinking or photo responsive switching of structure, or dipole moment.

The successful transfer of the concept was demonstrated by the conjugation of a P*n*BA block to a β-sheet forming $(Thr\text{-}Val)_5$ aggregator domain.[113] P*n*BA was chosen because of its interesting adhesion properties. Furthermore, the low T_g and the absence of crystallinity, make the interference *e. g.* polymer crystallization or glass formation with the peptide organization process not very likely. Ease of synthesis and controlled self-assembly was ensured by incorporating two switch-defect segments into the peptide aggregator domain (Figure 12, *1*-3 switch).[113] Later it was demonstrated that the number of switch segments can be reduced to one. These *single*-switch systems suppresses the aggregation tendency of a $(Thr\text{-}Val)_5$ aggregator domain sufficiently, while reducing the synthetic efforts strongly and allowing a binary OFF → ON switch (Figure 12, *single*-switch).[220]

The rearrangement in the switch segments proceeds in a clean manner, unaffected by the organic solvent (diethyl ether/methanol) and triggers the self-assembly of the conjugates.[113] It is noteworthy that the rate of switching can be carefully adjusted in organic solvents by the apparent pH-value or the addition rate of a base, allowing control over the aggregation kinetics. Because the hydrophobic effect (entropic effect) is usually absent in organic media the peptide organization is dominated by directed hydrogen bonding.[113] This leads to the formation of wound-tape structures with a left handed helical twist. Further reversible organization by defined entanglements results in soft, continuous organo-gels (*cf.* Figure 16).

Interestingly and somewhat surprisingly it was observed that small $(Thr\text{-}Val)_5$ aggregator domains could direct the self-assembly of even high molecular weight P*n*BA-blocks.[220] The peptide-guided microstructure formation of a set of P*n*BA-peptide conjugates was studied in organic solvents. The conjugates possess the same peptide segment, but differ in the molecular weight of the P*n*BA-block ($M_{n,P\textit{n}BA}$ = 2 k, 8 k, 14 k and 38 k g/mol). All systems assembled in fibrillar microstructures and the self-assembly could be correlated to the formation of a β-sheet peptide structure. Moreover, even for the high molecular weight system, evidence could be provided for the existence of a superhelical fine structure. Thus the organization of the peptide block has been recognized as crucial in the formation of the microstructure of a high molecular weight P*n*BA-block ($M_n \sim 38000$), even if the conjugate has a peptide content of only 3.5 *wt.*%.

The impressive capability of a peptide segment which is composed of only 11 amino acids to direct the organization of a synthetic polymer block with a $DP_n \sim 300$ reveals the remarkable driving forces present in peptide organization.[220] This fact allows further to envision that peptide-polymer conjugates might be of value beyond biomedical applications. The significant reduction of the expensive peptide compound way below 5 *wt.*% makes technical applications feasible *e. g. in situ* formation of fiber textures in glues during curing to enhance cohesion, or the formation of fiber scaffolds in melt-extruded plastics (self-assembly injection molding) to modulate mechanical properties.

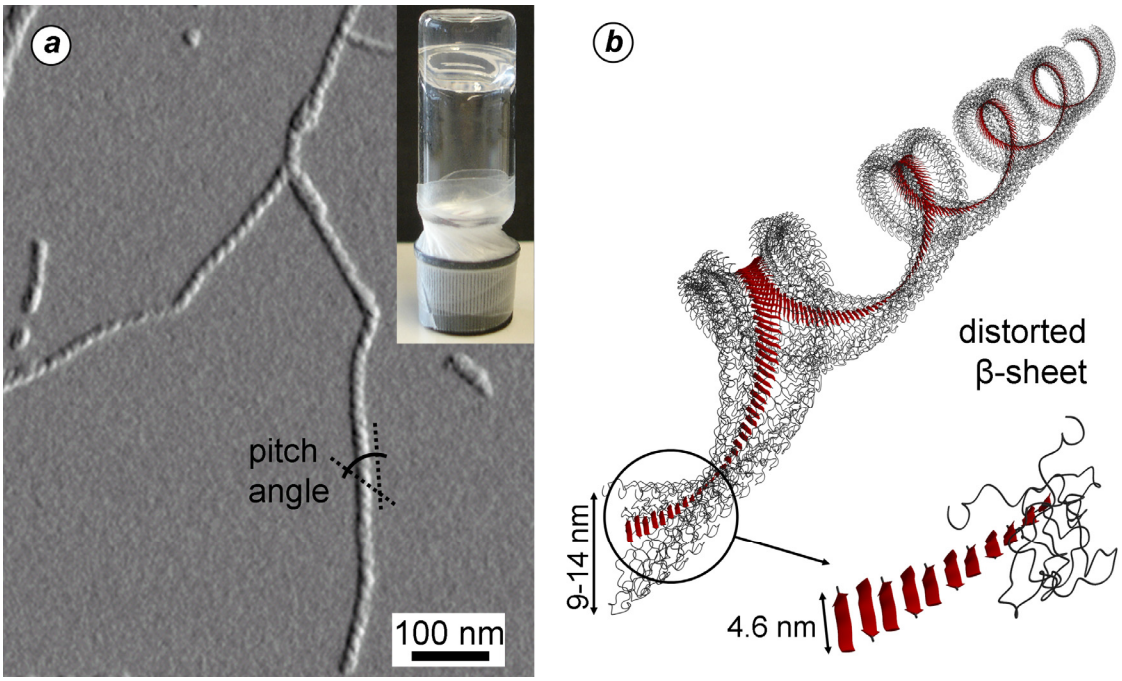


Figure 16. Superhelical P*n*BA-tapes exhibiting a left handed helical twist: a) AFM phase image (inset: self-supporting organo-gel formed by reversible entanglement of the superhelices) and b) idealized structure model of the structure elements showing the antiparallel β-sheet core (red) and the P*n*BA-shell (gray) (the β-sheet is distorted by 1.9° twist and 2.3° bend distortions) (inset: fist polymer coils were removed for clarity reasons).

3.2.5 Hierarchical assembly of fibrillar structure elements to mimic complex biomaterials

In the previous chapter it was demonstrated that peptide-polymer conjugates can be "programmed" to self-assemble into anisometric structures. The utilization of peptide segments with high propensities to adopt the β-sheet secondary structure motif leads to the formation of fibrillar, fiber-like or tape structures. Frequently, fibrillar structures can be found in biomaterials *e. g.* to construct hierarchical composites. The following paragraph will focus on studies, which elaborate on whether a basic concept can be derived from those biological fiber composite materials to allow structuring of synthetic fiber elements further into more complex, anisotropic materials.

Biomaterials are highly adapted to their purpose and in many cases superior to synthetic mimics.[221, 222] Frequently, this is a result of a defined hierarchical structure, formed via processes that are facilitated by peptides, proteins and - becoming more and more evident - also by polysaccharides.[223, 224] Understanding the underlying concepts of these controlled structure formation processes is an important field of bio(mimetic) materials sciences.[171, 223, 225] Moreover, it will contribute to nanochemistry, where the bottom-up synthesis of hierarchically structured materials makes the defined assembly of nanoscale building blocks mandatory.[226]

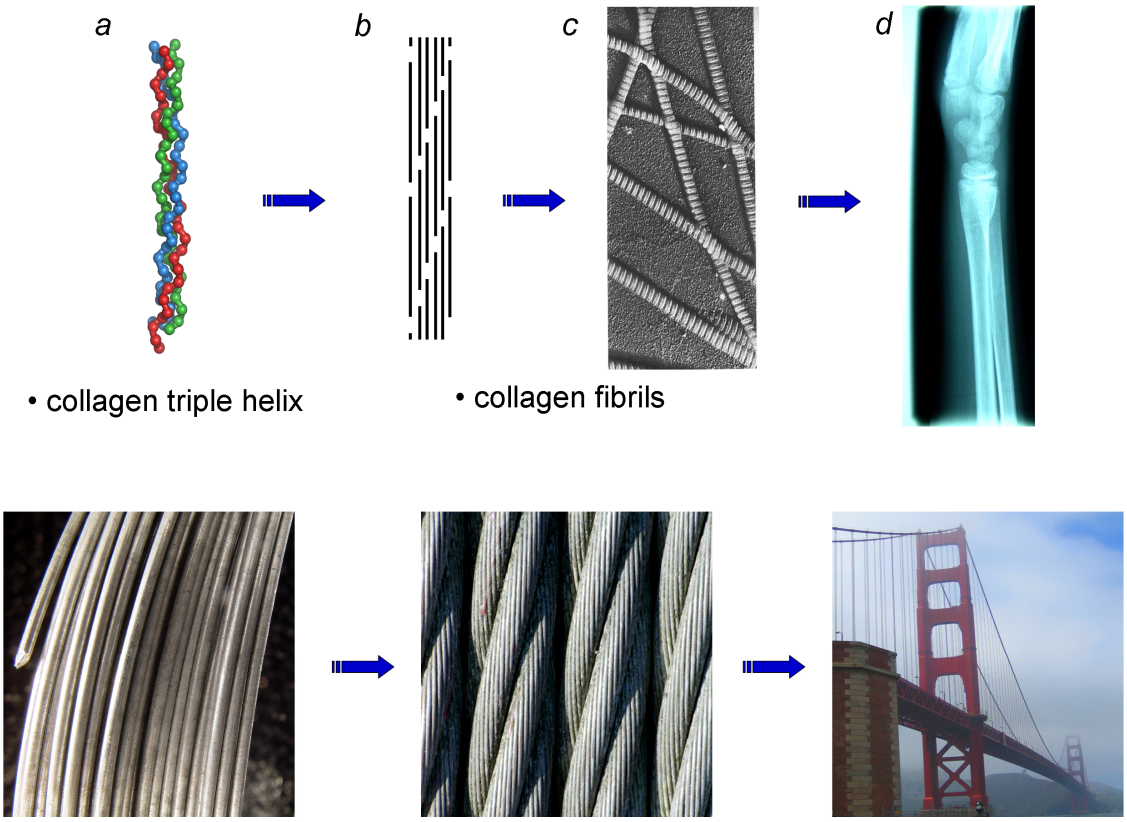


Figure 17. Bundling of uniform fiber elements as a concept, apparent in bio- and technical materials. Top: collagen triple helix (309 nm × 20 nm (length × width) (a), collagen fibrils 80-100 nm (b); fibrillar bundles of 1-5 µm (c)[227] and directed mineralization leading to macroscopic bone (d) and bottom: bundle formation of steel wires leads to high strength steel cables.

One universal concept to realize high performance materials in nature is the generation of fibrillar structure elements, which are used as nano- or sub-micrometer building blocks for further assembly to bundle structures with strongly anisotropic properties. This concept can be found in bones as well as in supportive plant tissues. Moreover, on the macroscopic scale engineers exploit it, too, making high strength stainless steel cables form small wires (Figure 17). From the point of view of an academic and also an applied researcher, it appears to be interesting to mimic this basic concept, learning how to design complex materials.[228]

Nature provides a breathtaking diversity of fiber reinforced or fiber-guided inorganic-bioorganic hybrid materials ranging from bones[229] to filaments of glass sponges.[230, 231] The underlying principles in the formation of these materials elucidate hierarchical self-assembly, combined with templating processes. Within these complex hybrid processes, information is translated across a range of length scales to control structures, functionality and function on each hierarchy level.[223]

Marine glass sponges like for instance the hexactinellid sponge *Euplectella sp.* are considered one of the most primitive animals in existence. However, they generate a hierarchically engineered cage structure, made of composite fibers that combine protein filaments and silica.[230, 232] These integrated fibers overcome the brittleness of glass, which is one major drawback of technical glass fibers. To realize this, multiple silica layers are laminated concentrically around a central protein filament. With ~3.2 GPa, bioglass fibers reach about half the indentation hardness of optical glass fibers, while being twice as elastic with a reduced indentation modulus of ~38 GPa.[232] An additional example is provided by the *Acanthoecaceae* from the family of protozoan. This unicellular microbe composes sub-micrometer hollow silica rods that are precisely glued together to cage structures (*lorica*), spanning tenths of micrometers.[173] Remarkably, the complex cage is formed within about three minutes by one microbe. This is indeed among the fastest biological silica fiber formation processes described.

These examples from biology show that high performance composite materials on the basis of glass can be rapidly formed. However, a complex design and inner structure is required to ensure the control of both mechanical and chemical interfaces between the different components to overcome the brittleness of its main constituent material, glass.[230]

In the formation of bioglass fibers (natural glass sponge spicules) proteins like silicateins[233] catalyze and guide the formation of silica from dilute silicic acid solution at neutral pH *in vivo* and *in vitro*.[234] While the structural and functional information required to direct the formation of the biological glass fiber is encoded in these protein filaments, a biomimetic process could be realized by using functional PEO-peptide nanotapes instead (*cf.* Figure 13 and Figure 14 a).[56, 160] This artificial filament was designed to generate functional surfaces with strong analogies to biological silica morphogenesis proteins, by exposing threonine rich β-sheet faces combined with cationic functionalities.[235, 236]

The addition of pre-hydrolyzed tetramethoxysilane ("silicic acid equivalent") to an ethanolic solution of these PEO-peptide nanotapes results in a rapid formation of macroscopic composite fibers within seconds. The biomimetic silicification process combines self-assembly of the polymer-peptide nanotapes with peptide-directed silicification. The resulting macroscopic composite fibers exhibit a hierarchically ordered inner structure, which spans length scales from the nanometer up to millimeters in their lateral and centimeters in their longitudinal direction (Figure 18). The cooperative nature of the artificial silicification process allows for the rapid production of complex composite fibers with six distinguishable levels of hierarchical order (Figure 18). These could be classified as the peptide-polymer conjugate (***hierarchy level I***), which forms nanotapes with a single β-sheet core (***hierarchy level II***)[160] and eventually a double β-sheet core (***hierarchy level III***, 15×1.4 nm cross-section). Furthermore, the composite materials are composed of proto-composite tapes ***(hierarchy level IV***, 15×3 nm cross-section), while anisotropic self-assembly of those leads to

proto-composite fibers (***hierarchy level V***, ~95 nm width), which tend to form bundles (***hierarchy level VI***, 0.1-2 μm width). The entanglement of such bundles leads to threads of up to 1 mm in width cannot be considered as a hierarchy level due to the absence of a uniform assembly motif.

The biomimetic composite fibers obtained proved to have excellent mechanical properties.[56] Nanoindentation experiments on the dried composite fibers revealed an indentation hardness of 0.99±0.2 GPa, which reaches about one third of the hardness of natural sponge spicules (*Rosella racovitzea*) and ~13% of optical glass fibers.[232] Moreover, a reduced indentation modulus of the biomimetic composite fibers of about 10 GPa results in a fiber with a four times higher elasticity, compared to sponge fibers or even approximately seven times higher elasticity, compared to synthetic glass fibers. These results highlight that the precise control of structure is a key-factor in bio- and biomimetic materials, allowing the modulation of material properties over a broad range.[7] It could be envisioned that a significant progress in structural control can lead to a dramatic change in material sciences. Future materials will probably be composites with hierarchical structures. As realized in many biomaterials, a change of properties would only require the adaptation of nano- and microstructure, but not a change in constituent components.

The hierarchical nature of the assembly process, as it is outlined in Figure 18 was verified by isolating the primary composite structures, referred to as proto-composite tapes (***hierarchy level IV***).[237] This could be achieved by strongly reducing the concentration of "silicic acid" that was added to the PEO-peptide nanotapes ($c[Si(OH)_4]_0$= 270 μM) and by allowing only short contact times of about 10 seconds. Despite these kinetic conditions, uniform silica nanocomposite tapes could be accessed with inorganic constituents, well-integrated into the organic structure. The nanocomposite tapes have a length of up to 5 μm, a rather uniform height of ~3.1 nm, while the width of the structure remains nearly constant at ~15 nm compared to the width of the PEO-peptide nanotapes. It is noteworthy, that the silicic acid concentration applied in this experiments corresponds only to about 3-times the equilibrium concentration of silicic acid in the oceans.[238] Taking this into account and considering the short contact time in the experiments a strong binding capability of the PEO-peptide nanotapes for silicic acid is evident. This reveals the regulation of the partitioning of silicic acid (local enrichment) as one of the key-parameters to direct the silicification process. Once identified, it is interesting to apply this concept to other systems *e. g.* in order to generate ultra thin, highly homogeneous silica layers as scratch resistant protective coatings or to integrate different functional inorganic components with analogue sol-gel processes leading to *e. g.* titania or barium titanate nanofibers.

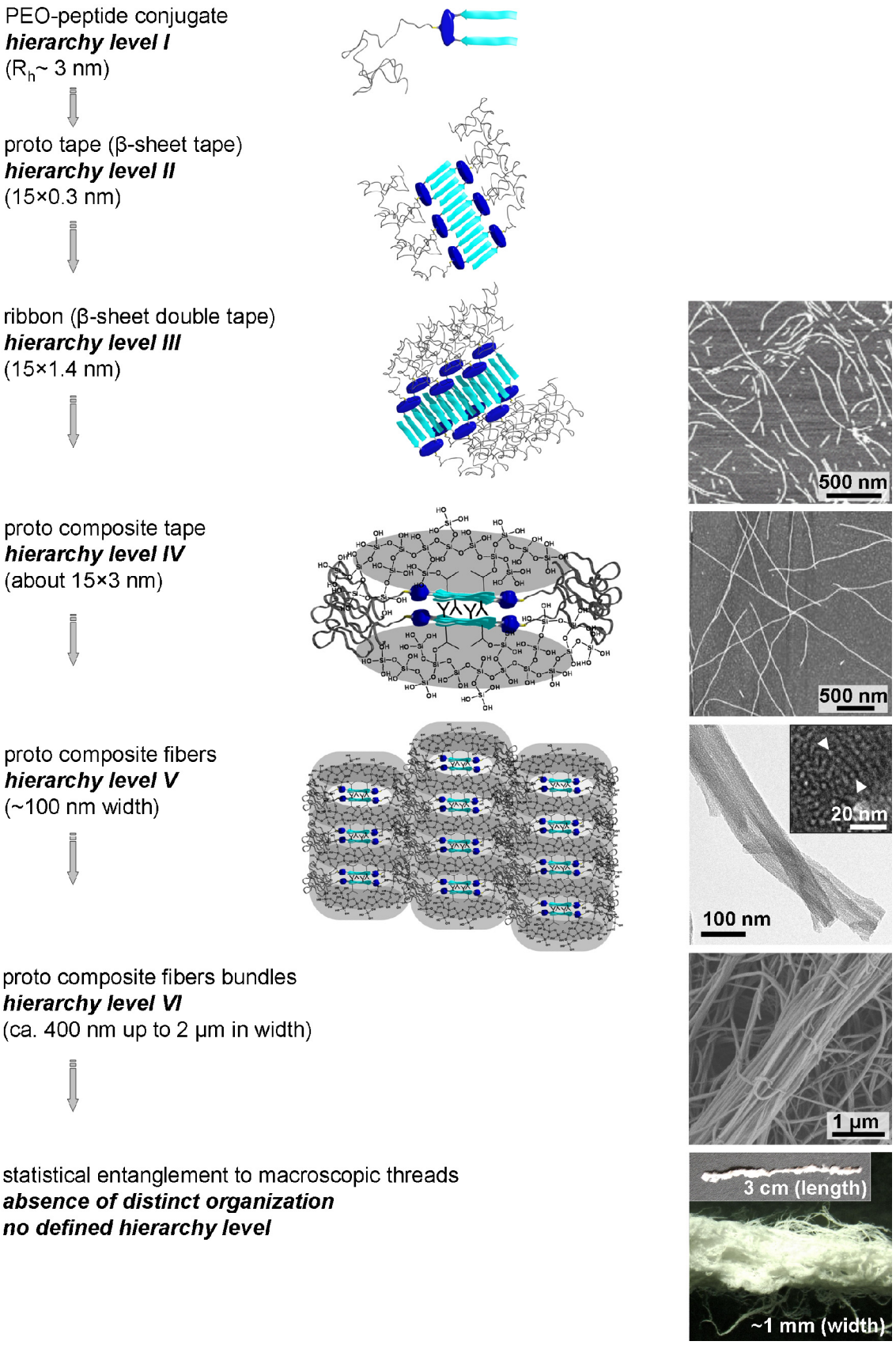


Figure 18. Hierarchically structured composite fibers formed by a hybrid process, combining self-assembly of polymer-peptide nanotapes with peptide-directed silicification (***Organic hierarchy levels*** are classified as peptide-polymer conjugate ***(I)***, nanotapes with single β-sheet core ***(II)*** and double β-sheet core ***(III)***. ***Composite hierarchy levels*** are specified as proto-composite tapes ***(IV)***, proto-composite fibers ***(V)*** and fiber bundles ***(VI)***).

The high rate of some of these biomimetic silica morphogenesis processes allows envisioning the combination of molecular self-assembly (a bottom-up method) with processing technologies known from top down approaches. The formation of the biomimetic glass fibers was exploited to establish a convenient 2D-plotting process, which enables the drawing of macroscopic networks of nanostructured silica composite fibers.[239] A similar route as described above combines peptide self-organization and directed silicification to rapidly generate networks of hierarchically structured composite fibers. However, the macroscopic form of these composite fibers could be plotted by local injection of PEO-peptide nanotapes into a thin layer of a solution of "silicic acid" (combined bottom-up & top-down approach). Depending on the rate of plotting, the line width of the composite fibers could be adjusted from 700 to 170 μm. Additionally, a clear effect of the orientation of both the composite fibers (nano- and micrometer structural elements) and the macroscopic network was evident. This makes the tuning of the network orientation from isotropic to anisotropic possible. Rapid plotting of composite fiber networks could be used in sophisticated production processes integrating inkjet printer, high speed spotters or 2D-plotting devices. Moreover, 3D-bio-plotting might be used to manufacture silica-based composite materials that exhibit smartly reinforced structures following the example of natural bone. More complex 2D or 3D mesoporous silica fabrics might be significant in the future for regenerative bone repair such as implantation of tailor made bone inlays.

This chapter has summarized how advanced control of functionality in peptide-polymer conjugates can be used to guide the formation of functional assemblies via directed secondary interactions between the building blocks. The (nano)structures obtained possess functionalities, which are precisely positioned on the surfaces of well-defined nanoobjects (β-sheet faces of nanotapes). Hence they can interact with inorganic or potentially with organic compounds, directing the controlled generation of hierarchically structured, integrated composite materials. It is noteworthy that the information, required to realize *e. g.* an inorganic-organic composite material with six distinguishable hierarchy levels, is encoded in a very simple amino acid sequence (*dm*Gly-Val-Thr-Val-Thr). During the formation of the composite the information is handed on across the different hierarchy levels. Thereby it is translated from a purely chemical code (chemical properties along the peptide chain) to a structure (core-shell nanotape) and further into a structure-functionality code (patches with defined functionalities). Interestingly, a similar development of linear chemical information with higher organization levels can be found in several biomacromolecules *i. e.* not only in proteins but also in DNA. For a chemist with the objective to mimic and rationaly design corresponding synthetic analogues, it is highly fascinating to learn about the processing of information in such complex molecular assemblies.

3.3 Positioning of chemical functionalities to generate functions

As discussed in the previous section, the positioning of chemical functionalities *e. g.* on a self-assembled tape structure, can be used to encode information into this structure that defines further organization. In the class of proteins this general structure-function concept is evident in a highly sophisticated manner. In proteins, such as receptors or enzymes, a complex scaffold is build-up by the folding process in order to position a few chemical functionalities in precise geometries.[37, 39] These are forming the active center of the protein *e. g.* to control selective substrate binding or to catalyze specific reactions. In these sophisticated systems, the design of the scaffolds is still too complex to be mimicked in a synthetic approach and the structure-function relationships cannot be generalized. However, already the positioning of chemical functionalities at specific sequence positions in a polymer chain enables the realization of advanced functions, frequently beyond those of common polymers. In this paragraph, our efforts to demonstrate the possibilities that arise from the control over the position of single moieties or short segments in a polymer chain are summarized.

3.3.1 Positioning of peptides in block copolymer assemblies to realize functional domains

To achieve control over the positioning of a functional peptide-domain in common block copolymer assemblies *e. g.* in micellar or vesicular aggregates, a functional, amphiphilic ABC-triblock copolymer has been synthesized.[154] This comprises a central monodisperse peptide segment, linking a PEO-block with a P*n*BA-block. The ABC-conjugate was obtained via RAFT polymerization of *n*BA, mediated by a PEO-*block*-peptide-CTA (*cf.* Figure 19 and Chapter 2.3.2). The resulting PEO-*block*-peptide-*block*-P*n*BA assembled in water into micellar aggregates (Figure 19). Considering the design of the conjugate, it is the amphiphilic segments which direct the self-assembly, while the expensive oligopeptide is probably positioned at the hydrophilic-hydrophobic interface, resulting in the formation of a domain with precisely controllable functionalities. The latter can be programmed during synthesis by incorporating a multitude of functionalities from the library of natural and non-natural α-amino acids as well as their fully synthetic analogues.

To demonstrate the concept, the polyvalent decaarginine (Arg_{10}) has been selected. Arginine is one of the most interesting naturally occurring α-amino acids. It contains a highly water soluble guanidino side chain functionality, which is non-nucleophilic and comparatively strongly basic ($pk_a \approx 12.5$). In protein and peptide systems this residue is utilized to establish soft cationic sites *e. g.* useful to modulate interactions with cell membranes, to generate membrane translocation or to allow nuclear location.[59] The resulting functional micelles are currently investigated in detail *e. g.* via NMR and ESR techniques, to verify the exact position of the peptide segment. Conceptionally, these assemblies are highly interesting, because as soft, globular structures they might allow binding and condensation of DNA as “artificial

histones". Moreover, the peptide domain at the hydrophilic-hydrophobic interface might be utilized to catalyze condensation reactions in water ("artificial enzymes"). Such a process could make use of an interfacial transport of polar substrates into the hydrophobic interior were the condensation of water can take place. The effective transport could for example be induced by the binding of the substrate to the functional peptide segment.

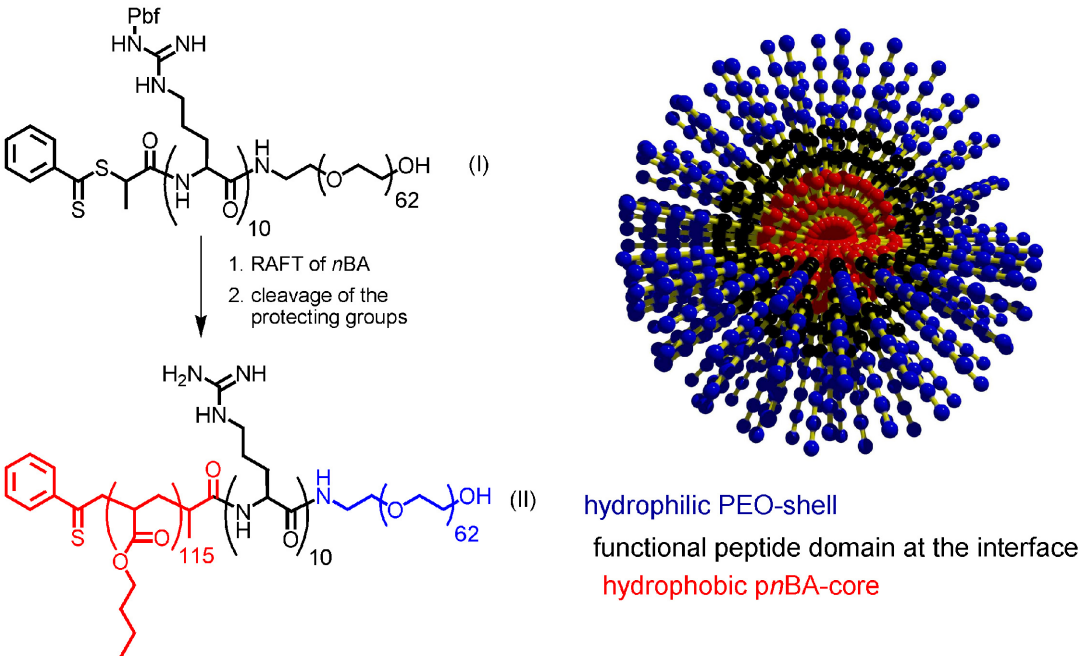


Figure 19. Synthesis of a PEO-Arg$_{10}$-P*n*BA (***II***) via RAFT, mediated by the PEO-Arg$_{10}$-CTA (***I***) (left) and schematic illustration of the formed aggregates, exhibiting a hydrophilic PEO-shell, a hydrophobic P*n*BA-core and a functional oligopeptide domain positioned at the interface (right).

3.3.2 Positioning of functionalities in PEO-PAA conjugates to realize functions

The applicability of conjugates, comprising a PEO block and a monodisperse pseudo-peptide domain (poly(amidoamine) (PAA)) was described in Chapter 3.1.1. The tunable complexation properties of these conjugates have been adjusted to actively compress *ds*DNA into compact, toroidal supercoils, which are highly demanded for DNA delivery purposes.[240]

While the design of many commonly applied, polymeric DNA carriers seems to focus on the compression of DNA, which is certainly required during transport and translocation (crossing of cell membrane barriers), the intracellular unpacking at the location of action is conceptionally not addressed. In this paragraph it is demonstrated that the capability of precise positioning of a functional moiety in the polymer chain enables the design of integrated carrier systems. These polymeric carriers combine both defined packing properties and programmable disassembly that may contribute to the intracellular unpacking and liberation of the cargo DNA.

To achieve this, cystamine was introduced as a convenient building block for the solid-phase supported synthesis of monodisperse PAA segments (*cf.* Table 2). The integration of cystamine leads to the positioning of a disulfide group in the PAA backbone. Disulfide bonds are stable in the oxidative environment of the extracellular fluids, but are rapidly degraded in the cell, due to an increased concentration of glutathione that causes a reductive intracellular environment.[241] These specific properties of disulfide bonds can be exploited to develop polymers, which not only undergo a controlled degradation after uptake into a cell, but also make the realization of a programmed fragmentation possible. Figure 20 shows the chemical structure of the conjugate, highlighting the single disulfide linkage that is positioned between the PEO block and the PAA segment. With the introduction of a digestible moiety, an advanced function was generated, realizing a PEO-SS-PAA carrier that exhibits a two-phase release process (Figure 20). To demonstrate this, the complexation behavior of the carrier with plasmid *ds*DNA was studied, comparing the structural and colloidal properties of the polyplexes with those, resulting after the addition of a synthetic reducing agent (Dithiothreitol, DTT). A distinct change of the polyplex properties was evident from the comparison of the untreated polyplexes and the polyplexes after reductive treatment. The programmed disassembly of the carrier polymer leads to a well-defined change in the polyplex structure. The removal of the sterically stabilizing PEO-block results in a compaction of the polyplex accompanied by the development of a positive net charge, preserving the colloidal stability of the system. An analogous disassembly of the DNA-carriers might also occur in the reductive environment of the endosomes. Judging from the literature, the intracellular generation of polyplexes with positive surface charges and compact structure indeed promotes endosomal escape and enhances nucleus localization. This is mainly due to an increased membrane interaction capability that enhances the intracellular passages of plasmid DNA to the nucleus.

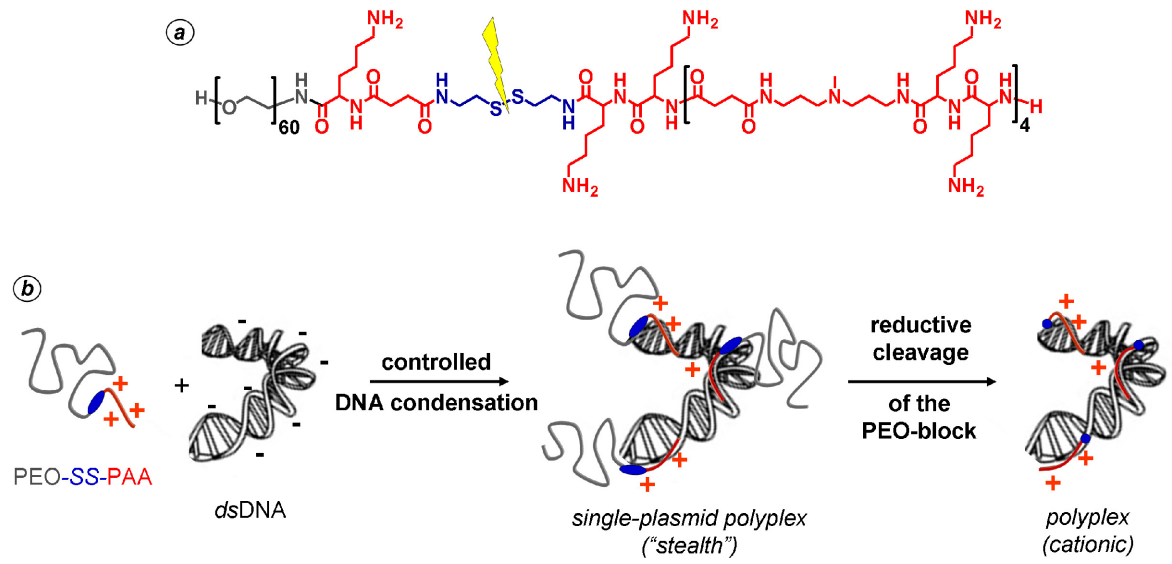


Figure 20. PEO-*block*-PAA conjugate with disulfide linker connecting both segments (a) and schematic illustration of the two stage delivery process (b), where the DNA is complexed by the carrier polymer, leading *i.* to PEO stabilized, single-plasmid polyplexes (middle) and subsequently cleavage of the PEO blocks leads *ii.* to polyplexes with effective cationic surface charges (right).

A programmable disassembly of polymeric carriers would certainly be of interest to address other important issues in polymer facilitated drug delivery such as the controlled release of pharmacologically active peptide segments or controlled biofade of the polymeric carriers. Moreover, the precise disassembly of the polymeric carrier might also contribute to the fundamental understanding of the mechanisms of polymeric drug delivery. With these high precision polymers biological properties can be closely correlated to polymeric structures and functions. This however is subject of ongoing *in vitro* cell culture and transfection experiments. Further interesting issues will be addressed in the design of future polymer carriers. The investigation of the intracellular processing of the carrier polymers and the polyplexes requires the introduction of fluorescence dye-quencher systems for FRET analysis. These systems will either mark the PEO/PAA pair or the PAA/DNA pair to learn about the intracellular distribution and function of the different compounds. Moreover, a systematic study that varies the length and cationic character of the PAA fragments is mandatory. For that, multiple disulfide linkages are required in a PAA segment. Certainly, it would be interesting to study whether PAA fragments with functionalities that correspond to spermidine or spermine show an optimum in the transfection activity, because these tri- and tetravalent polyamines are biological DNA transporters *e. g.* in the spermatozoon.

3.4 Generation of bioactivity (Materials that actively interact with biological systems)

Certainly, the combination of polymeric materials and peptides that possess biologically relevant sequences is of enormous interest for biotechnological and biomedical applications. The resulting materials promise the realization of synergistic properties far beyond those present in the isolated compounds. This is demonstrated by an example, where switchable bioactive surfaces have been achieved. Here, the modification of surfaces that enable cells to interact *e. g.* to adhere via specific interaction is targeted. However, adhesion should be reversible and detachment of the cells should proceed as a response to an external trigger. To realize this, a peptide-polymer conjugate was designed that integrates all necessary functions. Such conjugate was accessed in a straightforward manner by applying a "grafting from" strategy using the peptide-CTA approach (Chapter 2.3.2.2).

The peptide Gly-Gly-Arg-Gly-Asp-Ser (GGRGDS) corresponds to one of the minimal domains of the adhesive protein fibronectin and is known to mediate cell adhesion via selective integrin interactions.[242] GGRGDS was synthesized by solid-phase supported peptide synthesis techniques and modification of the N-terminus by coupling of S-1-dodecyl-S'-(R,R'-dimethyl-R''-acetic acid) gives the CTA-GGRGDS. This could be successfully used to mediate the polymerization of *N*-isopropylacrylamide (NIPAM). The hydrolysis of the CTA trithiocarbonate group of the resulting CTA-PNIPAM-GGRGD conjugate proceedes selectively by reductive means and provides a ω-thiol-α-peptide functionalized bioconjugate (HS-PNIPAM-GGRGDS).

To demonstrate the biorelevance of the obtained conjugate, HS-PNIPAM-GGRGDS was grafted onto planar gold surfaces for controlling cell adhesion.[243] Figure 21 (a) shows the effective adhesion of L929 mouse fibroblasts, cultivated at 37 °C on conjugate modified gold surfaces. The cells adhered fast and spread well after 18 h of cultivation. The observed adhesion rate is clearly faster compared to standard PNIPAM surfaces,[243, 244] indicating a beneficial effect of the GGRGDS segment. Upon temperature decrease to 25 °C, rapid cell rounding was evident within ~30 min. (Figure 21 b). In contrast to this, no cell rounding of spread fibroblasts occurs on unmodified gold substrates upon a temperature decrease from 37 °C to 25 °C. Thus, the polymer-modified Au surface exhibits a switchability from a dehydrated/cell attractant to a hydrated/cell repellent surface. Cell surface adhesion proceeds via specific integrin-GGRGDS interactions, as was suggested by a standard competition assay, where an excess of free GGRGDS was added to the cell culture medium. Under these conditions, the cells show at 37 °C, only weak surface contacts and spreading was suppressed successfully (Figure 21 c).

This study shows that the combination of functional polymers, displaying *e. g.* a lower critical solution temperature (LCST) with peptide adhesion labels leads to well-defined polymer-peptide conjugates, which can be applied to modify gold surfaces. As a result of the combination of a switchable polymer and a specific cell anchor peptide, a thermo-responsive surface has been generated in a rather straightforward manner. This study should be seen as an initial conceptional study, demonstrating the potentials of bioactive polymers as well as bioactive polymeric materials. Several further developments in the fields of polymer chemistry and engineering are required to address true applications. On the one hand, surface chemistry has to be optimized to realize "cell repellent" surfaces (not only not cell attractive surfaces), and the external triggers have to be focused locally to allow for instance the isolation of single cells from a 2D-tissue culture. On the other hand such switchable surfaces have to be effectively integrated into microfluidic devices to make advanced cell handling systems attainable.

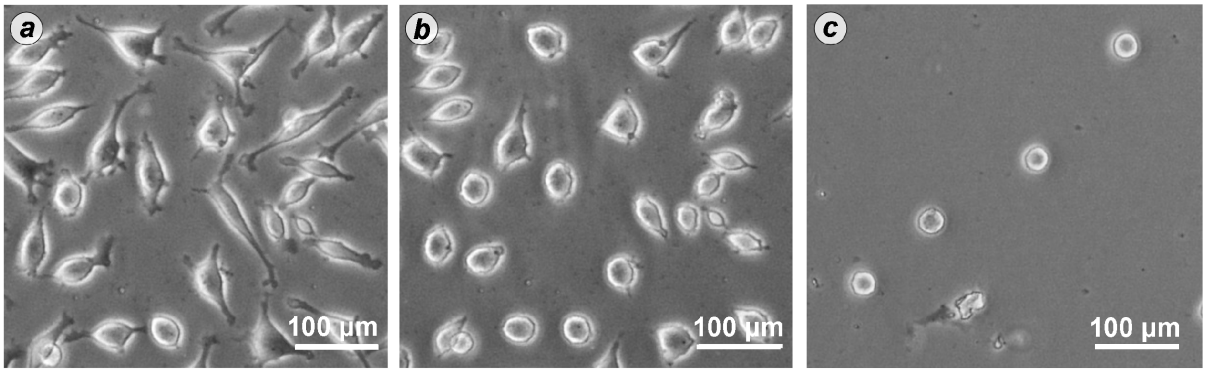


Figure 21. Representative micrographs of L929 mouse fibroblasts on PNIPAM-GGRGDS grafted Au surface: after 18 h cultivation at 37 °C (a), after 30 min of cooling at 25°C (b) and after 18 h of cultivation in a competition assay with free GGRGDS at 37 °C (c).

3.4.1 Positioning peptide domains on surfaces of fiber scaffolds

It has been demonstrated above, that peptide-polymer conjugates can be used to modify 2-dimensional, planar surfaces. Diverse studies have utilized microstructured surfaces and modified these very successfully with bioactive peptides or polymers and their mixtures.[245, 246] However, three dimensional scaffolds are mandatory to mimic the natural environments of cells. As these scaffolds should be multifunctional and biologically responsive (bioactive), the processing of peptide functionalized, sub-micrometer fibers has been addressed in a collaboration with R. Spontak (North Carolina State University).[58] Since the accessible surface area/volume ratio of fibers increases with decreasing fiber diameter, sub-microscale polymer fibers hold an excellent opportunity for efficient surface bio-functionalization. Moreover, the dimensions of these fibers coincide with those of biological systems and thus provide a promising interface between synthetic materials and biology. A single step process was realized, which integrates a process to functionalize fiber surfaces with peptides into an electrospin approach to access sub-micrometer fibers with peptide surface labels. This could be achieved by a convenient co-spinning strategy (electrospinning of a homogeneous two polymer mixture) combining an inexpensive, fiber-forming host polymer (PEO) with a polymer-peptide conjugate. The latter consists of a sequence-defined polypeptide segment and a poly(ethylene oxide) block that is compatible with the host polymer and thus serves as an anchor to the fiber bulk. It appears to be essential to the process that the peptide segment is strongly polarizable, compared to the host polymer. Under these conditions the peptide segment migrates to the fiber surface during electrospinning, probable because the surface charges are stabilized better by the peptides. Surface enrichment of the peptide segments by as much as an order of magnitude higher relative to the bulk conjugate concentration was achieved without additional treatment.

In the initial study, the co-electrospinning of a mixture of PEO and a $(Ser\text{-}Glu\text{-}Glu)_3$-PEO conjugate was investigated to proof the concept of field driven surface enrichment. Although PEO was selected because of its biocompatibility and ease of spinnability from aqueous solutions and the conjugate was a model compound with no biologic activity, the strategy can probably be generalized and is expected to be widely applicable to other polymer/conjugate pairs as well as to other pairs of materials. The utilization of the integrated process to access sub-micrometer fibers with functional surfaces appears not only interesting for biomedical applications, but also have potential in the field of inorganic-polymers hybrids for sensing, catalysis or even display technology.

4 Summary and Outlook

The main objective of the present thesis was to bridge the gap between common block copolymers and proteins. The latter provide a virtually infinite number of examples of how the precise control over secondary interactions in macromolecules allows generating functional nano- and microstructures. Thus, within this thesis, the question was addressed, whether basic concepts, abundant in the class of proteins, can be effectively transferred into the world of synthetic polymer science. In this respect, the efforts have been focused on design, synthesis and investigation of "*peptide-polymer conjugates*". These are linear block copolymers, composed of a common synthetic polymer block and a monodisperse oligopeptide segment with a defined monomer sequence. One of the main results of this work is the fact that already oligopeptides with up to 13 amino acids in length allow the transfer of the four basic concepts of functional protein structures to synthetic conjugates. Therefore, oligopeptides introduce the following fundamental properties to the synthetic polymer system:

i. *Defined secondary interactions along a monodisperse polymer chain*
ii. *Programmable formation of hierarchical structures with defined sub-structure*
iii. *Positioning of chemical functionalities to generate functions*
iv. *Capability to actively interact with biological systems*

Oligopeptides are a versatile platform that can be easily accessed in multigram scales by purely chemical means. This results in the possibility to expand the library of the 20 natural α-amino acids by several non-natural amino acids with synthetic side-chain functionalities (*cf.* Chapter 2.1). In this work, the incorporation of non-natural moieties at specific positions in a peptide segment has been utilized for instance to introduce initiator groups or orthogonally addressable functionalities. While the former allow growing a synthetic polymer from a specific peptide side, the latter enable regio-selective attachment of a polymer to a specific side of a peptide chain. Moreover, artificial adhesive groups were successfully introduced to "glue" peptides to metal/metal oxide surfaces or photo switchable side chain functionalities were positioned to change polarity of a peptide side on photo response. However, it is even the advantage of polymer chemistry not to be restricted to natural analogous. Hence, a library of completely synthetic monomers, which are common building blocks from polycondensation reactions, has been established. The sequential assembly of these monomers by means of fully automated solid-phase supported synthesis leads to monodisperse polyamides (i. e. pseudopeptides). These extend the nature of peptides by combining properties of sequence-defined oligopeptides with the diversity of synthetic polymers (*cf.* Chapter 2.2).

Conventional strategies to prepare peptide-polymer conjugates have almost exclusively focused on the introduction of poly(ethylene oxide) (PEO), because of its biomedical significance. In this thesis several novel access routes to peptide-polymer conjugates have been developed. These allow for the integration of monodisperse peptide segments into a

wide range of synthetic polymers with different properties, functionalities, and functions (Chapter 2.3). For that, both orthogonal coupling and protective group strategies have been investigated, demonstrating that end-functionalized polymers can be successfully attached to peptide segments in a sequence selective manner (*cf.* Chapter 2.3.1). While these coupling approaches are certainly limited to low molecular weight polymer blocks ($M_n < 2500$), a "grafting from" approach has also been established. This addresses the synthesis of conjugates with high molecular weight synthetic blocks and relies on the controlled growth of a synthetic polymer chain from a defined sequence position of a peptide (*cf.* Chapter 2.3.2). The most important methodologies of controlled radical polymerization (CRP), such as atom transfer radical polymerization (ATRP) and reversible addition fragmentation chain transfer radical polymerization (RAFT), have proved to be applicable and tolerant against the multifunctional character of peptides. Fully automated protocols to synthesize peptide macro-initiators and peptide macro-chain transfer agents for ATRP and RAFT have been established, respectively. Both systems are readily applicable without chromatographic purification steps and indeed allow the synthesis of peptide-polymer conjugates with adjustable molecular weights and low polydispersity indices.

The routes described provide materials on the gram or multigram scale. This is particularly important because the peptide-polymer conjugates have been subject of material science studies. These are summarized in the following paragraphs, which show how effective the fundamental concepts of proteins can be transferred and exploited for polymer sciences.

i.*) *Fine-tuning of secondary interactions by monodisperse segments

Controlling interactions in synthetic polymers as precisely as in proteins would certainly widen the potential of established polymer systems. This was investigated by combining a water soluble, non-interacting ("inert") poly(ethylene oxide) block with either a peptide or an analogous pseudopeptide segment as described in Chapter 3.1. The resulting PEO-(pseudo)peptide conjugates possess a monodisperse segment that can be fine-tuned by precisely adjusting the properties of each monomer position. This enables one to better define, understand and control interactions in such block copolymer systems.

For example it has been demonstrated that interaction capabilities of PEO-pseudopeptide conjugates can be tailor-made for the complexation of plasmid double stranded DNA (*ds*DNA). The resulting polymers are biocompatible, non-immunogenic and rationally designed to transport DNA, because they mimic biological tools for DNA packing. These synthetic carriers exhibited well-adjusted, soft polyion interactions with the *ds*DNA. It was found that a combination of secondary and primary amine functionalities in the pseudopeptide segment is required to induce the formation of highly compact, toroidal DNA supercoils. Moreover, the carrier shields the cargo DNA against enzymatic digestion and stabilizes the fragile DNA double helix, which are properties most suited for DNA delivery applications.

This approach is certainly not restricted to polymers that interact with DNA. The universal applicability of this concept has been further demonstrated by designing PEO-peptide conjugates with well-defined interactions to inorganic and organic crystal surfaces. In both cases a distinct modification of the crystal growth and the resulting crystal morphology could be observed. While in one study mesoporous crystalline calcium carbonate was formed in a soft templating/recrystallization process, in the other study interesting hollow tubes with quadratic cross sections were obtained from DL-alanine. In both examples the conjugates play multiple roles. Similar to biological morphogenesis proteins in bio-mineralization they stabilize amorphous precursor phases and guide or template both assembly and recrystallization processes. PEO-peptide conjugates provide easily adjustable, well-defined models with sharp sequence-property relationships and hence, contribute to the fundamental understanding of polymer-surface interactions.

Moreover, as the last example demonstrates, the functional peptide segment of PEO-peptide conjugates can be adapted to complex pharmacologically active compounds. A study investigated the interactions of *cis*-Platinum with a set of different PEO-peptide conjugates. The complex of the conjugates and the cytostatic drug, have proved to dramatically reduce the primary toxicity but preserve the pharmacological activity. This makes novel administration techniques feasible and allowing to address translymphatic chemotherapy, which specifically targets tumors that metastasize through the lymphatic system.

ii.*) *Programmable structure formation

This thesis shows that the peptide segments of polymer-peptide conjugates can be successfully utilized to program the formation of a variety of nano- and microstructures in solution (Chapter 3.2). A biomimetic concept of peptide-guided microstructure formation has been introduced. This concept relies on the fact that the formation of structure in polymer-peptide conjugates is dominantly directed by the self-assembly of the peptide-segments, which is driven by specific secondary interactions. An important contribution of this thesis is the establishment of means to effectively control the self-assembly kinetics of peptide-polymer conjugates. For that, temporary defect segments (*switch* defects) have been introduced into the peptide aggregator domain of a conjugate. These defects suppress the aggregation tendency. However, a pH-controlled rearrangement in the *switch* defects restores the undisturbed peptide and hence, triggers the self-assembly process. This procedure can be utilized to establish the peptide-guided microstructure formation of conjugates in both aqueous and organic media. Several examples are provided that use the diversity of the β-sheet secondary structure motif to access a multitude of microstructures. Depending on the peptide organizer unit, nanostructured tapes with a core-shell fine structure, nanosprings with distinct left-handed helical twist, core-shell nanotubes and microtapes with lengths of several millimeters could be realized. It is noteworthy that under certain conditions a peptide content

of 3.5 *wt.*% is sufficient to control the self-assembly of a conjugate into nanosprings, despite the high molecular weight of the synthetic polymer block of $M_n \sim 38000$ g/mol. Such nanosprings are of particular interest, because the aggregates reveal that the control over the structure can provide the possibility to generate a function (*biological structure to function concept*). In the present case, a nanosized damping element has been generated, which is currently subject to micromechanical and rheological investigations. Following examples from biological materials, the generated structure elements have been further organized to access more complex, integrated assemblies. Biomimetic strategies to co-assemble nanostructured tapes with silica were successfully developed, investigating the interplay with inorganic materials. Macroscopic composite fibers with six distinguishable levels of hierarchical order could be obtained, as the result of a complex hybrid process that combines self-assembly of the nanotapes and peptide-directed silicification. This process has been exploited on the one hand to rapidly obtain well-defined composite nanofibers and on the other hand to establish a convenient 2D-plotting process that enables the drawing of macroscopic composites using the nanotapes as ink.

It remains remarkable, that the characteristic feature of proteins to adopt hierarchical structures with well-defined sub-structures is, already inherently present in oligopeptides. This allows envisioning that the structural and functional diversity of proteins might be achieved with future polymer systems. In these synthetic systems, however, the ease of large scale accessibility and the chemical diversity of synthetic polymer chemistry should be preserved.

iii.) *Positioning of chemical functionalities to generate functions*

The positioning of highly defined chemical moieties such as a chemical functionality or a peptide domain at a specific position in a polymer-peptide conjugate provides the prospective to realize advanced functions in either macromolecules or macromolecular aggregates (Chapter 3.3). It has been demonstrated that the control over the molecular architecture of an ABC triblock conjugate allows the positioning of a peptide segment at the hydrophilic-hydrophobic interface of a block copolymer micelle. With this a functional domain has been realized that has precisely “programmable” functionalities and the resulting assemblies offer potentials in the fields of catalysis or for drug carrier design. Moreover, the positioning of a digestible chemical moiety like for instance a disulfide linkage between a poly(ethylene oxide) block and a cationic poly(amidoamine) pseudopeptide segment leads to a responsive two-stage delivery system for DNA. The PEO-pseudopeptide conjugates complex DNA, to provide single-plasmid polyplexes. These primary polyplexes have “stealth properties”, which are highly suited for the extracellular transport of DNA. However, the PEO block of the carrier can be selectively removed by reductive means *e. g.* in the reductive environment of the cytoplasm. This triggers a programmed fragmentation of the carrier and

leads to secondary polyplexes with effective cationic surface charges. It remains to be shown in ongoing studies that this novel delivery system enhances the *in vitro* and *in vivo* effectiveness of the transfection process. Nevertheless, the design of the carrier certainly exemplifies the possibility to generate advanced functions by sequence defined positioning of functionalities.

iv.*) *Generation of bioactivity

Besides the interesting self-assembly and chemical properties of polypeptides, they have the possibility to actively interact or even "communicate" with complex biological systems. This might be one of the most important properties of peptides. This potential for instance was exploited by integrating a peptide signaling domain into synthetic polymers, providing peptide-polymer conjugates that exhibit a biological meaning (Chapter 3.4). Well-defined conjugates have been accessed by combining either the biocompatible poly(oligo(ethylene glycol) acrylate) or the thermo-responsive poly(*N*-isopropylacrylamide) with the peptide GGRGDS, which allows for cell adhesion by specific interactions. For example a bioactive poly(*N*-isopropylacrylamide)-GGRGDS conjugate has proved to be useful for the modification of gold surfaces. The resulting thermo responsive surface coating leads to a reversible change of its properties from cell-attractive to cell-repellent with temperature decrease. Addressing the upcoming demands for bioactive materials that provide active interfaces to biological systems, a single-step process to generate sub-micrometer polymer fibers with peptide surface labels was developed. In the initial study the co-electrospinning of a mixture of PEO and a PEO-peptide conjugate was investigated. The electric field applied during the spin process could be successfully utilized to drive the peptide segments to the fiber surface. The integrated electrospin process that allows to access sub-micrometer fibers with functional surfaces can probably be generalized and is expected to be broadly applicable to obtain bioactive textiles and nonwoven fabrics.

Outlook: It is predictable that polymer chemistry with Schulz-Flory or Poisson distributions will evolve to macromolecular chemistry with precisely defined molecules. Hence fully synthetic, monodisperse polymers with a well-defined monomer sequence will be the upcoming challenge in synthetic polymer science. Completely non-natural polymer classes will be developed. These could combine novel units capable of specific molecular recognition and new monomer alphabets to fine-tune secondary interactions along linear polymer chains. Probably these polymers will be synthesized in a monodisperse and sequence-defined manner using fully automated, sequential monomer additions. Here for, the limitations of solid-phase supported synthesis have to be overcome.

Parallel to these elaborated synthesis techniques, analytical tools are essential, which enable the prediction of the rather complex relationship between monomer sequence, chain segment interactions and structure.

Such polymers allow envisioning progress in the fundamental understanding of the interactions between synthetic polymers and complex biological systems (*e. g.* with functional biomacromolecules (DNA or proteins), cells, tissues, organs or entire organisms). Moreover, design strategies of biomimetic materials (*e. g.* inorganic composites or biocomposites) might be changed, because monodisperse systems would enable one to encode information on the molecular level. This can then be used to control structure formation processes, positioning of functionalities and expression of functions.

To bridge the gap between poly- and monodisperse systems, precisely defined segments with monomer sequence control can be integrated into well-defined, but still polydisperse polymers. This approach represents an appropriate detour, allowing us to progressively understand the complex relationships between monomer sequence and both structural and functional properties. Our work and that of several other groups indicate that even oligomeric segments like for instance oligopeptides, oligonucleotides and oligosaccharides can provide us with a powerful toolbox *e. g.* to control structure formation and organization that is by far not fully exploited. Particularly, the class of sequence defined oligosaccharides offers practically untouched potential for fine-tuned secondary interactions, molecular recognition, directed self-assembly and bioactivity. With the recently established methodologies for fully automated synthesis of oligosaccharides, this exciting field is now open to investigations.

Taking into account that the synthesis of monodisperse segments (*e. g.* sequence-defined peptides) is still expensive and the amounts accessible are usually limited to gram scales, alternative access routes are mandatory. This direction of research addresses the predictable change of resources from petrochemical to renewable ones. Diverse sources are considered to have high potential. These include harvestable proteins (*e.g.* collagen, gluten *etc.*), enzymatically produced proteins (non ribosomal production), and genetically engineered proteins. Such precursor materials probably will have to be processed by chemo- or enzymatic modifications to yield the desired segments, which can be incorporated as functional blocks into bioconjugates.

5 Acknowledgements

I would like to express my gratitude to all those who gave me the possibility to accomplish this thesis!

Mein aller herzlichster Dank gilt meinem Mentor *Herrn Prof. Dr. Dr. Markus Antonietti* für die gewährten wissenschaftlichen Freiheiten, meine Arbeiten in Potsdam durchführen zu können. Für die enorme Unterstützung, die zahlreichen Diskussionen, die instantanen Problemlösungen und für die Komponierung meines persönlichen Songs möchte ich mich an dieser Stelle sehr herzlich bedanken!

Mein tiefster Dank gilt natürlich auch denjenigen, ohne deren kreative Forschung diese Arbeit nicht möglich gewesen wäre – meinen Doktorranden, Postdocs und Technikerrinnen, sowie den studentischen Hilfskräften. Danke für den Einsatz: Dr. Doreen Eckhardt, Dr. Hartmut Rettig, Dr. Mattijs ten Cate, Dr. Laura Hartmann, Emre Yagci, Stefanie Kessel, Jens Hentschel, Harald Hahn, Hans Kühnle, Rafael Gentsch und vor allem *DANKE* meinen beiden super Technikerrinnen: Jessica Brandt und Katharina Ostwald.

Für fruchtvolle Diskussionen, viele hilfreiche Anregungen, und die gute Zeit möchte ich mich ganz speziell bei Dr. *habil* Helmut Schlaad, Dr. Jean-Francois Lutz (FhG IAP), Priv.-Doz. Dr. *habil* Helmut Cölfen und Dr. Arne Thomas bedanken. Desweiteren natürlich auch bei Prof. Dr. Bernd Smarsly, Prof. Dr. Markus Niederberger, Dr. Reinhard Sigel und Priv.-Doz. Dr. *habil* Klaus Tauer.

Ich möchte mich an dieser Stelle auch bei Dr. Eberhard Krause (IMP Buch) für die massenspektrometrischen Untersuchungen unserer Konjugate; bei Prof. Jürgen Rabe und Dr. Nikola Severin (HU Berlin) für die AFM Untersuchungen sowie bei Dr. Peter Henklein (HU Berlin) und Dr. Michael Beyermann (IMP, Buch) für hilfreiche Tipps wärmend schwieriger Peptidsynthesen bedanken.

Für fruchtvolle Zusammenarbeiten möchte ich mich bedanken bei Prof. Regine Peschka-Süss, ihren Mitarbeitern und Prof. Rolf Schubert (Universität Freiburg), Prof. Jochen Werner und Dr. Anja Dünne (Philipps-Universität Marburg), Prof. Rich Spontak und Xiaoyu Sun (NCSU, USA), Prof. Dennis Discher (UPENN, USA), Dr. Claus Duschl, Dr. Andreas Lankenau und Oliver Ernst (FhG IBMT), sowie bei Prof. Andre Laschewski (FhG IAP), Dr. *habil* Gerald Brezesinski, Dr. Anna Muenter, Prof. Andreas Ferry und Dr. Himadri Gupta (MPIKG).

Nicht zuletzt danke ich allen Mitarbeitern des Institutes für die Unterstützung meiner Arbeit. Ganz besonders möchte ich Ines Below, Antje Völkel, Marlies Gräwert, Rona Pitschke, Heike Runge, Dr. Jürgen Harmann, Anne Heilig, Olaf Niemeyer, Birgit Schonert, Ingrid Zenke, Margit Barth, Günther Haseloff, Cliff Janiszewski, Annette Pape und Katharina Zesch danken.

Für die gute Zeit in Golm bedanke ich mich bei allen meinen Kolleginnen und Kollegen des MPI-KGFs. Speziell allerdings bei Dr. Andreas Taden, Dr. Matthijs Groenewolt und Dr. Thorsten Brezesinski.

An dieser Stelle möchte ich mich bei Prof. *em.* Dr. Walter Heitz (Philipps-Universität Marburg) bedanken, der mir die Freude am Arbeiten mit Makromolekülen vermittelt hat.

Mein Dank wäre nicht vollständig, wenn ich nicht meinen Geldgebern danken würde, die mich und meine Arbeitsgruppe sehr stark unterstützt haben: Der Deutschen Forschungsgemeinschaft mit dem Exzellenzprogramm Emmy-Noether (BO1762/1-3), der Max-Planck Gesellschaft, dem National Textile Center USA, dem MPG-FhG Projekthaus (bioaktive Surfaces) und der VW-Stiftung (biofunctional vesicels).

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7 Curriculum vitae

Name: **Dr. Hans G. Börner**
Date of birth: 15.09.1970 in Hannover, Germany

PROFESSIONAL BACKGROUND

4/2003-	**Max Planck Institute of Colloids and Interfaces, Potsdam** Group leader of an independent Emmy-Noether Group in the department of „Colloid chemistry“ (director: **Prof. Dr. M. Antonietti**) „*Bioorganic-Synthetic Hybrid Polymers as Molecular LEGO® - Bricks*“
1/2003- 3/2003	**University of New Hampshire, New Hampshire, USA** Visiting researcher in the group of **Prof. Dr. T. Laue** (Biochemistry) „*Protein stabilization of artificial spider silk and insulin by synthetic chaperons* “
8/2002- 10/2002	**Stellenbosch University, South Africa** Visiting Researcher in the group of **Prof. Dr. M. Rautenbach** (Biochemistry) „*Novel approaches for solid-phase supported peptide synthesis*“
10/2001- 4/2003	**Max Planck Institute of Colloids and Interfaces, Potsdam** Project leader in the department of „Colloid chemistry“ (**Prof. M. Antonietti**). „*Bioorganic-Synthetic Hybrid Polymers as Molecular LEGO® - Bricks*“
4/2000- 9/2001	**Carnegie Mellon University, Pittsburgh, USA** Postdoctoral Fellow in the group of **Prof. Dr. K. Matyjaszewski** „*Controlled radical polymerization to access molecular bottle brushes with complex architecture*“

AWARDS

4/2007	**Awarded by the Dr. Hermann-Schnell-foundation**
10/2006	**Selected for the membership in the Max-Bergmann Kreis**
4/2003	**Emmy-Noether-Scholarship**, German Research Foundation (DFG)
4/2000	**Research fellowship** of German Research Foundation (DFG)

EDUCATION

3/2000	**Defense of the Ph.D. thesis** „*Magna Cum Laude*“
10/1996- 2/2000	Ph.D. thesis in Polymer chemistry with **Prof. Dr. W. Heitz** (Philipps-University) „*Synthesis of phosphine-substituted block copolymers by anionic polymerization and their utilization to construct nano reactors*“
1/1996- 9/1996	Diploma thesis in macromolecular chemistry (Philipps-University) in cooperation with BASF AG (Dr. Jüngling), supervised by **Prof. Dr. W. Heitz**. „*Metal free anionic polymerization of acrylates and methacrylates utilizing the concept of large counter cations*“
12/1995	**Diploma in chemistry**: „*Sehr Gut*“ (Philipps-University in Marburg)
1993-95	Study of chemistry (Hauptstudium) at the **Philipps-University in Marburg**
9/1993	**Pre-Diploma in chemistry**: „*Sehr Gut*“ (Freie University Berlin)
1991-93	Study of chemistry (Grundstudium) at the **Freie University Berlin**
6/1991	General qualification for university entrance (Allgemeinen Hochschulreife)
1984-91	High school „Menzel-Oberschule in Berlin-Tiergarten“

8 List of publications (2004-2008)

In the following is given a list of publications made in the context of this thesis. The publications have been grouped by topics and categorized in the different subjects: *i.* reviews and monographs, *ii.* bioconjugate synthesis, *iii.* self-assembly and microstructure formation, *iv.* polymers with controlled secondary interactions, *v.* polymers with precisely positioned chemical functionalities and *vi.* miscellaneous.

- ***Reviews***

1. *Prog. Polym. Sci.* **2008***, 33,* 1-39
   Lutz, J.-F.; Börner, H. G.: "Modern Trends in Polymer Bioconjugates Design".

2. *Soft Matter* **2007**, *3,* 394-408
   Börner, H. G. ; Schlaad, H.: "Bioinspired functional block copolymers".

3. *Macromol. Chem. Phys.* **2007**, *208,* 124-130
   Börner, H. G.: "Functional Polymer-Bioconjugates as Molecular LEGO®-Bricks".

- ***Monographs***

4. Wiley-VCH, Weinheim 1 ed.; **2007**, *2,* 1307-1340, monograph
   Matyjaszewski, K.; Gananou, Y.; Leibler, L., Eds.
   Antonietti, M.; Börner, H. G.; Schlaad, H. in: "Macromolecular Engineering"; Elements of Macromolecular Structure Control; Bio-inspired complex polymer conjugates and their assembly.

- ***Synthesis routs toward peptide-polymer conjugates***

5. *Macromolecules* **2008**, *41,* 1073-1075
Hentschel, J.; Bleek, K.; Ernst, O.; Lutz, J.-F.; Börner, H. G.: "Easy Access to Bioactive Peptide-polymer Conjugates via RAFT".

6. *ACS Symposium Series: Progress in CLRP* **2006**, *944,* 198-214
Börner, H. G., ten Cate, M. G. J.: "Conjugates of polymers and sequence-defined polypeptides via controlled radical polymerization".

7. *Biomacromolecules* **2006**, *7,* 1239-1244
Hartmann, L.; Krause, E.; Antonietti, M.; Börner, H. G.: "Solid-Phase Supported Polymer Synthesis of Sequence Defined, Multifunctional Poly(amidoamines)".

8. *Macromolecules* **2005**, *38,* 10643-10649
ten Cate, M. G. J.; Rettig, H.; Bernhardt, K.; **Börner, H. G.:** "Sequence-Defined Polypeptide-Polymer Conjugates Utilizing Reversible Addition Fragmentation Transfer Radical Polymerization".

9. *Macromol. Rapid Commun.* **2004**, *25,* 1251-1256
Rettig, H.; Krause, E.; Börner, H. G.: "Atom transfer radical polymerization with polypeptide initiators: A general approach to block copolymers of sequence-defined polypeptides and synthetic polymers".

Programmed formation of nano- and microstructure (Peptide-guided organization)

- ***Structure formation in water***

10. *Langmuir* **2008***, 24*, 3306-3316
Muenter, A.; Hentschel, J.; Börner, H. G.; Brezesinski, G.: "Characterization of peptide-guided polymer assembly at the air/water interface".

11. *Macromolecules* **2008***, 41,* 1430-1437
Börner, H.G.; Smarsly, B.; Hentschel, J. Rank, A.; Schubert, R.; Geng, Y., Discher, D. E.; Hellweg, T.; Brandt A.: "Organization of Self-assembled Peptide-Polymer Nanofibers in Solution".

12. *J. Am. Chem. Soc.* **2006**, *128,* 7722-7723
Hentschel, J.; Krause, E.; Börner, H. G.: "Switch-peptides to Trigger the Peptide Guided Assembly of Poly(ethylene oxide)-Peptide Conjugates into Tape Structures"

13. *Macromolecules* **2006**, *39,* 7831-7838
ten Cate, M. G. J.; Severin, N.; Börner, H. G.: "Self-Assembling of Peptide-Polymer Conjugates Comprising *D,L*-Cyclopeptides as Aggregator Domains".

14. *Chem. Commun.* **2005**, 2814-2816
Eckhardt, D.; Groenewolt, M.; Krause, E.; Börner, H. G.: "Rational design of oligopeptide organizers for the formation of poly(ethylene oxide) nanofibers".

- ***Structure formation in organic solvents***

15. *Macromolecules* **2008**, *41,* 1073-1075
Hentschel, J.; ten Cate M. G. J.; Börner, H. G.: "Peptide-guided Organization of Peptide-polymer Conjugates: Expanding the approach from Oligo- to Polymers".

16. *J. Am. Chem. Soc.* **2006**, *128,* 14142-14149
Hentschel, J.; Börner, H. G.: "Peptide-Directed Microstructure Formation of Polymers in Organic Media".

- ***Complex structures and composite materials***

17. *Macromol. Rapid Commun.* **2008***, 29,* 316–320
Kessel, S.; Börner, H. G.: "Self-Assembled PEO-Peptide Nanotapes as Ink for Plotting Nonwoven Silica Nanocomposites and Mesoporous Silica Fiber Networks".

18. *Macromol. Rapid Commun.* **2008***, 29*, 419–424
Kessel, S.; Börner, H. G.: "High-Rate Silicification of Peptide-Polymer Assemblies toward Composite Nanotapes".

19. *Angew. Chem. Int. Ed.* **2007***, 46,* 9023-9026
Kessel, S.; Thomas, A.; Börner, H. G.: "Mimicking Biosilicification: Programmed Co-assembly of Peptide-Polymer-Nanotapes and Silica".

- ***Controlling secondary interactions***

20. *Cryst. Growth Des.* **2008***, 8*, 1792-1794
Page, M.G.; Nassif, N.; Börner, H. G.; Antonietti, M., Cölfen, H.: "Mesoporous calcite by polymer templating".

21. *Chem. Europ. J.* **2008**, *14,* 2025-2033
Hartmann, L.; Häfele, S.; Peschka-Süss, R.; Antonietti, M.; Börner, H. G.: "Tailor-made poly(amidoamine)s for controlled complexation and condensation of DNA".

22. *Soft Matter* **2008**, *4,* 534 - 539
Hartmann, L.; Bedard, M.; Sukhorukov, G. B.; Börner, H. G.; Möhwald, H.*; Sukhorukov, G. B.; Antonietti, M.: "CO_2-switchable oligoamine patches based on amino acids and their use to build polyelectrolyte containers with intelligent gating".

23. *Anti Cancer Res.,* **2007**, *27,* 3935-3940
Dünne, A. A.; Börner, H. G.; Kukula, H.; Schlaad, H.; Werner, J. A.; Antonietti, M.: "Block copolymer carrier systems for translymphatic chemotherapy of lymph node metastases".

24. *Chem. Eur. J.* **2006**, *12,* 7682-7688
Ma, Y.; Börner, H. G.; Hartmann, J.; Cölfen, H.: "Synthesis of DL-alanine hollow tubes and core-shell mesostructures".

- ***Positioning of Chemical Functionalities***

25. *Macromol. Rapid. Commun.* **2008**, *209*, 1455-1460
Sun, X.-Y.; Nobles, L. R.; Börner, H. G.; Spontak, R. J.: "Field-Driven Surface Segregation of Biofunctional Species on Electrospun PMMA/PEO Nanofibers".

26. *Macromol. Chem. Phys.* **2007**, *208,* 1437-1446
ten Cate M. G. J.; Börner, H. G.: "Synthesis of ABC-triblock peptide–polymer conjugates to position peptide segments within block copolymer aggregates".

27. *Macromolecules* **2007***, 40,* 7771-7776
Hartmann, L.; Häfele, S.; Peschka-Süss, R.; Antonietti, M.; Börner, H. G. : "Sequence positioning of disulfide linkages to program the degradation of monodisperse poly(amidoamines)".

28. *Adv. Mater.* **2007**, *19,* 87-91
Sun, X.-Y.; Shankar, R.; Börner, H. G.; Ghosh, T. K.; Spontak, R. J.: "Field-Driven Biofunctionalization of Polymer Fiber-Surfaces during Electrospinning".

- ***Additional publications to related subjects***

29. *Chem. Commun.* **2007,** 1894-1895
Klawonn, T.; Gansäuer, A.; Winkler, I.; Lauterbach, T.; Franke, D.; Nolte, R. J. M.; Feiters, M. C.; Börner, H. G.; Hentschel, J.; Dötz, K. H.: "A Tailored Organometallic Gelator with Enhanced Amphiphilic Character and Structural Diversity of Gelation".

30. *Aust. J. Chem.* **2007**, 60, 410-413
Lutz, J.-F.; Börner, H. G.; Weichenhan, K.: "“Click” Bioconjugation of a Well-defined Synthetic Polymer and a Protein Transduction Domain".

31. *Macromol. Rapid Commun.* **2006**, 27, 1660-1664
Yagci, Y. E.; Antonietti, M.; **Börner, H. G.**: "Synthesis of poly(tartar amides) as bio-inspired antifreeze additives".

32. *Macromolecules* **2006**, 39, 6376-6383
Lutz, J.-F.; Börner, H. G.; Weichenhan, K.: "Combining ATRP and click chemistry: a Promising Platform Towards Functional Biocompatible Polymers and Polymer Bioconjugates".

33. *Macromol. Rapid Commun.* **2005,** 26, 514-518
Lutz, J.-F.; Börner, H. G.; Weichenhan, K.: "Combining ATRP and Click Chemistry: An "Universal" Method for Preparing End-Functional Polymers".

34. *Angew. Chem. Int.. Ed.* **2008**, *47,* 5666-5668
Wischerhoff E., Uhlig K., Lankenau A., Börner H. G., Laschewsky A., Duschl C, Lutz J.-F.: "Controlled Cell Adhesion on PEG-based Switchable Surfaces".