Protein-Inspired Materials: Synthetic Concepts and Potential Applications**

Harm-Anton Klok*

Introduction

Natural materials in many cases surpass their synthetic counterparts developed by mankind. Enzymes, for example, can catalyze chemical reactions under physiological conditions with yields and stereoselectivities unmet by synthetic catalysts. From a synthetic point of view, the structural and functional diversity of natural proteins is particularly impressive if one recalls that they are prepared from a limited pool of α-amino acids. Over many years of evolution, nature has developed ingenious methods to prepare perfectly monodisperse polypeptides with excellent control of the primary structure. After synthesis, the linear chains fold and assemble through a number of hierarchical steps into stable and active proteins. This process, as well as the structure and properties of the final protein, are largely determined by the peptide's primary structure.

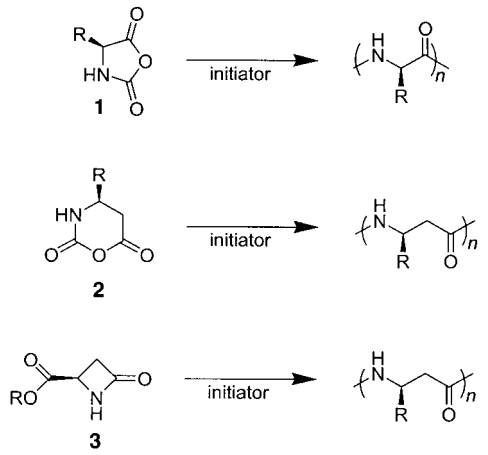
From a materials science point of view polypeptides offer many advantages over conventional synthetic polymers. In particular, their inherent ability to adopt stable conformations and self-assemble into precisely defined structures might allow unprecedented control over materials morphology and properties. Synthetic strategies for the preparation of polypeptides can be divided in three classes:

- ring-opening polymerization,
- solid-phase synthesis,
- protein engineering.

Recent advances in these methods have allowed the preparation of (poly)peptides and (poly)peptide hybrids, which can be assembled in a controlled fashion into supramolecular architectures and materials that mimic the structure and function of proteins. This article will highlight some recent examples and discuss the current scope and limitations of the different synthetic methods for the preparation of protein-inspired materials.

Ring-Opening Polymerization

Polypeptides can be prepared by ring-opening polymerization of α- and β-amino acid *N*-carboxyanhydrides (α- and β-NCAs, **1** and **2**, respectively)[1] and β-lactams **3**[2] (Scheme 1). Whereas polymerization of **1** affords poly(α-amino acid)s, **2** and **3** yield poly(β-amino acid)s. Generally, the ring-opening polymerizations can be performed following well-established protocols, thus allowing a straightforward synthesis of high molecular weight polypeptides. These polymers, however, are neither monodisperse, nor possess the sequence specificity of natural proteins. In addition, the polymerizations are usually plagued by side reactions, which make it difficult to prepare optically pure polypeptides with predictable molecular weights and narrow molecular weight distributions and also hamper the formation of well-defined block copolymers.



Scheme 1. Strategies for the preparation of polypeptides by ring-opening polymerization.

A number of the drawbacks mentioned above have been overcome since the discovery of a number of transition metal initiators including for example, [bpyNi(cod)] (cod = cycloocta-1,5-dienyl, bpy = 2,2′bipyridyl) and $[Co(PMe_3)_4]$, which have allowed the synthesis of α-amino acid homo- and block copolymers with unprecedented control of the chain length and very narrow polydispersities.[3] β-NCAs (**2**) can also be polymerized using nickel amidoamidate com-

[*] Dr. H.-A. Klok
Max-Planck-Institute for Polymer Research
Ackermannweg 10, 55128 Mainz (Germany)
Fax: (+49) 6131-379-100
E-mail: hak@mpip-mainz.mpg.de

[**] The author is grateful to the Deutsche Forschungsgemeinschaft for financial support within the Emmy Noether Programm and to Prof. Klaus Müllen for his continuous interest and support.

  1433-7851/02/4109-1509 $ 20.00+.50/0

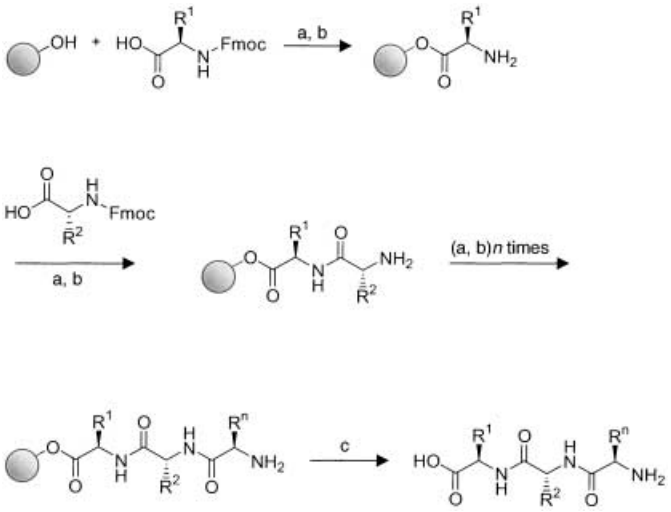
plexes, however, precipitation of the polymers from the reaction mixture was found to limit the molecular weight.[4] Generally, high molecular weight poly(β-amino acid)s are only accessible by ring-opening polymerization of β-lactams.[1a, 2] Very recently, it was shown that [Sc(N(TMS)$_2$)$_3$] (TMS = trimethylsilyl) can initiate the living polymerization of certain β-lactams, and allows the preparation of unprecedented di- and triblock poly(β-amino acid)s in high yields and with narrow molecular weight distributions.[5, 6]

The advances in α-NCA polymerization have found application in the biomimetic synthesis of ordered silica structures. Well-defined block copolymers of L-cysteine and L-lysine were found to self-assemble in ordered aggregates, which simultaneously could hydrolyze tetraethoxysilane (TEOS) and direct the morphology of the resulting silica structures (Figure 1).[7] The design of these block copolymers, which mimic the protein silicatein, was based on the rationale that their amphiphilic character would provide the self-assembling properties while the L-cysteine residues would enable hydrolysis of the TEOS precursor at a neutral pH value. Cross-linking of the block copolymers, by oxidation of the SH groups, affects the self-assembly process and provides an additional means to direct the mineralization process.

Solid-Phase Synthesis

Solid-phase peptide synthesis (SPPS, Scheme 2) is a powerful method for the preparation of small and medium-sized peptides.[8] In contrast to the NCA polymerization, SPPS allows the preparation of monodisperse peptides with precise control of the primary structure. However, although the yields in each of the reaction steps are very high (>98%), they are not quantitative, which results in the formation of deletion sequences. The concentration of these defects increases exponentially with chain length, and for very large peptides they can constitute the major part of the crude product. As a consequence, small and medium-sized peptides are easily obtained in high yields and purities, however, yields rapidly drop and purification becomes more difficult with increasing chain length.

One way to overcome the limitations of SPPS with respect to the size of the peptides that can be prepared is the thioester-mediated native chemical ligation (NCL) of unprotected peptide segments.[9] This method (Scheme 3) relies on the chemoselectivity of the reaction between a peptide-α-thioester and another segment containing an N-terminal



Scheme 2. Solid-phase peptide synthesis: a) coupling, b) deprotection, c) cleavage from resin (Fmoc = 9-fluorenylmethoxycarbonyl).

cysteine residue. NCL has been successfully used for the total chemical synthesis of a variety of proteins. Since the peptide segments, which typically contain 50–60 α-amino acids, are prepared by standard SPPS, NCL is a very versatile method that allows the site-specific incorporation of unnatural structures that cannot be introduced or are only difficult to introduce by other techniques.

Recently, protocols have been developed that allow NCL to be carried out on solid supports.[10] This procedure does not only simplify the purification of the resin-bound product, but also provides a facile route for the synthesis of proteins composed of a larger number of peptide segments. Another very recent highlight is the development of the 1-phenyl-2-sulfanylethyl auxiliary group which obviates the need for a cysteine residue in one of the peptide segments.[11] In this way, the total synthesis of cytochrome b562, a 106 α-amino acid sequence lacking cysteine, has yielded the correctly folded and functional protein.[12]

Chemoselective ligation of unprotected peptide segments has also been used for the preparation of template-assembled synthetic proteins (TASPs)[13] and multiple antigen peptides (MAPs).[14] The TASP concept focuses on bypassing the protein folding problem by ligating secondary structural elements onto regioselectively addressable topological templates in a predetermined packing arrangement.[13] MAPs are composed of a large number of peptide epitopes ligated onto a dendritic scaffold and have attracted attention for the development of vaccines.[14]

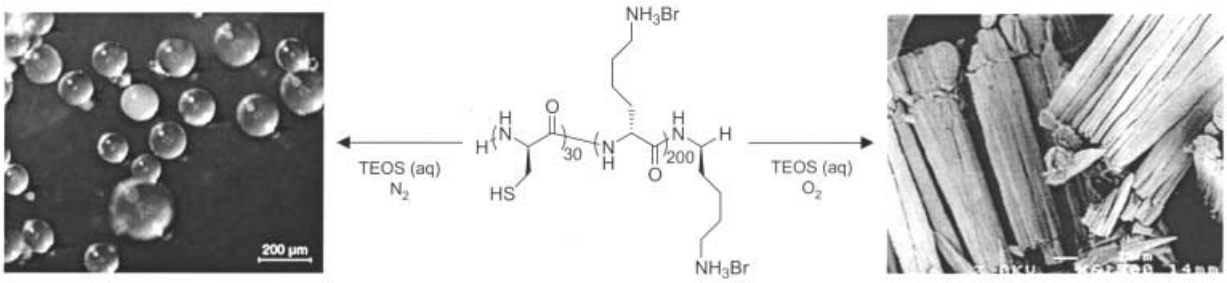


Figure 1. Biomimetic synthesis of ordered silica structures. The size and shape of the silica particles is controlled by the peptide block copolymer.

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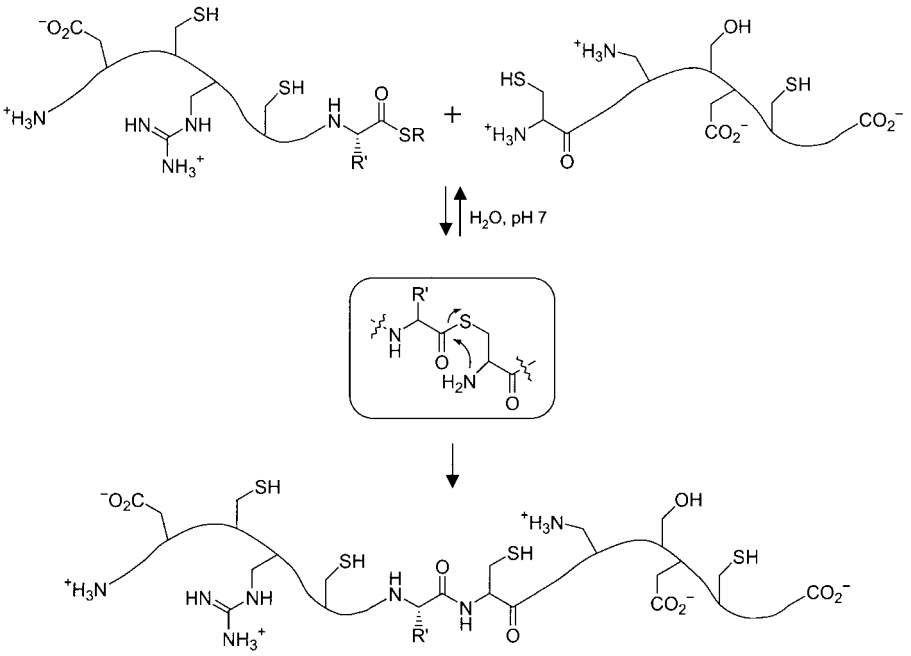

Scheme 3. Coupling of deprotected peptide segment by NCL.

SPPS has also gained interest for the synthesis of peptide building blocks for biologically inspired supramolecular architectures. Examples include cyclic peptides, which can self-assemble into nanotubes,[15] and linear oligopeptides that form fibrillar β-sheet structures.[16] In some cases, the self-assembly of β-sheet-forming peptides can be induced by changes in ionic strength and lead to the formation of hydrogels.[17] Very recently, the self-assembly properties of such peptides have been combined with the release properties of certain stimuli-sensitive liposomes to allow temperature- or light-induced formation of hydrogels.[18] Exposure of an aqueous suspension of $CaCl_2$-loaded liposomes and the β-sheet-forming peptide to near-infrared light or an increase in the suspension's temperature to 37 °C triggers release of $CaCl_2$ from the liposomes and results in rapid gelation.[18]

SPPS is also useful for the synthesis of hybrid molecules containing, for example, hydrophobic alkyl chains. Such peptide amphiphiles are interesting since their self-assembly is not only determined by the peptide's primary structure but also by the overall molecular geometry. This effect was nicely demonstrated in a very recent report which describes the self-assembly of peptide amphiphiles into nanofibrous scaffolds with diameters of 6 – 7 nm.[19] The formation of the nanofibers was found to be a reversible process that could be controlled by the pH value. Oxidation of the cysteine SH groups in the peptide sequence covalently captured the supramolecular object and resulted in a polymeric nanofiber with a molecular weight of about 2×10^8 Da. The fibers were able to direct the mineralization of hydroxyapatite in the same way as that known for collagen fibrils in bone.

Protein Engineering

Although strategies for cloning and expression of genes have been established for many years and are routinely used in biochemistry and molecular biology, these methods have been recognized as interesting tools for the preparation of polypeptide materials only in the past 5 – 10 years.[20] An overview of the different steps involved in the synthesis of polypeptides by bacterial expression of artificial genes is shown in Figure 2. This strategy, which is also referred to as protein or genetic engineering, is very attractive, since it allows the preparation of high molecular weight and perfectly monodisperse polypeptides with a precisely defined primary structure. Such materials cannot be prepared using the ring-opening polymerization and solid-phase methods discussed above. Protein engineering has been successfully used both for the synthesis of structural proteins such as silk, collagen, and elastin, as well as for the preparation of de novo designed proteins, that is, proteins which are not based on a natural α-amino acid sequence but are designed from scratch.[20]

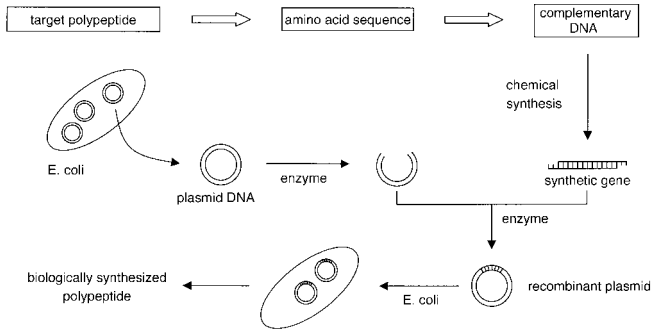

Figure 2. Protein biosynthesis by bacterial expression of artificial genes.

Protein engineering is not restricted to proteogenic α-amino acids. Various methods are available that allow the incorporation of unnatural analogues.[21] In this way, it has become possible to prepare artificial proteins that carry a variety of nonproteogenic functional groups. A very interesting example is the incorporation of trifluoroleucine (Tfl) in leucine-zipper proteins.[24] The primary structure of a leucine-zipper protein is characterized by a heptad periodicity *-abcdefg-*, where positions *a* and *d* are occupied by hydrophobic α-amino acids and leucine (Leu) is the predominant α-amino acid at position *d*. Leucine-zipper proteins can form amphiphilic α-helical secondary structures, in which the α-amino acids at positions *a* and *d* constitute the hydrophobic face of the α-helix. Aggregation of two or more of these proteins in a superhelical fashion through the formation of a hydrophobic interhelical interface results in a structural motif referred to as a coiled coil. The folding and assembly of leucine-zipper proteins into coiled-coil structures is a reversible process, which can be controlled by temperature and/or pH values. The replacement of the proteogenic Leu by Tfl

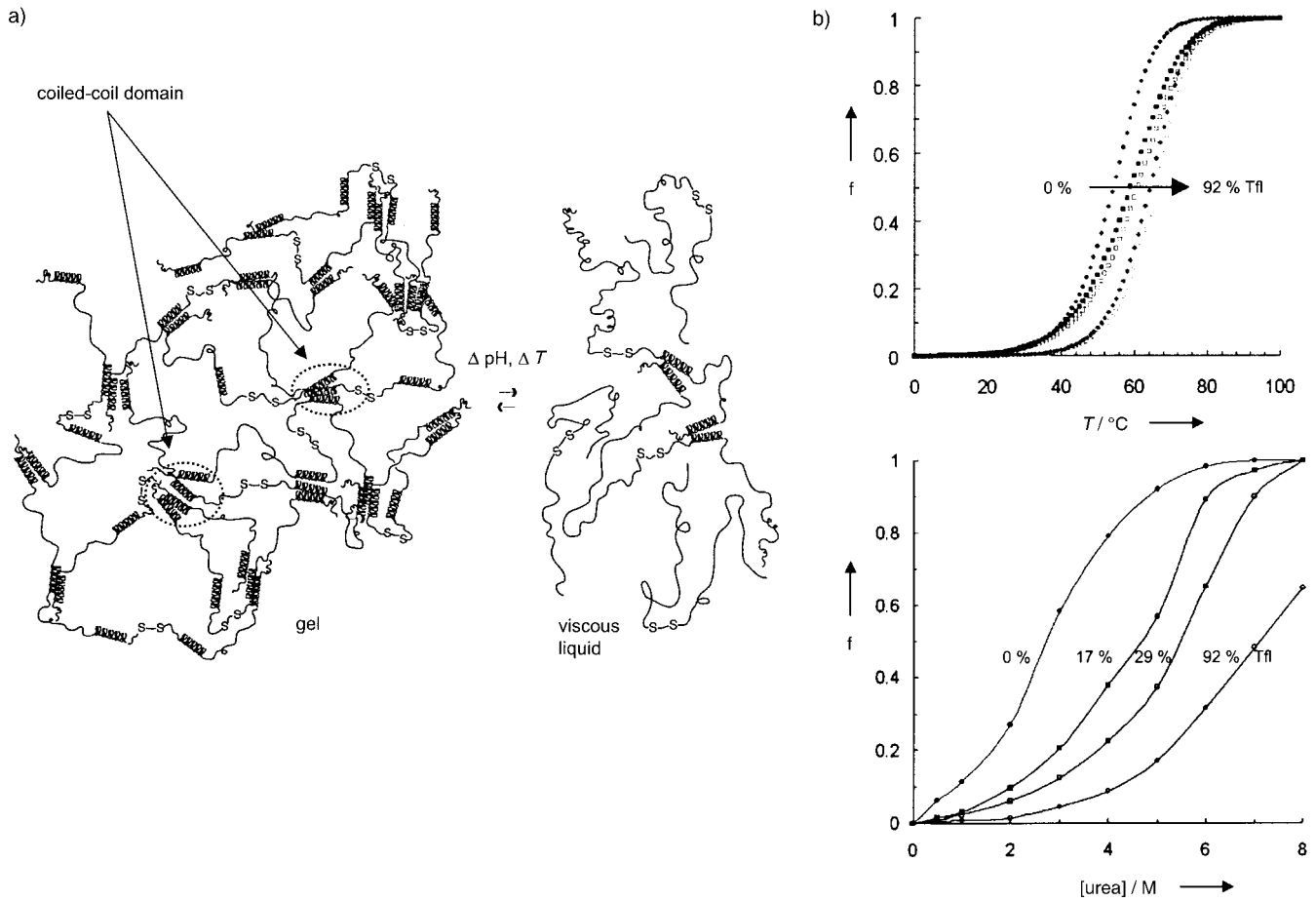

Figure 3. a) Reversible network formation by aggregation of leucine-zipper domains in artificial proteins. b) The thermal (top) and chemical (bottom) stability of the leucine-zipper domains can be enhanced by substitution of leucine for trifluoroleucine (Tfl). (f = fraction unfolded.)

resulted in an elevation of the thermal unfolding temperature by up to 13 °C and an increased resistance against chemical denaturation by urea (Figure 3).[24] This observation is of particular interest in the context of an earlier finding that artificial protein triblock copolymers containing terminal leucine-zipper domains can form reversible hydrogels.[25] It is clear that the incorporation of unnatural α-amino acids such as Tfl offers unprecedented opportunities to adjust the sol–gel behavior of these polymers and allows a precise tailoring of the materials properties for specific applications.

Although the control over folding, assembly, and properties exhibited by natural proteins remains to be surpassed, the advances in chemical and biological synthesis outlined above allow hitherto unprecedented control over composition, structure, and organization of artificial proteins and peptide hybrid materials. Undoubtly, these developments and future advances will allow the integration of biological design concepts in materials science and lead to protein-inspired materials with a level of structural control approaching that of natural proteins.

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Of Molecular Gyroscopes, Matroshka Dolls, and Other "Nano"-Toys

Christoph A. Schalley*

At the outset, let us stand up for playing. Often mistaken for a kid's domain, playing opens up new fields of experience even for the "grown-ups". Possibly, it is one of the prerequisites of the creative process, in the sense that dealing playfully with known ideas provides new ways of thinking. Scientists are no exception. On the contrary, in the area of supramolecular chemistry, with all the model-like realizations of macroscopic objects at a molecular level, the drive to play seems to be followed strongly.

That said, it is no suprise that second-sphere supramolecular complexes, that is, the inclusion of a guest in a host which is already encapsulated in an even larger host, have been compared to russian Matroshka dolls. However, such architectures have been realized only rarely so far, probably because of the difficulties inherent in the synthesis of appropriately sized host molecules which can incorporate an entire host – guest system. Several early examples have been observed by Vögtle and Müller, who described the co-crystallization of γ-cyclodextrin with coronates and cryptates in 1:1 und 2:1 ratios.[1] These so-called "cascade complexes" contain an alkali metal ion bound inside a crown ether or a cryptand, which—as later shown by X-ray crystal structure analyses[2]—are surrounded by one or two cyclodextrin molecules. Recently, a few other examples have been added, for example, hydrogen-bonded capsules which self-assemble reversibly in solution around an encapsulated cryptate (Figure 1)[3] as well as onionlike arrangements of fullerenes and carbon nanotubes.[4]

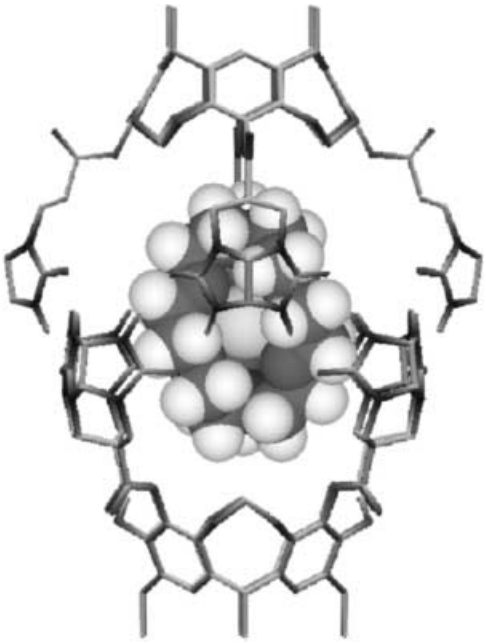

Figure 1. A dimeric, hydrogen-bonded capsule with an interior volume of almost 1000 Å^2 which reversibly encapsulates different cryptates in solution. The guest is depicted as a space-filling model (central sphere = K^+ ion inside the [2.2.2]cryptand), while the capsule is shown as tubes (for clarity, the solubilizing side chains have been omitted).

A particularly beautiful example has just been published by Day, Blanch, and co-workers.[5] This "gyroscane" (Figure 2) consists of a cucurbit[5]uril that is surrounded by a larger analogue, cucurbit[10]uril.[6] The particular aesthetics of this complex arise from the fact that both shells belong to the same class of compounds and thus provide a highly symmetrical compound. Upon crystallization from concentrated hydro-

[*] Dr. C. A. Schalley
Kekulé-Institut für Organische Chemie und Biochemie
Universität Bonn
Gerhard-Domagk-Strasse 1, 53121 Bonn (Germany)
Fax: (+49) 228-735662
E-mail: c.schalley@uni-bonn.de

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