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Author(s):
Wennemers, Helma

Publication date:
2021-06

Permanent link:
https://doi.org/10.3929/ethz-b-000497738

Originally published in:
Chimia 75(6), https://doi.org/10.2533/chimia.2021.525

doi:10.2533/chimia.2021.525 Chimia 75 (2021) 525–529 © H. Wennemers

Peptides – Molecular Allrounders

Helma Wennemers*

Abstract: The enormous structural and functional diversity available through combining different amino acids into peptides offers numerous exciting opportunities. This article summarizes recent research highlights from my laboratory in the areas of asymmetric catalysis, supramolecular chemistry, and chemical biology. This scope includes the development of bioinspired peptide catalysts, synthetic collagen peptides, supramolecular porous assemblies, and cell-penetrating peptides.

Keywords: Asymmetric catalysis · Chemical biology · Collagen · Porous materials · Supramolecular chemistry

Introduction

In nature and our everyday life, peptides fulfill manifold different roles. Peptides serve as hormones, neurotransmitters, snake and frog toxins, artificial sweeteners, anti-wrinkle additives and drugs against major diseases. This wide palette of different functions is enabled by the large structural and functional diversity of peptides that can be easily accessed by linking different amino acids with each other. For example, a diversity of $20^6 = 6.4 \times 10^7$ different hexapeptides is generated by combining 20 different amino acids randomly in any possible combination. Effective and, often, automated synthetic methods facilitate straightforward access to peptides, including peptides that contain non-proteinogenic amino acids. Combined with combinatorial methods, millions of linear and cyclic peptides consisting of essentially any desired amino acid building block are accessible.

The resulting opportunities for scientists are vast. Already decades ago, the benefits for drug development were evident and resulted in many peptide-based therapeutics that are important drugs (*e.g.* cyclosporine, octreotide, glucagon) up to the present day. With ever more challenging drug targets and remedies against caveats of peptide therapeutics, such as proteolytic degradation of peptides composed of natural α-amino acids, the number of peptide-based drugs is steadily increasing.[1] The rise of peptides is, however, not limited to drug development. Numerous other disciplines are recognizing the opportunities offered by the large structural and functional diversity of peptides combined with their comparative ease in synthesis. As a result, peptides have expanded the fields of asymmetric catalysis[2] and material sciences and are invaluable for chemical biology. Over the past two decades my laboratory has been intrigued by the versatility of peptides and contributed to those fields. Herein, I present recent highlights from our research on peptidic catalysts, synthetic collagen peptides, cell-penetrating peptides, selective hosts for signaling molecules, as well as peptidic supramolecular assemblies and frameworks.

Peptides as Asymmetric Catalysts

Despite the numerous roles that peptides fulfill not a single peptide with catalytic activity is known in nature. Our group is therefore intrigued by the question whether short peptides can function as effective stereoselective catalysts. With a combination of combinatorial screening and rational design we established tripeptides of the general type H-Pro-Pro-Xaa as effective catalysts for C–C bond forming reactions that proceed *via* an enamine intermediate.[3–7] Their modular nature allowed us to develop highly stereoselective catalysts for a range of aldol and conjugate addition reactions including even 'difficult' electrophiles that yielded, *e.g.* access to products with quaternary stereogenic centers with exquisite yields and stereoselectivities.[3–7] The peptidic catalysts are so robust that addition reactions with immobilized H-DPro-Pro-Glu-NH_2 can be performed in a flow reactor on a >100 g (>400 mmol) scale.[8] Furthermore, the reactivity of the peptidic catalysts is so high that a loading of as little as 0.05 mol% is feasible,[4c,9] which is the lowest catalyst loading so far achieved for C–C forming reactions relying on an enamine-based mechanism. The low catalyst loadings were enabled by detailed conformational analyses and mechanistic investigations using kinetic, NMR-spectroscopic, and ESI-MS studies.[9–14] These studies provided deep insight into the key features of the peptide catalyst structure and the catalytic cycle. They showed, for example, that the *trans*/*cis* ratio of the DPro–Pro bond, the pyramidalization of the enamine nitrogen, and an intramolecular salt-bridge between the CO_2H group and the secondary amine are critical for the performance of the peptidic catalysts.[12–14] Most importantly, these investigations provided principles for catalyst design.

In a recent example, we used those guidelines to reverse the diastereoselectivity of addition reactions between aldehydes and nitroolefins from *syn* to *anti*.[15] Numerous secondary amines had previously been reported for this transformation that provides direct access to γ-nitroaldehydes and downstream compounds such as pyrrolidines and γ-butyrolactams, which are key moieties in many bioactive molecules. All reported chiral amine-based organocatalysts follow Seebach's topological rule[16] and provide the product with *syn*-diastereoselectivity, but many target molecules require access to the *anti*-diastereomer. We succeeded to inverse the diastereoselectivity by installing substituents at C^δ of the N-terminal pyrrolidine moiety to enforce the preferential formation of s-*cis* enamines as reactive intermediates and their reaction in a (*Re*/*Si*)-attack with *E*-configured nitroolefins (Fig. 1).[15] Further careful tuning of the conformational features resulted in optimized catalyst **1** that enables access to a broad range of different products with remarkably high *anti*-diastereoselectivities and excellent enantioselectivities. Mechanistic studies showed that the reaction proceeds *via* a Curtin-Hammett scenario, which enables the amplification of the s-*cis*/s-*trans* enamine ratio and the *anti*/*syn*-diastereoselectivity of the products.

*Correspondence: Prof. H. Wennemers, E-mail: helma.wennemers@org.chem.ethz.ch
Laboratorium für Organische Chemie, ETH Zürich, D-CHAB, Vladimir-Prelog-Weg 3, CH-8093 Zurich

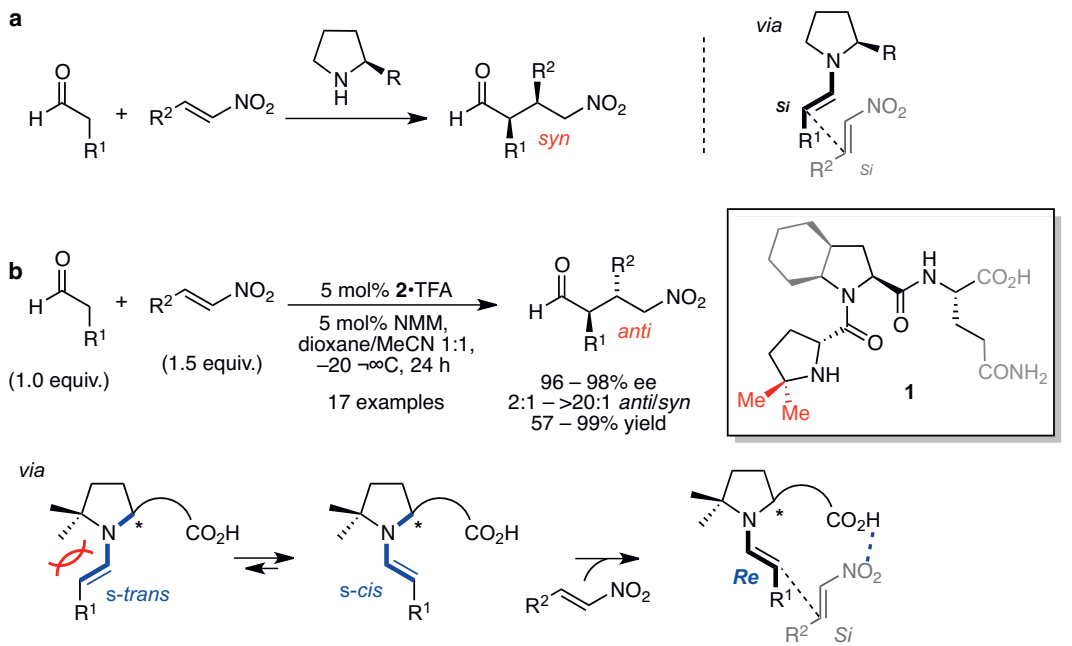

Fig. 1. Conjugate addition reaction of aldehydes to nitroolefins. a) *Syn*-selectivity following Seebach's topological rule. b) *Anti*-selective conjugate addition reaction catalyzed by tripeptide 2 *via* s-*cis* enamines.

These studies highlight the versatility of peptides for the development of effective catalysts and how insight into the conformational properties, the modularity and ease of synthesis can enable the development of practical catalysts. Our results also showed that catalytic activity of amino acid-based compounds is not limited to enzymes, which in turn suggests that peptides played a crucial role in the evolution of enzymes.

Synthetic Collagen Peptides

Collagen, the most abundant protein in mammals, is key to the stability of skin, bones and connective tissue and involved in many signaling events that control cellular activities.[17] Collagen's unique triple helical structure has intrigued scientists across different disciplines for decades and a lot of research has been dedicated to understanding the factors that are responsible for the high structural stability of collagen on the molecular level.[17] Many of these studies used collagen mimetic peptides (CMPs) that form discrete homotrimeric collagen triple helices.[17] Heterotrimeric collagen triple helices are common in nature but are significantly more difficult to access synthetically since the three single strands assemble with a one-residue stagger.[18] Three different collagen strands can therefore form a total of $3^3 = 27$ trimers that differ in their composition and register.

Research in our group led to a 'toolkit' of functionalizable synthetic CMPs that have different propensities for self-assembly into collagen triple helices.[19] These peptides include lipidated and cross-linked CMPs that form hyperstable triple helices as well as pH-responsive synthetic collagen.[20–22] Recently, the obtained insights also enabled the design of tailored CMPs that fold selectively into A_2B and even ABC-type heterotrimers (Fig. 2).[23] Key for this selective assembly is the formation of interstrand salt bridges between (4*S*)-aminoproline (Amp) and aspartic acid (Asp) residues that direct the composition and register-specific assembly of the synthetic collagen heterotrimers.

These studies open exciting prospects for creating synthetic collagen materials and functional CMPs that can integrate into wounds and promote the repair process.

Cell Penetrating Peptides and Selective Hosts of Small Molecules

Cells protect themselves from large or abiotic molecules with their cell membrane. The penetration of this obstacle is therefore key for the efficacy of therapeutics and imaging agents with intracellular targets. Cationic peptides are an attractive tool for this purpose.[24] Many examples of such cell penetrating peptides (CPPs), including proline-rich peptides have been developed and used to deliver cargo into cells.[24] The mechanism(s) with which CPPs pass the cell membrane remains under debate. Our laboratory used oligoprolines – peptides that form already at chain lengths of six residues a defined polyproline II (PPII) helix[25] – with cationic charges arising from guanidinium groups to probe the effect of preorganized versus undefined cationic charge display on cell penetration (Fig. 3a).[26] Careful comparison with flexible cationic peptides revealed that charge localization in distances of ~9 Å

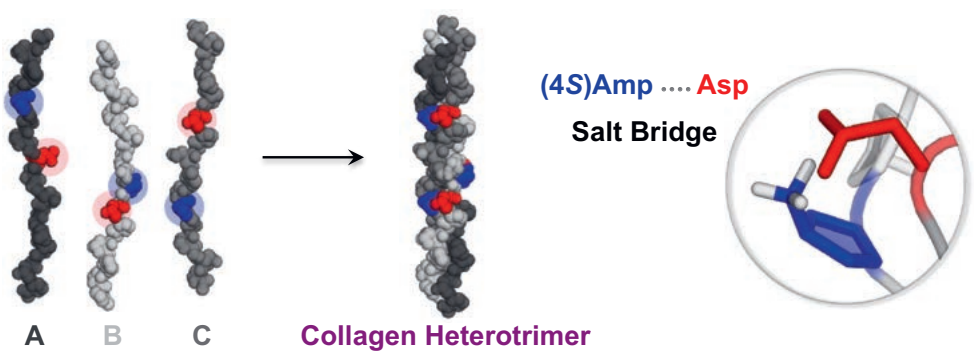

Fig. 2. Selective assembly of three CMPs bearing Amp and Asp residues at defined positions to allow for the controlled formation of interstrand salt bridges. Figure adapted from ref. [23].

enables more efficient cellular entry into different cancer cell lines (HeLa, MCF7, and HT-29). This lateral distance between the cationic moieties corresponds to the distance between negatively charged sulfate moieties of cell surface glycans such as heparan sulfate. This ionic interaction between the peptide and the glycan likely contributes to the efficacy of cell penetration, as corroborated by binding studies with heparin and assays with heparan-sulfate-deficient CHO-K1 cells.[26]

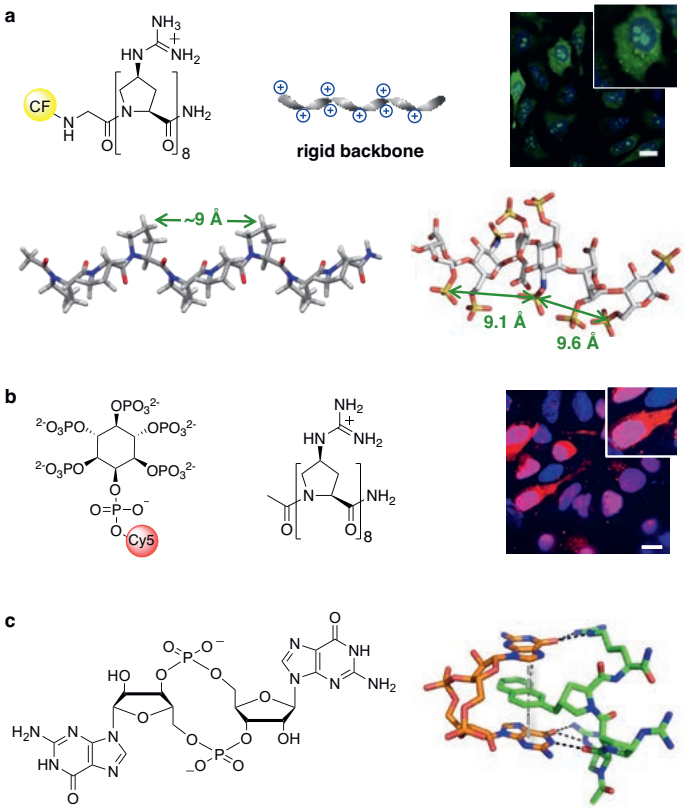

Fig. 3. a) Structure of fluorescently labeled cationic oligoproline (CF = 5(6)-carboxyfluorescein) with corresponding confocal microscopic image of HeLa cells (top) and model of PPII helical oligoproline with segment of the crystal structure of heparin (bottom). b) Structure of fluorescently labeled inositol hexakisphosphate ($InsP_6$) and confocal microscopic image of HeLa cells. c) Structure of bis-(3',5')-cyclic dimeric guanosine monophosphate (c-di-GMP) and complex with cationic peptide Ac-GupGupNapArg-NH_2 (Gup = guanidino proline, Nap = naphthylmethyl proline). Figure adapted and modified from refs [26-28]

More recently we showed that such cationic oligoprolines bind and deliver inositol hexakisphosphate ($InsP_6$), a central member of the inositol phosphate messengers in eukaryotic cells, into the cell nucleus (Fig. 3b).[27] Again, the rigid, helical structure of the oligoprolines proved key to this finding that opens opportunities for the functional studies with $InsP_6$ as well as other inositol phosphates.

Cationic proline-rich peptides that bear an aromatic moiety were also identified in a combinatorial screening as selective binders of bis-(3',5')-cyclic dimeric guanosine monophosphate (c-di-GMP), an important signaling molecule in bacteria that is involved, for example, in the formation of biofilms.[28] Spectroscopic, molecular modeling, and SAR studies showed that the binding is driven by a combination of π,π-stacking, H-bonding, and electrostatic interactions between the peptide and c-di-GMP (Fig. 3c). The peptides were found to inhibit the formation of biofilm by the opportunistic pathogen *P. aeruginosa*, a result that could become valuable for the development of novel antibiotics.

Peptidic Porous Supramolecular Assemblies

Supramolecular assemblies with cavities are fascinating since they provide spatially defined and confined compartments for, for example, selective encapsulation and catalysis.[29] Extended frameworks, as well as discrete capsules and cages, are typically built from aromatic building blocks that serve as rigid scaffolding moieties.[29] Peptides are attractive alternatives since they allow for the facile introduction of functional groups that are needed for the selective recognition and/or transformation of guest molecules. Together with their large structural diversity, chirality, modularity, facile synthesis, biocompatibility and intrinsic chirality, peptidic building blocks have therefore the potential to significantly enlarge the versatility of supramolecular assemblies with cavities. Yet, drawbacks such as a lack of symmetry and high conformational flexibility have hampered the development of peptidic frameworks. Our group used oligoprolines to overcome these challenges. These peptidic scaffolds enabled the formation of a triaxial supramolecular weave and peptide-metal frameworks.[30,31]

To create the triaxially woven material – an architecture that is common for macroscopic woven materials but that was unprecedented on the nanoscopic level – we built on prior supramolecular assemblies of oligoproline-chromophore conjugates,[32] and installed perylene monoimide (PMI) moieties at the two penultimate positions of an oligoproline 9-mer (**2**, Fig. 4a). This molecular design ensured that **2** forms fibers *via* head-to-tail π–π stacking of the N and C terminal chromophores (similarly to previously studied conjugates)[32] with voids at regular intervals that enable entwining and the establishment of crossing points. The self-assembled hexagonal structure is held together by a combination of non-covalent π–π stacking and CH–π interactions and extends into the micrometer regime (average diameter ~3 μm), as revealed by TEM and AFM analyses (Fig. 4a). The weaving takes place in two dimensions, but the material extends into the 3rd dimension as a result of dispersion interactions between the individual layers. On average, the hexagonal domains have a width of 3 μm and a height of 170 nm.[30]

More recently we used an oligoproline hexamer that bears carboxylates as metal coordination sites at the N- and C-termini (**3**) to form crystalline networks with Zn/K or Zn/Rb (Fig. 4b).[31] The overall architecture consists of stacks of 2D pleated nanosheets with channels that comprise ~12 volume% of the crystalline coordination framework (Fig. 4b). This structure arises from an asymmetric unit cell that consists of one Zn^{2+} and one K^+ (or Rb^+) cation as well as two oligoproline ligands, one with a *trans*-configured ('*trans*-**2**') and the other with a *cis*-configured ('*cis*-**2**') N-terminal amide bond. The 2D pleated nanosheets emerge from the coordination of each *trans*-oligoproline ligand to Zn^{2+} and K^+ (or Rb^+) ions with the N- and C-terminal carboxylate groups in a zig-zag arrangement. Since the *cis*-amide bond creates a kink in the *cis*-oligoprolines these ligands are shorter than the *trans*-oligoprolines and can therefore coordinate only with their N-terminal carboxylate to the Zn^{2+} and K^+ (or Rb^+) ions. They coordinate alternatingly from underneath and above and serve as 'glue', by filling part of the space between the *trans*-oligoproline layers (Fig. 4b). The rest of the space remains as pores filled with disordered DMF molecules.[31]

These findings show the propensity of rigid peptides to serve as building blocks and/or ligands to access extended supramolecular assemblies. They open exciting prospects for expanding the realm of porous peptidic materials and utilizing them for molecular recognition and catalysis.

Conclusions and Outlook

The examples highlight the broad spectrum of science that is currently explored with peptides and evidence that the traditional borders of peptide chemistry have been widely expanded. The crosstalk between scientists from different fields and backgrounds will certainly broaden the horizon of peptide science and open up

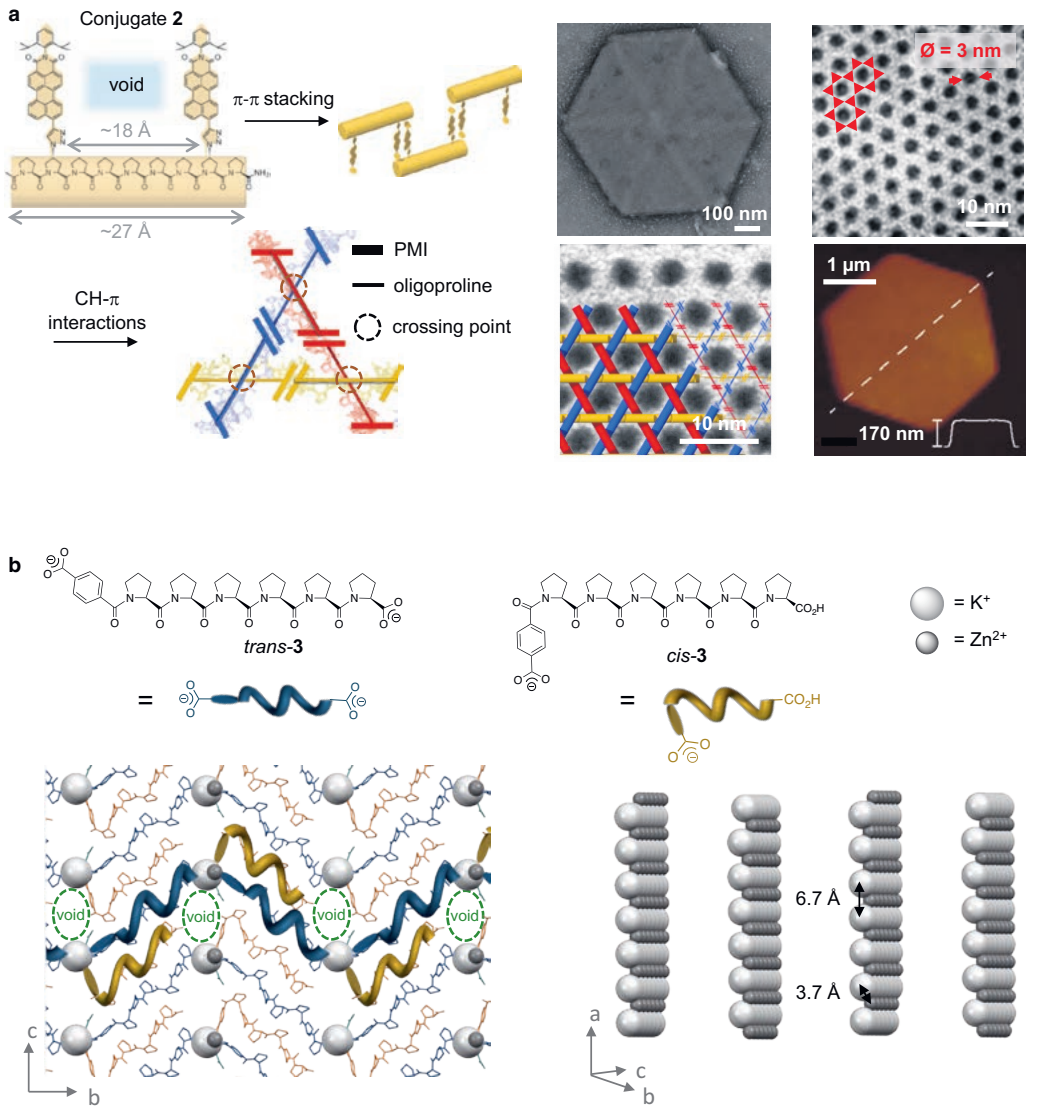

Fig. 4. a) Triaxial supramolecular weave: Molecular structure of conjugate **2** and self-assembly into triangular connecting point. TEM images at different magnifications and superimposition of the woven topology on the micrograph. AFM image with height profile. b) Zn–K–oligoproline network formed by oligoproline ligand **3** and K^+ (or Rb^+) and Zn^{2+}: View along the 'a' axis of the crystal structure with the stacked pleated sheets (left) and arrangement of the metal cations within the network. Figure adapted and modified from refs [30,31]

further exciting avenues. Clearly, the future of peptide science is bright and an exciting arena for practitioners!

Acknowledgements

None of the results would have been possible without a fabulous team of coworkers. I sincerely thank the members of the Wennemers group, past and present, for their dedication, creativity, and team spirit! I am also grateful to the University of Basel where some of the described projects started and the ETH Zurich for providing an excellent research environment, and I thank the Swiss National Science Foundation for continuous financial support.

Received: May 14, 2021

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