Review

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Peptides as asymmetric catalysts and templates for the controlled formation of Ag nanoparticles†‡

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The question whether peptides can fulfill functions for which nature utilizes large macromolecules is an overarching theme of the research in the Wennemers laboratory. The Zervas Award Lecture summarized our research on the development of peptides as asymmetric catalysts and templates for the controlled formation of silver nanoparticles. Tripeptides of the general type Pro-Pro-Xaa (Xaa = acidic amino acid) were presented that effectively catalyze aldol and conjugate addition reactions. These peptides are not only highly active, robust, and stereoselective catalysts but have also remarkable chemoselectivities. In the second part, short peptides that allow for the generation of silver nanoparticles in distinctly different sizes were presented. Copyright © 2012 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: asymmetric catalysis; conjugate addition reactions; peptides; nanoparticles; silver

Background

In nature and everyday life, peptides are widespread. They serve as hormones, neurotransmitters, drugs against numerous different diseases, toxins, artificial sweeteners, and antiwrinkle agents to name just a few of their functions [1]. In light of these numerous different roles, it is somewhat surprising that not a single catalytically active peptide is known in nature. In addition, nature utilizes also for the directed growth of inorganic materials such as, e.g. bones or metal nanoparticles, mainly proteins and not peptides [2]. Part of my laboratory is exploring the questions whether short peptides can function as effective asymmetric catalysts and templates for the controlled formation of metal nanoparticles.

Tripeptides as Asymmetric Catalysts for Enamine Catalysis

Already early examples by Inoue [3] and Juliá [4] demonstrated that the rational design of peptidic catalysts is not trivial [5,6]. Rational design is a challenge for any type of catalyst because our understanding of the necessary parameters for catalysis is still limited; however, it is arguably even more challenging for peptidic catalysts because of the typically large degree of conformational flexibility of peptides [7]. We therefore started our research program by developing the combinatorial screening method of 'catalyst–substrate co-immobilization' that allows for the identification of catalytically active compounds within the members of a split-and-mix library for essentially any bimolecular reaction [8]. To probe the question whether short peptides consisting of only three amino acids can be efficient catalysts, we chose aldol and related reactions. This choice was driven by the value of such reactions for organic synthesis and also with a view to the possibility of comparing features of the peptidic catalysts with those of natural aldolases.

Combinatorial screenings using the catalyst–substrate co-immobilization method led to the identification of the general motive H-Pro-Pro-Xaa (Xaa = acidic amino acid) as versatile catalysts for aldol and related reactions (Figure 1) [9,10]. For example, tripeptide H-Pro-Pro-Asp-NH_2 (**1**) is a highly active and stereoselective catalyst for direct aldol reactions [9–14], and the closely related peptide H-D-Pro-Pro-Glu-NH_2 (**2**) proved to be an effective catalyst for conjugate addition reactions between aldehydes and nitroolefins [15–19].

These peptidic catalysts have remarkable properties with respect to reactivity, substrate scope, stereoselectivity, and chemoselectivity, as well as chemical robustness. For example, in the presence of as little as ≤1 mol% of tripeptide **2**, a broad range of different aldehydes and nitroolefins react readily with each other to form the desired γ-nitroaldehydes in excellent yields and stereoselectivities (Scheme 1) [16–19]. This is a remarkably low catalyst loading compared with other chiral amine-based organocatalysts with which typically 10–30 mol% are required to obtain the desired products in acceptable yields and stereoselectivities [20]. The substrate scope is broad and includes even aldehydes and nitroolefins bearing functional groups [16–19]. Thus, synthetically useful γ-nitroolefins with diverse functionalities that can be easily converted into, for example, chiral γ-amino acids or butyrolactams are easily accessible using the peptidic catalyst **2**.

Even nitroethylene that is known for its high tendency to polymerize reacts readily with aldehydes in the presence of 1 mol% of **2** (Scheme 2) [19]. This methodology provides a direct asymmetric route to monosubstituted γ-nitroaldehydes and

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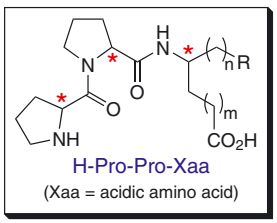
Biography

Helma Wennemers studied chemistry at the University of Frankfurt and obtained her PhD from Columbia University (mentor W. C. Still), New York in 1996. Following postdoctoral studies at Nagoya University (mentor H. Yamamoto) she moved to the University of Basel in 1999 where she was holding the Bachem endowed professorship until she became Professor of Organic Chemistry at the ETH Zurich in 2011. Her research interests are synthetic organic chemistry and chemical biology and include the use of peptides as asymmetric catalysts, as templates for the controlled formation of metal nanoparticles, and the development of synthetic collagen.



aldol reactions H-Pro-Pro-Asp-NH_2 **1**

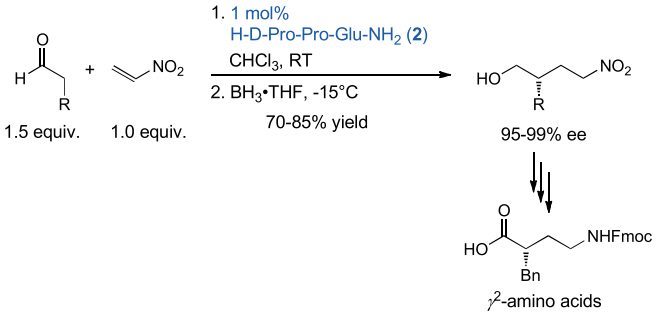
1,4-addition reactions of aldehydes with:

H-D-Pro-Pro-Glu-NH_2 **2**

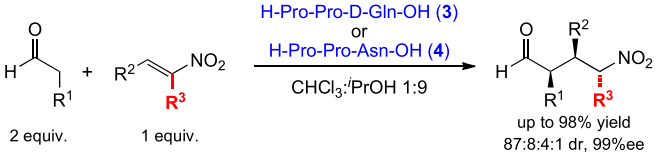
H-Pro-Pro-D-Gln-OH **3**

H-Pro-Pro-Asn-OH **4**

Figure 1. Peptidic catalysts for aldol and conjugate addition reactions.



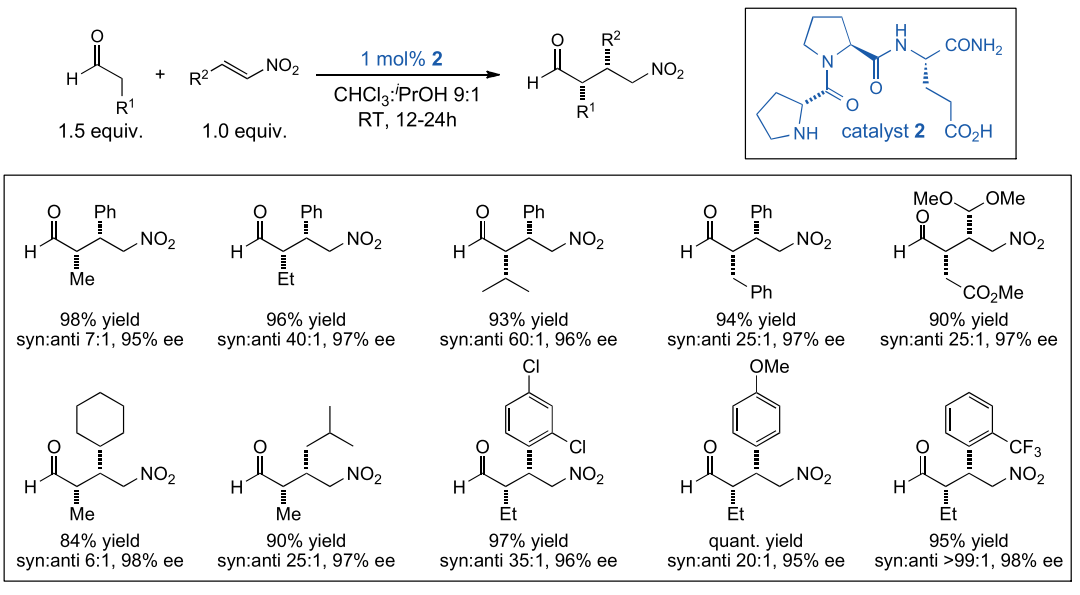
Scheme 2. A direct asymmetric catalysis route to γ^2-amino acids [19].



Scheme 3. A direct asymmetric catalysis route to γ-nitroaldehydes with three stereogenic centers [21].

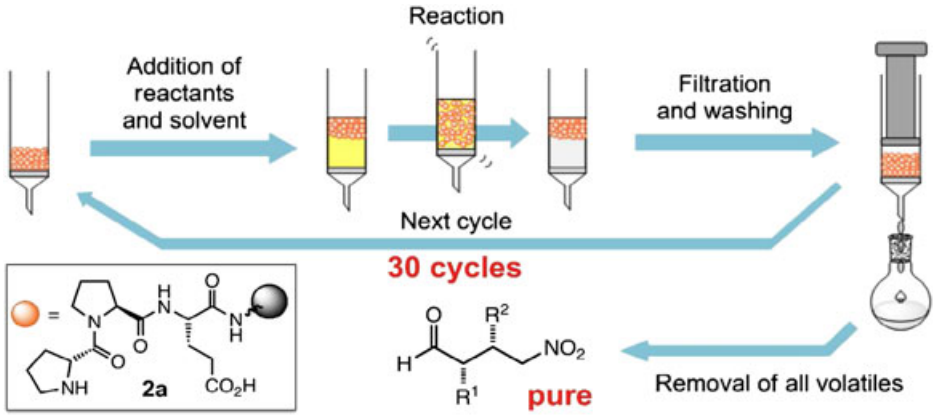
via straightforward further transformations to monosubstituted γ-amino acids that were thus far only accessible using chiral auxiliaries.

Noteworthy is also the chemoselectivity of peptide **2**. Whereas with other secondary amine-based catalysts, products from homo-aldol reactions are commonly observed, peptide **2** has a high selectivity for 1,4-addition reactions over 1,2-addition reactions [16]. This high chemoselectivity is particularly remarkable because the closely related peptide H-Pro-Pro-Asp-NH_2 is a good catalyst for aldol reactions [9–14]. The finding shows that the chemoselectivity of the tripeptidic catalysts can be easily modified by variations in their structure. With this in mind, we probed whether peptidic catalysts of the type H-Pro-Pro-Xaa (Xaa = acidic amino acid) can be developed for conjugate addition reactions between aldehydes and α,β-disubstituted nitroolefins. The additional substituent on the nitroolefin



Scheme 1. Conjugate addition reactions between aldehydes and β-substituted nitroolefins catalyzed by peptide **2** [16,17].

Journal of PeptideScience

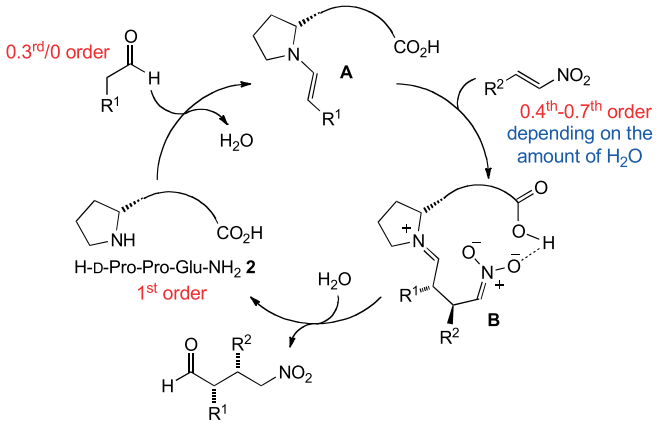


Scheme 4. Conjugate additions with the immobilized catalyst **2a** [22].

leads to a significantly reduced electrophilicity compare to β-monosubstituted nitroolefins. As a result, homo-aldol reactions are even more favored over the desired conjugate addition reaction and the desired γ-nitroaldehydes that are synthetically highly valuable compounds with three consecutive stereogenic centers are difficult to obtain by direct asymmetric catalysis. Reassuringly, a screening of 15 tripeptides of the type Pro-Pro-Xaa allowed for the identification of the peptides H-Pro-Pro-D-Gln-OH (**3**) and H-Pro-Pro-Asn-OH (**4**) as catalysts for this reaction [21]. In the presence of slightly higher but still acceptable amounts of 5 mol% of the peptidic catalysts, a range of different trisubstituted γ-nitroaldehydes formed in good yields and stereoselectivities (Scheme 3).

All of the described reactions are conveniently carried out with the trifluoroacetic acid salts of the peptides and an equivalent amount of a tertiary amine (e.g. *N*-methylmorpholine) as a base to liberate the N-terminal secondary amine. This is convenient because the peptides are easily accessible on multigram scales by standard solid-phase peptide synthesis using the Fmoc/tBu protocol or by solution-phase chemistry. However, the reactions proceed with the same product yields and stereoselectivities in the absence of trifluoroacetic acid and *N*-methylmorpholine using the 'desalted' peptidic catalysts [16]. Thus, no additives are necessary for the catalysis. This feature renders the peptides ideal for their use as immobilized catalysts. Indeed, the immobilized catalyst H-D-Pro-Pro-Glu-NH-TentaGel (**2a**) does not only provide the desired conjugate addition products in excellent yields and stereoselectivities but is also readily reusable (Scheme 4) [22]. It can be reused for at least 30 reaction and recovery cycles without loss in catalytic activity or stereoselectivity after a simple filtration from the reaction products. Because of the high chemoselectivity of the peptidic catalyst, the reactions proceed so cleanly that the conjugate addition products are obtained in such high purities that removal of all volatiles from the filtrates allowed for the isolation of analytically pure products, as judged by not only NMR spectroscopic inspection but also elemental analysis [22].

A plausible mechanism involves reaction of the catalyst with the aldehyde to form an enamine (**A**) that reacts with the nitroolefin followed by protonation and hydrolysis of the resulting imminium-nitronate (**B**) (Scheme 5). Studies with analogs of H-D-Pro-Pro-Glu-NH_2 in which the carboxylic acid is replaced by an amide or ester demonstrated that the carboxylic acid is important for the catalytic activity as well as the stereoselectivity [16]. This suggests that the intramolecular carboxylic acid



Scheme 5. Proposed catalytic cycle with rate orders [18].

of the peptidic catalysts coordinates to the imminium-nitronate and ultimately protonates the imminium-nitronate intermediate. This assumption is also supported by our studies on the addition reactions of aldehydes to α,β-disubstituted nitroolefins, where mechanistic studies demonstrated that the protonation of the carbon bearing the nitro group is controlled by the catalyst [21]. Kinetic studies showed that not the step in which the aldehyde reacts with the catalyst but the C-C bond formation step with the nitroolefin is rate limiting [18]. In addition, the reaction rate is fastest when the water amount is kept to a minimum, and only the water that is generated during the enamine formation step is allowed to be present.

These insights provided a clear guide for improving the reaction conditions further. An excess of the nitroolefin with respect to the aldehyde combined with the use of anhydrous solvents allowed to reduce the catalyst loading by a factor of 10 to 0.1 mol% and still isolate the conjugate addition products in high yields and stereoselectivities [18]. This catalyst loading is to the best of our knowledge the lowest so far achieved with an organocatalyst for enamine catalysis. In comparison with the activity of most enzymes, a turnover number of ~990 is low. However, the peptide is a small molecule with a molecular weight of 340 g mol^{-1} that is readily accessible in multigram quantities, whereas aldolases consist of at least 300 amino acids.

Initial studies to gain insight into the conformational properties of the peptidic catalysts [16] suggest that peptides of the type H-Pro-Pro-Xaa (Xaa = acidic amino acid) are rigidified to a certain extent but still have enough conformational flexibility to

accommodate to different geometries of the transition states within the catalytic cycle.

In summary, the research showed that short-chain peptides of the type H-Pro-Pro-Xaa (Xaa = acidic amino acid) are robust, highly active as well as chemoselective and stereoselective catalysts for aldol and related reactions. The structure of the peptidic catalysts proved to be easily tunable to accommodate the steric and stereoelectronic properties of a given substrate and allow for chemoselective and stereoselective transformations. Combined with the modular nature and facile synthesis of short peptides, these features render peptides very attractive for asymmetric catalysis. The research also illustrated that peptidic catalysts have features that are typically assigned to synthetic catalysts (e.g. broad substrate scope) and others that are considered to be typical for enzymes (e.g. conformational flexibility). Peptides might therefore have played a role in the chemical evolution of enzymes. These conclusions are based on the results obtained with peptidic catalysts for aldol and related reactions. It is, however, likely that the general lessons would have been the same or very similar if a different type of reaction had served to evaluate the properties of peptidic catalysts [5,6].

Peptides as Templates for the Controlled Formation of Ag Nanoparticles

Silver nanoparticles (AgNPs) are important for a wide range of different applications including imaging, catalysis, and the development of antimicrobial coatings [23,24]. They are typically prepared by chemical reduction of Ag^+ salts in the presence of additives. The additive is crucial for stabilizing the AgNPs and influencing the initial nucleation of the AgNP. Because the properties of Ag and other metal nanoparticles depend largely on their size and shape, a lot of research has focused on the development of additives and conditions that allow for the controlled formation and stabilization of AgNPs [24]. Peptides are promising additives [23] because a large structural and functional diversity can easily be accessed by linking different amino acids to a simple tripeptide. However, the rational design of a suitable peptidic additive is not trivial because of the still limited understanding of the factors that are required for controlling the defined formation of AgNPs. To address this challenge, we developed a screening method that allows for the identification of compounds that enable for the generation and stabilization of AgNPs among the members of combinatorial split-and-mix libraries [25]. The distinct coloration of AgNPs that depends on their size and shape provided for an easy tool to identify active library members. Using a library in which the amino acids serine (Ser), aspartate (Asp), histidine (His), and tyrosine (Tyr) were linked by both rigid as well as flexible spacer molecules, peptides were identified that control the formation of AgNPs from Ag^+ ions either by light or sodium ascorbate as a reducing agent (Figure 2) [25].

Moreover, in the assay using sodium ascorbate to reduce the Ag^+ ions, different peptides were identified that generate AgNPs in distinctly different sizes [25]. This is qualitatively indicated by the different coloration of the beads ranging from yellow to dark red in the combinatorial assay (Figure 2) and verified by scanning electron microscopy analysis. For example, peptides such as His-Ahx-Asp were on the red beads and induce the formation of AgNPs with an average diameter of ~50 nm that can agglomerate to larger assemblies of ≤200 nm. AgNPs on the yellow beads bearing peptides such as Ser-Ahx-Tyr are significantly smaller with an average diameter of ~10 nm. These studies demonstrate that short-chain peptides are highly attractive additives to not only control the generation but also the size of AgNPs [25].

More recently, we showed that the average diameter of AgNPs formed in the presence of aldehyde-functionalized oligoprolines correlates linearly with the lengths of the oligoprolines [26]. Thus, a molecular property of the additives is directly reflected in a nanoscopic property of the metal nanoparticles. These are important insights for the rational design of not only further peptidic templates but also other additives to control the dimensions of metal nanoparticles.

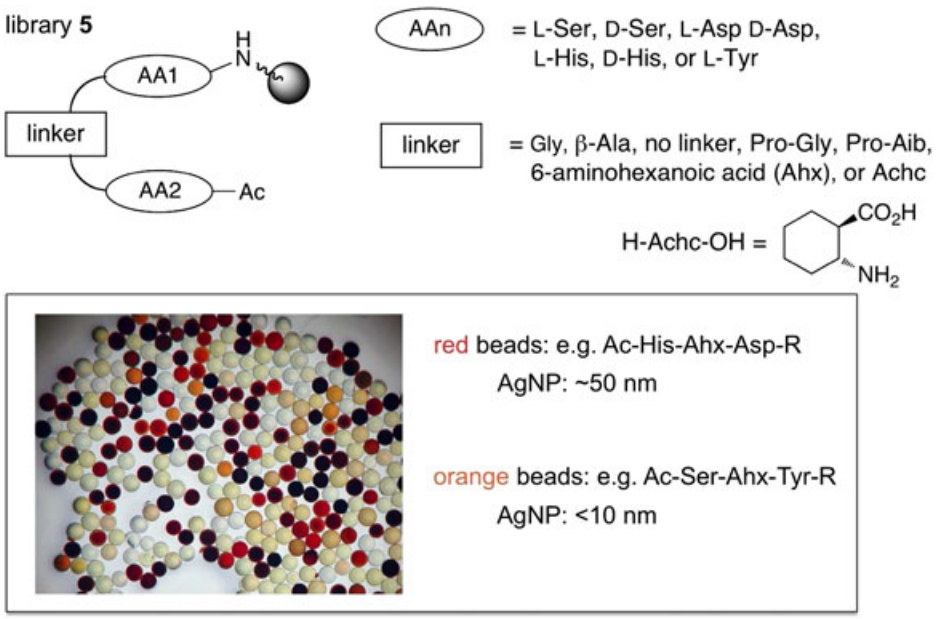


Figure 2. Silver nanoparticle (AgNP) formation within the assay of library **5** after complexation with Ag^+ ions and reduction with sodium ascorbate [25].

Journal of PeptideScience

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