Molecular Machines

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A Supramolecular Peptide Synthesizer**

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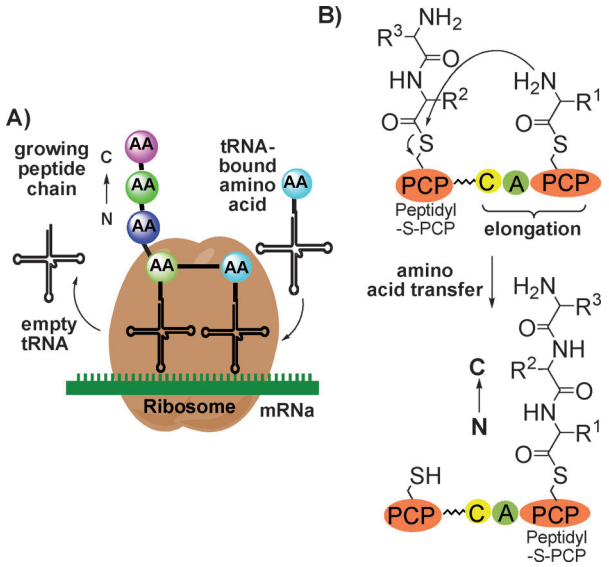
The synthesis of peptides has been an active area of research for chemists for many decades. Hallmarks in this area include the first synthesis of a peptide in solution by Fischer and Fourneau in 1901,[1] Merrifield's peptide synthesis on the solid support,[2] and the development of ligation reactions, especially native chemical ligation (NCL) by Kent et al.,[3] for the assembly of unprotected peptides and proteins of synthetic or biotechnological origin. Solid-phase peptide synthesis (SPPS) developed from these revolutionary concepts and has become the method of choice; the growing peptide is immobilized to an insoluble resin, and in this way the scalable synthesis of polypeptides with up to 40–50 amino acids can be achieved in high yields. SPPS follows a stepwise protocol to add amino acids one at a time to a growing peptide chain. Importantly, each amino acid building block has to be activated and orthogonally protected at its N-terminus and functional side chains. The selective removal of the N-terminal protecting group and subsequent coupling with the next amino acid in a synthetic protocol are steps that can easily be automated for the sequence-specific incorporation of both natural and unnatural amino acid building blocks. Despite many improvements in the area of SPPS the general concept is still valid: The synthesis proceeds—in contrast to peptide biosynthesis—from the C-terminus to the N-terminus and the specificity is ensured by the reaction of a *selected* activated amino acid building block with an N-terminally unprotected solid-supported peptide of choice.

Very recently in early 2013, Leigh and co-workers reported a conceptually new approach to peptide synthesis, in which the amino acids are *preorganized* in a supramolecular architecture for the synthesis of small peptides.[4] For reactions in this artificial molecular machine, close analogies can be drawn to natural ribosomal and nonribosomal peptide biosynthesis. This effort can be considered as a milestone in the design of biologically inspired supramolecular machines.[5]

The most interesting aspect of this work is not only the capability of the molecular machine to synthesize peptides but also how the design makes use of nature's approach: There are two biosynthetic pathways in nature for the assembly of amino acids to form polypeptides, which rely either on ribosomal peptide synthesis (RPS)[6] or on nonribosomal peptide synthesis (NRPS).[7] Ribosomal peptides are synthesized by translation of the messenger RNA (mRNA) (Scheme 1 A), thereby taking advantage of several molecular components of the cell; these include the aminoacyl tRNA-synthetase (aaRS) for the selection and loading of a cognate amino acid to the tRNA, which transfers the activated amino acid to the ribosome, and of course the ribosome itself, where the decoding and peptide synthesis take place. In contrast, the nonribosomal machinery for peptide synthesis uses large multi-enzyme complexes as an assembly line to catalyze the peptide condensation in a stepwise manner (Scheme 1 B). In analogy to RPS, an enzyme (A-domain) selects the cognate amino acids and activates them as amino acyl adenylate, much like the aaRS. The activated amino acid is then transferred to a peptidyl carrier protein



Scheme 1. A) Ribosomal peptide synthesis (RPS); B) nonribosomal peptide synthesis (NRPS). AMP = adenosine monophosphate, PCP = peptide carrier protein.

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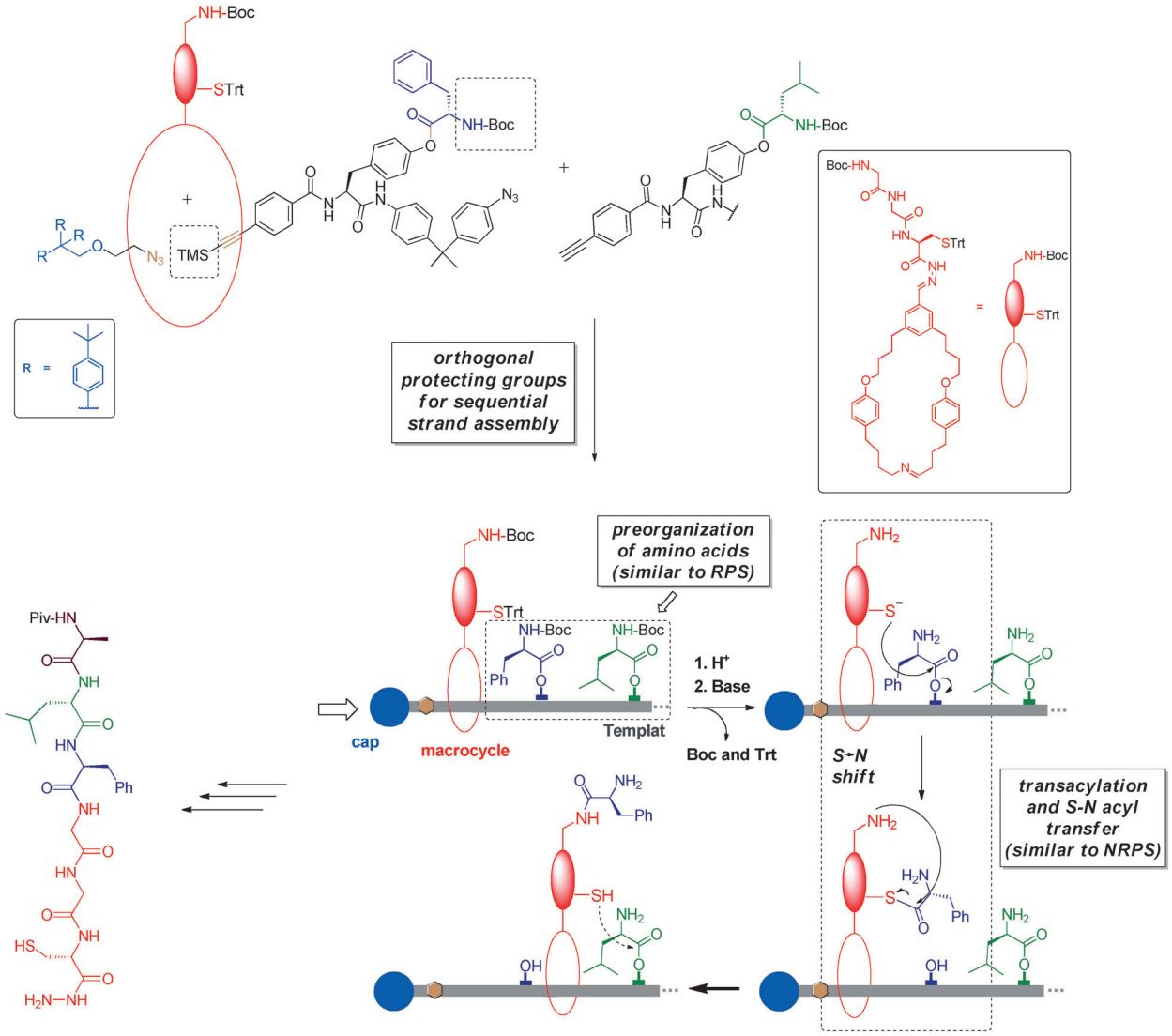
(PCP), which assumes a function similar to that of tRNA. Very importantly (and also of relevance for the Leigh paper) this domain contains a reactive thiol that forms a thioester after reaction with the activated amino acids. The condensation domain (C-domain) finally catalyzes the formation of the peptide, similar to the ribosome in RPS. Nevertheless, several differences between the two biosynthetic pathways are apparent. For instance, nonribosomal peptides (NRPs) are not restricted to the canonical amino acids that are required in RPS. Another important difference is the way the peptide sequence is encoded; in NRPS this information comes from the A-domain, since each nonribosomal peptide synthetase can synthesize only one type of peptide. This ensures that NRPS is highly specific and leads to a single peptidic product. In RPS it is the mRNA that encodes the peptide sequence and alterations can be achieved by simple manipulation of a (few) codon(s), whereas NRPS requires extensive genetic engineering to incorporate changes.

In Leigh's molecular machine some features of the biosynthesis can also be found (Scheme 2). For instance, an analogy to RPS can be found in the strand that organizes activated electrophilic amino acid to predetermine the later peptide sequence *before* the synthesis occurs, analogous to the mRNA and the assembled tRNA–mRNA complex during RPS. Additionally, the way in which peptide bonds are formed proceeds similar to that in NRPS: in the Leigh system a reactive thiol on a macrocycle rotaxane imitates the previously mentioned PCP domain (Scheme 2). However, the similarities to well-known peptide (bio-)synthesis concepts do not end here. For the synthesis of the molecular machine itself, orthogonal protecting groups are required in analogy to SPPS, since the strand synthesis follows a stepwise conjugation of individual amino acid building blocks that need to be orthogonally protected to achieve the desired predetermined peptide sequence. Finally, the peptide coupling proceeds through a capture–rearrangement step and a final S→N acyl shift as utilized during NCL.[8] In other



Scheme 2. A rotaxane-based molecular machine for the synthesis of small peptides. Boc = *tert*-butoxycarbonyl, Piv = pivaloyl, TMS = trimethylsilyl, Trt = triphenylmethyl.

words, Leigh's team has combined the best features of each method and come up with a molecular machine capable of stepwise intramolecular reactions to finally afford small peptides. And how do all the components do the job? The concept relies on well-known chemical reactions. For the strand synthesis, the copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction is used in which a terminal TMS-alkyne on each preactivated amino acid ensures selective triazole formation.[9] Also for the rotaxane synthesis, CuAAC is used for the attachment of a macrocycle and a final cap, which prevents the macrocycle from slipping backwards off the axle. Finally, the peptide chain with a thiol-containing cysteine is added by hydrazone formation.

Once the rotaxane-based molecular machine is preassembled, peptide synthesis is initiated by acidic removal of the protecting groups of the reactive arm and the strand and by addition of a nonnucleophilic base. The thiol on the macrocycle undergoes transacylation with the first amino acid similar to the capture step in NCL. The formed thioester is then able to rearrange by means of an S→N shift to transfer the amino acid to the elongation site of the reactive arm. This capture–rearrangement step allows the peptide macrocycle to move along the strand to pick up the next amino acid in line. Once the macrocycle has converted the last amino acid, it is released from the axle and the target peptide can be detached from the macrocycle by hydrolysis. Remarkably, no starting material, deletions, or unexpected sequences were observed by HPLC-MS analysis and tandem MS.

Several important molecular features ensure the impressive chemical reaction sequence in this molecular machine. For instance, the strand contains not only the amino acids but also rigid spacers to avoid the interaction of the reactive arm with other amino acids out of the sequence. The transacylation reaction of the amino acids is possible due to electrophilic phenolic ester bonds that are used for the attachment to the strand. Additionally, the reactive arm contains a Gly-Gly dipeptide motif between the cysteine and the elongation site which then facilitates the S→N acyl transfer via a favored 11-membered ring for the conjugation of the first amino acid.[10] Finally, once started the molecular machine runs automatically and its small size (one-tenth of a ribosome's) allows the production of peptides on a scale of tens of milligrams.

Although the major accomplishment of this work—the design and function of this molecular machine—is indisputable, this approach is still far from being synthetically competitive with current protocols in peptide synthesis or from mimicking the whole process by which peptides are synthesized biosynthetically. The ribosome can, for example, put together 15 building blocks in one second whereas the molecular machine assembles each amide bond in 12 hours.[6] Furthermore, the size of the target peptide is another problem as only small peptides are reported. Since the elongation proceeds by an S→N acyl shift, it is hard to imagine that longer peptides, not to mention a full-length protein could be synthesized, since structural preorganization of the growing peptide chain will hamper the capture step, for which the ring size will increase with each additionally added amino acid. In addition, in contrast to the ribosome, the macrocycle-reactive arm "destroys" the code it reads, thereby calling for a setup similar to an aaRS in RPS that can reload the amino acids on the strand. Finally, this strategy would be problematic for the incorporation of unprotected amino acids with nucleophilic side chains or with azido and alkyne moieties, since they either interfere with the capture–rearrangement step or the presented synthesis route for the strand assembly.

Nevertheless, despite these limitations, this new molecular machine can be considered a major breakthrough in the area of supramolecular chemistry that opens up new avenues for organic synthesis using artificial nanomachines. Making molecules using molecular templates might lead to promising synthetic routes towards polymer materials, plastics, carbohydrates, natural products, and even molecules that have never been seen before.

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[1] E. Fischer, E. Fourneau, *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 2868–2877.
[2] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.
[3] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. Kent, *Science* **1994**, *266*, 776–779.
[4] B. Lewandowski, G. De Bo, J. W. Ward, M. Papmeyer, S. Kuschel, M. J. Aldegunde, P. M. E. Gramlich, D. Heckmann, S. M. Goldup, D. M. D'Souza, A. E. Fernandes, D. A. Leigh, *Science* **2013**, *339*, 189–193.
[5] W. R. Browne, B. L. Feringa, *Nat. Nanotechnol.* **2006**, *1*, 25–35.
[6] A. Yonath, *Angew. Chem.* **2010**, *122*, 4438–4453; *Angew. Chem. Int. Ed.* **2010**, *49*, 4340–4354.
[7] R. Finking, M. A. Marahiel, *Annu. Rev. Microbiol.* **2004**, *58*, 453–488.
[8] C. P. R. Hackenberger, D. Schwarzer, *Angew. Chem.* **2008**, *120*, 10182–10228; *Angew. Chem. Int. Ed.* **2008**, *47*, 10030–10074.
[9] V. Aucagne, D. A. Leigh, *Org. Lett.* **2006**, *8*, 4505–4507.
[10] F. K. Hansen, K. Ha, E. Todadze, A. Lillicotch, A. Frey, A. R. Katritzky, *Org. Biomol. Chem.* **2011**, *9*, 7162–7167.