Catalysis of amide bond formation by enzyme-like miniproteins towards a novel method of peptide ligation

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The expanded structure of miniproteins, oligomers with a molecular weight below 10 kDa and stable tertiary structure, provides numerous possibilities for constructing molecules with fascinating functions. The advancements in de novo design strategies based on computer-aided approaches have significantly increased the potential of this class of compounds. The incorporation of demanding tasks like the inhibition of protein-protein interactions became feasible. However, developing enzyme-like catalysts based on miniproteins remains a significant challenge. A few examples of catalytic miniproteins have been published.

This proposal concerns the elaboration of new classes of highly conformationally stable miniproteins. Moreover, the development of miniprotein-based enzyme-like catalysts of amide formation is planned.

The obtained miniproteins will show unprecedented structure, high conformational stability, and the possibility of further rational engineering toward functional structures. The proposed methodology of catalyst development has several advantages over known approaches. In particular, newly created catalysts will combine the benefits of enzymes (extended structures, high efficiency, and selectivity) with synthetic availability and the possibility of various modifications.

De novo-designed miniproteins composed of both native and beta-amino acid-containing helices will contain cavities suitable for the construction of the active site of artificial enzymes. Their tertiary structures will be stabilized mainly by hydrophobic interactions. Both newly obtained and known miniproteins will be further used for the creation of new catalysts. Computer-aided iterative modifications of substrate binding cleft and distant residues will give access to efficient catalysts. Various catalysts for amide bond formation will be developed to transform low-molecular-weight compounds, to derivatize and to ligate peptides.

The project will address one of the major challenges of modern chemistry – catalyst development, and its results will significantly impact this field in various ways. Newly created miniproteins could serve as scaffolds for constructing several other catalysts. New synthetic methodologies of valuable compounds will show numerous advantages, including good atom economy, lack of organic solvents, low temperature, and mild conditions. The elaborated methods for peptide derivatization and ligation will open new possibilities for synthesizing compounds that were hardly available previously, including proteins.