Summary of the doctoral thesis

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Title: Peptide foldamers with aldolase-like activity

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One of the main challenges of the modern chemistry is to obtain catalysts for various non-natural reactions with the enzyme-like properties, namely the high activity in conditions of a normal pressure, temperature, pH and high specificity towards substrate and reaction. Growing interest in the field of *de novo* enzyme design is also the result of well-grounded knowledge about the enzymatic catalysis and the ease of access to many *in silico* tools aiding the process of the design. Up to now most of the studies focused on artificial enzymes obtained *via* directed evolution or transition state analog immunization (catalytic antibodies) which results in structurally complex and sensitive to working conditions proteins. Much less attempts to rational enzyme design including smaller than proteins scaffolds were reported. Peptide foldamers are excellent starting points for incorporation of function because they fold to a stable and well-defined conformation which can serve as a scaffold for placing functional groups in a predefined geometry. Moreover, their limited size make them synthetically available with a broad range of building blocks.

The goal of this study is the development of a rational approach towards catalysts with use of the peptide foldamers on the example of aldolases class I mimetics. The choice of the model enzyme was dictated mainly by the well-known mechanism of aldol reaction catalysis but it is also worth to underline that the reaction of breaking or forming a C-C bond is useful in the context of chemical synthesis.

The implementation of the goal comprised of: *in silico* design of peptide sequences, synthesis on a solid phase, conformational analysis with use of CD (in some cases also 2D NMR) and aldolase activity tests using fluorogenic substrate – methodol.

The thesis was divided into two parts. In the first part the rigidified scaffolds built from α,β-peptides were used for the construction of aldolase mimetics. Three different tertiary structures were *de novo* designed from a single experimental structure of 9/10/9/12 helix: helix-loop-helix, helix-turn-helix and structure comprising three helices. Then the natively occurring active site, namely Lys/Glu/Lys and Lys/Tyr/Lys were incorporated onto the prepared scaffolds by substituting proper α-residue positions in starting sequences. One of the obtained peptide was proven to accelerate aldol reaction by 1000 times in pH 8. The structure-activity relationship (SAR) has confirmed that in this case the catalysis is the result of the proper active site geometry rather than the exposure of primary ammonium groups.

In the second part of the thesis the construction of aldolase mimetics was based on α-peptide sequence of C-terminal domain of mini-protein MvaT. This mini-protein folds into stable tertiary structure comprising two α-

helices and three β-strands and because of its small size (43 amino acid residues) is synthetically available. In this case the catalytic residues were incorporated in the stepwise manner eventually giving an active site encompassing Lys/Tyr/Tyr/Phe residues. As the mechanism of action of aldolases was calculated, the optimization of the active site was then conducted by a properly distribution of charged residues around the catalytic site. This charge incorporation was designed to facilitate proton transfer between the catalytic residues which was recognized as the highest energy barrier step of the aldol reaction catalysis. Two of the optimized peptides were highly active. The acceleration of the aldol reaction was determined as around 5000 times.

It should be emphasized that there is no previous reports on rational optimization of artificial enzymes by taking into account the second sphere of coordination.