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De novo Design and AI-supported Flow Synthesis of Catalytic Miniproteins

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Introduction

Miniproteins are bridging the gap between peptides and proteins. The combination of a stable tertiary structure with the feasibility of synthesizing miniproteins provides a significant advantage for the development of demanding functions.[1,2] Recent significant progress in the computational design of proteins has also impacted the field of miniproteins, opening the way to create structures of any desired geometry and size. Several examples of *de novo*-designed miniproteins have been published.[3,4] These also included miniproteins incorporating non-canonical amino acids, in particular backbone-modifying ones. Moreover, it has been shown that miniproteins constitute a unique scaffold for the construction of biologically active molecules. Published examples confirm their usefulness for creating binders of difficult molecular targets, as numerous well-defined intermolecular interactions can be feasibly designed.[5] In particular, inhibitors of various protein-protein interactions were developed using miniproteins. However, constructing miniproteins with catalytic activity remains challenging, and only a few examples have been reported to date.[6]

The goal

Development of rational methodology of catalytic miniprotein construction.

Results

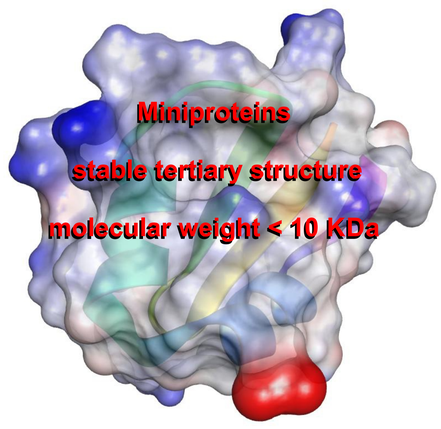
Using the FastDesign protocol from the Rosetta software suite, new miniproteins with cavities were designed. The improved conformational stability was achieved by incorporating conformationally constrained β-amino acids. To efficiently synthesize miniproteins with long and difficult sequences, we have developed a fully automated flow peptide synthesizer. Online control of synthesis progress using a UV detector enabled direct, fully automatic adjustment of several synthesis parameters. In particular, the optimal coupling time is determined using an AI model trained on numerous experimental datasets. The cavity-containing miniproteins were used for the construction of various enzyme-like catalysts. Several catalytic miniproteins that act according to Michaelis-Menten kinetics and significantly increase reaction rates were developed.

Synthesis

Poster 276

Application of an Automated System for Flow Solid Phase Peptide Synthesis

Manish Kumar



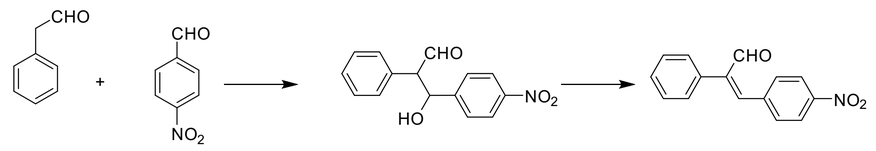
Structure

Poster 472

The NMR Structures of De Novo-designed Novel Miniproteins Containing 2-Aminocyclopentane-carboxylic Acid Residues

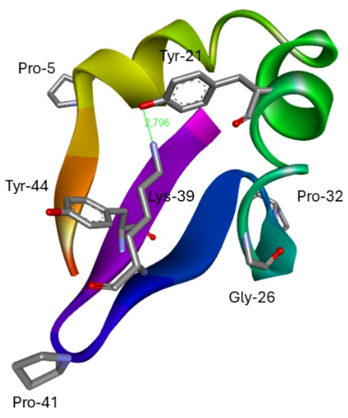
Ewa Rudzińska-Szostak

Catalysis

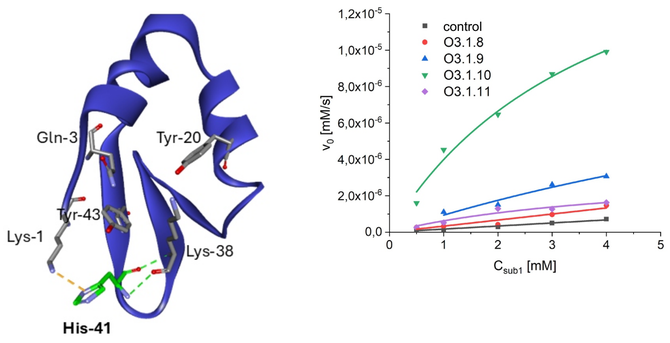


De novo-designed scaffold with active motiff

HHEEE

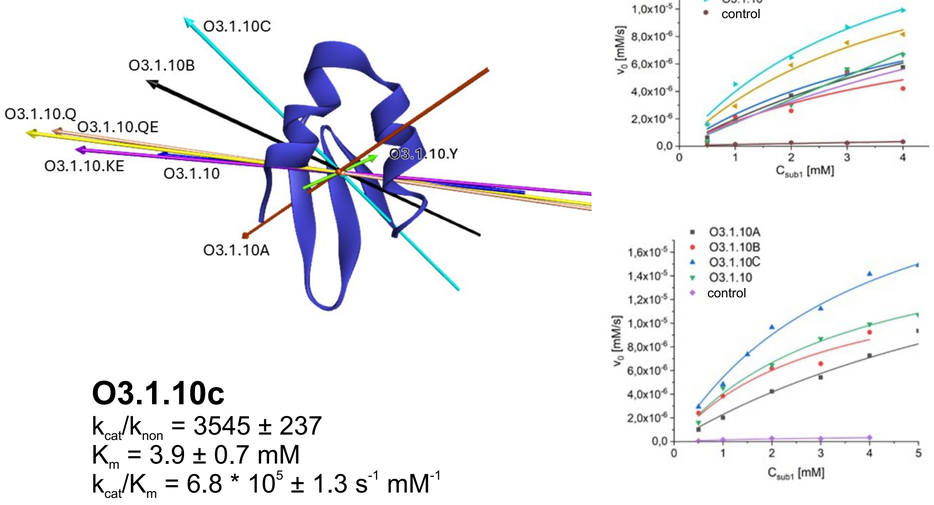


Optimisation of first sphere of interactions



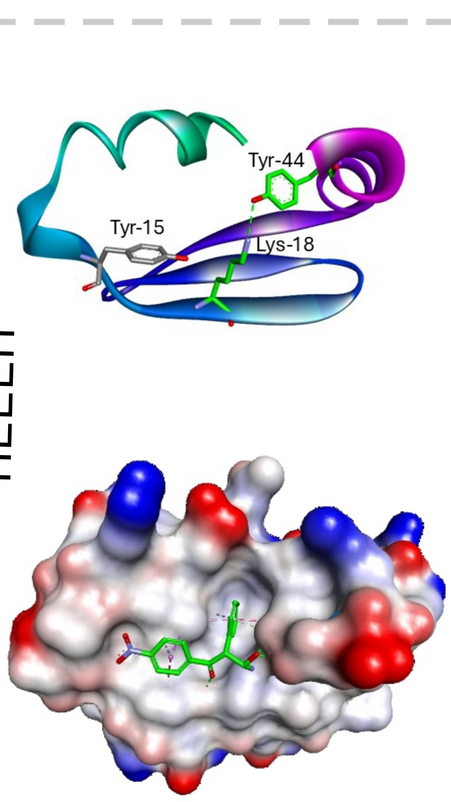
O3.1.10
k_{cat}/k_{non} = 2765 ± 291
K_m = 4.4 ± 1.9 mM
k_{cat}/K_m = 4.8 * 10^5 ± 2.1 s^{-1} mM^{-1}

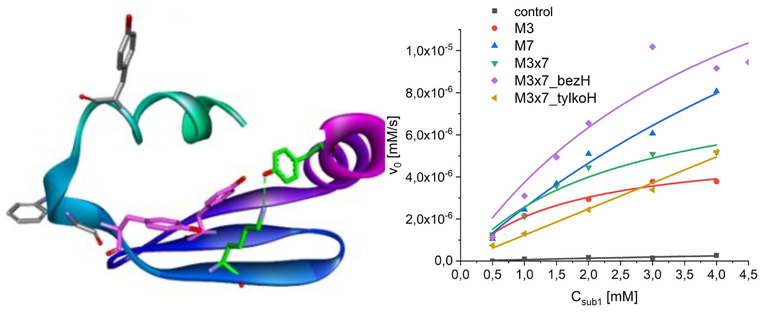
Global dipol moment-based optimisation



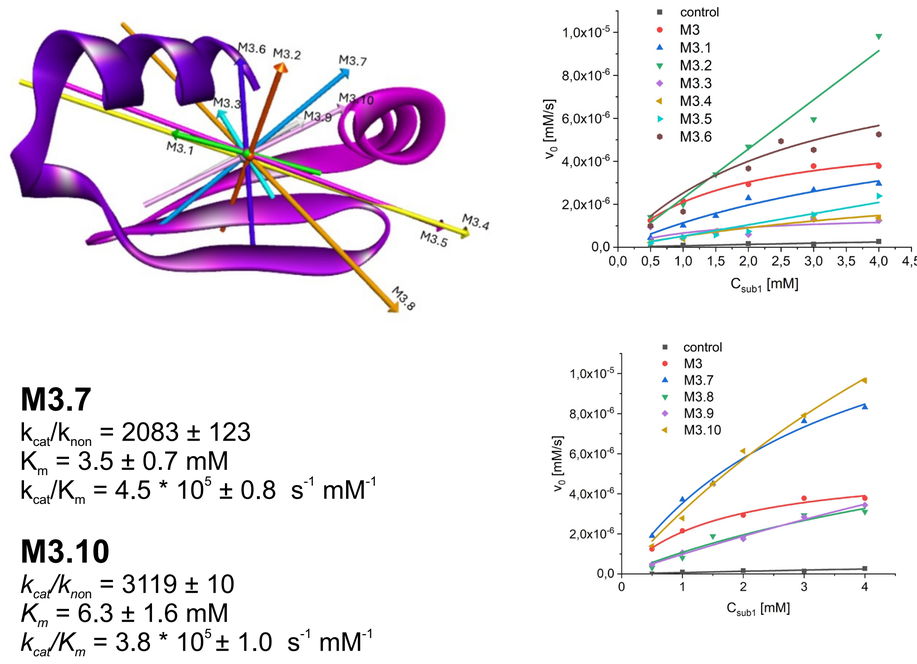
O3.1.10c
k_{cat}/k_{non} = 3545 ± 237
K_m = 3.9 ± 0.7 mM
k_{cat}/K_m = 6.8 * 10^5 ± 1.3 s^{-1} mM^{-1}

HEEEH





M3x7_bezH
k_{cat}/k_{non} = 2581 ± 157
K_m = 4,4 ± 2,6 mM
k_{cat}/K_m = 4,6 * 10^5 ± 1,9 s^{-1} mM^{-1}



M3.7
k_{cat}/k_{non} = 2083 ± 123
K_m = 3.5 ± 0.7 mM
k_{cat}/K_m = 4.5 * 10^5 ± 0.8 s^{-1} mM^{-1}

M3.10
k_{cat}/k_{non} = 3119 ± 10
K_m = 6.3 ± 1.6 mM
k_{cat}/K_m = 3.8 * 10^5 ± 1.0 s^{-1} mM^{-1}

References

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