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Design of a Thermostable WW Domain Scaffold

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Introduction

The current goal of research in the field of synthetic chemistry is the design and production of peptide- and protein structures that function as biological synthesis machines [1]. At the beginning of the *de novo* design of peptide catalysts, the focus was set on α-helical structural motifs such as the coiled coil [2]. Based on the fact that these are relatively rigid and self-assembling scaffolds, current scientific efforts are on the design of smaller single chain β-sheet motifs such as the WW domain [3-7].

In this project, the WW domain was selected as potential scaffold for the design of miniaturized enzymes due to its properties as a small independently folding protein motif. It is a protein interaction module with 34-40 amino acid residues and has a flexible binding site [8] (Figure 1).

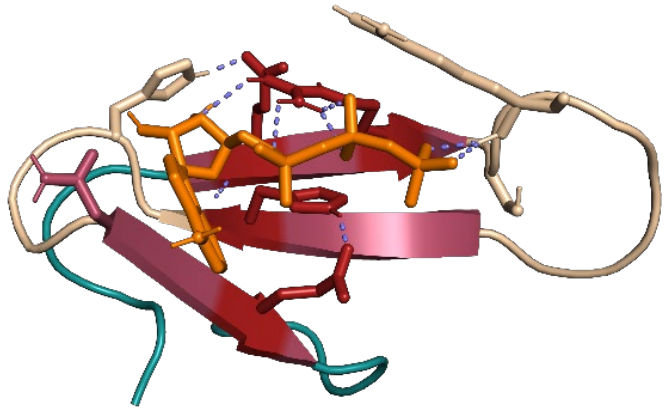

Fig. 1. Visual representation of a designed ATP-binding WW domain [9]. The graph was created in PyMol, the N-&-C-terminus are displayed in green, the β-sheets in raspberry, the loops in wheat color and the ATP in orange. The important binding residues of the WW domain peptide are shown in red, whereas the polar contacts are violet.

Results and Discussion

To establish the WW domain as a scaffold, it is important to deepen the knowledge of the sequence-to-structure-to-function relationships and to investigate the possibilities of binding pocket engineering. Thus, binding- and structure relevant amino acid residues on variable sequence positions were identified based on the generation of a consensus sequence by aligning the sequences of 85 WW domains. This consensus sequence served as a starting point for the design of a temperature-stable WW domain based basic scaffold. 9 CD thermal denaturation examinations of the most promising candidate exhibited a melting temperature of 89 °C and a K_D value in micromolar range in fluorescence-based binding studies (Figure 2).

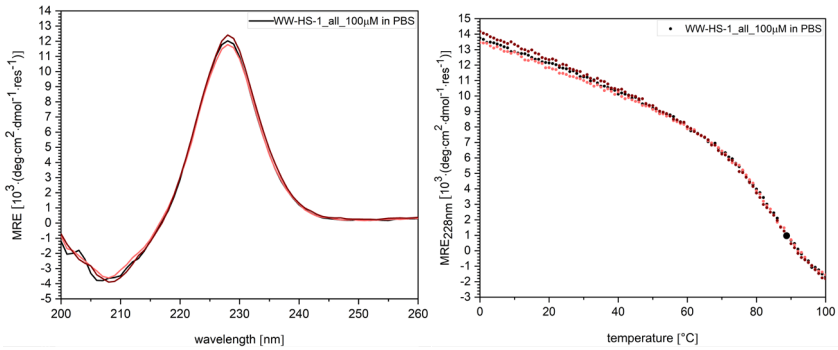

Fig. 2. Left CD spectra of WW-hs1 at 20 °C (n=3). Right CD thermal denaturation curves (n=3). T_M= 89 °C.

Conclusion

According to the findings, the WW domain is robust to mutations in terms of structural stability. In addition, there was a successful identification of structure-relevant and stabilizing amino acid residues in strand and loop regions, which led to the generation of a thermostable WW domain with a melting temperature of 89 °C, compared to 55 °C for the natural reference peptide $hPin1_{WW}$.

Outlook

The future aim is to construct different binding properties onto the basic WW domain template, leading to the design of mini phosphate receptors and phosphatases (Figure 3).

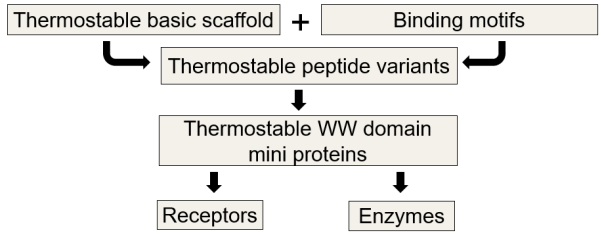

Fig. 3. Overview over the future steps towards mini receptors and enzymes.

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