Navigating the structural and stability landscape of *de novo* TIM barrels by protein design and engineering

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The ability to create and engineer stable proteins with custom functions is a fundamental goal in biochemistry, with practical relevance for our environment and society. Protein stability can be fine-tuned by modifying diverse structural features such as hydrogen-bond networks, salt bridges, hydrophobic cores, disulfide bonds, loop extension, or protein-protein interfaces, among others. One of the most abundant topologies in nature and a common functional scaffold that is of interest in this context is the $(\beta/\alpha)_8$-barrel or TIM-barrel fold [1]. Therefore, we designed and engineered a collection of stable *de novo* TIM barrels (DeNovoTIMs) using a computational fixed-backbone and a modular approach based on improved hydrophobic packing [2] and the introduction of salt-bridges [3]. DeNovoTIMs were subjected to thorough biochemical and folding analyses using computational, biophysical, structural, and thermodynamic methods to explore their structure and stability. We found that DeNovoTIMs navigate a region of the stability landscape previously uncharted by natural proteins, with variations spanning 60 degrees in melting temperature and 22 kcal per mol in conformational stability throughout the designs. Significant non-additive or epistatic effects were observed in their stability and structural features when stabilizing mutations from different barrel regions were combined (Fig. 1). Salt-bridge variants from some DeNovoTIMs exhibit important differences in comparison with the parental proteins, both in conformational stability and structural properties (Fig. 2). The engineering of stable proteins increases the applicability of *de novo* proteins and provides crucial information on the molecular determinants of the sequence-structure-stability relationships, with this study being an essential step towards fine-tuned modulation of protein stability by protein design.

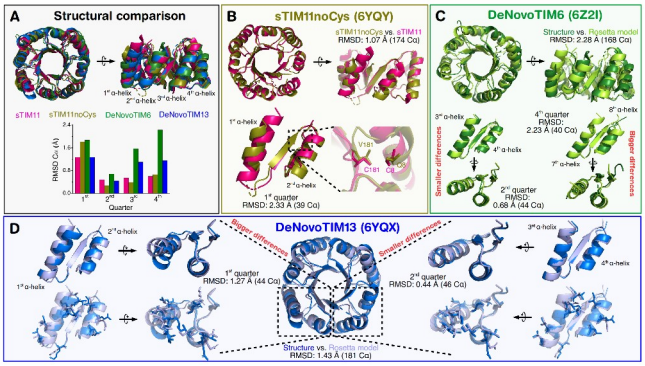

Figure 1. Structural properties observed in the DeNovoTIM collection [2].

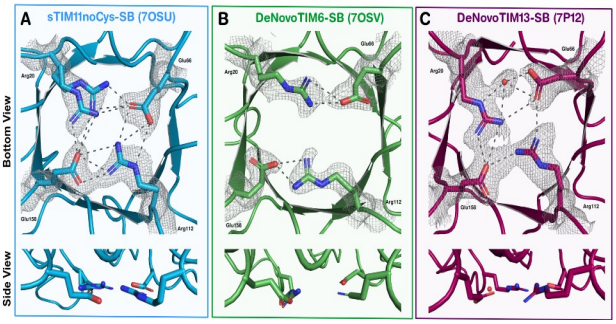

Figure 2. Structural conformations of the salt bridge interactions in the DeNovoTIM-SB variants [3].

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