The bright-side and the dark-side of computational protein stabilization

M. Marek[1,2,*], K. Markova[1], A. Kunka[1,2], K. Chmelova[1], M. Havlasek[1], P. Babkova[1], S. M. Marques[1,2], M. Vasina[1,2], J. Iglesias Planas[1], R. Chaloupkova[1], D. Bednar[1,2], Z. Prokop[1,2], and J. Damborsky[1,2]

[1]Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic,
[2]International Clinical Research Center, St. Anne's University Hospital Brno, Pekarska 53, 65691 Brno, Czech Republic,

martin.marek@recetox.muni.cz

The functionality of a protein depends on its unique three-dimensional structure, which is a result of the folding process when the nascent polypeptide follows a funnel-like energy landscape to reach a global energy minimum. Computer-encoded algorithms are increasingly employed to stabilize native proteins for use in research and biotechnology applications [1].

Here, we reveal a unique example where the computational stabilization of a monomeric α/β-hydrolase fold enzyme (T_m = 73.5°C; ΔT_m > 23°C) affected the protein folding energy landscape. Introduction of eleven single-point stabilizing mutations based on force field calculations and evolutionary analysis yielded catalytically active domain-swapped intermediates trapped in local energy minima. Crystallographic structures revealed that these stabilizing mutations target cryptic hinge regions and newly introduced secondary interfaces, where they make extensive non-covalent interactions between the intertwined misfolded protomers [2]. The existence of domain-swapped dimers in a solution is further confirmed experimentally by data obtained from SAXS and crosslinking mass spectrometry. Unfolding experiments showed that the domain-swapped dimers can be irreversibly converted into native-like monomers, suggesting that the domain-swapping occurs exclusively *in vivo* [2].

Our findings uncovered hidden protein-folding consequences of computational protein design, which need to be taken into account when applying a rational stabilization to proteins of biological and pharmaceutical interest.

[1] Markova K., Chmelova K., Marques S. M., Carpentier P., Bednar D., Damborsky J., Marek M. (2020). Decoding the intricate network of molecular interactions of a hyperstable engineered biocatalyst. Chemical Science 11, 11162-11178.

[2] Markova K., Chmelova K., Marques S. M., Carpentier P., Bednar D., Damborsky J., Marek M. (2021). Computational protein stabilization can affect folding energy landscapes and lead to domain-swapped dimers. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.13634021.v1

Keywords: Computational protein design, enzyme, folding energy landscape, X-ray crystallography, domain-swapping

This work was supported by MSCA Marie Sklodowska - Curie Actions (792772) and GAMU (MUNI/H/1561/2018).