Improving protein thermostability by identifying and reinforcing structural weak spots

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Mesophilic enzymes are often rarely capable of enduring high temperatures that occur during industrial processes [1]. Generating proteins with higher thermostability is therefore necessary but still interminable and expensive, if experimental techniques are used. Finding promising mutation sites by the use of *in silico* methods presents a challenging alternative.

Increased thermostability of proteins has been linked to an enhanced structural rigidity of the folded native state [2]. In that, rigid proteins unfold later in the thermal denaturation process compared to more flexible ones. Thus, analyzing the flexibility of a protein during thermal unfolding may point to less stable regions ("weak spots"). Reinforcing those regions should lead to a higher thermostability of the protein at the same temperature. In the present study, proteins are analyzed as constraint networks in order to identify such weak spots [1]. For this, changes in protein rigidity upon thermal unfolding are determined using the Floppy Inclusion and Rigid Substructure Topography (FIRST) method [3]. The thermal unfolding is simulated by diluting the hydrogen bond network of the folded structure. For every dilution step, the global molecule rigidity is computed. A phase transition referring to the folded-unfolded transition of a protein is then observed and protein network representations before and after this transition are used for identifying weak spots. Finally, computational mutagenesis guided by evolutionary information is performed.

A comparison to experimental data on 7 stabilizing mutants in thermolysin-like protein (TLP) and 3-isopropylmalate-dehydrogenase (IPMDH) demonstrates that the effect of mutants at weak points can be correctly described and that the increase of the protein melting temperature triggered by the mutations can be reproduced.

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