Large-scale analysis of protein thermostability and detergent tolerance

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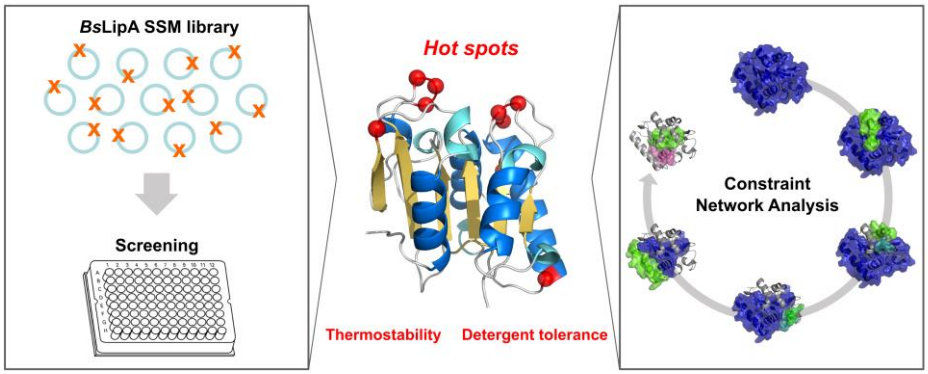
Understanding how structure and activity of proteins are related to their (thermo-)stability [1-2] or tolerance against solvents [3] and detergents [4] is of utmost importance in protein engineering. However, until now, the role and impact of the location and type of substitution on protein properties has been predominantly studied from analyses of only a few protein variants or large-scale studies over data sets of many proteins, only contributing a few variants each. Furthermore, most analyses considered only one protein property at a time. Here, for the first time, we exhaustively characterize changes in thermostability *and* detergent tolerance for a complete site-saturation mutagenesis (SSM) library containing 3439 single variants of the *Bacillus subtilis* lipase A (*Bs*LipA). To establish a set of generally applicable guidelines regarding improved protein thermostability *and* / *or* detergent tolerance, we identified *hot spot* residues, i.e. those with a high likelihood to yield stabilized variants, and probed if they can be predicted based on protein structural characteristics or via rigidity theory-based Constraint Network Analysis (CNA) [5]. The main outcome is that CNA can precisely predict *hot spots* for rational protein design aiming at improved protein thermostability *and* / *or* detergent tolerance.



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