Supplementary data

PoPMusic is an evaluating algorithm for determining stability changes of a protein with linear combinations of database-derived potentials under all possible *in silico* single site mutations in a sequence, globally, or locally as specified by the user (ΔΔG computed = ΔGmutant _ ΔGwild-type) Since Tm (thermal stability) depends on the both ΔH and ΔS parameters, ΔΔG (thermal stability) is also related to Tm (thermal stability). Introducing a mutation to the protein structure can change the ΔH and ΔS and as a result of ΔΔG. Assessing protein stability using ΔG is popular because all forms of stabilizing processes can be associated with this parameter. Using the systematic mutation method, amino acids which were present at the active site and have increased the thermostability of the protein were selected.

Briefly, the LGA is a hybrid of genetic algorithm (GA) method with adaptive local search (LS) method. In the first step, it finds the lowest fitness function values and in the second step maps the calculated values against their respective genotypes. It has a higher performance and can resolve problems with more degree of freedom. The MolDock scoring function in this study was:

MolDock algorithm combines cavity prediction algorithm with differential evolution. Working on the basis of piecewise linear potential (PLP), the MolDock scoring function takes the charges and directionality of the hydrogen bonds into account.

$E_{score} = E_{inter} + E_{intra}$

While, E_{inter} and E_{intra} correspond to the ligand-protein interaction energy and internal energy of the ligand.

E_{inter} is calculated as follows:

$$E_{\text{inter}} = \sum_{i \in ligand} \sum_{j \in protein} \left[E_{PLP}(r_j) + 332.0 \frac{q_i q_j}{4 r_j^2} \right]$$

Where, EPLP is a piecewise linear potential. The summation encompasses all the heavy atoms in the protein and the ligand as well as any cofactor atoms and water molecule atoms. The second term points to the electrostatic interactions between charged atoms. Describing the electrostatic interactions between charged atoms, E_{intra} is calculated as follows:

$$E_{intra} = \sum_{i \in ligand} \sum_{j \in ligand} E_{PLP}(r_j) + \sum_{\substack{flexible \\ bonds}} A[1 - \cos(m.\theta - \theta_0] + E_{clash}$$

Where θ and E_{clash} correspond to the torsional angle of the bonds and the punishment for infeasible ligand conformations, respectively. E_{clash} assigns a penalty of 1000 for distances less than 2.0 A between the two heavy atoms.