INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	Debamitra Chakravorty
Roll Number	:	10610619
Programme of Study	:	Ph.D.

Thesis Title: Attaining Protein Thermostability: A Rationalized Approach

Name of Thesis Supervisor(s)	:	Dr. Sanjukta Patra, Dr. Vishal Trivedi
Thesis Submitted to the Department/ Center	:	Biosciences & Bioengineering
Date of completion of Thesis Viva-Voce Exam	:	15th September, 2016
Key words for description of Thesis Work	:	Mutation, Thermostability, Model, Ranking, Rational

SHORT ABSTRACT

In proteins natural mutation occurs such that it tries to maintain maximum stability while retaining functionality indicating that nature may be rationalizing the way of attaining such mutations. Thermophilic proteins are examples of natural mutations that lead to stability of proteins at extreme of temperatures. Chapter I of the dissertation work unravels that the mechanism of thermostability has been attributed to the cumulative effect of numerous factors. However a guided approach to attain thermostabilizing mutations was still elusive. The intention of this work was to fill this caveat of attaining thermostabilizing mutations, by developing a rational approach of predicting thermostabilizing mutations. Chapter II of the thesis is related to data collection and creation of a database for thermostable proteins which can be accessed through www.extreme-stabledb.in. Feature collection and analysis was accomplished and it was observed that majority of the thermostable proteins were hydrolases. In Chapter III, therefore, thermostable lipases which are hydrolases were analysed for the features that render them thermostable. For the first time it was observed that γ-turn increases not only in thermostable lipases but in all thermostable proteins also. It was also realized that structural features were more important in understanding thermostability. Chapter IV deals with prioritizing these features according to their role in contributing towards thermostability and a model has been generated which can predict multiple mutations leading to thermostability. The ranking was developed based on an elaborate analysis of a set of 17 quantitative structural features on a final dataset of 127 pairs of thermostable and mesostable protein structures. Ionic interaction and main-chain to main-chain hydrogen bonds were the features showing the highest priority vectors for thermostability. To find generic appropriateness of these results, this method was subjected to multiple blind tests. Further a rank value of 0.54 for a protein undergoing mutations was predicted to be thermostabilizing and the accuracy of the method was calculated to be 91%. The ranks were used to develop a tool; RankProt. In Chapter V, further validatation was carried out by deriving at stabilizing mutations for mesostable *Bacillus subtilis* lipase A with rank value <0.54 (mut 1) and another double mutant with rank value >0.54 (mut 2). The resulting double mutants were further evaluated to be stabilizing by performing 30ns molecular dynamics simulations, molecular docking and intra-protein contact map analysis. In all these study mut 2 was found to be a better mutant as compared to mut 1, thus validating RankProt. The double mutants were then engineered through multi-site-directed mutagenesis and mut 2 was found to be thermostable with a temperature optimum of 55°C and T_m of 66°C whereas mut 1 was stable at 40°C with a T_m of 63°C. Conclusively, it can be said that the method is empirical, rational and provides an insight into the structural changes brought about by the mutations, which has led to the increase in the rank value of the proteins and thus thermostability. The aim to obtain at a predictable method for thermostabilizing mutations was successfully achieved through this dissertation.