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On the Other Side of Shining Mercury: In Silico Prediction of Cold Stabilizing Mutations in Serine Endopeptidase from Bacillus lentus

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Abstract : Cold-adapted proteases enhance wash performance in low-temperature laundry resulting in a reduction in energy consumption and wear of textiles and are also used in the dehairing process in leather industries. Unfortunately, the possible drawbacks of using cold-adapted proteases are their instability at higher temperatures. Therefore, proteases with broad temperature stability are required. Unfortunately, wild-type cold-adapted proteases exhibit instability at higher temperatures and thus have low shelf lives. Therefore, attempts to engineer cold-adapted proteases by protein engineering were made previously by directed evolution and random mutagenesis. The lacuna is the time, capital, and labour involved to obtain these variants are very demanding and challenging. Therefore, rational engineering for cold stability without compromising an enzyme's optimum pH and temperature for activity is the current requirement. In this work, mutations were rationally designed with the aid of high throughput computational methodology of network analysis, evolutionary conservation scores, and molecular dynamics simulations for Savinase from Bacillus lentus with the intention of rendering the mutants cold stable without affecting their temperature and pH optimum for activity. Further, an attempt was made to incorporate a mutation in the most stable mutant rationally obtained by this method to introduce oxidative stability in the mutant. Such enzymes are desired in detergents with bleaching agents. In silico analysis by performing 300 ns molecular dynamics simulations at 5 different temperatures revealed that these three mutants were found to be better in cold stability compared to the wild type Savinase from Bacillus lentus. Conclusively, this work shows that cold adaptation without losing optimum temperature and pH stability and additionally stability from oxidative damage can be rationally designed by in silico enzyme engineering. The key findings of this work were first, the in silico data of H5 (cold stable savinase) used as a control in this work, corroborated with its reported wet lab temperature stability data. Secondly, three cold stable mutants of Savinase from Bacillus lentus were rationally identified. Lastly, a mutation which will stabilize savinase against oxidative damage was additionally identified.

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