484a Application of the Consensus Concept for Increased Thermostability of Glucose Dehydrogenase

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Enzyme mediated reductions are of prime interest for the synthesis of chiral compounds (e.g., alcohols, hydroxyl acids, etc.), key to the development of novel pharmaceuticals. A crucial distinction of many redox enzymes is the need of cofactors (i.e., NAD(P)(H)) which accept or donate chemical equivalents for reduction or oxidation. The high price of cofactors prohibit their stoichiometric use and thus require regenerating systems. The steps en route to the synthesis of pharmaceuticals often require nonnative conditions of solvent, pH, and temperature, limiting enzyme applicability and efficacy.

For this, we aim to increase glucose dehydrogenase (GDH) temperature stability via the consensus concept. GDH is a homotetramer capable of accepting NAD+ or NADP+ with high specific activity, making it an attractive target. We have been able to predict mutations that have been proven to increase thermostability of GDH from various Bacilli sp. Using this approach we have identified nine amino acids with potential to increase thermostability. Currently we have successfully cloned, expressed, and purified the GDH encoding gene from Bacillus subtilis and Geobacillus stearothermophilus in Escherichia coli. In this presentation we seek to discuss the applicability of the consensus method to the development of a thermostable GDH and progress to date.