484e Increasing the Synthetic Utility of Penicillin G Acylase by Rational and Directed Evolution

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Penicillin G acylase (PGA) is an important enzyme in the semi-synthetic production of β-lactam antibiotics. Despite many years of research, the enzyme is still lacking in stability and substrate specificity. Improvements to these properties would increase the utility of the enzyme and decrease the cost of antibiotic production. We set out both to improve the thermostability and to improve the kinetics of PGA.

The PGA enzyme is a large, hetereodimeric enzyme which is difficult to evolve using truly random methods. Therefore, we began our efforts with an extensive bioinformatic analysis in an effort to limit the search space for protein engineering. For improvement of thermostability, we targeted specific residues using a consensus-based sequence comparison approach. Additional work focuses on using directed evolution to improve the synthetic utility of the enzyme by mutagenesis targeted to the segments of the α- and β- chains that are in contact with the substrate binding pocket. To facilitate directed evolution, we have obtained and characterized the kinetics of the penicillin G acylase genes from 4 different organisms, each with different stabilities and catalytic characteristics. In the presentation, we will cover both the approach as well as the results to date.