

Unearthing Enzyme Promiscuity with Cheminformatics to Design Biosynthetic Pathways Towards Novel Biomolecules

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Project Goals: Enzyme promiscuity, where an enzyme may catalyze a range of side reactions in addition to its main reaction, is a widely recognized yet largely unexplored phenomenon in biological systems. Cheminformatics workflows that can learn from native enzymatic reactions, enumerate novel reactions based on relevant enzymatic transformations, assess predicted biosynthetic pathways, as well as visualize predicted pathways in a user-friendly way, is crucial for pushing the boundaries of biomanufacturing. This could open up vast possibilities for bioproduction of valuable chemicals not natively produced by biology, where biosynthetic pathways can be constructed based on enzymes with desired promiscuous reactions. On the other hand, this also enables us to understand the implications of introducing heterologous enzymes on host organisms.

We have developed the enzymatic reaction ruleset “JN1224min”, an open-source retrobiosynthesis package Pickaxe v2.0, and the novel metabolite explorer Metabolic In-silico Network Expansions (MINEs) database v2.0. These are tools that incorporate enzyme promiscuity into the design of novel biosynthetic pathways. “JN1224min” is a minimal yet comprehensive set of 1224 enzymatic reaction rules that describes all common enzymatic transformations. These reaction rules, which specify reaction-center transformations of enzymatic reactions, can enumerate the largest possible number of reactions using the least number of rules. Pickaxe v2.0 utilizes these reaction rules to generate novel enzymatic reaction networks iteratively over many generations, in order to find promising pathways that lead from feedstocks to valuable products. We have updated Pickaxe to allow for on-the-fly filtering of enumerated reactions, based on criteria including chemical similarity, molecular weight, thermodynamics, as well as any custom filters. These implementations allow us to explore the largest possible enzymatic reaction space with improved efficiencies. MINE v2.0 is an update of the original MINE v1.0, a resource that

allows users to query novel metabolites that could be products of a promiscuous reactions from KEGG, E.coli, and yeast metabolites. MINEs are deployed as a graphical user interface, which allows users to easily propose candidate structures from untargeted metabolomics. Using our updated ruleset, MINE v2.0 features more than 15 times the number of potential metabolite structures compared to v1.0. Therefore, we have provided powerful tools that enable the metabolic engineering community to utilize enzyme promiscuity and improve pathway design towards biomanufacturing of a wider array of novel biomolecules.

This poster is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018249.