Directed Evolution Toward Nonstandard Amino Acids by a Decarboxylative Aldol Reaction

**Significance:** Buller and co-workers disclose an enantioselective synthesis of \( \gamma \)-hydroxy amino acids catalyzed by engineered variants of a pyridoxal 5’-phosphate (PLP)-dependent enzyme. Various products of C–C bond-forming reactions were obtained in moderate to excellent yields and enantioselectivities. The optimized enzyme UstD\(^{2.0} \) is efficient in a whole-cell biocatalysis format, thereby eliminating the need for enzyme purification in gram-scale syntheses.

**Comment:** Unlike other classic aldolases, UstD is capable of decarboxylating the side chain of pyridoxal-bound \( L \)-aspartate to form a putative nucleophilic enamine intermediate, which renders the enantioselective C–C bond-forming reaction effectively irreversible. An X-ray crystal-structure analysis of UstD\(^{2.0} \) revealed the active site and provides a foundation for probing the UstD mechanism. A broader scope of gram-scale whole-cell applications is anticipated.