Directed Evolution toward an Iron-Heme Enzyme for Asymmetric C–H Amination

**Significance:** Arnold and co-workers report the directed evolution from iron-heme P450\textsubscript{BM3} to P411\textsubscript{CHA} for the highly enantioselective intermolecular amination of benzylic C–H bonds with up to 1300 catalytic turnovers. The authors suggest that the reaction proceeds through a commonly accepted iron nitrenoid intermediate, which undergoes nitrene insertion to afford valuable benzyl amines in up to 87% yield and >99.5:0.5 er.

**Comment:** The authors discovered that P-4, a P450\textsubscript{BM3} variant with 17 mutations from the wild-type, catalyzes the benzylic C–H amination of 4-ethylanisole, albeit with low enantioselectivity. Through sequential rounds of site-selective mutagenesis, P-411\textsubscript{CHA} was found to dramatically improve the yield and enantioselectivity of the reaction for a wide range of electronically-differentiated substrates. X-ray crystallography showed that all of the beneficial mutations lie within the active site of the enzyme.