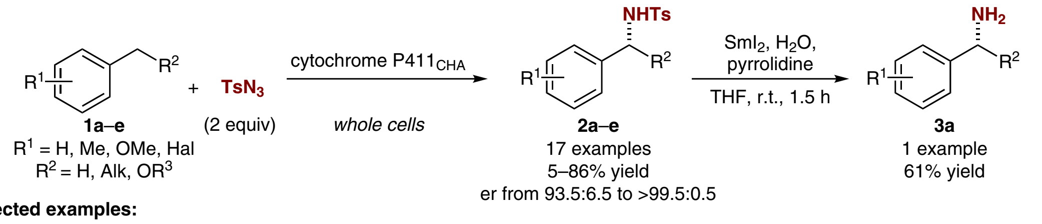
C. K. PRIER, R. K. ZHANG, A. R. BULLER, S. BRINKMANN-CHEN, F. H. ARNOLD* (CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, USA)
Enantioselective, Intermolecular Benzylic C–H Amination Catalysed by an Engineered Iron-Haem Enzyme
Nat. Chem. **2017**, *9*, 629–634.

Category
Organo- and Biocatalysis

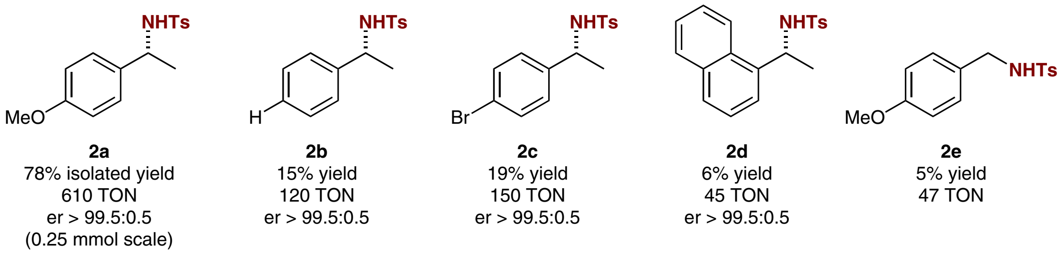
Key words
cytochrome P411
C–H amination
directed evolution
iron-heme enzyme
benzylamines

Synfact of the month

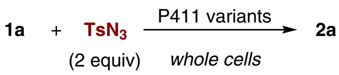
Directed Evolution toward an Iron-Heme Enzyme for Asymmetric C–H Amination



Selected examples:

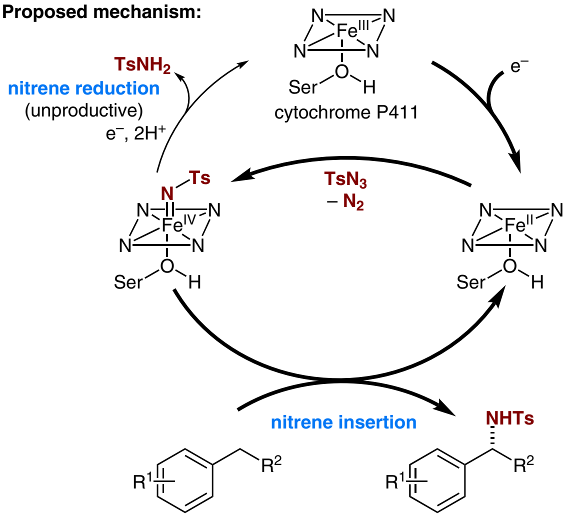


Directed evolution for C–H amination:



variant	yield	er	TON
P-4	11 ± 1%	43:57	310
P-4 A82L	51 ± 3%	88.5:11.5	1000
P-4 A82L A78V	66 ± 2%	90:10	1200
P-4 A82L A78V F263L	66 ± 2%	>99.5:0.5	*NA*
P-4 A82L A78V F263L E267D ($P411_{CHA}$)	66 ± 3%	>99.5:0.5	1000

Proposed mechanism:



Significance: Arnold and co-workers report the directed evolution from iron-heme $P450_{BM3}$ to $P411_{CHA}$ 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 $P450_{BM3}$ 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, $P\text{-}411_{CHA}$ 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.

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