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

Significance: Arnold and co-workers report the directed evolution from iron-heme P450BM3 to P411CHA for the highly enantioselective intermolecular amination of benzyl 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 P450BM3 variant with 17 mutations from the wild-type, catalyzes the benzyl C–H amination of 4-ethylanisole, albeit with low enantioselectivity. Through sequential rounds of site-selective mutagenesis, P-411CHA 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.

Selected examples:

1a: MeO
2a: 78% isolated yield, 610 TON, er > 99.5:0.5
3a: 1 example, 61% yield

1b: H, 15% yield, 120 TON, er > 99.5:0.5
1c: Br, 19% yield, 150 TON, er > 99.5:0.5
1d: MeO, 6% yield, 45 TON, er > 99.5:0.5
1e: Br, 5% yield, 47 TON

Directed evolution for C–H amination:

P411 variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Yield (%)</th>
<th>ER</th>
<th>TON</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-4</td>
<td>11 ± 1%</td>
<td>43.57</td>
<td>310</td>
</tr>
<tr>
<td>P-4 A82L A78V</td>
<td>51 ± 3%</td>
<td>88.5:11.5</td>
<td>1000</td>
</tr>
<tr>
<td>P-4 A82L A78V F263L</td>
<td>66 ± 2%</td>
<td>90:10</td>
<td>1200</td>
</tr>
<tr>
<td>P-4 A82L A78V F263L (P411CHA)</td>
<td>66 ± 3%</td>
<td>&gt;99.5:0.5</td>
<td>1000</td>
</tr>
</tbody>
</table>

Proposed mechanism:

NHTs

1a–e: R1 = H, Me, OMe, Hal
R2 = H, Alk, OR3

Category: Organo- and Biocatalysis

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