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:

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>product</th>
<th>yield</th>
<th>er</th>
<th>TON</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeO</td>
<td></td>
<td>2a</td>
<td>78%</td>
<td>&gt;99.5:0.5</td>
<td>60 TON</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>2b</td>
<td>15%</td>
<td>&gt;99.5:0.5</td>
<td>120 TON</td>
</tr>
<tr>
<td></td>
<td>Br</td>
<td>2c</td>
<td>19%</td>
<td>&gt;99.5:0.5</td>
<td>150 TON</td>
</tr>
<tr>
<td></td>
<td>MeO</td>
<td>2d</td>
<td>15%</td>
<td>&gt;99.5:0.5</td>
<td>150 TON</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2e</td>
<td>5%</td>
<td>&gt;99.5:0.5</td>
<td>47 TON</td>
</tr>
</tbody>
</table>

Proposed mechanism:

1a + TsN3 → 2a

1 example 61% yield 17 examples er from 93.5:6.5 to >99.5:0.5

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 (P411CHA) | 66 ± 3% | >99.5:0.5 | 1000 |

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