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 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 P450BM3 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-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>78% isolated yield</th>
<th>15% yield</th>
<th>19% yield</th>
<th>6% yield</th>
<th>5% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeO</td>
<td>H</td>
<td>610 TON er &gt; 99.5:0.5</td>
<td>120 TON er &gt; 99.5:0.5</td>
<td>150 TON er &gt; 99.5:0.5</td>
<td>45 TON er &gt; 99.5:0.5</td>
<td>47 TON er &gt; 99.5:0.5</td>
</tr>
</tbody>
</table>

Directed evolution for C–H amination:

\[
\text{P-4} + \text{TsNH}_2 (2 \text{ equiv}) \rightarrow \text{1a} \rightarrow \text{2a}
\]

\[
\text{yield} \quad \text{er} \quad \text{TON}
\]

<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 \pm 1%</td>
<td>43.57</td>
<td>310</td>
</tr>
<tr>
<td>P-4 A82L</td>
<td>51 \pm 3%</td>
<td>88.5:11.5</td>
<td>1000</td>
</tr>
<tr>
<td>P-4 A82L A78V</td>
<td>66 \pm 2%</td>
<td>90:10</td>
<td>1200</td>
</tr>
<tr>
<td>P-4 A82L A78V F263L</td>
<td>66 \pm 2% &gt;99.5:0.5</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>P-4 A82L A78V F263L (P411CHA)</td>
<td>66 \pm 3% &gt;99.5:0.5</td>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

Proposed mechanism:

\[
\text{TsNH}_2 + e^- + \text{H}^+ \rightarrow \text{TsN} + \text{NH}_2 \quad \text{nitrene reduction (unproductive)}
\]

\[
\text{TsN} + e^- + \text{H}^+ \rightarrow \text{TsNH}_2 \quad \text{nitrene insertion}
\]

\[
\text{SmI}_2, \text{H}_2\text{O}, \text{pyrrolidine} \rightarrow \text{TTHF, r.t., 1.5 h}
\]

Selected examples:

\[
\begin{align*}
\text{2a} & \quad 78\% \text{ isolated yield} \\
\text{2b} & \quad 15\% \text{ yield} \\
\text{2c} & \quad 19\% \text{ yield} \\
\text{2d} & \quad 6\% \text{ yield} \\
\text{2e} & \quad 5\% \text{ yield}
\end{align*}
\]

17 examples 5–86% yield er from 93.5:6.5 to >99.5:0.5

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