Ph.D. DISSERTATION DEFENSE

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Overwriting Natural Evolution by Inverting the Chemoselectivity of an Engineered Tryptophan Synthase β-subunit

Buller Group

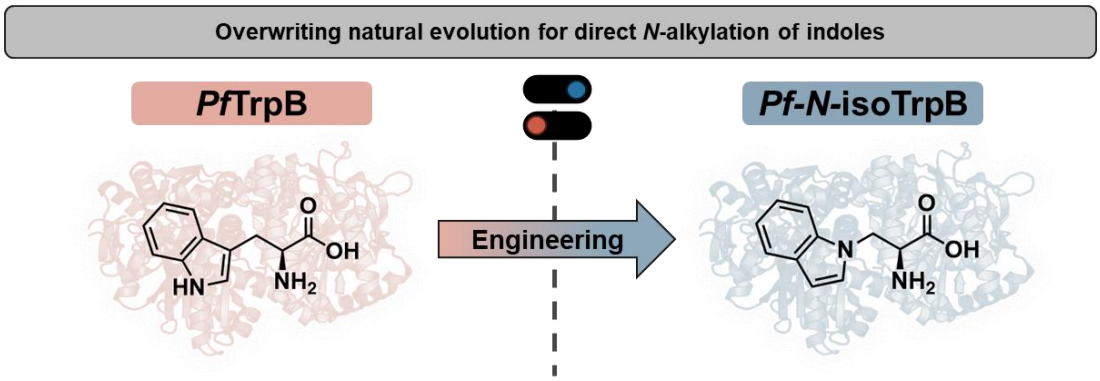
Friday, July 25, 2025, at 2:00 PM

Chemistry Building Rm 1315

Zoom Meeting ID: 921 6336 1641 Passcode: 995101

Directed evolution offers a powerful route to overcome reaction reversibility and product degradation in biocatalysis. The β-subunit of tryptophan synthase from *Pyrococcus furiosus* (*Pf*TrpB) performs both *C*- and *N*- alkylation, however its β-lyase activity can degrade products from β-*N*-substitution. Our first work engineered *Pf*TrpB which natively turns over indoline, for efficient β-*N*-substitution. This enabled gram-scale synthesis of 2,3-dihydro-l-tryptophan (DIT), expanding biocatalytic *N*-alkylation and efficient access to α,β-diamino acids.

Building on this, our current work directly synthesizes *N*-alkylated indoles, such as *N*-iso-Trp, a desirable motif in medicinal chemistry for treating anxiety and mood disorders. Despite previous chemoenzymatic routes from DIT, *N*-iso-Trp isolation was challenging. To achieve one-step access, we conducted a multi-round directed evolution campaign, overcoming the evolutionary legacy of *Pf*TrpB for β-*C*-substitution. Mechanistic investigations revealed that a single mutation can bias the TrpB conformational ensemble towards more open states, dictating chemoselectivity. This work provides a direct route to *N*-alkylated indole analogs of TrpB and deepens our understanding of how enzyme dynamics govern catalysis.



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