Two-step Whole-cell Biocatalytic Cascade with *in-situ* Smart Amine Donor Synthesis

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Transaminases (TAs) are highly regarded in the biocatalytic production of chiral building blocks for pharmaceuticals due to their high enantioselectivity and specificity. One approach to overcome the equilibrium limitations of TA reactions is the application of „smart amine donors," such as cadaverine. After cadaverine donates one amine group, an irreversible and spontaneous cyclization of 5-aminopentanal to 1-piperdine effectively pulls the equilibrium forward. Cadaverine is a natural polyamine generated from L-lysine by the native *E. coli* lysine decarboxylase CadA, an enzyme associated with the acid-stress response system in cells. In this study, a two-step whole-cell biocatalytic cascade was developed to leverage externally supplied L-lysine for *in situ* production of the smart amine donor, cadaverine. The cascade couples the autologous lysine decarboxylase CadA with the recombinant TA, using distinct pH controls to optimize enzyme activity at each step.

Using Benzaldehyde as a model acceptor substrate for the TA, we showcase the first reported *in situ* cadaverine generation from L-lysine directly coupled to transamination reaction in whole cells via a simple pH switch, achieving up to 60% overall yields in 24 hours on a 20 mM scale. This integrated setup reduces reliance on externally supplied amine donors and supports greener process design through improved atom economy and mild reaction conditions.