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Transaminases for chiral amine synthesis

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Amine transaminases are important biocatalysts for the synthesis of chiral primary amines. Unlike many enzymes that have been employed for the synthesis of optically active amines, amine transaminases are capable of asymmetric synthesis and do not rely on costly cofactors that must be regenerated *in situ*. However, their application as general catalysts for the preparation of amines is hampered by a limited substrate scope, substrate and (co)product inhibition and difficulties associated with displacing challenging reaction equilibrium. There has been important progress made to overcome these challenges, including the development of enzymes with broader substrate scope and the design of methodology to effectively displace the reaction equilibrium. Amine transaminases are also being applied in an increasing range of (chemo)enzymatic cascades and immobilized for applications in flow.

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Introduction

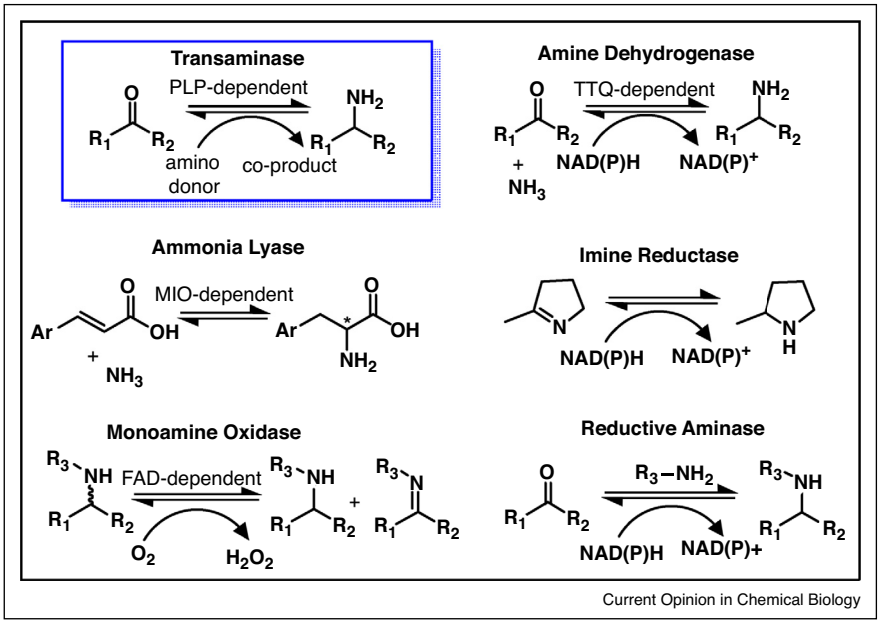
Chiral amines are prevalent in active pharmaceutical ingredients, agrochemicals and bioactive natural products and are important pharmaceutical building blocks. As such, the development of broadly applicable biocatalytic strategies for their synthesis is of great interest. A number of enzymes have been employed for the synthesis of chiral amines, including transaminases, amine dehydrogenases, imine reductases, ammonia lyases, monoamine oxidases [1] and the more recently discovered reductive aminase [2••] (Figure 1). Transaminases (TAs) belong to fold types I and IV of pyridoxal 5′-phosphate (PLP) dependent enzymes and catalyse the reversible transfer of an amino group from a suitable donor to a carbonyl acceptor. Two types of PLP-dependent TAs have been identified and are grouped according to the type of substrate they convert [3]. α-TAs exclusively convert α-amino and α-keto acids whereas ω-TAs can accept substrates with a distal carboxylate group. Importantly, a subgroup of ω-TAs, known as amine TAs (ATAs) are capable of accepting substrates that completely lack a carboxylate group and these enzymes have received considerable attention in recent years due to their potential for the synthesis of chiral primary amines from the corresponding prochiral ketones.

Despite the enormous potential of ATAs, challenges associated with substrate and co-product inhibition, difficulties displacing reaction equilibria and substrate restrictions have contributed to their slow uptake by both the academic and industrial communities. There are a number of research groups addressing these challenges through enzyme engineering and process development and this review will focus on the most significant developments in the field over the past two years.

Expanding their substrate scope

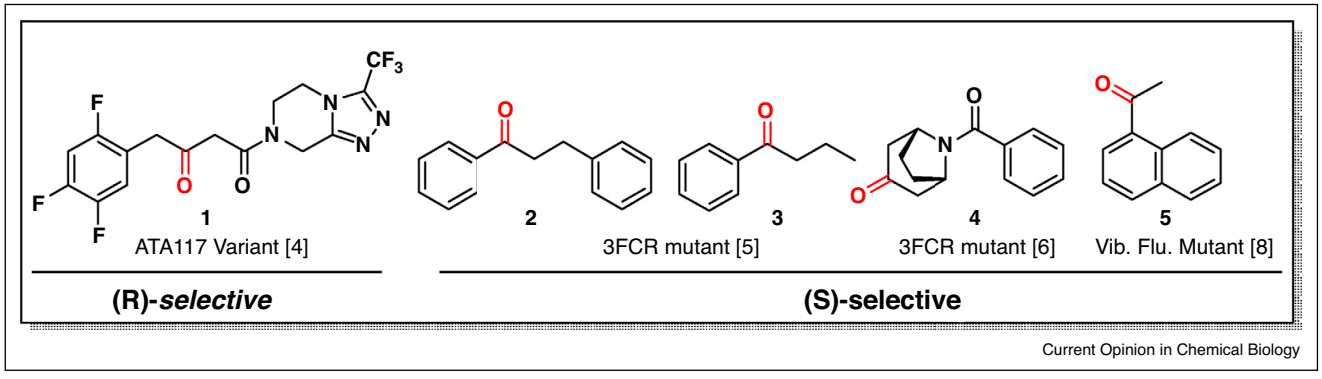
The substrate scope of ATAs can be considered relatively broad, as they are capable of catalysing the amination of a wide range of aldehydes and ketones. However, the active site of these enzymes contains a small and large binding pocket and therefore wild-type enzymes are restricted to ketones bearing at least one 'small' substituent (methyl or ethyl typically). Considerable effort has been directed towards increasing the capacity of the small binding pocket to enable these enzymes to accept ketones with two bulky substituents. The most high-profile example of such an engineering effort was reported in 2010, where an evolved (*R*)-selective ω-TA replaced a rhodium-catalysed hydrogenation step in the conversion of bulky ketone **1** (Figure 2) to the anti-diabetic drug, sitagliptin [4]. The biocatalytic route offers dramatic improvements in yield, purity and selectivity compared to the traditional metal-catalysed approach and this landmark example has inspired subsequent engineering projects (Figure 2). Bornscheuer and co-workers have made substantial progress towards developing ATAs that accept substrates with two sterically demanding substituents. They recently reported an (*S*)-selective ω-TA variant from *Ruegeria* sp. TM1040 (3FCR) that is capable of converting bulky ketones (including **2** and **3**) to the corresponding chiral amines with good to excellent yields and enantiomeric excess [5••]. Interestingly, the variant contains only four mutations and the approach has been shown to be transferrable to other fold class I ω-TA templates with similar sequence identities. Despite the impressive activity of this variant towards bulky

Figure 1



A selection of enzymes that can be used for the synthesis of chiral amines.

Figure 2



Engineered ATAs that act on bulky substrates **1–5** [4–6,8].

substrates, relatively modest substituent changes are not tolerated well by the mutant and this highlights the challenges associated with developing TAs with broad activity. Additionally, the variants have not been shown to work effectively at high concentrations. The ATA from *Ruegeria* sp. TM1040 has also been engineered to accept the sterically demanding bridged ketone, exo-3-amino-8-aza-bicyclo[3.2.1]oct-8-yl-phenylmethanone **4** [6], and variants of an AT from *Vibrio fluvialis* (PDB-ID: 4E3Q) showed activity towards branched-chain bulky–bulky ketones [7]. Moody and co-workers also developed a variant of 43EQ capable of mediating the conversion of the bulky substrate, 2-acetylbiphenyl **5**, having identified no residual activity in the wild-type enzyme [8]. Targeting mutations in the small binding pocket to increase capacity is not the only effective approach. Shin and co-workers reported that mutation of the L57 residue in the large binding pocket of a TA from *Ochrobactrum anthropi* dramatically enhanced activity towards bulky arylalkylamines and alkylamines [9].

Displacing reaction equilibria

A significant obstacle to the application of TAs for the synthesis of chiral amines is the reversible nature of the enzymatic reaction [10]. Achieving good conversion of ketone to amine requires removal of the carbonyl co-product in order to prevent the reverse reaction from competing and compromising conversion/yield (Figure 3)