**Imine Reductases (IREDs)**

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***Highlights***

* Imine reductases (IREDs) are NAD(P)H-dependent oxidoreductases that enable the asymmetric synthesis of secondary and tertiary amines from the corresponding imines.
* The development of annotated sequence databases has led to the discovery of new IREDs from bacteria and fungi.
* The characterisation of new IREDs has resulted in an expansion of the substrate scope and the design of biocatalytic cascades.
* Structural studies of IREDs have provided initial insights into their mechanism of action although further work is required to elucidate the detail of catalysis.
* Reductive amination has been demonstrated using IREDs, although with low efficiency, and represents an important area of investigation for the future.

***Abstract***

Imine reductases (IREDs) have emerged as a valuable new set of biocatalysts for the asymmetric synthesis of optically active amines. The development of bioinformatics tools and searchable databases has led to the identification of a diverse range of new IRED biocatalysts that have been characterized and employed in different synthetic processes. This review describes the latest developments in the structural and mechanistic aspects of IREDs, together with synthetic applications of these enzymes, and identifies ongoing and future challenges in the field.

***Introduction***

Imine reductases (IREDs) are NADPH-dependent oxidoreductases that catalyse the asymmetric reduction of prochiral imines to the corresponding amines [1–3]. The reduction of C=N bonds constitutes a physiological reaction present in a number of biosynthetic pathways leading to a variety of metabolites including folate, siderophores and antibiotics. The imine intermediates in these pathways are structurally very distinct and hence functionally different IREDs, often unrelated by sequence, have evolved to catalyse imine reduction. Dihydrofolate reductase (DHFR), for example, catalyses the NADPH-dependent reduction of 7,8-dihydrofolate **1** to yield 5,6,7,8-tetrahydrofolate **2** in the folate biosynthesis pathway (Figure 1B) [4]. Pip2C/Pyr2C reductases have been shown to catalyse the reduction of cyclic imino acids Δ1-piperideine-2-carboxylate (Pip2C) **3a** and Δ1-pyrroline-2-carboxylate (Pyr2C) **4a** to the corresponding amino acids L-pipecolate **3b** and L-proline **4b** respectively in the pipecolate pathway (Figure 1C)[5]. Other biosynthetic IREDs include PchG from *Pseudomonas aeruginosa* and its homologue, Irp3 from *Yersinia enterocolitica*, which catalyse the reduction of the thiazoline ring of intermediates in the biosynthesis of the siderophores pyochelin and yersiniabactin respectively (Figure 1D) [6,7]. In morphine biosynthesis, an imine reduction step has been identified in the inversion of (*S*)-reticuline **7** to (*R*)-reticuline **7**, in which the iminium ion intermediate 1,2-dehydroreticuline **8** is reduced by dihydroreticuline reductase (DRR) (Figure 1E) [8••,9••]. Although these IREDs have been well studied for their physiological and biomedical relevance, they have to date found limited synthetic applications due to their narrow substrate scope.

In 2010 Mitsukura *et al.* reported the imine reducing activity of two NADPH-dependent oxidoreductases, (*R*)- and (*S*)-IRED from *Streptomyces* sp. GF3587 and Streptomyces sp. GF3546 respectively, on the synthetic substrate 2-methylpyrroline **10** (Figure 1F) [10••,11]. The application of these enzymes in the asymmetric reduction of a variety of imines and iminium ions, together with the incorporation of these IREDs in biocatalytic cascades, was thereafter described by Turner and co-workers [12–16]. These studies spurred interest in this class of enzyme and several IRED homologues have now been characterised by other groups [17–24]. The purpose of this review is to give an overview of the recent advances in the discovery, characterization and application of IREDs.



**Figure 1.** **A** Overview of transformations catalyzed by IREDs. **B**-**E** Imine reduction in metabolic pathways. **F** Reduction of imine **10** by IREDs from *Streptomyces* sp.

***Structural and mechanistic features of IREDs***

The first structure of an IRED, for which the ability to reduce 2-methyl pyrroline **10** had been established, was that of Q1EQE0 from *Streptomyces kanamyceticus* [25••].As suggested by initial solution studies by Mitsukura *et al.* [11], the IRED enzyme is a dimer (Figure 2A), and forms what now appears to be a conserved IRED fold. The N-terminal region of each monomer forms a Rossman domain for NADP(H) binding, and is connected to a C-terminal helical bundle by a long helix. The monomers participate in reciprocal domain sharing that gives rise to the dimer and the formation of the active site, in which NADP(H) is bound at the dimer interface. Q1EQE0 displayed structural similarities to enzymes in the hydroxyisobutyrate dehydrogenase (HIBDH) family such as 2CVZ [26], although domain sharing is not observed in those enzymes. Furthermore, the lysine in HIBDH, which acts as the proton acceptor/donor in the reduction/oxidation of its substrate, was replaced in the IRED with an aspartate (Asp) in position 187. Subsequent structures of IREDs from *Streptomyces* sp.,[27•] *Bacillus* sp. (*Bc*IRED) and *Nocardiopsis* sp. (*Nh*IRED)[28], which are all (*S*)-selective in the reduction of **10**, revealed a tyrosine (Tyr) residue in this position.

It was hypothesised that Asp or Tyr may act as a proton donor in imine reduction, although in the first case, the distance of 8 Å of the Asp carboxylate to the C4 atom of NADP(H) that delivers hydride, was much further than that observed in other reductases between the proton donor and the cofactor, suggesting the possible involvement of a water molecule. However, the distance from the Tyr phenol to C4 of NADPH in *Bc*IRED was only 5 Å, which is more in line with Tyr acting as a direct proton donor. Mutation of Asp to Ala [18,25], or Tyr to Phe [25] or Ala [18] in Asp- or Tyr-containing IREDs respectively, gave rise to enzymes of greatly reduced activity. However, the requirement for a proton donor in the IRED-catalysed reaction is not certain, as imine substrates should be protonated at the operating pH of IREDs, and moreover IREDs with a non-protic residue (Asn, Phe) at this position have subsequently been shown to be active [29•,30]. A review of IRED sequences by Pleiss and Hauer [19•] identified two superfamilies, with opposite stereopreference, defined by distinct active site motifs. However, the stereoselectivity of IREDs is highly variable, and a series of related [28,30], or even identical [29•], substrates can be reduced with different selectivity depending on the state of the enzyme, suggesting that more complex classification systems may be required.

The following structures of the IRED from *Amycolatopsis orientalis* (*Ao*IRED) [29•], which has an Asn residue, Asn171, at the Asp/Tyr position, have recently shed new light on ligand binding in IREDs: an *apo*-form with no cofactor, a complex with NADP(H), and also a ternary complex with NADP(H) and the (*R*)- product of the reduction of 1-methyl dihydroisoquinoline to (*R*)-methyltetrahydroisoquinoline [(*R*)-MTQ]. These structures reveal a great mobility of secondary elements, and also quaternary organization, upon ligand binding, suggesting a significant role for protein dynamics in IRED catalysis. In the ternary complex (Figure 2B), a pronounced closure of the active site is observed, resulting in a discrete binding site for the amine product.

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**Figure 2**. Structure and active site of IREDs as exemplified by *Ao*IRED **A.** *Ao*IRED in complex with NADP(H) and (*R*)-MTQ at 2.1 Ångstrom resolution (PDB code 5FWN). Monomers are shown in green and coral. NADP(H) is shown in cylinder format at the dimer interface. **B.** Active site of 5FWN at the dimer interface, showing binding of (*R*)-MTQ. Bonding interaction distances are given in Ångstroms. Asn171 is replaced by Asp, Tyr and other residues in different IREDs.

In this complex, the amine nitrogen is 4.4 Å from the phenol of Tyr179 and 3.4 Å from the side-chain of Asn241. The chiral carbon atom of MTQ, which corresponds to the electrophilic atom of the imine substrate, is approximately 3.5 Å from the C4 of NADP(H), an ideal position to receive or donate hydride.However, a comparative analysis of IRED structures currently available strongly suggests that imine binding and reduction may be assisted by different types of residues in different enzymes.

***Recent advances in synthetic applications of IREDs***

Mining of protein databases coupled with bioinformatics have resulted in the discovery and characterisation of a number of novel IREDs [18]. An annotated sequence database of more than a thousand putative IREDs from fourteen superfamilies (https://ired.biocatnet.de/) has also been created [19•] which has resulted in a significant expansion of the IRED toolbox, leading to new synthetic applications. Scheller and Nestl reported the characterisation of two (*R*)-selective IREDs from *Streptosporangium roseum* DSM43021 and *Streptomyces turgidiscabies* and one (*S*)-selective IRED from *Paenibacillus elgii* [31], and interestingly showed that the (*S*)-selective IRED is unusually thermostable (70% activity after 7 days at 50 °C), whereas the other IREDs lose most of their activity after 24 hours at 30 °C. All three novel IREDs showed some activity against stable exocyclic imines, such as *N*-benzylidenemethylamine, phenylethylideneaniline and benzophenoneimine.

The vast majority of IREDs described so far are NADPH-dependent, although there are some examples of NADH-dependent IREDs in biosynthetic pathways [5,32]. Furthermore, Gand *et al.* have engineered an IRED with improved NADH affinity and demonstrated preparative-scale reactions with this variant [17].

The *Ao*IRED reported by Aleku *et al*. [29•] catalyses the reduction of prochiral imines and iminium ions and displays unique stereoselective properties. For example, the enzyme was shown to exhibit a switch in stereoselectivity towards MTQ depending on the state of the enzyme. Wetzl *et al.* carried out successful reductions of a wide range of cyclic imines employing a selection of bacterial IREDs including preparative-scale biotransformations with feeding of the substrate to reduce inhibition [30]. Li and coworkers have recently reported the asymmetric preparation of a variety of substituted indolines using enantiocomplementary IREDs from *Paenibacillus lactis* in good conversions and stereoselectivities [33,34]. Finally, Maugeri *et al.* have reported the application of IREDs in micro-aqueous systems by testing different enzymes from *Streptomyces* strains and an IRED from *Paenibacillus elgii* B69 against different model substrates in efforts to address the poor solubility of organic compounds in aqueous systems [35].

The chemoselectivity of IREDs for the reduction of C=N bonds, in the presence of C=O containing compounds, has enabled the application of these enzymes in multi-enzyme cascade reactions. Recently, France *et al.* developed a one-pot cascade employing a carboxylic acid reductase (CAR), an *ω*-transaminase (*ω*-TA) and the (*R*)- or (*S*)-IRED from *Streptomyces* sp. to access chiral mono- and disubstituted piperidines and pyrrolidines starting from simpler keto-acids (Figure 3A) [14•]. Interestingly, it has recently come to light that this *de novo* designed pathway mimics a biosynthetic route for the formation of piperidine-containing natural products [36]. Other examples include an amine oxidase-IRED cascade for the deracemisation of racemic piperidines and pyrrolidines (Figure 3B) and a putrescine transaminase-IRED cascade for the synthesis of nitrogen heterocycles from diamine precursors (Figure 3C) [15].



**Figure 3.** Application of IREDs in multi-enzyme biocatalytic cascades. *ω*-TA = transaminase, CAR = carboxylic acid reductase, LDH = lactate dehydrogenase, GDH = glucose dehydrogenase.

***Reductive amination***

The IRED-mediated asymmetric reduction of C=N bonds now offers an alternative approach for biocatalytic chiral amine synthesis and complements existing approaches based upon the use of transaminases [37–39], amine oxidases [40–42], ammonia lyases [43,44], amine dehydrogenases [45,46] and norcoclaurine/strictosidine synthases [47,48]. One approach that is however currently underdeveloped is the asymmetric reductive amination of ketones to generate a wide range of chiral 2o and 3o amines. Several different enzyme classes have been shown to catalyse reductive aminations, including octopine dehydrogenases (OctDHs) [49], amino acid dehydrogenases (AADHs) [50], amine dehydrogenases (AmDHs) [45] and *N*-methyl-amino acid dehydrogenases (NMAADHs) [51]. Although these systems often display high catalytic activity there are severe restrictions in substrate scope with respect to both ketone and amine. On the face of it IREDs seem unsuitable for reductive amination as most exocyclic imines are unstable with respect to hydrolysis in aqueous media. Nevertheless, in 2014 Müller and co-workers reported the first example of reductive amination using the (*S*)-selective IRED from *Streptomyces* sp*.* GF3546 [27•]. The conversions obtained were very low (< 10%) and high concentrations of both amine donor (212 mM) and enzyme (2.5 mg mL-1) were required. Nestl and co-workers subsequently explored the scope of reductive amination of the (*R*)-selective IRED from *Streptosporangium roseum*, whichexhibited activity for several different carbonyl compounds and was able to use ammonia, methylamine and benzylamine as amine donors [52]. By employing NMR studies, they observed that imine formation for benzaldehyde in water is favoured under basic conditions (pH > 9). However, for the compounds reported, only moderate conversions (~50%) of ketones (10 mM) could be achieved at high amine:ketone ratios (50:1) and enzyme loadings (10 mg mL-1), although good to excellent enantiomeric excesses (78-98% *ee*) were exhibited in all cases.

Wetzl *et al.* have recently reported an extensive screen of 28 IREDs towards reductive amination of cyclic, aromatic and aliphatic prochiral ketones using ammonia and small aliphatic primary amines as donors (20 mM substrate, 250 mM amine) [53••]. All products were produced by at least one of the IREDs employed, with conversions that varied from poor to excellent depending upon the ketone-amine combination. Preparative scale reactions were also demonstrated using two of the IREDs, with the corresponding amine products obtained in moderate to good yields (50-71%) and excellent enantio- and diastereoselectivities (> 94%).

**Table 1. IRED-catalysed reductive amination of ketones.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Substrate | Amine | IRED | Conversion (%) | *e.e.*/*d.e.* (%) |
|  | MeNH2 | *Streptomyces sp.* GF3546 | 9 a | n. d. |
|  | MeNH2 | *Streptosporangium roseum* | 16 b | 87 (*R*) |
|  | NH3 | *Streptosporangium roseum* | 53 b | 78 (*R*) |
|  | MeNH2 | *Streptomyces tsukubaensis* | 88 c | 96 (*R*) |
|  | MeNH2 | *Nitratireductor pacificus* | 90 c | n. a. |
|  | BuNH2 | *Nocardia cyriacigeorgica* GUH-2 | 42 c | n. a. |
|  | MeNH2 | *Mesorhizobium sp.* L48C026A00 | 96 c | n. d. |
|  | MeNH2 | *Verrucosispora maris* | 94 c | 98 (*S*,*R*) |

a Reaction performed with 20 mM substrate, 212 mM amine donor and 2.5 mg mL-1 purified IRED [27•]. b Reactions performed with 10 mM substrate, 500 mM amine donor and 10 mg mL-1 purified IRED [52]. c Reactions performed with 20 mM substrate, 250 mM amine donor and 0.6 mg mL-1 purified IRED [53••]. n.d.: not determined. n.a.: not applicable.

***Conclusions and outlook***

The development of an online database based on IRED-specific sequence motifs has identified a large number of putative IRED sequences for investigation. The structural characterisation of several of these has revealed some of the large domain movements required for catalysis and also cast doubt on the identity of residues previously connected with a particular enantiopreference. Although certain motifs have been suggested to control enantioselectivity, classification of these enzymes as (*R*) or (*S*) is not straightforward. Furthermore, the precise mechanism of imine reduction is also still unknown and represents an ongoing area for further studies.

In parallel, the substrate scope of IREDs has expanded beyond cyclic imines to a variety of exocyclic imines and even carbonyl/amine donor pairings for which imine formation is disfavoured in aqueous solution. The question of whether certain IREDs are capable of catalysing not only imine reduction, but also imine formation, as observed in amino acid dehydrogenases, has yet to be resolved. Such ‘reductive aminase’ enzymes could rival currently available biocatalysts for amine synthesis by offering a far broader substrate scope.

Finally, IREDs have successfully been combined with other enzymes to synthesise valuable amine products. The design of new cascade systems that exploit the unique chemo-and stereoselectivity of IREDs and the implementation of IRED-catalysed processes in industry offer new opportunities in research for the future.

***Acknowledgements***

We thank the industrial affiliates of the Centre of Excellence for Biocatalysis, Biotransformations and Biomanufacture (CoEBio3) for studentships to G.A.A. and H.M., and Pfizer, Johnson Matthey and AstraZeneca for studentships to S.P.F., S.L.M. and J.I.R. respectively. We also acknowledge the UK Biotechnology and Biological Sciences Research Council for funding (Grant BB/M006832/1) and N.J.T. thanks the Royal Society for a Wolfson Research Merit Award.

***References and recommended reading***

Papers of particular interest, published within the period of review have been highlighted as:

• of special interest

•• of outstanding interest

1. Leipold F, Hussain S, France SP, Turner NJ: **Imine Reductases**. In *Science of Synthesis: Biocatalysis in Organic Synthesis 2*. Edited by Faber K, Fessner W, Turner NJ. Georg Thieme Verlag; 2015:359–382.

2. Grogan G, Turner NJ: **InspIRED by Nature: NADPH-Dependent Imine Reductases (IREDs) as Catalysts for the Preparation of Chiral Amines**. *Chem. - A Eur. J.* 2016, **22**:1900–1907.

3. Schrittwieser JH, Velikogne S, Kroutil W: **Biocatalytic Imine Reduction and Reductive Amination of Ketones**. *Adv. Synth. Catal.* 2015, **357**:1655–1685.

4. Posner BA, Li L, Bethell R, Tsuji T, Benkovic SJ: **Engineering specificity for folate into dihydrofolate reductase from *Escherichia coli***. *Biochemistry* 1996, **35**:1653–1663.

5. Muramatsu H, Mihara H, Kakutani R, Yasuda M, Ueda M, Kurihara T, Esaki N: **The putative malate/lactate dehydrogenase from *Pseudomonas putida* is an NADPH-dependent Δ1-piperideine-2- carboxylate/Δ1-pyrroline-2-carboxylate reductase involved in the catabolism of D-lysine and D-proline**. *J. Biol. Chem.* 2005, **280**:5329–5335.

6. Meneely KM, Lamb AL: **Two structures of a thiazolinyl imine reductase from *Yersinia enterocolitica* provide insight into catalysis and binding to the nonribosomal peptide synthetase module of HMWP1**. *Biochemistry* 2012, **51**:9002–9013.

7. Meneely KM, Ronnebaum TA, Riley AP, Prisinzano TE, Lamb AL: **Holo Structure and Steady State Kinetics of the Thiazolinyl Imine Reductases for Siderophore Biosynthesis**. *Biochemistry* 2016, **55**:5423–5433.

8. •• Winzer T, Kern M, King AJ, Larson TR, Teodor RI, Donninger SL, Li Y, Dowle AA, Cartwright J, Bates R, *et al.*: **Morphinan biosynthesis in opium poppy requires a P450-oxidoreductase fusion protein**. *Science* 2015, **349**:309–312.

The discovery and characterisation of the enzyme in morphine biosynthesis that catalyses the conversion of (*S*)- to (*R*)-reticuline via the corresponding iminium ion (see also reference 9).

9. •• Farrow SC, Hagel JM, Beaudoin G a W, Burns DC, Facchini PJ: **Stereochemical inversion of (*S*)-reticuline by a cytochrome P450 fusion in opium poppy**. *Nat. Chem. Biol.* 2015, **11**:728–732.

10. •• Mitsukura K, Suzuki M, Tada K, Yoshida T, Nagasawa T: **Asymmetric synthesis of chiral cyclic amine from cyclic imine by bacterial whole-cell catalyst of enantioselective imine reductase.** *Org. Biomol. Chem.* 2010, **8**:4533–4535.

This paper suggested that IREDs were in fact more prevalent in Nature than previously imagined and has encouraged groups to search for novel homologues based upon sequence similarities.

11. Mitsukura K, Suzuki M, Shinoda S, Kuramoto T, Yoshida T, Nagasawa T: **Purification and Characterization of a Novel (*R*)-Imine Reductase from *Streptomyces* sp. GF3587**. *Biosci. Biotechnol. Biochem.* 2011, **75**:1778–1782.

12. Hussain S, Leipold F, Man H, Wells E, France SP, Mulholland KR, Grogan G, Turner NJ: **An (*R*)-imine reductase biocatalyst for the asymmetric reduction of cyclic imines**. *ChemCatChem* 2015, **7**:579–583.

13. Heath RS, Pontini M, Hussain S, Turner NJ: **Combined Imine Reductase and Amine Oxidase Catalyzed Deracemization of Nitrogen Heterocycles**. *ChemCatChem* 2016, **8**:117–120.

14. • France SP, Hussain S, Hill AM, Hepworth LJ, Howard RM, Mulholland KR, Flitsch SL, Turner NJ: **One Pot Cascade Synthesis of Mono- and Disubstituted Piperidines and Pyrrolidines using Carboxylic Acid Reductase (CAR), *ω*-Transaminase (*ω*-TA) and Imine Reductase (IRED) Biocatalysts**. *ACS Catal.* 2016, **6**:3753–3759.

Demonstration that IREDs can be used in cascade reactions with transaminases and carboxylic acid reductases to synthesis a wide range of substituted chiral piperidines on a preparative scale.

15. Slabu I, Galman JL, Weise NJ, Lloyd RC, Turner NJ: **Putrescine Transaminases for the Synthesis of Saturated Nitrogen Heterocycles from Polyamines**. *ChemCatChem* 2016, **8**:1038–1042.

16. Leipold F, Hussain S, Ghislieri D, Turner NJ: **Asymmetric reduction of cyclic imines catalyzed by a whole-cell biocatalyst containing an (*S*)-imine reductase**. *ChemCatChem* 2013, **5**:3505–3508.

17. Gand M, Thöle C, Müller H, Brundiek H, Bashiri G, Höhne M: **A NADH-accepting imine reductase variant: Immobilization and cofactor regeneration by oxidative deamination**. *J. Biotechnol.* 2016, **230**:11–18.

18. Scheller PN, Fademrecht S, Hofelzer S, Pleiss J, Leipold F, Turner NJ, Nestl BM, Hauer B: **Enzyme Toolbox: Novel Enantiocomplementary Imine Reductases**. *ChemBioChem* 2014, **15**:2201–2204.

19. • Fademrecht S, Scheller PN, Nestl BM, Hauer B, Pleiss J: **Identification of imine reductase-specific sequence motifs**. *Proteins Struct. Funct. Bioinforma.* 2016, **84**:600–610.

A searchable database containing more that 1,000 putative IRED sequences.

20. Gamenara D, de Maria P: **Enantioselective imine reduction catalyzed by imine reductases and artificial metalloenzymes**. *Org. Biomol. Chem.* 2014, **12**:2989–2992.

21. Gand M, Müller H, Wardenga R, Höhne M: **Characterization of three novel enzymes with imine reductase activity**. *J. Mol. Catal. B Enzym.* 2014, **110**:126–132.

22. Lenz M, Scheller PN, Richter SM, Hauer B, Nestl BM: **Cultivation and purification of two stereoselective imine reductases from *Streptosporangium roseum* and *Paenibacillus elgii***. *Protein Expr. Purif.* 2016, doi:10.1016/j.pep.2016.05.003.

23. Mitsukura K, Kuramoto T, Yoshida T, Kimoto N, Yamamoto H, Nagasawa T: **A NADPH-dependent (*S*)-imine reductase (SIR) from *Streptomyces* sp. GF3546 for asymmetric synthesis of optically active amines: Purification, characterization, gene cloning, and expression**. *Appl. Microbiol. Biotechnol.* 2013, **97**:8079–8086.

24. Kohls H, Steffen-Munsberg F, Höhne M: **Recent achievements in developing the biocatalytic toolbox for chiral amine synthesis**. *Curr. Opin. Chem. Biol.* 2014, **19**:180–192.

25. •• Rodriguez-Mata M, Frank A, Wells E, Leipold F, Turner NJ, Hart S, Turkenburg JP, Grogan G: **Structure and activity of NADPH-dependent reductase Q1EQE0 from *Streptomyces kanamyceticus*, which catalyses the *R*-selective reduction of an imine substrate**. *ChemBioChem* 2013, **14**:1372–1379.

This paper reports the first structure of an IRED and discusses possible active-site residues which are important for catalysis and controlling stereoselectivity.

26. Lokanath NK, Ohshima N, Takio K, Shiromizu I, Kuroishi C, Okazaki N, Kuramitsu S, Yokoyama S, Miyano M, Kunishima N: **Crystal structure of novel NADP-dependent 3-hydroxyisobutyrate dehydrogenase from *Thermus thermophilus* HB8**. *J. Mol. Biol.* 2005, **352**:905–917.

27. • Huber T, Schneider L, Präg A, Gerhardt S, Einsle O, Müller M: **Direct reductive amination of ketones: Structure and activity of *S*-selective imine reductases from *Streptomyces***. *ChemCatChem* 2014, **6**:2248–2252.

This paper reports the first IRED-mediated reductive amination reactions.

28. Man H, Wells E, Hussain S, Leipold F, Hart S, Turkenburg JP, Turner NJ, Grogan G: **Structure, activity and stereoselectivity of NADPH-dependent oxidoreductases catalysing the *S*-selective reduction of the imine substrate 2-methylpyrroline**. *ChemBioChem* 2015, **16**:1052–1059.

29. • Aleku GA, Man H, France SP, Leipold F, Hussain S, Toca-Gonzalez L, Marchington R, Hart S, Turkenburg JP, Grogan G, *et al.*: **Stereoselectivity and Structural Characterization of an Imine Reductase (IRED) from *Amycolatopsis orientalis***. 2016, **6**:3380–3889.

This paper reports the in depth characterisation of one specific IRED and highlights some unusual changes in stereoselectivity with structurally similar substrates.

30. Wetzl D, Berrera M, Sandon N, Fishlock D, Ebeling M, Müller M, Hanlon S, Wirz B, Iding H: **Expanding the Imine Reductase Toolbox by Exploring the Bacterial Protein-Sequence Space**. *ChemBioChem* 2015, **16**:1749–1756.

31. Scheller PN, Nestl BM: **The biochemical characterization of three imine-reducing enzymes from *Streptosporangium roseum* DSM43021, *Streptomyces turgidiscabies* and *Paenibacillus elgii***. *Appl. Microbiol. Biotechnol.* 2016, **100**:10509–10520.

32. Garweg G, von Rehren D, Hintze U: **L-Pipecolate Formation in the Mammalian Brain. Regional Distribution of Δ1-Pyrroline-2-carboxylate Reductase Activity**. *J. Neurochem.* 1980, **35**:616–621.

33. Li H, Luan ZJ, Zheng GW, Xu JH: **Efficient Synthesis of Chiral Indolines using an Imine Reductase from *Paenibacillus lactis***. *Adv. Synth. Catal.* 2015, **357**:1692–1696.

34. Li H, Zhang G-X, Li L-M, Ou Y-S, Wang M-Y, Li C-X, Zheng G-W, Xu J-H: **A Novel (*R*)-Imine Reductase from *Paenibacillus lactis* for Asymmetric Reduction of 3 H -Indoles**. *ChemCatChem* 2016, **8**:724–727.

35. Maugeri Z, Rother D: **Application of Imine Reductases (IREDs) in Micro-Aqueous Reaction Systems**. *Adv. Synth. Catal.* 2016, **358**:2745–2750.

36. Peng H, Wei E, Wang J, Zhang Y, Cheng L, Deng Z, Qu X: **Deciphering Piperidine Formation in Polyketide-Derived Indolizidines Reveals a Thioester Reduction, Transamination, and Unusual Imine Reduction Process**. *ACS Chem. Biol.* 2016, doi:10.1021/acschembio.6b00875.

37. Savile CK, Janey JM, Mundorff EC, Moore JC, Tam S, Jarvis WR, Colbeck JC, Krebber A, Fleitz FJ, Brands J, et al.: **Biocatalytic Asymmetric Synthesis of Sitagliptin Manufacture**. *Science* 2010, **329**:305–310.

38. Pavlidis IV, Weiß MS, Genz M, Spurr P, Hanlon SP, Wirz B, Iding H, Bornscheuer UT: **Identification of (*S*)-selective transaminases for the asymmetric synthesis of bulky chiral amines**. *Nat. Chem.* 2016, **8**:1076-1082.

39. Simon RC, Richter N, Busto E, Kroutil W: **Recent developments of cascade reactions involving *ω*-transaminases**. *ACS Catal.* 2014, **4**:129–143.

40. Ghislieri D, Green AP, Pontini M, Willies SC, Rowles I, Frank A, Grogan G, Turner NJ: **Engineering an enantioselective amine oxidase for the synthesis of pharmaceutical building blocks and alkaloid natural products**. *J. Am. Chem. Soc.* 2013, **135**:10863–10869.

41. Heath RS, Pontini M, Bechi B, Turner NJ: **Development of an *R*-selective amine oxidase with broad substrate specificity and high enantioselectivity**. *ChemCatChem* 2014, **6**:996–1002.

42. Yasukawa K, Nakano S, Asano Y: **Tailoring D-amino acid oxidase from the pig kidney to *R*-stereoselective amine oxidase and its use in the deracemization of α-methylbenzylamine**. *Angew. Chemie - Int. Ed.* 2014, **53**:4428–4431.

43. Weise NJ, Parmeggiani F, Ahmed ST, Turner NJ: **The bacterial ammonia lyase EncP: A tunable biocatalyst for the synthesis of unnatural amino acids**. *J. Am. Chem. Soc.* 2015, **137**:12977–12983.

44. Parmeggiani F, Lovelock SL, Weise NJ, Ahmed ST, Turner NJ: **Synthesis of D- and L-Phenylalanine Derivatives by Phenylalanine Ammonia Lyases: A Multienzymatic Cascade Process**. *Angew. Chemie - Int. Ed.* 2015, **54**:4608–4611.

45. Abrahamson MJ, Vázquez-Figueroa E, Woodall NB, Moore JC, Bommarius AS: **Development of an amine dehydrogenase for synthesis of chiral amines**. *Angew. Chemie - Int. Ed.* 2012, **51**:3969–3972.

46. Knaus T, Bohmer W, Mutti FG: **Amine dehydrogenases: Efficient biocatalysts for the reductive amination of carbonyl compounds**. *Green Chem.* 2016, doi:10.1039/C6GC01987K.

47. Stöckigt J, Barleben L, Panjikar S, Loris EA: **3D-Structure and function of strictosidine synthase - the key enzyme of monoterpenoid indole alkaloid biosynthesis**. *Plant Physiol. Biochem.* 2008, **46**:340–355.

48. Bonamore A, Rovardi I, Gasparrini F, Baiocco P, Barba M, Molinaro C, Botta B, Boffi A, Macone A: **An enzymatic, stereoselective synthesis of (*S*)-norcoclaurine**. *Green Chem.* 2010, **12**:1623.

49. Dairi T, Asano Y: **Cloning, nucleotide sequencing, and expression of an opine dehydrogenase gene from *Arthrobacter* sp. strain 1C**. *Appl Env. Microbiol* 1995, **61**:3169–3171.

50. Ye LJ, Toh HH, Yang Y, Adams JP, Snajdrova R, Li Z: **Engineering of amine dehydrogenase for asymmetric reductive amination of ketone by evolving *Rhodococcus* phenylalanine dehydrogenase**. *ACS Catal.* 2015, **5**:1119–1122.

51. Mihara H, Muramatsu H, Kakutani R, Yasuda M, Ueda M, Kurihara T, Esaki N: ***N*-methyl-L-amino acid dehydrogenase from *Pseudomonas putida*: A novel member of an unusual NAD(P)-dependent oxidoreductase superfamily**. *FEBS J.* 2005, **272**:1117–1123.

52. Scheller PN, Lenz M, Hammer SC, Hauer B, Nestl BM: **Imine Reductase-Catalyzed Intermolecular Reductive Amination of Aldehydes and Ketones**. *ChemCatChem* 2015, **7**:3239–3242.

53. •• Wetzl D, Gand M, Ross A, Müller H, Matzel P, Hanlon SP, Müller M, Wirz B, Höhne M, Iding H: **Asymmetric Reductive Amination of Ketones Catalyzed by Imine Reductases**. *ChemCatChem* 2016, **8**:2023–2026.

In this paper the Roche group report an extensive screen of IRED-mediated reductive amination reactions, with different amines and ketones, and also show that in some cases the reactions can be carried out on a preparative scale.