A Mechanism for Reductive Amination Catalyzed by Fungal Reductive Aminases (RedAms)

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ABSTRACT Reductive Aminases (RedAms) catalyze the asymmetric reductive amination of ketones with primary amines to give secondary amine products. RedAms have great potential for the synthesis of bioactive chiral amines, however, insights into their mechanism are currently limited. Comparative studies on reductive amination of cyclohexanone with allylamine in the presence of RedAms, imine reductases (IREDs) or NaBH3CN support the distinctive activity of RedAms in catalyzing both imine formation and reduction in the reaction. Structures of *At*RedAm from *Aspergillus terreus*, in complex with NADPH and ketone and amine substrates, along with kinetic analysis of active-site mutants, reveal modes of substrate binding, the basis for the specificity of RedAms for reduction of imines over ketones, and the importance of domain flexibility in bringing the reactive participants together for the reaction. This information is used to propose a mechanism for their action and also to expand the substrate specificity of RedAms using protein engineering.

KEYWORDS Oxidoreductase, Amine, Imine Reductase, Reductive Amination, NADPH

**Introduction**

Reductive amination is the process whereby aldehydes or ketones are transformed into amines through nucleophilic attack of an amine onto the carbonyl group, followed by reduction of the subsequently formed iminium ion intermediate (Scheme 1a).1



**Scheme 1**. **a**: General scheme for a reductive amination reaction; **b**-**d**: Reductive amination reactions catalyzed by amino acid dehydrogenases (AADHs), amine dehydrogenases (AmDHs) and opine dehydrogenases (OpDHs) respectively; **e**: Reaction catalyzed by reductive aminases (RedAms) and imine reductases (IREDs).

When accomplished asymmetrically, it becomes a powerful tool for the production of high-value chiral amines for pharmaceutical and agrochemical synthesis.2,3 Asymmetric reductive amination can employ transition metal-catalysis,3 using, for example, iridium,4 iron5 or cobalt6 however, challenges persist, including reduction of the carbonyl starting material, the formation of product mixtures and inefficient aminations with weakly basic amines. Enzymatic methods for reductive amination have thus become a focus for research because, in addition to addressing some of these challenges, they present both sustainable chemistry credentials and high selectivity.7 ω-Transaminases (ω-TAs),8-10 which catalyze amination of ketones using an ammonia donor, such as isopropylamine, catalyze a formal equivalent of a reductive amination reaction using the cofactor pyridoxal phosphate (PLP). In addition, amino acid dehydrogenases (AADHs),11 such as leucine DH and phenylalanine DH, which transfer ammonia to keto-acid precursors (Scheme 1b), have been engineered to become amine dehydrogenases (AmDHs, Scheme 1c) for the transformation of structurally related ketones into amines.12-15 A separate group of natural AmDHs with similar activity has also recently been discovered.16 However, the application of both ω-TAs and AmDHs is limited to the production of primary amines, necessitating subsequent alkylation chemistry for the formation of chiral secondary amines.

Enzyme-catalyzed reductive amination reactions would be especially appealing for the preparation of secondary amines, as they would permit the transformation of prochiral ketones to chiral amines in one enzymatic step. Genuine enzymatic reductive aminations would involve the binding and reactive coupling of a ketone and an amine, presented in stoichiometric amounts, followed by nicotinamide cofactor (NAD(P)H)-dependent reduction of the formed iminium ion, all within the enzyme active site. Opine dehydrogenases (OpDHs, (Scheme 1d), which catalyze the reversible coupling of amino acids and keto acids such as pyruvate and 2-oxo glutarate17 have been engineered to remove the requirement for the carboxylate functional group in each substrate, permitting the reductive amination of ketones with small amine partners to form chiral secondary amines.18 Imine reductases (IREDs, Scheme 1e),19,20 which catalyze the asymmetric NADPH-dependent reduction of preformed imines, have also been applied to reductive aminations,21-26 although it appears that IREDs catalyze only imine reduction and not imine formation, since large excesses of amine (typically 10-50 equivalents) are required to achieve quantitative conversions. As part of our ongoing studies into IREDs, we have recently reported a homolog from the fungus *Aspergillus oryzae*, which, in addition to enabling the formation of amines such as the anti-Parkinson’s agent (*R*)-rasagiline **1a** directly from ketone **1** and amine **a,** supplied in a 50-fold excess (Scheme 2a), also catalyzed the reductive amination of a limited range of ketones with small amine donors supplied in a 1:1 ratio.27



**Scheme 2**. **a**: Synthesis of (*R*)-rasagiline using *Asp*RedAm from *Aspergillus oryzae;* **b**: Ketone and amine substrates used in this study.

The distinctive activity of this enzyme prompted us to name it a ‘reductive aminase’ (*Asp*RedAm). The structure of *Asp*RedAm, determined in complex with the amine product (*R*)-rasagiline, permitted the first observations of interactions between active site residues and an amine product.27 However, further experiments are required to provide information that would inform tailoring of these enzymes for synthetic applications.

Here, we first describe reaction studies with RedAms that establish their catalysis of accelerated reductive amination activity over and above either IREDs or the chemical reductant NaBH3CN. We then explore this distinctive reactivity through structural studies on *At*RedAm from *Aspergillus terreus*, in complex with cofactors and ketone and amine substrates, which illustrate modes of substrate binding and the role of domain flexibility at different stages of the reaction. The structures of *At*RedAm, combined with kinetics studies on this enzyme and also *Ad*RedAm from *Ajellomyces dermatitidis*, have informed mutagenesis studies that result in both the formulation of a mechanistic hypothesis for fungal RedAms and also the alteration of their substrate specificity.

**Fungal RedAms catalyze reductive amination much faster than either IREDs or NaBH3CN.**

We have focused on two *Asp*RedAm homologs derived from *Aspergillus terreus* (*At*RedAm) and *Ajellomyces dermatitidis* (*Ad*RedAm).The sequences of each enzyme (Section S2, Figure S1) contained residues D169, N93, Y177 and Q240 (*Asp*RedAm numbering) that were identified within the active site of *Asp*RedAm.27 Details of the cloning and expression of the genes encoding *At*RedAm and *Ad*RedAm (Section S2), the determination of kinetic constants for reductive aminations of ketone **4** with allylamine **b** (Section S3), and also data pertaining to the reductive amination of a range of ketones with amine partners by *At*RedAm and *Ad*RedAm (Scheme 2b, Section S4-S5), are found in the Supporting Information. Products of enzyme-catalysed reductive amination reactions were compared against standards prepared using sodium triacetoxyborohydride in dry THF under nitrogen as described in the Supporting Information, and were typically obtained with yields of between 35 and 74%. To summarise the biotransformation data, cyclohexanone **4** was aminated with one equivalent of either **a**, **b** and **c** by each enzyme with conversions of between 79% and 90%, which could be improved to 95% if two equivalents of amine were used. One equivalent of benzylamine **e** yielded only 26% and 3% conversion to the secondary amine for *Ad*RedAm and *At*RedAm respectively. Ester **2** was aminated with five equivalents of **a**, **b**, **c**, by both enzymes with conversions of between 64% and 97%; however the longer ester **3**  gave lower conversions with **b**, with 37% and 8% conversion to the product amine for *Ad*RedAm and *At*RedAm respectively. In the cases of **2** and **3**, e.e.s of between 34 and 91% were observed. Octan-2-one **5**, and decan-2-one **6** were aminated by each enzyme with five equivalents of **b** to give conversions of 43-44%; 4-phenyl-butan-2-one **7** was aminated by both enzymes with five equivalents of methylamine **f** to give (*R*)-configured products of 62-68% e.e. Hexan-2-one **8** gave secondary amines with five equivalents of amines **a-g**, with conversions of 49-74% and e.e.s of (*R*)-configured products between 62 and 94%. Overall small hydrophobic amines with pi-character proved to be the best amine partners. Crucially, neither of the enzymes catalyzed the reduction of ketone substrates to alcohol products.

Both *At*RedAm and *Ad*RedAm catalyzed the synthesis of secondary amines such as **4b** when provided with cyclohexanone **4** and allylamine **b** in a ratio of 1:1, suggestive of the capacity of these enzymes to catalyze both imine formation and imine reduction, and thus to constitute true RedAm activities. To provide further evidence of rate acceleration of imine formation by RedAms, the rate of formation of **4b** by *At*RedAm and *Ad*RedAm was compared to that achieved using the (*S*)-IRED from *Streptomyces* sp.GF3546 and also the chemical reductant NaBH3CN,in the absence of an enzyme (Section S6). The results clearly showed that the rate of conversion by the RedAms was significantly greater, either at equimolar concentrations of ketone and amine, for which conversion was 73% and 45% after 3 h *vs* 6% and 4% for (*S*)-IRED and NaBH3CN, or where a ten-fold excess of amine was supplied (Figure S4). The results suggest that (*S*)-IRED is acting only as an imine reductase, recruiting imine formed in solution for reduction, whereas the RedAms, with relevant substrates, actively bind ketone and amine partner substrates and then catalyze their coupling to form the iminium ion for reduction.

**Crystal structures of *At*RedAm in complex with cofactors and ketone and amine substrates.**

Having obtained evidence for the distinctive catalytic activity of fungal RedAms as described above, we proceeded to explore the determinants of substrate binding and catalysis using X-ray crystallography. Although a structure of *Asp*RedAm with the amine product (*R*)-rasagiline had previously been determined,27 this complex was not informative for the purposes of defining the determinants of ketone or amine binding *en route* to imine formation. *At*RedAm proved to be a superior target protein for crystallization overall, and we had recently determined its structure in complex with a dibenzazepine imine substrate and a redox-inactive cofactor analog, NADPH4.28 Herein, we report the *At*RedAm structure determined in complex with the cofactor NADPH alone, and also ternary complexes featuring NADPH with either ethyl levulinate **2**, or ethyl 5-oxohexanoate **3** (Scheme 2b; for data collection and refinement statistics see Section S7, Table S11). In common with IREDs21,29-31 and also *Asp*RedAm,27 the *At*RedAm monomer features an N-terminal Rossmann domain attached to a C-terminal helical bundle by an inter-domain helix. Two monomers form a dimer through domain sharing (Figure 1A), in which the active site is formed at the interface of the N- and C-terminal domains of neighbouring monomers.



**Figure 1.** Crystal structures of *At*RedAm. **A:** Structure of active *At*RedAm dimer showing overall fold; **B**: Superimposition of open (green) and closed (coral) chains of *At*RedAm showing domain closure upon NADPH binding with the hinge region between residues 174 and 176; axis of rotation is depicted in blue at the crosshair. **C**: Crystal structures of *At*RedAm in complex with NADPH and C5 keto-ester **2** and; **D** a C6 keto-ester **3**. Backbone and carbon atoms of subunits A and B are shown in green and coral respectively. NADPH and esters are shown in cylinder format with carbon atoms in grey and purple respectively. Electron density corresponds to the 2*Fo-Fc* and *Fo-Fc* omit maps in blue and green at levels of 1*σ* and 3*σ* respectively, obtained after building the protein, cofactor and water molecules and prior to refinement of the ligand.

Earlier structures of IREDs showed that the active site cleft is wider in *apo*- structures and NADPH complexes, and narrower in ternary complexes with amine products bound,21,29-31 suggestive of domain closure during catalysis. The structure of *At*RedAm-NADPH featured four dimers in the asymmetric unit, and this permitted the observation of different conformations of the dimers, perhaps representative of some of these modes. Analysis of the most ‘open’ (*apo*-) and ‘closed’ (NADPH-bound) subunits within the structure using DynDom,32 revealed a rotation of 14° around the hinge residues D175 and L176 within the inter-domain helix (Figure 1B). Complexes of *At*RedAm with the keto-ester ethyl levulinate **2,** and also its C6 homolog ethyl 5-oxohexanoate **3,** were then obtained. *At*RedAm-NADPH-**2** is again representative of a more ‘closed’ complex of the enzyme, in which the dimensions of the active site cavity are reduced (Figure 1C). A relocation of loop 231-240 to cover the base of the active site is accompanied by the progressive movement of M244 to cover the front. The overall effect of this closure is to provide a smaller and more hydrophobic environment, which would presumably be required to stabilize the iminium ion intermediate in RedAm reactions, but also to constrain the geometry of the active site for conferring prochiral selectivity in the reduction reaction. The ketone carbonyl group of ethyl levulinate **2** is secured by the phenolic hydroxyl of Y183, placing the carbon atom of the C=O group 4.5 Å from the C4 atom of NADPH. This appears to be too distant for hydride transfer between the two, and offers a partial explanation for the inability of *At*RedAm to catalyze the reduction of the ketone carbonyl of **2** to the corresponding alcohol product.

The binding mode of ester **2** is in marked contrast to that of the longer ketoester **3**. In the *At*RedAm-NADPH-**3** complex, **3** is rotated 180° in the active site with the ester carbonyl at the front, again approximately 4.5 Å from the C4 atom of NADPH and too far away from the proposed catalytic residues Y183, D175 or N98, in what is, presumably, an unproductive conformation (Figure 1D), with the ketone carbonyl group at the rear of the active site. A comparison of the *At*RedAm complexes with **2** and **3**, reveals that the side chain of (B) Y222 is displaced by the C6 keto-ester **3** to form an interaction with the side-chain of D126. The different conformation of **2** and **3** in the active site suggests a reason for the lower conversions of the latter in biotransformations by *At*RedAm.

In an effort to obtain a quaternary complex of *At*RedAm, in which ketone, amine and cofactor might be trapped in the active site prior to imine formation, we attempted complexes with the redox-inactive NADPH analogue NADPH4,33 with cyclohexanone **4** and allylamine **b**, partners in one of the reductive amination reactions catalyzed by *At*RedAm. In these experiments, *At*RedAmwas co-crystallized with NADPH4 (the synthesis is described in Section S8) and cyclohexanone **4**, and allylamine **b** was then soaked into crystals of the ternary complex. The resulting *At*RedAm-NADPH4-**4**-**b** complex provided further evidence for the role of Y183 in ketone binding, with the cyclohexanone carbonyl in contact with a water molecule that is coordinated to the side chain of Y183 (Figure 2A).

The omit map also revealed density adjacent to D175 and N98, consistent with the presence of allylamine **b**. The amine nitrogen of **b** interacts with the side chains of these residues at distances of 2.8 Å each, and the allyl group is bound in a pocket formed by the side-chains of L96 and I123, with the pi-system of the double bond interacting with the backbone amide carbonyl and amine groups of these residues respectively (Figure 2B). Hydrogen bonds between pi-systems and backbone residues are well-documented34,35 and, crucially, the identification of an amine binding site may offer a partial explanation as to why smaller amines with pi character (**a**, **b**, **c**, sometimes **e**) are favored over similar sized amines, such as isopropylamine **d**, which is not accepted.



**Figure 2.** Crystal structures of *At*RedAm in complex with NADPH4, cyclohexanone **4** and allylamine **b**. **A**: Binding of NADPH4 and cyclohexanone at the dimer interface with allylamine bound to side-chains of D175 and N98; **B**: Detail of allylamine binding site. Backbone and carbon atoms of subunits A and B are shown in green and coral respectively. NADPH4, cyclohexanone and allylamine are shown in cylinder format with carbon atoms in grey, purple and blue respectively. Electron density corresponds to the 2*Fo-Fc* and *Fo-Fc* omit maps in blue and green at levels of 1*σ* and 3*σ* respectively, obtained after building the protein, cofactor and water molecules and prior to refinement of the ligand. Selected interactions are indicated by dashed black lines with distances shown in Å.

**Reduced activity in point mutants at the active sites of *At*RedAm and *Ad*RedAm.**

The structures of *At*RedAm complexes informed the generation of mutants of *At*RedAm and *Ad*RedAm, designed to explore the roles of active site residues in the catalytic mechanism. Thus, mutants Y183F, D175A and N98A (*At*RedAm) and Y177F, N94A and D169A (*Ad*RedAm) were prepared (Section S9) and subjected to kinetic analysis (Table 1) using the reductive amination of cyclohexanone **4** with allylamine **b** in reactions where the concentration of **b** was varied at constant concentrations of **4** and *vice-versa*.

Table 1. Kinetic constants of WT *At*RedAm and *Ad*RedAm and variants, and the (*S*)-selective IRED [(*S*)-IRED] from *Streptomyces* sp. GF354636,37 for cyclohexanone **4** and allylamine **b**.

|  |  |  |  |
| --- | --- | --- | --- |
| Enzyme variants | *K*m 4(mM) | *k*cat (s-1) | *k*cat/*K*m (s-1 mM-1) x 10-2 |
| *At*RedAm WT | 2.1 ± 0.6 | 0.11 ± 0.01 | 5.2 ± 1.1 |
| *At*RedAm Y183F | 12 ± 4 | 0.10 ± 0.02 | 0.8 ± 0.3 |
| *Ad*RedAm WT | 3.8 ± 0.3 | 2.1 ± 0.1 | 55 ± 5 |
| *Ad*RedAm Y177F | 12 ± 2 | 0.12 ± 0.01 | 1.0 ± 0.2 |
| *Ad*RedAm N94A | 7.6 ± 2.3 | 0.08 ± 0.01 | 1.1 ± 0.3 |
| (*S*)-IRED | 3.0 ± 1.3 | 0.02 ± 0.01 | 0.7 ± 0.4 |
| Conditions: 0.2-60 mM cyclohexanone **4**; 100 mM allylamine **b**; 0.4 mM NADPH, 1% (v/v) dimethylsulfoxide and 5–100 μg of purified *At*RedAm variant in 100 mM Tris-HCl pH 9.0 buffer. The activities of *At*RedAm D175A, N98A and *Ad*RedAm D169A were too low to be measured. | | | |

Overall, wild-type *Ad*RedAm was 7-fold more active than *At*RedAm, but each was significantly more active than the known (*S*)-selective IRED from *Streptomyces* sp. GF3546 [(*S*)-IRED],36,37 previously reported to demonstrate poor reductive amination activity,27 which was 83-fold and 8-fold less active than *Ad*RedAm and *At*RedAm respectively.All the RedAm mutants were disabled with respect to activity (Table 1), but especially D175A (*At*RedAm) and D169A (*Ad*RedAm), for which activities were not measurable. The *K*M values for ketone **4** with the Y183F (*At*RedAm) and Y177F (*Ad*RedAm) mutants were increased approximately six and three-fold respectively, supportive of a role in the binding of the ketone, as suggested by crystallographic observations. *K*M values for the amine were determined by varying the concentration of **b**, and found to be approximately 30 mM for each wild-type RedAm (Supporting Information Table S2), however, the activity of the mutants D175A and N98A (*At*RedAm) and D169A/N98A (*Ad*RedAm) were again too low to provide reliable kinetic data, suggestive of a significant role in amine recognition, especially for the aspartate residues. Reductive aminations of **4** using **b** were carried out using *Ad*RedAm mutantsto corroborate the results of kinetics (Section S10, Table S13). Mutants Y177F and N94A displayed approximately 40% of the wild-type activity after 24 h reaction time, but the D169A mutant was not active.

**Proposed mechanism for fungal RedAms.**

To rationalize the data from the ligand complexes and mutagenesis studies, a mechanism is proposed for *At*RedAm based on that of the enzyme phenylalanine dehydrogenase (PheDH),38 which catalyzes the reductive amination of phenylpyruvate with ammonia. Brunhuber and co-workers established that a residue of high p*K*a, K78, was required to coordinate the carbonyl group of phenylpyruvate, and to protonate the hydroxyl group of a carbinolamine intermediate in the formation of the imine. In addition, a side-chain of low p*K*a, D118, was required to deprotonate ammonia for attack at the carbonyl group to form the carbinolamine. The data reported for *At*RedAm herein support some shared aspects of mechanism between the two enzymes. First, the sequential order of binding of cofactor, ketone and amine to the enzyme, with ordered release of amine product and NADP+, previously determined for *Asp*RedAm27 is the same *Ter*-*Bi* mechanism reported for PheDH.

In the case of RedAms, the carbonyl group of a ketone substrate is coordinated either directly, or through a water molecule, by Y177 (*Asp*RedAm numbering; Scheme 3), as suggested by both the structures and the increase in *K*m upon its mutation to alanine.



**Scheme 3.** Proposed mechanism for reductive amination of prochiral ketones by fungal RedAms. Sequential binding of cofactor, ketone and amine to the *apo-*(**I**) triggers progressive closure of the enzyme active site. The carbonyl group of the substrate is secured by the side-chain of Y177 (*Asp*RedAm numbering) and the amine substrate is bound by the side-chains of N93 and D169 (**II**). D169 deprotonates the amine substrate which then couples to the ketone to give a carbinolamine intermediate (**III**). Elimination of water forms the iminium ion (**IV**), which is then reduced by the NADPH cofactor to give the final amine product (**V**).

Following ketone binding, the amine is deprotonated by the side chain of D169, the interaction between which is again supported by both crystallography and the absence of reductive amination activity in aspartate-alanine mutants at this position. The deprotonated amine may then attack the carbonyl group of the ketone to yield a carbinolamine intermediate. The structure in Figure 2 does not show the amine in position to attack the carbonyl, but this complex was obtained by soaking the ternary complex of (*At*RedAm-NADPH4-**4**) with allylamine **b**, and given the limitations of protein mobility within the crystal, may not represent the true quaternary state *en route* to imine formation. In the next step**,** D169 protonates the hydroxyl group of the intermediate, creating water as a leaving group, as the phenolate of Y177 deprotonates the amine. These events yield the prochiral iminium ion intermediate (IV), which is then reduced by the NADPH hydride at one prochiral face of the C=N bond, yielding the secondary amine product (V).

**Engineering ketone and amine specificity through structure-guided mutagenesis.**

Complexes of *At*RedAm with ketones **2** and **3** revealed a possible role for Y222 in imposing steric constraints on ketone specificity, with this residue observed to reorient to accommodate the longer substrate (Figure 1C and 1D). In order to explore this role, *At*RedAm Y222A was expressed and purified, and applied to the reductive amination of **3** with **b** using 5 equivalents of amine. As predicted, an increase in conversion from 8% using the wild-type, to 19% with the mutant, was observed, presumably as a result of removing the constraint on ketone length, but also the competitor binding site for the ketone carbonyl (Section S11, Table S14). With longer ketones such as 2-octanone **5** and 2-decanone **6**, Y222A performed better than the wild-type, with an increase of 4% to 53% conversion observed in the reaction of **5** with **b**. This phenomenon was further explored through mutation of H215 in *Ad*RedAm to alanine, which occupies the position equivalent to Y222 in *At*RedAm. Reductive aminations of ketones using *Ad*RedAm-H215A were carried out using allylamine **b** as amine partner (Table S15) and this mutant again performed better than the wild-type enzyme. With **3**, an increase in conversion from 37% to 57% was observed, and for 6 a higher conversion was also recorded (up from 18% to 34%). For the reaction of 4-phenyl-2-butanone **7** with **b**, similar conversions were obtained with the wild-type and H215A variant, but with an increase in e.e. of the (*R*)- product from 28% to 86%. Kinetic constants for the *Ad*-H215A mutant were obtained, using 4-phenyl-2-butanone **7** and 2-octanone **5** as substrates, with allylamine **b** as the amine donor at constant concentration (Table S16). For both substrates a decrease in *K*M was observed (from 3.8 mM to 0.8 mM for **5** and from 2.1 to 0.7 mM for **3**) supporting a role for this position in the unproductive binding of substrates as suggested by the *At*RedAm-NADPH-3 complex.

The *At*RedAm-NADPH4-**4**-**b** complex suggested that the amine binding site lined with residues I123 and L96 may also impose constraints on amine binding, explaining the preference of RedAms for small amines with pi-bond character. In order to explore these constraints, mutants N98A, L96A and I123A and double mutant L96A/I123A were created and their specific activity determined against a panel of amines (**b**, **d**-**j**) using cyclohexanone **4** as the ketone substrate in each case (Table S17). *At*RedAmmutants N98A and L96A displayed 2.3 and 4.3 fold lower activity than the wild-type with allylamine respectively. Lower activities were also recorded with these mutants and methylamine **f** and pyrrolidine **i**. However, mutation I123A gave a variant with activity toward **b**, isopropylamine **d**, **f** and even ammonia **g**. In addition the activity towards larger amines was improved, with activity toward hexylamine **j** and pyrrolidine **i** increased 5.5 and 4.0 fold respectively, and even some activity recorded towards aniline, which was not transformed by the wild-type enzyme, suggesting that it may be possible to increase specificity for larger amines in equimolar reductive amination reactions.

**Conclusion**

The importance of chiral secondary amines as building blocks for the synthesis of pharmaceuticals and agrochemicals highlights a potentially key role for engineered RedAms in the preparation of these compounds. RedAms catalyze, in the best cases, the synthesis of chiral secondary amines from ketone and amine substrates provided in a stoichiometric ratio, with associated benefits for the atom economy of these reactions. The identification of ketone and amine binding modes presented suggests that, in cases where the delivery of stoichiometric equivalents of substrates are important, scope exists for engineering RedAms to accommodate a broader range of ketone and amine partners, leading to higher yields and improved or inverted stereochemistry. In the future it is expected that, in addition to comprehensive engineering studies on RedAms, further mechanisms of reductive amination in enzymes of related sequence will be discovered.

ASSOCIATED CONTENT

Supporting Information. Amino acid sequences, PCR primers, gene cloning, expression and protein purification protocols; experimental details for substrate and product synthesis, including NADPH4; biotransformations and analysis of products; Protein crystallization, data collection and refinement statistics can be found in the supporting information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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