

This is a repository copy of *An Engineered Biocatalyst for Enantioselective Hydrazone Reduction*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/225654/>

Version: Published Version

Article:

Grogan, Gideon James orcid.org/0000-0003-1383-7056, Hutton, Amy, Zhao, Fei et al. (7 more authors) (2025) An Engineered Biocatalyst for Enantioselective Hydrazone Reduction. *Angewandte Chemie International Edition*. e202424350. ISSN 1433-7851

<https://doi.org/10.1002/anie.202424350>

Reuse

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:

<https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

A Journal of the Gesellschaft Deutscher Chemiker

Angewandte Chemie

GDCh

International Edition

www.angewandte.org

Accepted Article

Title: An Engineered Biocatalyst for Enantioselective Hydrazone Reduction

Authors: Anthony P. Green, Amy E. Hutton, Fei Zhao, Elizabeth Ho, Jack Domenech, Vanessa Harawa, Murray J. B. Brown, Gideon Grogan, Phillip D. Clayman, and Nicholas J. Turner

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Angew. Chem. Int. Ed.* **2025**, e202424350

Link to VoR: <https://doi.org/10.1002/anie.202424350>

An Engineered Biocatalyst for Enantioselective Hydrazone Reduction

Amy E. Hutton^[a,b], Fei Zhao^[a], Elizabeth Ho^[c], Jack Domenech^[c], Vanessa Harawa^[d], Murray J. B. Brown^[d], Gideon Grogan^{[c]*}, Phillip D. Clayman^{[e]*}, Nicholas J. Turner^{[a]*} and Anthony P. Green^{[a]*}

[a] Dr Amy E. Hutton, Dr Fei Zhao, Prof. Nicholas J. Turner, Prof. Anthony P. Green
Manchester Institute of Biotechnology and Department of Chemistry
University of Manchester
131 Princess Street, Manchester, M1 7DN, UK
E-mail: Nicholas.turner@manchester.ac.uk; Anthony.green@manchester.ac.uk

[b] Dr Amy E. Hutton
Disyn Biotech Ltd.
33 Matlock Close, Great Sankey, Warrington, England, WA5 3PZ, UK

[c] Elizabeth Ho, Jack Domenech, Prof. Gideon Grogan
Department of Chemistry
University of York
Heslington, York, YO10 5DD, UK
Email: Gideon.grogan@york.ac.uk

[d] Dr Vanessa Harawa, Dr Murray J. B. Brown
GSK, Medicine Development & Supply
GSK
GSK Medicines Research Centre, Stevenage, SG1 2NY, UK

[e] Dr Phillip D. Clayman
GSK, Medicine Development & Supply
GSK
Collegeville, PA, USA
Email: Philip.d.clayman@gsk.com

Supporting information for this article is given via a link at the end of the document.

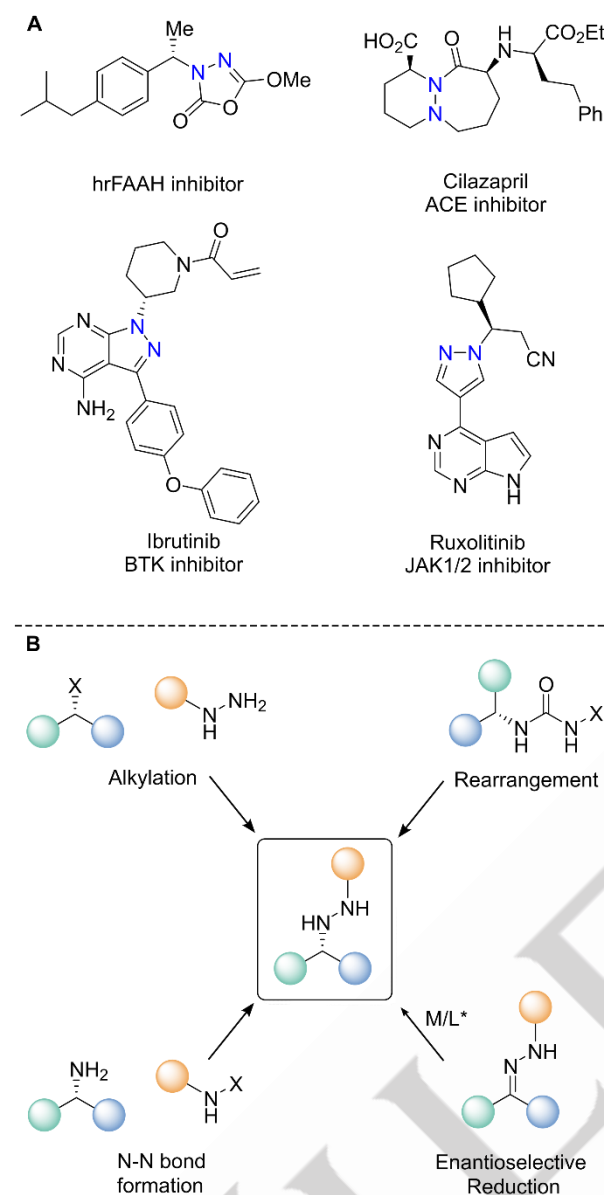
Abstract: Enantioselective reduction of hydrazones provides a convergent and versatile route to synthesize hydrazine-containing motifs that are commonly found in pharmaceuticals and agrochemicals. However current methods require the use of precious metals, costly chiral ligands and/or forcing reaction conditions. Here, we report the development of a biocatalytic approach for enantioselective hydrazone reduction using engineered imine reductases. Following evaluation of an in-house panel of >400 IRED sequences, we identified a single IR361 I127F L179V variant that promotes reduction of Cbz-protected hydrazones. Introduction of an additional two mutations *via* directed evolution afforded HRED1.1 that is 20-fold more active than the parent template and promotes reduction of a variety of protected hydrazones in high yields and selectivities (>99% *e.e.*), including in preparative scale biotransformations. Structural analysis of HRED1.1 provides insights into the origins of its unique hydrazone reductase activity. This study offers a powerful biocatalytic route to synthesize valuable chiral hydrazine products and further expands the impressive range of transformations accessible with engineered imine reductases.

Chiral hydrazines are key structural motifs that are found in many natural products, agrochemicals and clinically relevant pharmaceuticals (Scheme 1A).^[1–5] These N-N bond containing compounds can be synthesized from chiral precursors using a

variety of methods (Scheme 1B), including alkylation of hydrazines, N-N bond formation using chloramines and rearrangement of halogenated ureas.^[6–11] Recently, asymmetric hydrogenation of hydrazones has emerged as a valuable alternative which doesn't rely on the use of optically enriched substrates.^[12–21] Although powerful, this approach requires the use of transition metal catalysts involving costly chiral ligands along with precious and/or toxic metals. Furthermore, these transformations typically require forcing reaction conditions, including elevated temperatures and high pressures of hydrogen, which hinders their broader application.

Increasingly, biocatalysis is viewed as an attractive alternative to more traditional synthetic methods due to the high catalytic efficiencies and exacting specificities associated with enzymes. Furthermore, the development of powerful enzyme engineering techniques such as directed evolution allows the properties of biocatalysts to be adapted to meet target applications, including adaption of substrate preferences, improvements in kinetic parameters and enhanced stability under process conditions.^[22–24] Imine reductases (IREDs) are one such enzyme class that have become widely adopted as biocatalysts for the preparation of chiral amines.^[25–31] These nicotinamide adenine dinucleotide phosphate-dependent enzymes catalyze enantioselective reductions of prochiral imines to a diverse array of amine products,

COMMUNICATION



Scheme 1. A. Representative compounds containing chiral hydrazine motifs. **B.** Synthetic strategies towards chiral hydrazines.

making them attractive targets for industrial biocatalysis. For example, engineered IREDs have been developed for the synthesis of the chiral LSD1 inhibitor GSK2879552^[32] and for the manufacture of the JAK1 inhibitor Acrocitinib.^[33]

Although hydrazones have been generated using an artificially designed enzyme^[34] and have been shown to be substrates for transaminases,^[35] reports of enzymatic hydrazone reduction are scarce. To our knowledge there are only two reported cases involving (i) the selective coupling of 3-cyclopentyl-3-oxopropanenitrile with hydrazine hydrate,^[36] and (ii) a preliminary study using the IRED from *Myxococcus stipitatus*, although no data on conversion or enantioselectivity was provided.^[37] Given the significant safety concerns associated with the use of

hydrazine hydrate as a reagent for large scale synthesis,^[38] together with a need to identify enzymes capable of reducing C=N bonds in a broader range of functional groups, there remains considerable interest in developing more broadly applicable biocatalytic hydrazone reduction methodologies for applications in asymmetric synthesis. In addition to improved safety, the protected hydrazine products are commonly desired as they facilitate downstream synthetic manipulations. Here we report the discovery and engineering of a hydrazine reductase (HRED) biocatalyst that furnishes a variety of chiral hydrazine products with high levels of stereocontrol.

We selected biocatalytic reduction of hydrazone **1**, derived from condensation of commercial (benzyloxycarbonyl)hydrazine (Cbz-hydrazine) and acetophenone, as an initial synthetic target (Figure 1A). To identify a suitable starting template for enzyme engineering, an in-house panel of >400 IREDs was evaluated for activity towards the target transformation. Biotransformations were performed using cell-free extracts along with an established NADPH cofactor recycling system using glucose dehydrogenase (GDH). Of the enzymes tested, only one IRED sequence was identified with observable activity towards the conversion of **1** to **1a**, namely an I127F L179V double mutant of IR361 (henceforth named HRED1.0) that was recently engineered by our lab for reductive amination processes involving cyclic secondary amines as substrates.^[39] Notably, neither the wild-type IRED361, nor the variants with a single mutation, I127F or L179V, displayed any observable activity under these assay conditions.

To enhance hydrazone reduction activity, HRED1.0 was subjected to a single round of evolutionary optimization. We targeted 22 positions within the active site, including those in close proximity to the NADPH cofactor and in the putative substrate-binding pocket, which were individually randomized using NNK degenerate codons. Individual library variants were arrayed in 96-well plates and evaluated as clarified cell lysate using a UPLC assay monitoring the conversion of **1** to **1a**. Analysis of >2000 variants led to the identification of four point mutations (M125F, M125W, H250L, A100T) displaying increased activity. Substitution of Met 125 to either Phe or Trp gave a substantial 6-fold and 5-fold increases in reaction conversion, respectively, under assay conditions used for evolution (Figure 1C and Table S1), with more modest ca. 1.5-fold improvements observed with the H250L and A100T mutations (Figure S1 and Table S1). These beneficial mutations were subsequently combined by DNA shuffling, leading to the identification of a M125W H250L double mutant (HRED1.1) which displays a significant 20-fold improvement in reaction conversion, and a 19-fold improvement in reaction rate, over the parent HRED1.0 template (Figures 1B and 1C and Figure S2 and Table S1).

Using HRED1.1 as a biocatalyst, complete conversion of **1** (5 mM) to **1a** can be achieved within 18 h (Figure 2 and Table S1). For comparison, under identical assay conditions only 5% conversion is observed with HRED1.0. Furthermore, HRED1.1 displays exceptional levels of stereocontrol, affording the (*R*)-enantiomer of **1a** in >99% e.e. (Figure 1D). The synthetic utility of HRED1.1 was demonstrated in a preparative scale biotransformation to yield 123 mg of (*R*)-**1a** (98% conversion, 92% isolated yield)(Table S1). Optimization of reaction conditions (Figures S3-

COMMUNICATION

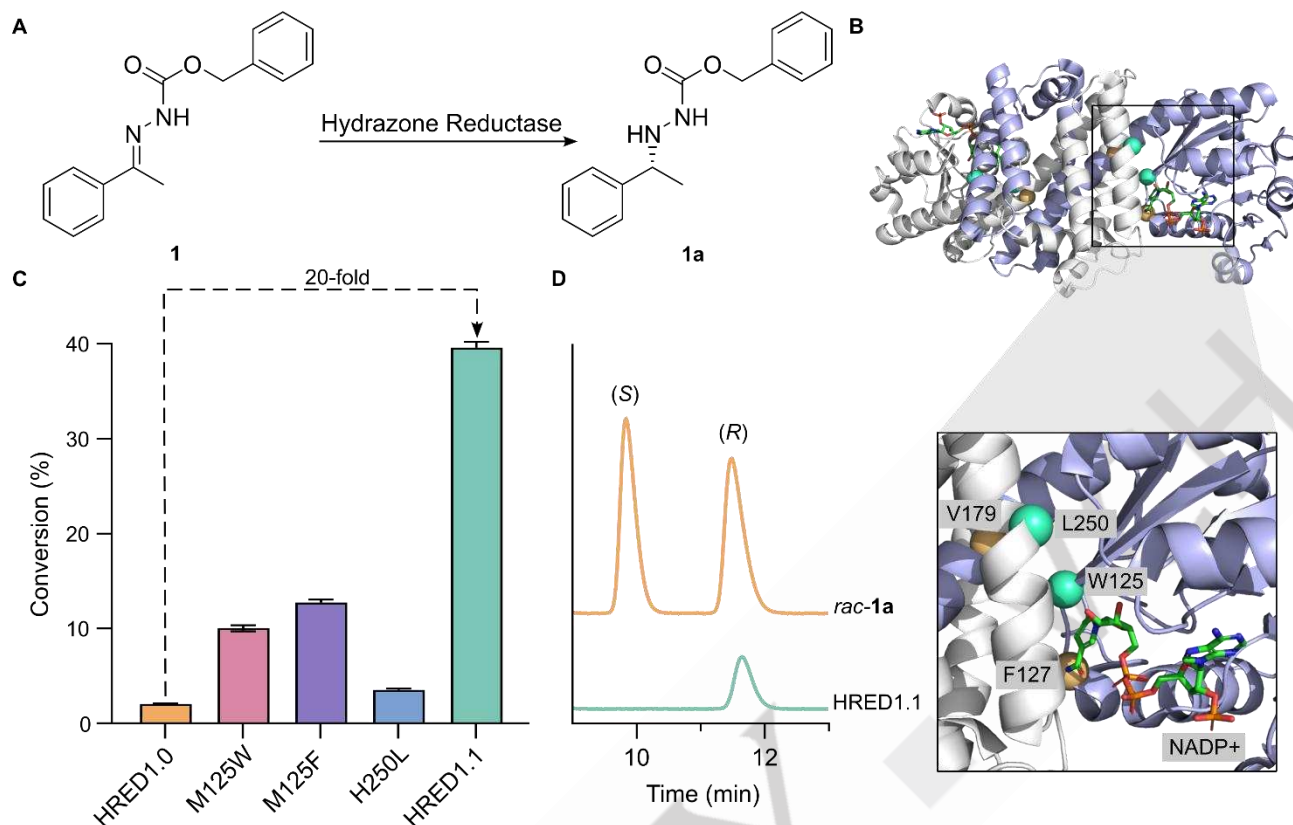


Figure 1. A. Target transformation for the reduction of hydrazone **1** into chiral hydrazine **1a**. B. Structure showing the amino acid positions mutated in HRED1.1. The mutations introduced are represented as spheres at the C α mapped onto the crystal structure of the homo-dimer IR361 (PDB: 7OSN).^[40] I127F and L179V mutated in IR361 to afford HRED1.0 are in orange, M125W and H250L to afford HRED1.1 are in green. The NADPH cofactor is shown as atom-coloured sticks with green carbons. C. Bar chart showing the improvement in activity achieved with selected point mutations to HRED1.0 and the combination of two mutations to afford HRED1.1. Biotransformations were performed using **1** (5 mM) and enzyme (25 μ M) in 100 mM KPi pH 7.0 with NADP⁺ (500 μ M), GDH (1 mg/mL), D-glucose (50 mM) and 5% (v/v) DMSO as a cosolvent. Reactions were analyzed following 25 h incubation at 30 °C. Error bars represent the standard deviation of measurements made in triplicate. D. Chiral HPLC trace of *rac*-**1a** and the product of a biotransformation of HRED1.1 under the same conditions as Figure 1C.

9) afforded a 10-fold reduction in NADP⁺ and GDH-901 concentrations compared to those used during evolution without significant reduction in conversion. The high yields achieved by HRED1.1 are particularly notable given that the biotransformations are heterogeneous due to the low solubility of both hydrazone **1** and hydrazine **1a**. Beyond reduction of preformed hydrazones, HRED1.1 can also achieve reductive coupling of acetophenone and Cbz-hydrazine with modest conversions and exceptional stereocontrol (Figure 2).

To further explore its synthetic utility, HRED1.1 was evaluated for activity towards a range of protected hydrazones **1–11** (Figure 2 and Table S2). In all cases, HRED1.1 achieves exceptional levels of stereocontrol (>99% e.e.). Derivatives of **1** containing *ortho*-, *meta*- or *para*-fluoro substituents (**2–4**) are all well tolerated, albeit with reduced conversions achieved with the *para*-derivative **4**. Analogues **5** and **6** containing pharmaceutically relevant pyridyl motifs are also substrates for HRED1.1.^[41,42] Substrates containing larger aryl-substituents are not accommodated by HRED1.1 due to steric constraints within the active site pocket (Figure S10). Notably the scope of HRED1.1 is not limited to aromatic hydrazones, with substrates **7** and **8**, derived from the

aliphatic ketones pentanone and *tert*-butyl methyl ketone respectively, efficiently converted with high yields. Finally, we explored the activity of HRED1.1 with a selection of substrates derived from commercial hydrazine reagents featuring orthogonal benzoyl (**9–10**) and Boc (**11**) protecting groups. Importantly, these modifications are also well tolerated by HRED1.1, thus further expanding the synthetic utility of our hydrazone reductase biocatalyst.

The improved ability of HRED1.1 to accept the hydrazone **1** was investigated using X-ray crystallography and modelling. The structure was determined to a resolution of 2.77 Å, with two molecules, A and B, in the asymmetric unit comprising one dimer. The structure was very similar overall to that of wild-type IR361 (PDB 7OSN)^[40] displaying the canonical IRED fold in which an N-terminal Rossmann fold domain is attached to a C-terminal helical bundle through a long interdomain helix. Two active sites are formed at the interfaces of the N-terminal domain of one monomer and the C-terminal bundle of the other through reciprocal domain sharing. The crystals contained a large proportion of water (71%) and the modest resolution was accompanied by poor electron density in the N-terminal domain of monomer B, leading to some

COMMUNICATION

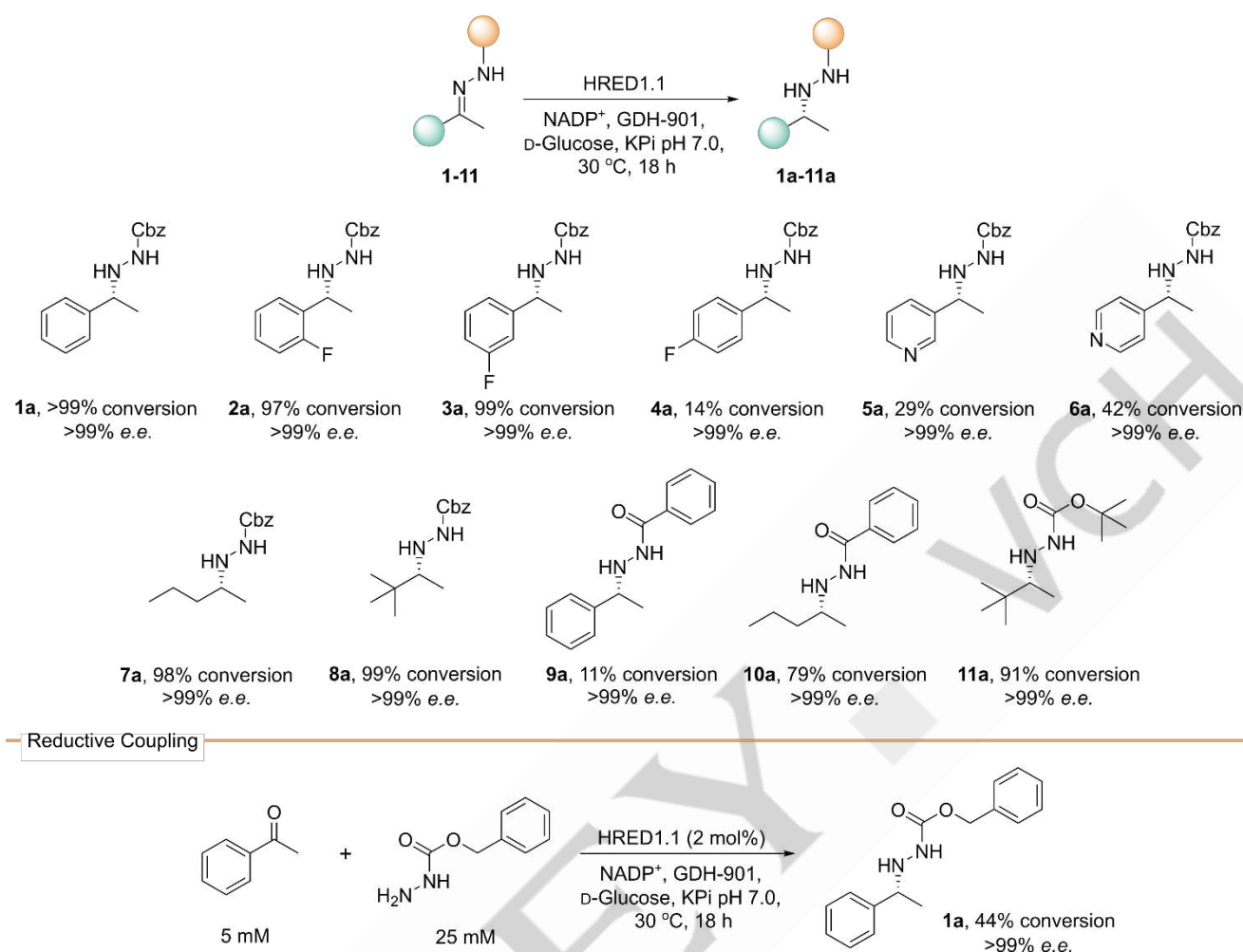


Figure 2. HRED1.1 promotes the reduction of a range of substrates with high conversions and excellent selectivity. Biotransformations were performed using substrate (5 mM) and HRED1.1 (2-5 mol%) in 100 mM KPi pH 7.0 with NADP⁺ (500 μM), GDH (1 mg/mL), D-glucose (50 mM) and 5% (v/v) DMSO as a cosolvent. Reactions were analyzed following 18 h incubation at 30 °C. The absolute stereochemistry of **1a** was assigned by comparison to optical rotation values reported in the literature.^[7] The stereochemistry of **2a-8a** and **10a-11a** was assigned by analogy to HRED1.1-derived (*R*)-**1a**. The stereochemistry of **9a** was assigned by comparison of HPLC retention times to those reported in reference 45. HRED1.1 (2 mol%) promotes the reductive coupling between acetophenone (5 mM) and benzyloxycarbonylhydrazine (25 mM) with modest conversion and exceptional stereocontrol.

zero-occupancy residues and many side-chains not being modelled in that region. However, the density within monomer A and the C-terminal bundle of B was good, permitting the building of side chains and an NADP⁺ molecule within the active site at the interface of the domains. Omit density for the more structurally consequential mutations (M125W and I127F) was also clear (Figure S11). Superimposition of the HRED1.1 structure with that of wild-type IR361 revealed the consequences of the mutations (Figure 3A). The substitution of I for F in position 127 at the bottom of the active site as presented in Figure 3A has, counterintuitively, made more space in the active site through the displacement of the side chain of (B)Y221. Mutation of M125 to W at the top of the active site has again created more space, despite being a larger residue, as it projects upwards, and makes a new stabilizing interaction with the side chain of D176 through the N-atom on the indole ring. Further stabilization of this conformation may be provided by a stacking interaction between the indole and the

mutated 179 position from L to V, which lies on the interdomain helix on the other side of the active site cavity. (B)H250L is situated at the interface between the N-terminal domain of one monomer and the C-terminal bundle of its neighbour, and may provide stabilizing hydrophobic interactions in a mode that has been observed for other IREDs in engineering studies.^[29,32,33,43] The structure of HRED1.1 was modelled with the substrate **1** (Figure 3B) using Autodock VINA.^[44] One of the lowest energy conformations positioned the ligand with the electrophilic carbon of the C=N bond at a suitable approximate distance (~3.8 Å) from the C4 of the nicotinamide ring of NADP⁺ to receive a hydride, and on the correct *si* prochiral face to furnish the experimentally observed (*R*)-enantiomer of **1a**. The Cbz-group was observed to project backwards into the active site into the space made accessible by the M125W and I127F mutations described above. The F127 residue may also participate in productive π-π interactions with the Cbz aromatic ring. It appears therefore that

COMMUNICATION

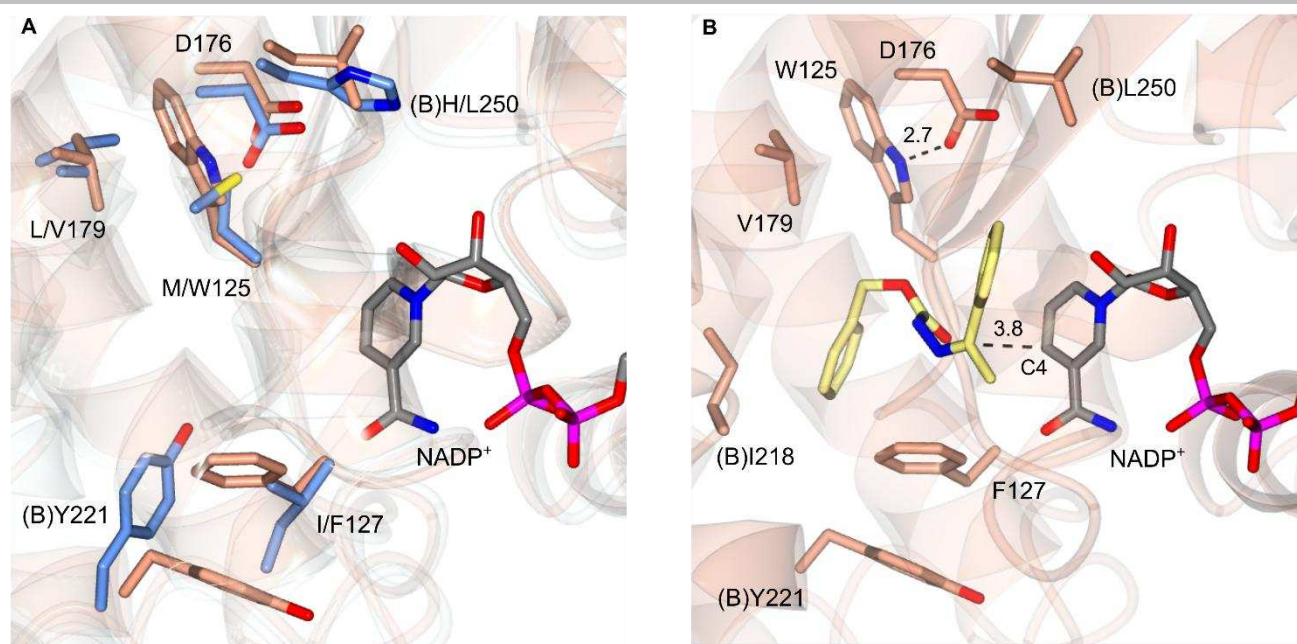


Figure 3. Crystal structure and active site residues of HRED1.1. **A.** Superimposition of active sites of wild-type IR361 (PDB 7OSN)^[40] with HRED1.1. Carbon atoms are in blue and pink respectively. **B.** Active site of HRED1.1 modelled with hydrazone **1** (carbon atoms in yellow). Selected interaction is shown as a black dashed line with the distance in Ångstroms.

the improved activity of the mutant towards **1** may derive from better accommodation of the Cbz group.

In summary, we have established a biocatalytic platform for selective reduction of hydrazones to afford valuable chiral hydrazine motifs that are common precursors for pharmaceutical and agrochemical synthesis. Our analysis of >400 IRED sequences suggests that hydrazone reduction is a rare activity within the imine reductase family. Nevertheless, introduction of only a handful of mutations into IR361 has led to the development of an engineered HRED biocatalyst that achieves exceptional selectivities across a range of hydrazone substrates, with high reaction yields also achievable with elevated enzyme loadings (2 mol%). Moving forward, more extensive engineering will undoubtedly deliver more potent HRED biocatalysts, including those that are tailored towards specific target products and/or adapted to operate under viable process conditions. In contrast to existing methods of hydrazone reduction, our biocatalytic approach is not reliant on precious/toxic metals, costly ligands or forcing reaction conditions, and therefore offers a more sustainable strategy for synthesizing valuable chiral N-N containing products.

Supporting Information

The authors have cited additional references within the Supporting Information.^[46–53]

Acknowledgements

We are grateful to the Future Biomanufacturing Hub (EP/S01778X/1), the Henry Royce Institute for Advanced Materials (financed through EPSRC grant nos. EP/R00661X/1, EP/S019367/1, EP/P025021/1 and EP/P025498/1) for access to their facilities, Manchester SYNBIOCHEM Centre (BB/M017702/1) and to M. Dunstan (Manchester Institute of Biotechnology) for guidance on automating directed-evolution workflows. We thank Mr Sam Hart and Dr Johan P. Turkenburg for assistance with X-ray data collection, and the Diamond Light Source Didcot UK for access to beamline I03 under proposal mx32736.

Keywords: Biocatalysis, Directed Evolution, Hydrazines, Protein Engineering, Oxidoreductases

- [1] L. M. Blair, J. Sperry, *J. Nat. Prod.* **2013**, 794–812.
- [2] J. Z. Patel, T. Parkkari, T. Laitinen, A. A. Kaczor, S. M. Saario, J. R. Savinainen, D. Navia-Paldanius, M. Cipriano, J. Leppänen, I. O. Koshevoy, A. Poso, C. J. Fowler, J. T. Laitinen, T. Nevalainen, *J. Med. Chem.* **2013**, 56, 8484–8496.
- [3] D. L. Hughes, *Org. Process Res. Dev.* **2016**, 20, 1855–1869.
- [4] G. Le Goff, J. Ouazzani, *Bioorg. Med. Chem.* **2014**, 22, 6529–6544.
- [5] G. R. Humphrey, J. T. Kueth, *Chem. Rev.* **2006**, 106, 2875–2911.
- [6] U. Ragnarsson, *Chem. Soc. Rev.* **2001**, 30, 205–213.
- [7] P. Yang, C. Zhang, Y. Ma, C. Zhang, A. Li, B. Tang, J. S. Zhou, *Angew. Chem. Int. Ed.* **2017**, 56, 14702–14706.
- [8] D. E. Polat, D. D. Brzezinski, A. M. Beauchemin, *Org. Lett.* **2019**, 21, 4849–4852.

COMMUNICATION

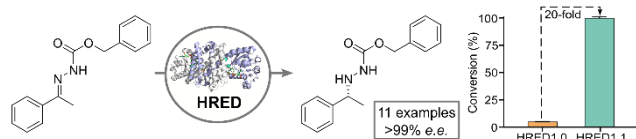
- [9] Q. Guo, Z. Lu, *Synthesis* **2017**, 49, 3835–3847.
- [10] A. Bredihhin, U. M. Groth, U. Mäeorg, *Org. Lett.* **2007**, 9, 1097–1099.
- [11] A. Bhattacharya, N. C. Patel, R. E. Plata, M. Peddicord, Q. Ye, L. Parlanti, V. A. Palaniswamy, J. A. Grosso, *Tetrahedron Lett.* **2006**, 47, 5341–5343.
- [12] C. H. Schuster, J. F. Dropinski, M. Shevlin, H. Li, S. Chen, *Org. Lett.* **2020**, 22, 7562–7566.
- [13] B. Li, D. Liu, Y. Hu, J. Chen, Z. Zhang, W. Zhang, *European J. Org. Chem.* **2021**, 2021, 3421–3425.
- [14] D. Fan, Y. Hu, F. Jiang, Z. Zhang, W. Zhang, *Adv. Synth. Catal.* **2018**, 360, 2228–2232.
- [15] Q. Hu, Y. Hu, Y. Liu, Z. Zhang, Y. Liu, W. Zhang, *Chem. Eur. J.* **2017**, 23, 1040–1043.
- [16] Z. P. Chen, S. B. Hu, M. W. Chen, Y. G. Zhou, *Org. Lett.* **2016**, 18, 2676–2679.
- [17] Z. P. Chen, S. B. Hu, J. Zhou, Y. G. Zhou, *ACS Catal.* **2015**, 5, 6086–6089.
- [18] K. Yu, W. Meng, X. Feng, H. Du, *Org. Lett.* **2023**, 25, 3607–3610.
- [19] M. Chang, S. Liu, K. Huang, X. Zhang, *Org. Lett.* **2013**, 15, 4354–4357.
- [20] Y. Hu, Z. Zhang, J. Zhang, Y. Liu, I. D. Gridnev, W. Zhang, *Angew. Chem. Int. Ed.* **2019**, 58, 15767–15771.
- [21] M. J. Burk, J. E. Feaster, *J. Am. Chem. Soc.* **1992**, 6266–6267.
- [22] A. O'Connell, A. Barry, A. J. Burke, A. E. Hutton, E. L. Bell, A. P. Green, E. O'Reilly, *Chem. Soc. Rev.* **2024**, 53, 2828–2850.
- [23] E. L. Bell, W. Finnigan, S. P. France, A. P. Green, M. A. Hayes, L. J. Hepworth, S. L. Lovelock, H. Niikura, S. Osuna, E. Romero, K. S. Ryan, N. J. Turner, S. L. Flitsch, *Nature Reviews Methods Primers* **2021**, 1, DOI 10.1038/s43586-021-00044-z.
- [24] S. Wu, R. Snajdrova, J. C. Moore, K. Baldenius, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, 60, 88–119.
- [25] J. R. Marshall, P. Yao, S. L. Montgomery, J. D. Finnigan, T. W. Thorpe, R. B. Palmer, J. Mangas-Sanchez, R. A. M. Duncan, R. S. Heath, K. M. Graham, D. J. Cook, S. J. Charnock, N. J. Turner, *Nat. Chem.* **2021**, 13, 140–148.
- [26] J. Mangas-Sanchez, S. P. France, S. L. Montgomery, G. A. Aleku, H. Man, M. Sharma, J. I. Ramsden, G. Grogan, N. J. Turner, *Curr. Opin. Chem. Biol.* **2017**, 37, 19–25.
- [27] M. Lenz, N. Borlinghaus, L. Weinmann, B. M. Nestl, *World J. Microbiol. Biotechnol.* **2017**, 33, DOI 10.1007/s11274-017-2365-8.
- [28] A. K. Gilio, T. W. Thorpe, N. Turner, G. Grogan, *Chem. Sci.* **2022**, 13, 4697–4713.
- [29] A. K. Gilio, T. W. Thorpe, A. Heyam, M. R. Petchey, B. Pogranyi, S. P. France, R. M. Howard, M. J. Karmilowicz, R. Lewis, N. Turner, G. Grogan, *ACS Catal.* **2023**, 13, 1669–1677.
- [30] J. Citoler, V. Harawa, J. R. Marshall, H. Bevinakatti, J. D. Finnigan, S. J. Charnock, N. J. Turner, *Angew. Chem. Int. Ed.* **2021**, 60, 24456–24460.
- [31] G. D. Roiban, M. Kern, Z. Liu, J. Hyslop, P. L. Tey, M. S. Levine, L. S. Jordan, K. K. Brown, T. Hadi, L. A. F. Ihnken, M. J. B. Brown, *ChemCatChem* **2017**, 9, 4475–4479.
- [32] M. Schober, C. MacDermid, A. A. Ollis, S. Chang, D. Khan, J. Hosford, J. Latham, L. A. F. Ihnken, M. J. B. Brown, D. Fuerst, M. J. Sanganee, G. D. Roiban, *Nat. Catal.* **2019**, 2, 909–915.
- [33] R. Kumar, M. J. Karmilowicz, D. Burke, M. P. Burns, L. A. Clark, C. G. Connor, E. Cordi, N. M. Do, K. M. Doyle, S. Hoagland, C. A. Lewis, D. Mangan, C. A. Martinez, E. L. McInturf, K. Meldrum, R. Pearson, J. Steflik, A. Rane, J. Weaver, *Nat. Catal.* **2021**, 4, 775–782.
- [34] L. Ofori Atta, Z. Zhou, G. Roelfes, *Angew. Chem. Int. Ed.* **2023**, 62, e202214191.
- [35] E. M. Carter, F. Subrizi, J. M. Ward, T. D. Sheppard, H. C. Hailes, *ChemCatChem* **2021**, 13, 4520–4523.
- [36] A. Huang, X. Zhang, Y. Yang, C. Shi, B. Zhang, X. Tuo, P. Shen, X. Jiao, N. Zhang, *J. Org. Chem.* **2024**, 89, 11446–11454.
- [37] N. Borlinghaus, D. Calabrese, L. Lauterbach, B. M. Nestl, *ChemBioChem* **2024**, DOI 10.1002/cbic.202400700.
- [38] J. K. Niemeier, D. P. Kjell, *Org. Process Res. Dev.* **2013**, 17, 1580–1590.
- [39] A. J. Burke, T. M. Lister, J. R. Marshall, M. J. B. Brown, R. Lloyd, A. P. Green, N. J. Turner, *ChemCatChem* **2023**, 15, e202300256.
- [40] V. Harawa, T. W. Thorpe, J. R. Marshall, J. J. Sangster, A. K. Gilio, L. Pirvu, R. S. Heath, A. Angelastro, J. D. Finnigan, S. J. Charnock, J. W. Nafie, G. Grogan, R. C. Whitehead, N. J. Turner, *J. Am. Chem. Soc.* **2022**, 144, 21088–21095.
- [41] A. R. Dwivedi, S. Jaiswal, D. Kukkar, R. Kumar, T. G. Singh, M. P. Singh, A. M. Gaidhane, S. Lakhanpal, K. N. Prasad, B. Kumar, *RSC Med. Chem.* **2024**, DOI 10.1039/d4md00632a.
- [42] S. De, A. Kumar S K, S. K. Shah, S. Kazi, N. Sarkar, S. Banerjee, S. Dey, *RSC Adv.* **2022**, 12, 15385–15406.
- [43] E. J. Ma, E. Sirola, C. Moore, A. Kummer, M. Stoeckli, M. Faller, C. Bouquet, F. Eggimann, M. Ligibel, D. Huynh, G. Cutler, L. Siegrist, R. A. Lewis, A. C. Acker, E. Freund, E. Koch, M. Vogel, H. Schlingensiepen, E. J. Oakeley, R. Snajdrova, *ACS Catal.* **2021**, 11, 12433–12445.
- [44] J. Eberhardt, D. Santos-Martins, A. F. Tillack, S. Forli, *J. Chem. Inf. Model* **2021**, 61, 3891–3898.
- [45] T. Chen, Y. Hu, X. Tang, Y. Zou, L. Wei, Z. Zhang, W. Zhang, *Org. Lett.* **2024**, 26, 769–774.
- [46] K. E. Atkin, R. Reiss, N. J. Turner, A. M. Brzozowski, G. Grogan, *Acta Crystallogr. Sect. F. Struct. Biol. Cryst. Commun.* **2008**, 64, 182–185.
- [47] W. Kabsch, *Acta Crystallogr. D. Biol. Crystallogr.* **2010**, 66, 125–132.
- [48] P. Evans, in *Acta Crystallogr. D. Biol. Crystallogr.*, **2006**, pp. 72–82.
- [49] G. Winter, *J. Appl. Crystallogr.* **2010**, 43, 186–190.
- [50] A. Vagin, A. Teplyakov, *J. Appl. Crystallogr.* **1997**, 30, 1022–1025.
- [51] P. Emsley, K. Cowtan, *Acta Crystallogr. D. Biol. Crystallogr.* **2004**, 60, 2126–2132.

COMMUNICATION

[52] G. N. Murshudov Alexe, A. Vagin, E. J. Dodson, *Acta Crystallogr. Sect. D. Biol.*, **1997**, 53, 240-255.

[53] F. Long, R. A. Nicholls, P. Emsley, S. Gražulis, A. Merkys, A. Vaitkus, G. N. Murshudov, *Acta Crystallogr. D. Struct. Biol.* **2017**, 73, 112–122.

Entry for the Table of Contents



Here we report the discovery and engineering of a hydrazone reductase (HRED) biocatalyst that promotes efficient reduction of protected hydrazones with exceptional stereocontrol. These biotransformations offer a sustainable strategy for synthesizing valuable chiral N-N containing products that are commonly found in pharmaceuticals and agrochemicals.