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Biocatalytic Reduction of Activated Cinnamic Acid Derivatives

Asymmetric reduction of C=C double bonds using Johnson Matthey enzymes

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The asymmetric reduction of C=C double bonds is a sought-after chemical transformation to obtain chiral molecules used in the synthesis of fine chemicals. Biocatalytic C=C double bond reduction is a particularly interesting transformation complementary to more established chemocatalytic methods. The enzymes capable of catalysing this reaction are called ene-reductases (ENEs). For the reaction to take place, ENEs need an electron withdrawing group (EWG) in conjugation with the double bond. Especially favourable EWGs are carbonyls and nitro groups; other EWGs, such as carboxylic acids, esters or nitriles, often give poor results. In this work, a substrate engineering strategy is proposed whereby a simple transformation of the carboxylic acid into a fluorinated ester or a cyclic imide allows to increase the ability of ENEs to reduce the conjugated double bond. Up to complete conversion of the substrates tested was observed with enzymes ENE-105 and *ENE-69.

1. Introduction

The use of enzymes for the asymmetric reduction of activated C=C double bonds can be a viable and straightforward alternative to asymmetric hydrogenation. Traditionally, whole cell microorganisms were used for this purpose but a recent increase in the number of isolated and characterised ENEs means that recombinantly-expressed enzyme preparations are now generally favoured over whole cells, as a number of recent publications demonstrate (1–10).

Double bond 'activation' to facilitate ENEs mediated reduction can be achieved in many cases by alpha substituted functional groups including aldehydes, ketones or nitro moieties. Carboxylate derivatives (such as esters, lactones and anhydrides) can also act as activating groups but their ability to sufficiently activate the C=C bond in the absence of other groups is less evident (11, 12). The traditional approach in these cases is to turn to chemocatalytic hydrogenation (see (13–15) for reviews focused on industrial applications). Herein we describe a new approach to activate α,β-unsaturated carboxylic acids for the reduction with ENEs using a substrate engineering approach.

2. Experimental

2.1 General

All reagents and solvents were purchased from Sigma-Aldrich and Alfa Aesar, Thermo Fisher Scientific. They were of the highest available purity and were used without further purification. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded using a Bruker 400 MHz Avance III HD equipped with SMART probe (Bruker Corporation, USA) where spectra are referenced to deuterated chloroform ($CDCl_3$) 7.26 ppm, shifts are recorded in parts per million and *J* values in hertz. The NMR results can be found in the Supplementary Information.

2.2 Enzyme Preparations

Genes coding for Johnson Matthey, ENEs (ENE-101, ENE-102, ENE-103, ENE-104, ENE-105, *ENE-69 and GDH-101) were ordered codon-optimised from GeneArt (Thermo Fisher Scientific) and cloned into T5 vector pJEx401 (ATUM). Enzymes were expressed recombinantly in *Escherichia coli* BL21 in both shake flasks and fed batch fermentations, whereby induction was carried out with isopropyl β-D thiogalactopyranoside (IPTG) at 30°C. Harvested biomass was resuspended in 100 mM potassium phosphate buffer (pH 7) and cells were broken up either by sonication or homogenisation. The so-obtained cell lysate was clarified by centrifugation and filtrated prior to lyophilisation. Protein expression was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and chromatographic activity assays.

Enzymes ERED-103, ERED-110, ERED-112, ERED-207, ERED-P1-A04, ERED-P1-E04 and ERED-P1-H09 were purchased from Codexis.

2.3 2,2,2-Trifluoroethyl Cinnamate (3a) and 3-Phenyl-Acrylic Acid 2,2,2-Trifluoro-1-Trifluoromethyl-Ethyl Ester (5a)

Cinnamic acid **1a** (5 g, 33.75 mmol) and oxalyl chloride (2.85 ml, 33.75 mmol) in dichloromethane (5 ml) were stirred at 25°C for 2 h before adding the fluorinated alcohol-trifluoro ethanol for **3a** (2.47 ml, 33.75 mmol) and 1,1,1,3,3,3-hexafluoropropan-2-ol for **5a** (3.50 ml, 33.75 mmol). The reaction was then stirred at room temperature overnight before being quenched by addition of saturated aqueous $NaHCO_3$ (20 ml) and extracted with dichloromethane (2 × 20 ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the corresponding fluorinated esters **3a** and **5a** in quantitative yield.

2.4 3-Phenyl-Acrylic Acid 2,2,3,3,4,4,4-Heptafluoro-Butyl Ester (6a) and (Perfluorophenyl)Methyl Cinnamate (7a)

Cinnamoyl chloride (0.75 g, 4.50 mmol) and the corresponding fluorinated alcohols – 2,2,3,3,4,4,4-heptafluorobutan-1-ol for **6a** (0.98 g, 4.50 mmol) and pentafluoro benzyl alcohol for **7a** (0.89 g, 4.50 mmol) – in dichloromethane (2.5 ml) were stirred at room temperature overnight. The reaction was then quenched by addition of saturated aqueous $NaHCO_3$ (20 ml) and extracted with dichloromethane (2 × 20 ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the corresponding fluorinated esters **6a** and **7a** in 95% to 99% yield.

2.5 1-Cinnamoylpyrrolidin-2-one (9a)

Cinnamoyl chloride (5 g, 30.01 mmol), pyrrolidinone (2.3 ml, 36.01 mmol) and triethylamine (13 ml, 90.03 mmol) in dichloromethane (50 ml) were stirred at room temperature overnight. The reaction was quenched by addition of water (20 ml), the organic layer was separated and washed with saturated aqueous NaCl (20 ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford **9a** in 81% yield.

2.6 3-Cinnamoyloxazolidin-2-one (8a)

Cinnamic acid **1a** (5 g, 33.56 mmol) and oxalyl chloride (2.85 ml, 33.56 mmol) in dichloromethane (5 ml) were stirred at room temperature overnight before removing the solvent under reduced pressure. The reaction crude was dissolved in anhydrous tetrahydrofuran (THF) (20 ml) and *n*-butyllithium (1.6 M in hexane, 21 ml, 33.56 mmol, one equivalent) was added dropwise over 30 min. The cinnamoyl chloride solution was then added dropwise to a solution of oxazolidinone (2.92 g, 33.56 mmol) in anhydrous THF (100 ml) at 0°C before stirring at room temperature overnight. The reaction was quenched with water (50 ml), extracted with ethyl acetate (EtOAc) (2 × 100 ml), washed with saturated aqueous $NaHCO_3$ (20 ml) and saturated aqueous NaCl (20 ml). The solvent was removed under reduced pressure and the solid was recrystallised from a 1:1 mixture EtOAc:heptane (20 ml). The solid was filtered and washed with hexane (10 ml) to give crystals of **8a** in 80% yield.

2.7 (*E*)-1-(2-Methyl-3-Phenylacryloyl)Pyrrolidin-2-one (10a) and (*E*)-1-(2,3-Diphenylacryloyl)Pyrrolidin-2-one (11a)

(*E*)-2-methyl-3-phenylacrylic acid (5 g, 30.86 mmol) was converted to the corresponding

acid chloride by addition of oxalyl chloride (1.4 ml, 30.86 mmol) in dichloromethane (5 ml). The reaction was stirred at room temperature for 3 h. Pyrrolidinone (2.82 ml, 37.03 mmol) and triethylamine (13 ml, 92.58 mmol) were added before stirring the reaction overnight. The reaction was quenched by addition of water (20 ml) and saturated aqueous NaCl (20 ml). The solvent was removed under reduced pressure and the solid was dissolved in EtOAc and treated with activated charcoal (1 g), filtered through Celite® and concentrated. The solid was recrystallised from heptane (10 ml) to give **10a** in 55% yield.

Following an identical procedure, **11a** was synthesised in 53% yield from (*E*)-2,3-diphenylacrylic acid (10 g, 44.64 mmol).

2.8 Small Scale Screening Reactions

Substrates **1a–9a** (0.025 mmol) and enzymes ENE-101, ENE-102, ENE-103, ENE-104, ENE-105 or *ENE-69 (2.5 mg), were added to reaction vials containing 500 µl of aqueous media at pH 7 (250 mM potassium phosphate buffer pH 7, 1.1 mM $NAD(P)^+$, 100 mM D-glucose, 10 U ml^{-1} GDH-101) to give a final concentration of substrate of 50 mM. The vials were shaken at 400 rpm, 30°C for 18 h. For high-performance liquid chromatography (HPLC) analysis, the reactions were quenched with acetonitrile (MeCN) (1 ml), vortexed, centrifuged and aliquoted. For gas chromatography (GC) analysis, samples were extracted with EtOAc (2 × 0.5 ml), dried over $MgSO_4$ and analysed directly. For NMR analysis, the reactions were extracted with $CDCl_3$ and analysed directly.

2.9 Preparative Scale Screening Reactions

Reactions were scaled up using three-neck round bottom flask equipped with stir bar and pH titrator (10 M NaOH). To the flask was weighed 100–500 mg substrate (40–100 mM final concentration) and 5 mg ml^{-1} enzyme which was suspended in aqueous media at pH 7 (250 mM potassium phosphate buffer pH 7, 1.1 mM $NAD(P)^+$, 100–200 mM D-glucose (two equivalent), 10 U ml^{-1} GDH-101) the reactions were stirred at 30°C, 400 rpm for 18 h.

2.10 Analytical Methods

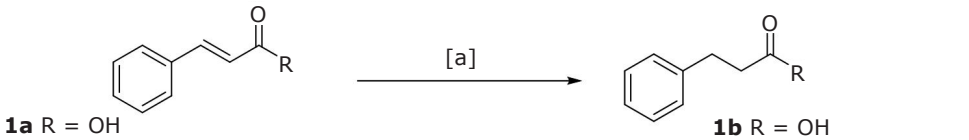
HPLC analysis of conversion was conducted on an 1260 Infinity II LC system (Agilent, USA) using a C18 SunFire Column (Waters Corporation, USA, 150 × 4.6 mm, 3.5 µm) with an isocratic method (MeCN:Water, 30:70 + 0.1% trifluoroacetic acid) and a flow rate of 1 ml min^{-1}.

Chiral HPLC analysis was performed on a Varian ProStar series (Agilent) with a CHIRALCEL® OD-H column (Chiral Technologies, USA, 250 × 4.6 mm, 5 µm) with an isocratic method A (heptane:isopropyl alcohol (IPA), 88:12) and a flow rate of 1 ml min^{-1} or isocratic method B (heptane:IPA, 98:2).

GC analysis of conversion was performed on a Varian CP-3800 (Agilent) using γ-DEX™ 225 capillary column (Sigma-Aldrich, 30 m × 0.25 mm × 0.25 µm) and using helium as carrier gas. Percentage conversion was measured by integration of the product peak in the GC (uncorrected area under curve (AUC)), values below 100% indicate that unreacted starting material was detected. No side products were detected in any of the reported reactions. GC program parameters: injector 250°C, flame ionization detector (FID) 250°C, 80°C for 3 min then 5°C min^{-1} up to 160°C, hold 1 min (total time 20 min), constant flow 5 ml min^{-1}.

3. Results and Discussion

It has been found that a particular ENE in Johnson Matthey's collection, a homologue from the tobacco ENE reductase fold (16), ENE-105, was capable of reducing methyl ester **2a** (**Figure 1**), albeit in a very low yield of 3% (Entry 2, **Table I**). By comparison, cinnamic acid **1a** was a poor substrate and showed no conversion to the reduced product **1b** at pH 7.0 (Entry 1, **Table I**). The pKa of cinnamic acid **1a** is 4.4 and therefore, at pH 7.0, the carboxylic acid should be deprotonated affecting its ability to bind to the enzyme active site. This observation is in line with other literature examples where carboxylates were found to be poor activating groups (17). Encouraged by this initial result, we turned our efforts towards the use of more activated esters. It was envisaged that converting the alkyl chain in the ester moiety to a more EWG could lead to an increase in double bond activation. A similar approach has been reported previously by BASF SE for the lipase-catalysed kinetic resolution of racemic amines and alcohols, where the choice of acylating agent proved critical (18). We chose trifluoroethyl ester **3a** as a starting point which was reduced by ENE-105 and *ENE-69 in 6% and 12% conversion respectively (Entry 3, **Table I**) suggesting that the addition of an EWG had a positive activating-effect on the reduction. To consolidate this theory, ethyl ester **4a** was tested



1a R = OH
2a R = OCH_3
3a R = OCH_2CF_3
4a R = OCH_2CH_3
5a R = $OCH(CF_3)_2$
6a R = $OCH_2CF_2CF_2CF_3$
7a R = $OCH_2C_6F_5$

1b R = OH
2b R = OCH_3
3b R = OCH_2CF_3
4b R = OCH_2CH_3
5b R = $OCH(CF_3)_2$
6b R = $OCH_2CF_2CF_2CF_3$
7b R = $OCH_2C_6F_5$

Fig. 1. Reduction of cinnamic acid and cinnamoyl esters. [a] = **1–7a** (50 mM concentration), ENE-105 or ENE-69 (5 mg ml^{-1}), 500 µl buffer (250 mM KP_i, pH 7, 1.1 mM $NAD(P)^+$, 100 mM D-glucose, 10 U ml^{-1} GDH-101), 400 rpm, 30°C, 18 h

Table I Reduction of Cinnamoyl Esters at 50 mM Substrate Concentration, pH 7, 30°C, 18 h

		Conversion, %[a]	
Entry	**Substrate**	**ENE-105**	***ENE-69**
1[b]	**1a**	0	0
2	**2a**	3	1
3	**3a**	6	12
4	**4a**	<0.5	<0.5
5[c]	**5a**	0	0
6[d]	**6a**	<0.5	<0.5
7[d]	**7a**	0	0

[a]Integration of the product peak in the GC (uncorrected AUC), values below 100% indicate that unreacted starting material was detected; no side products were detected for these reactions

[b]Integration of the product peak in the HPLC (achiral method, uncorrected AUC), values below 100% indicate that unreacted starting material was detected; no side products were detected for these reactions

[c]10% cinnamic acid observed

[d] Conversion calculated by ^{1}H NMR

Table II Reduction of Cinnamoyl Cyclic Imide Derivatives at 50 mM Substrate Concentration, pH 7, 30°C, 18 h

		Conversion, %	
Entry	**Substrate**	**ENE-105**	***ENE-69**
1[a]	**8a**	51	39
2[b]	**9a**	>95	>95

[a]Integration of the product peak in the HPLC (chiral method, uncorrected AUC), values below 100% indicate that unreacted starting material was detected; no side products were detected for these reactions

[b]Conversion calculated by ^{1}H NMR

with the novel ENEs; only a trace of reduction was observed <0.5% (Entry 4, **Table I**).

Other EWGs such as hexafluoroethyl in compound **5a**, heptafluorobutyl in **6a** and pentafluorobenzyl in **7a** could also activate the double bond in the same way, so **5a**, **6a** and **7a** were prepared by reacting cinnamoyl chloride with the corresponding fluorinated alcohols and these substrates were subsequently tested with the ENEs. Hexafluoro **5a** was not reduced by ENE-105 or *ENE-69 (Entry 5, **Table I**), instead, a significant amount of hydrolysis product (cinnamic acid **1a**, 10%) was observed. Heptafluorobutyl **6a** and pentafluoro **7a** were poor activating groups with **6a** showing only a trace amount of product **6b** (Entry 6, **Table I**) and **7a** giving no conversion (Entry 7, **Table I**).

With only limited success with the fluorinated activating groups, our efforts turned towards cyclic imides since activated substrates **8a** and **9a** have been shown to be highly activated towards Michael addition reactions (19, 20, 21) (**Figure 2**). Compounds **8a** and **9a** were synthesised and tested with enzymes ENE-105 and *ENE-69. Pleasingly, oxazolidinone **8a** was successfully reduced by both ENEs (51% and 39% conversion to **8b**, Entry 1, **Table II**) and pyrrolidinone **9a** was reduced to **9b** in >95% conversion (Entry 2, **Table II**), proving to be an excellent activating group. The ^{1}H NMR shift of the alkene proton alpha to the carbonyl for pyrrolidinone **9a** is shifted down field (7.92 ppm) compared to cinnamic acid **1a** (6.46 ppm), therefore supporting the electron-withdrawing nature of the activating group.

The enzymes were then tested for their ability to reduce α-substituted cinnamic acid derivatives such as α-methyl **10a** and α-phenyl **11a** (**Figure 3**). Encouragingly, the tri-substituted double bond in **10a** was reduced to **10b** in >95% conversion by ^{1}H NMR analysis (Entry 2, **Table III**). However, bulkier substrate **11a**, was not tolerated so well on an analytical scale due to solubility issues causing mass-transfer limitations (Entry 3, **Table III**). The reaction was repeated on a larger scale with stirring (Entry 4, **Table III**) and >95% conversion

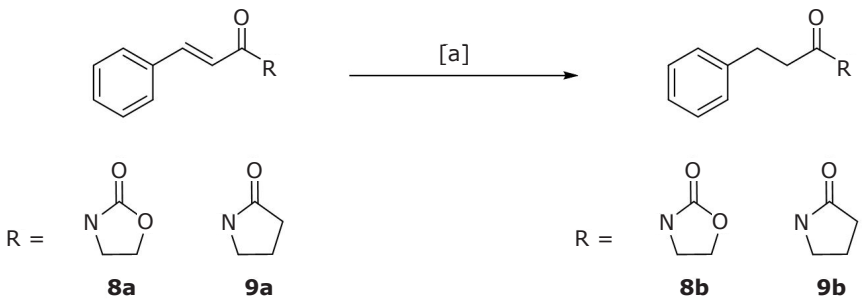
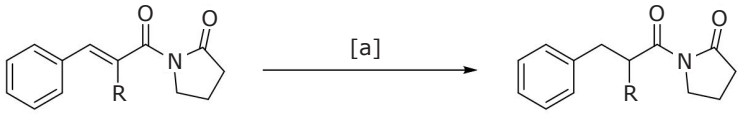


Fig. 2. Cinnamoyl cyclic imide derivatives. [a] = **8a–9a** (50 mM concentration), ENE-105 or ENE-69 (5 mg ml^{-1}), 500 µl buffer (250 mM KP_i, pH 7, 1.1 mM $NAD(P)^+$, 100 mM D-glucose, 10 U ml^{-1} GDH-101), 400 rpm, 30°C, 18 h



9a R = H
10a R = Me
11a R = Ph

9b R = H
10b R = Me
11b R = Ph

Fig. 3. Reduction of α-substituted cinnamoyl pyrrolidinones. [a] = **9a–11a** (40–100 mM concentration), ENE-105 or ENE-69 (5 mg ml^{-1}), buffer (250 mM KP_i, pH 7, 1.1 mM $NAD(P)^+$, two equivalent D-glucose, 10 U ml^{-1} GDH-101), 400 rpm, 30°C, 18 h

Table III Reduction of α-Substituted Cinnamoyl Pyrrolidinones at 50 mM Substrate Concentration, pH 7, 30°C, 18 h

		Conversion, %[a]	
Entry	**Substrate**	**ENE-105**	***ENE-69**
1	**9a**	>95	>95
2	**10a**	>95	>95
3	**11a**	24	2
9[b]	**11a**	>95	–

[a]Conversion calculated by ^{1}H NMR
[b]100 mg scale with stirring

was achieved. **10b** and **11b** were obtained as racemic mixtures.

With a successful activating group found, the reaction was repeated on a preparative scale to test reproducibility and scalability (**Table IV**). Pyrrolidinone **9a** was successfully reduced using enzyme ENE-105 at 130 mg scale with the desired product **9b** being obtained in 95% conversion by ^{1}H NMR (Entry 1, **Table IV**). 72% conversion to **10b** was achieved after 20 h (Entry 3, **Table IV**) on the reduction of pyrrolidinone **10a** at 500 mg scale.

Having found enzymes in Johnson Matthey's collection that could successfully reduce masked carboxylic acids, other commercially available enzymes were tested as a comparison on the reduction of **10a** (**Table V**). Six enzymes from Johnson Matthey collection (Entries 3 to 6, **Table V**) and seven enzymes purchased from Codexis (Entries 7 to 13, **Table V**) were compared with ENE-105 and ENE-69* (Entries 1 and 2, **Table V**). It was found that, despite the extra activation of the C=C double bond, none of the tested enzymes could reduce cinnamic acid derivative **10a**, highlighting the unique ability of ENE-105 and *ENE-69 within the focused library (13 enzymes) screened.

In summary, we have shown that cinnamic acid derivatives activated as fluorinated esters or as cyclic imides can be reduced using Johnson Matthey enzymes ENE-105 or *ENE-69. The concept of 'substrate engineering' as opposed to 'enzyme engineering', offers a complimentary and faster approach to developing a bioprocess, making difficult transformations possible. The reduced products can be subsequently converted to the parent carboxylic acids by LiOH hydrolysis (22, 23) and the potential re-use of these activating groups will be investigated in the future. It is envisaged that the work will lead to further examples of activated acids or esters being reduced by ENEs.

Table IV Reduction of Cinnamoyl Pyrrolidinones by ENE-105 at pH 7[b] and 30°C

Entry	Substrate	Scale, mg	Concentration, mM	Time, h	Conversion, %[a]
1	**9a**	130	40	16	>95
2	**10a**	500	100	4	33
3	**10a**	500	100	20	72

[a]Conversion calculated by ^{1}H NMR

[b]pH controlled with NaOH titration (10 M)

Table V Reduction of Cinnamoyl Pyrrolidinone 10a at 50 mM Substrate Concentration, pH 7, 30°C, 18 h

Entry	Enzyme	Conversion, %[a]
1	ENE-105	>95
2	*ENE-69	>95
3	ENE-101	<0.5
4	ENE-102	1
5	ENE-103	0
6	ENE-104	0
7	ERED-103	0
8	ERED-110	0.5
9	ERED-112	0
10	ERED-207	<0.5
11	ERED-P1-A04	1
12	ERED-P1-E04	0
13	ERED-P1-H09	0

[a]Conversion calculated by ^{1}H NMR

4. Conclusions

The biocatalysed reduction of the double bond of cinnamic acid derivatives is strongly influenced by the nature of the EWG. While no conversion was observed on the biocatalysed reduction of cinnamic acid **1a**, an enzyme in Johnson Matthey's collection, ENE-105, was capable of reducing methyl ester derivative **2a** in low conversion. By replacing the alkyl chain in the ester moiety by a more EWG, such as fluorinated alkanes, and in the presence of enzymes ENE-105 and *ENE-69, we were able to significantly increase conversion to the reduced product. Furthermore, other electronegative derivatives such as cyclic imides proved to be even better activating groups, allowing the reduction of challenging substituted double bonds such as substrates **10a** and **11a**.

In summary, by 'masking' the carboxylic acid moiety into a fluorinated alkyl ester or a cyclic imide, following a straightforward synthetic procedure, and in combination with the right enzyme, it was possible to biocatalytically reduce the conjugated double bond of cinnamic acid and substituted derivatives.

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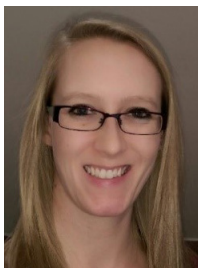
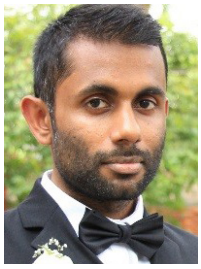
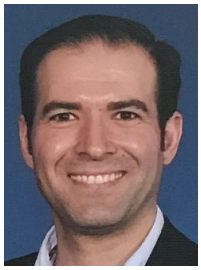


Samantha Staniland graduated from The University of Manchester, UK, in 2011 with an MChem in Chemistry with Industrial Experience, while carrying out her industrial placement at Pfizer, UK, in Medicinal Chemistry. In 2011–2015, Sam did a PhD in the groups of Professor Jonathan Clayden and Professor Nicholas Turner on the biocatalytic asymmetric synthesis of atropisomers. Sam joined Johnson Matthey in 2015 as a research chemist in catalysis.

Tommaso Angelini completed his PhD in Chemical Science in 2010 from University of Perugia, Italy, working on the development of environmentally friendly synthetic protocols. During his postdoctoral studies, he finalised his work designing new continuous flow devices for the use of solid supported catalyst in low E-Factor transformations. Later, he gained experience in developing active pharmaceutical ingredient (API) production process at Procos (Italy). In 2015, he joined Johnson Matthey as Research Chemist, designing new enantioselective synthetic process for the preparation of APIs. He is now a Research Expert at Evotec Verona (Italy), working on the production of preclinical and Phase 1 API candidates.



Ahir Pushpanath obtained his PhD in Birkbeck College (University of London, UK) working on the engineering of enzymes for industrial biofuel production. With a biochemistry background, he specialises in the use of bioinformatics and computational biology in the rational design of new enzyme variants. Ahir joined Johnson Matthey in 2013 as a Senior Biologist and was instrumental in demonstrating the utility of computational techniques for rapid enzyme discovery through genome mining, in silico design and targeted enzyme engineering. He currently leads the enzyme development arm of biocatalysis, continuing to develop faster, more effective methods for 'predictive biocatalysis'.



Amin Bornadel studied chemical engineering and received a PhD in biotechnology from Lund University in Sweden. For postdoctoral work, Amin went to Germany, where he carried out research within biocatalysis at University of Dresden and Technical University of Hamburg. In 2016, Amin joined Johnson Matthey to work as a biocatalysis researcher. He is currently a senior scientist working in the Biotech team.

Elina Siirola completed her PhD in 2012 from the University of Graz, Austria, where she worked on biocatalytic C=C bond hydrolysis. After a postdoctoral position in enzyme engineering at the Max Planck Institute for Coal Research, Germany, she joined Johnson Matthey in 2013, where she worked on biocatalysis research and development (R&D). Since 2017 she is a Principal Scientist in the Bioreactions group at Novartis Pharma in Basel, Switzerland.

Serena Bisagni completed her MSc in Industrial Biotechnology from the University of Pavia, Italy, in 2010 and then moved to Lund University, Sweden, for her postgraduate studies. In 2014 she obtained her PhD in Biotechnology in which she focused on the identification of new Baeyer-Villiger monooxygenases for fine chemicals synthesis within the Marie Curie Innovative Training Networks (ITN) 'Biotrains'. In 2015 Serena joined Johnson Matthey. Her main interests are enzyme screening for synthesis of active pharmaceutical ingredients and fine chemicals and identification of novel biocatalysts.

Antonio Zanotti-Gerosa studied in Milano, Italy, completing his PhD in 1994 (organometallic chemistry). His academic experience include secondments to Imperial College, UK (Professor S. V. Ley), Nagoya University, Japan (Professor R. Noyori) and postdoctoral research at the University of Lausanne, Switzerland (Professor C. Floriani). Since 1997 he has been working on industrial applications of homogeneous catalysis. In 2003 he joined Johnson Matthey and, as R&D Director, he is leading the chemocatalysis group in the Cambridge laboratories.

Beatriz Domínguez gained her PhD in Synthetic Organic Chemistry from the University of Vigo, Spain, and then moved to the UK where she worked with Professor Tom Brown at the University of Southampton, UK, and with Professor Guy Lloyd-Jones at the University of Bristol, UK. In 2002 she joined Synetix, soon to become Johnson Matthey Catalysts and Chiral Technologies and has worked at Johnson Matthey's facilities in Cambridge since. Beatriz has gained broad experience in the application of metal catalysis and biocatalysis, working closely with fine chemicals companies to deliver optimal catalysts for chemical processes.