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Pioneering Women Shaping the Field of Biocatalysis

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In this perspective article, we celebrate the accomplishments of female-led research groups in biocatalysis. Through this initiative, we aim to showcase the breadth and excellence of women's research and increase their visibility within the catalysis community. The authors wish to emphasize that this perspective article represents only a small selection of the extraordinary women who have shaped the field of biocatalysis over time. Among them are scientists who have directly or significantly influenced and inspired the authors' scientific journeys.

1. Introduction

The field of biocatalysis has experienced remarkable growth and transformation over the past few decades, fueled by an ever-deepening understanding of enzymatic processes and innovative catalyst design strategies. These advancements have not only pushed the boundaries of scientific knowledge but have also opened up new possibilities for industrial applications, environmental sustainability, and biomedical advancements. However, while the story of scientific progress is often told through its groundbreaking discoveries and technological innovations, the researchers behind these achievements are equally central to this narrative. In particular, the contributions of women have been pivotal in advancing science, yet they often remain underrepresented and receive lower visibility.

Despite recent initiatives to improve representation and awareness, women in science still face significant challenges in achieving a successful career while maintaining a healthy work–life balance.[1] To build on these advances, supportive policies—such as enhanced parental leave, flexible schedules, and accessible childcare—remain essential for fostering a more equitable and inclusive scientific environment. Addressing these challenges is crucial for fostering a more inclusive scientific environment, and it underscores the resilience and determination of female scientists.

This resilience is evident throughout history, as women have consistently pushed the limits of scientific research, introducing new ideas, methodologies, and perspectives that have shaped the trajectory of various fields. In biocatalysis, female scientists have made great contributions, pioneering innovative approaches to enzyme engineering, catalysis, and sustainable chemistry. By acknowledging the groundbreaking work of women scientists, we can help bridge the gap and ensure that their contributions are given the visibility they deserve.[2] This also sends a strong message to the next generation of scientists, illustrating that gender does not limit one's ability to contribute meaningfully to the advancement of science.

This perspective article seeks to shine a well-deserved spotlight on the exceptional contributions made by female researchers in biocatalysis. By highlighting the breadth, diversity, and impact of their work, we aim not only to celebrate their achievements but also to inspire a broader recognition of the crucial role women play in shaping the future of science. From developing new enzyme-based methods that enable sustainable chemical transformations to designing novel biocatalytic pathways with broad industrial applications, women have consistently demonstrated brilliance, leadership, and resilience. Their work has redefined key areas of biocatalysis, providing solutions to some of the most pressing challenges in sustainability, medicine, and industry.

In addition to celebrating individual accomplishments, this perspective article highlights the broader importance of diversity and inclusion in science. Gender diversity brings a wealth of perspectives, fostering creativity, innovation, and collaboration. These diverse viewpoints lead to more comprehensive solutions to complex scientific challenges, enriching the process of discovery and application.[3] By showcasing the work of female scientists in biocatalysis, we aim to spark further dialogue about the need for a more equitable scientific community, recognizing talent, creativity, and excellence regardless of gender. Moreover, we hope this initiative encourages other fields to undertake similar efforts, promoting diversity across all areas of research and development.

Ultimately, this perspective article acknowledges the role that women have played—and continue to play—in the field of biocatalysis. By celebrating their contributions, we not only honor their achievements but also help lay the foundation for future generations of women to pursue their scientific passions. We aim to empower the next generation of female researchers to challenge boundaries, push the frontiers of knowledge, and contribute to the ever-growing body of scientific work in this dynamic field. Through increased visibility and recognition, we can help in building a more inclusive and innovative future for science.

Below are snapshots of key contributions by leading women scientists who have directly or significantly influenced and inspired the authors' scientific journeys in four distinct areas: enzyme discovery and engineering, enzyme mechanism and characterization, applied biocatalysis, and computational tools.

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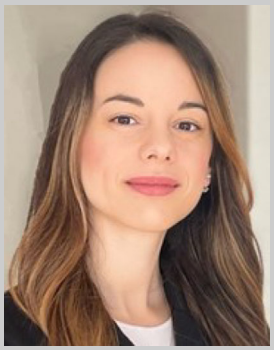
Dedicated to the next generation of women in science—may you continue to break barriers and inspire change. And to Camilla—may you grow up in a world that is fair, inclusive, and full of opportunities, where gender equality is not a goal but a reality.

An additional section is dedicated to recognize the essential role of female mentorship in the field.

2. Enzyme Discovery and Engineering

Enzyme discovery and engineering are undoubtedly two key areas that have contributed significantly to the successful adaptation of biocatalytic transformations in chemistry, medicine, and food technology. Only in the last five years, the discovery of enzymes with useful activities and properties has been accelerated by the increasing number of available protein sequences and the development of data-driven tools that enable faster enzyme discovery. In addition, the application of protein engineering and directed evolution to tailor enzymatic properties has been further advanced by the implementation of machine learning (ML), automation, and novel high-throughput screening capabilities. These developments not only enable accelerated development of enzymes tailored to the desired application but also create unprecedented opportunities to use enzymes for reactions that are new-to-nature, while expanding the available toolbox of biocatalysts.

Frances H. Arnold (Caltech, USA) has pioneered the use of directed evolution to design new enzymes, combining compu-



Ana I. Benítez-Mateos is SNSF Ambizione Group Leader at ETH Zürich since 2023. She obtained her PhD in 2019 with Prof. Fernando López-Gallego at CICbiomaGUNE (Spain). She then joined the group of Prof. Francesca Paradisi as a postdoctoral fellow, first at the University of Nottingham and later at the University of Bern. Her research has been focused on enzyme immobilization, from the study of enzymatic reactions at single-particle level to the integration of immobilized enzymes in flow reactors. Currently, her research aims to develop more stable and sustainable biocatalysts harnessing the unique properties of extremotolerant organisms.

Caroline E. Paul received her Honours BSc and MSc degrees in biological chemistry at the University of Toronto with Prof. M. Nitz, and her PhD degree at the University of Oviedo in bioorganic chemistry in the group of Prof. V. Gotor with Prof. V. Gotor-Fernández and Prof. I. Lavandera within the EU project "Biotrains." After postdoctoral work at TU Delft and Wageningen University pursuing her research interests on biomimetic cofactors, she was appointed as assistant professor in biocatalysis at TU Delft in 2018 and associate professor in 2023. Her research focus includes the design and engineering of artificial cofactors in biocatalytic reactions, and exploring the full synthetic potential of enzymes.

Sandy Schmidt is an associate professor (Rosalind Franklin Fellow) at the Groningen Research Institute of Pharmacy of the University of Groningen. She completed her Ph.D. in 2015 in the group of Prof. Uwe Bornscheuer at the University of Greifswald. After a research stay at Delft University of Technology as postdoctoral fellow within the group of Prof. Frank Hollmann, she was working as group leader at Graz University of Technology. Her research interests include the discovery, design, and exploitation of iron-dependent biocatalysts and the development of photo-biocatalytic approaches for applications in organic chemistry.

Martina L. Contente is an associate professor at the University of Milan. She earned her PhD in medicinal chemistry under the mentorship of Prof. Francesco Molinari. She then joined Francesca Paradisi group as a visiting postdoctoral researcher at UCD (Ireland) and later at the University of Nottingham (UK) as a Marie-Curie Fellow. She then joined the University of Bern as a visiting researcher, working on a Roche-funded project, before returning to her alma mater to establish her independent career. Her research focuses on the discovery of novel enzymes, their stabilization via enzyme immobilization and the development of continuous processes to enhance efficiency, sustainability, and cost-effectiveness of chemical transformations. All four co-authors are co-founders and/or organizers of the annual NextGen-Biocat symposium dedicated to promoting early-career researchers in biocatalysis.

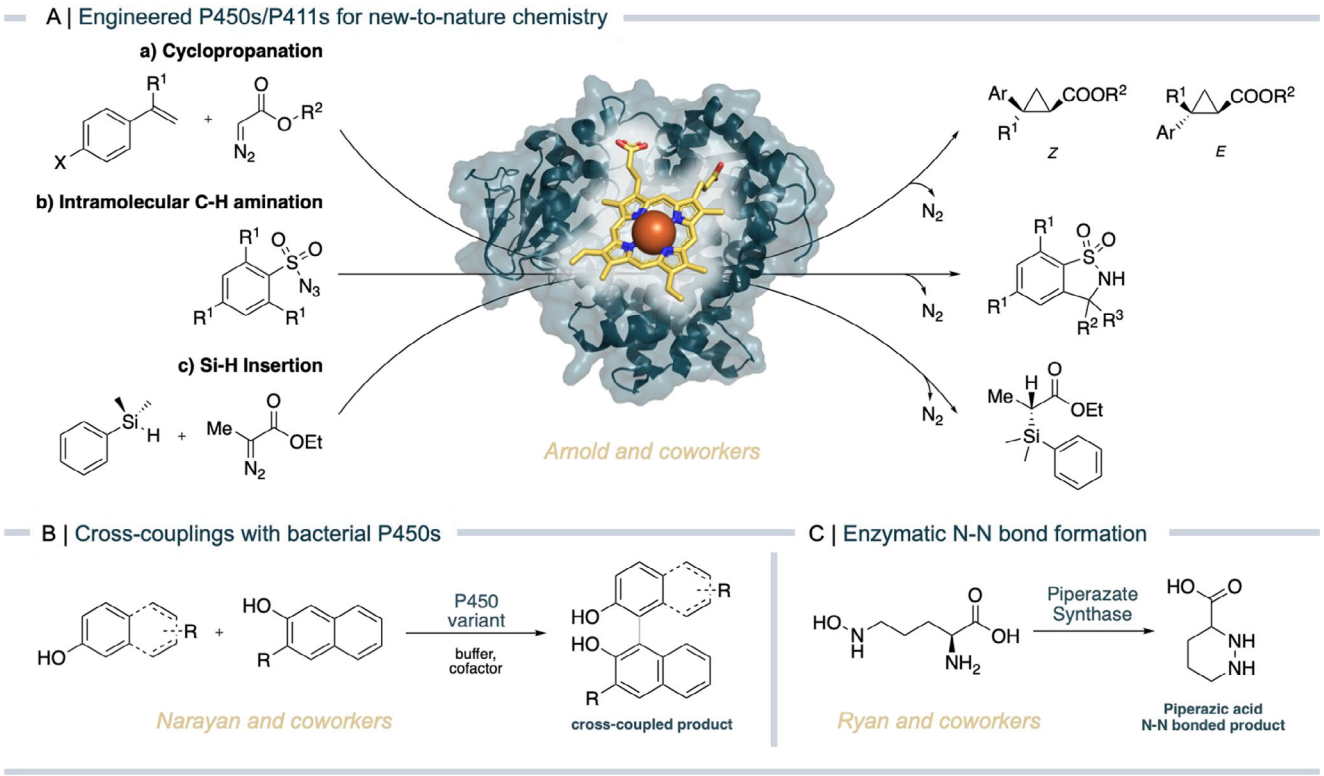


Figure 1. Representative examples of groundbreaking achievements in the field of enzyme discovery and engineering. (A) New-to-nature transformations catalyzed by engineered cytochrome P450s/P411s, including cyclopropanation, intramolecular C—H animation, and Si—H insertion, (B) biocatalytic cross-coupling reactions through oxidative C—C bond formation catalyzed by bacterial P450 enzymes, and (C) discovery and characterization of a piperazate synthase forming the N—N bond in the natural product kutzneride 2. Figure 1A was reproduced from Ref. [16] with permission from the Royal Society of Chemistry.

tational and evolutionary design approaches to create enzymes for applications ranging from pharmaceutical synthesis and biofuels to sensors and diagnostics. She has received numerous awards recognizing her achievements, including the 2018 Nobel Prize in Chemistry. Among numerous contributions, her group has pioneered the engineering of cytochrome P450s for new-to-nature chemistry. For example, Coelho et al. used the transition metal-catalyzed carbene transfer reactions developed by synthetic chemists as inspiration to engineer P450s to catalyze carbene transfer reactions (Figure 1A).[4] Indeed, variants of P450 BM3 (CYP102A1) from *Bacillus megaterium* catalyze the cyclopropanation of styrene in vitro. To further optimize this new catalytic function, a cytochrome "P411" with a serine-heme ligation was designed that catalyzes selective cyclopropanations in vivo with >65000 total turnover number (TTN).[5] This and other P450 enzymes have been further engineered to also enable challenging nitrene transfer reactions, for example, to catalyze intramolecular C—H amination reactions.[6] Based on these pioneering studies, many other novel reactivities have been unlocked from engineered P450s, including N—H insertion,[7] S—H insertion,[8] and Si—H insertion.[9] These studies demonstrate how the scope of chemical reactions accessible to enzymatic catalysis is expanded, such as C—Si and C—B bond-forming activities.[10]

Alison Narayan (University of Michigan, USA) has made outstanding contributions to the discovery and engineering of enzymes from natural product biosynthetic pathways and the demonstration of their potential applications in challenging chemical transformations. For example, she has been instrumental in reviving interest in Rieske oxygenases and their application to the functionalization of complex molecules. One of her seminal papers[11] describes the identification and characterization of three Rieske oxygenases involved in the biosynthetic pathway of saxitoxin, providing unprecedented insight into the natural assembly and further elaboration of this famous molecule. She has also made significant contributions to expanding the scope of biocatalytic C—C bond formation. For example, her group demonstrated how sequence similarity networks combined with enzyme engineering provide powerful tools to screen a subset of cytochrome P450s for non-natural oxidative cross-coupling for biaryl bond formation (Figure 1B).[12] By exploiting the promiscuity of the well-known enzyme KtnC and directed evolution of this enzyme, several cross-coupling reactions on a panel of phenolic substrates were achieved with the desired reactivity, site selectivity, and atroposelectivity.

Anna Fryszkowska (Merck and Co., Inc., USA) has had a major impact on the development of biocatalytic processes in the pharmaceutical industry. Her outstanding contributions have focused on enzyme discovery and evolution, organic synthesis, and (chemo)enzymatic cascade design. One of the striking examples she contributed to was the design of an in vitro biocatalytic cascade for the production of islatravir, an investigational

HIV treatment.[13] Together with Codexis, the Merck team performed directed evolution of five cascade enzymes to act on non-natural substrates. These five core cascade enzymes were combined with four auxiliary enzymes to stereoselectively form islatravir from 2-ethynylglycerol in three steps with an overall yield of 51%. This cascade has undoubtedly set a new benchmark for the adoption of cascade biocatalysis as a strategy for the sustainable synthesis of complex non-natural molecules such as pharmaceuticals. In addition, she has applied her expertise in directed evolution and biocatalysis to the development of a chemoenzymatic strategy for the site-selective functionalization of native peptides and proteins.[14] In an unprecedented way, this work highlights directed evolution as a key tool for reprogramming enzymes to perform site-specific conjugation to a peptide, thereby providing an efficient enzymatic methodology for challenging ligation of native proteins or other large molecules.

Katherine Ryan (University of British Columbia, Canada) has had a major impact on the discovery and characterization of novel enzymes for biocatalytic applications. Specifically, she and her team focus on isolating novel biosynthetic pathways from microbes, engineering enzymes to catalyze novel reactions, and generating natural product derivatives through combinatorial engineering and chemo-enzymatic synthesis. Her group has contributed to the understanding of the biosynthesis of many natural products. In particular, they have identified enzymes involved in the biosynthesis of N—N bond-containing natural products. A notable example is the identification and characterization of a heme b-dependent piperazate synthase that constructs the hydrazine bond of L-piperazate in *Kutzneria* sp. 744 (Figure 1C).[15] This is noteworthy because a large number of bioactive molecules are derived from the structural scaffold of this building block, which is found in hundreds of non-ribosomal peptide synthetase-derived secondary metabolites. In addition, the biosynthetic strategies that nature uses to construct N—N bonds have only been elucidated in the last decade, and the Ryan group has contributed significantly to this increased knowledge.

Joelle Pelletier (University of Montreal, Canada) has devoted her scientific endeavors to understanding structure-function relationships in enzymes, engineering and characterizing biocatalysts, and using experimental and computational methods to study and modify proteins for health applications. In the area of biocatalysis, her group's recent efforts have focused on accelerating the discovery of substrate promiscuity in biocatalytic oxidations, exemplified by the development of an indigo-based high-throughput screening methodology that can serve as a predictor of promiscuous hydroxylation of non-native aromatic substrates in cytochrome P450 BM3.[17] The powerful workflow includes site-saturation mutagenesis, screening for indigo production on solid media, linking indigo-positive/negative phenotype to its genotype, expression of individual variants under optimized conditions, a hit map of well-expressed indigo-positive variants, and determination of promiscuous activity of variants for hydroxylation of non-native aromatic substrates. Substitution at positions in indigo-positive variants, or hotspot residues, may provide a general mechanism for increasing aromatic hydroxylation activity. This research demonstrates that indigo production enables effective screening of P450 monooxygenases, accelerating enzyme engineering in an unprecedented manner.

Emily Balskus (Harvard University, USA) has made significant advances in our understanding of microbes and microbiomes by pioneering approaches that integrate microbiology and chemistry. Her research has contributed to the discovery of functionally unique enzymes by studying how microbes make structurally unusual natural products. For example, her team identified the cylindrocyclophane, lomaiviticin, bartoloside, and cremeomycin biosynthetic gene clusters and their encoded enzymes,[18] which revealed intriguing reactivities, including enzymes that halogenate unactivated carbon centers, link aromatic rings with alkyl halides, and construct N—N bonds. In one of the group's recent studies,[19] they discovered a new class of aminoacyl radical enzymes (AAREs) that form stable α-carbon radicals when activated by partner activating enzymes. Mechanistic studies indicate that the AAREs can install either an alanyl, serinyl, or threonyl radical upon activation. The formation of these protein-centered radicals represents post-translational modifications that have not been previously observed. Overall, this study not only expands the known repertoire of radical chemistry in nature but may also provide novel enzymes for biocatalysis and chemical synthesis.

Jennifer Littlechild (University of Exeter, UK) has made an enormous contribution to our understanding of the structural and mechanistic features of a wide range of enzymes. She has not only contributed to the elucidation of, for example, C—C bond-forming enzymes (transketolases and aldolases), Baeyer–Villiger monooxygenases, aminoacylases, transaminases, and others, but has also discovered many new enzyme candidates from extreme environments, leading to industrial applications of these enzymes with favorable properties. For example, novel thermophilic limonene-1,2-epoxide hydrolases were discovered from hot spring metagenomic libraries and subsequently characterized.[20] The new hydrolases exhibit higher optimum temperatures and apparent melting temperatures, and subsequent structural analysis has provided insights into the mechanism, substrate specificity and stereoselectivity of these new enzymes, further supported by mutagenesis studies.

3. Enzyme Mechanisms and Characterization

Exploring and understanding the mechanism of enzymes is key to further developing them for synthetic applications. The characterization of enzymes is not always systematically investigated, yet thanks to incredible advances in recombinant expression, purification, stopped-flow spectrophotometry, crystallization, modeling, and other techniques, we can now better than ever explore their substrate scope, kinetic parameters, chemical reactivity, and versatility. In this section, we highlight pioneering work on mechanistic aspects as well as chemical reactivity of different enzyme families essential for biocatalytic applications.

Pimchai Chaiyen (VISTEC, Thailand) a world-renowned flavinologist, earned her expertise on flavoprotein monooxygenases

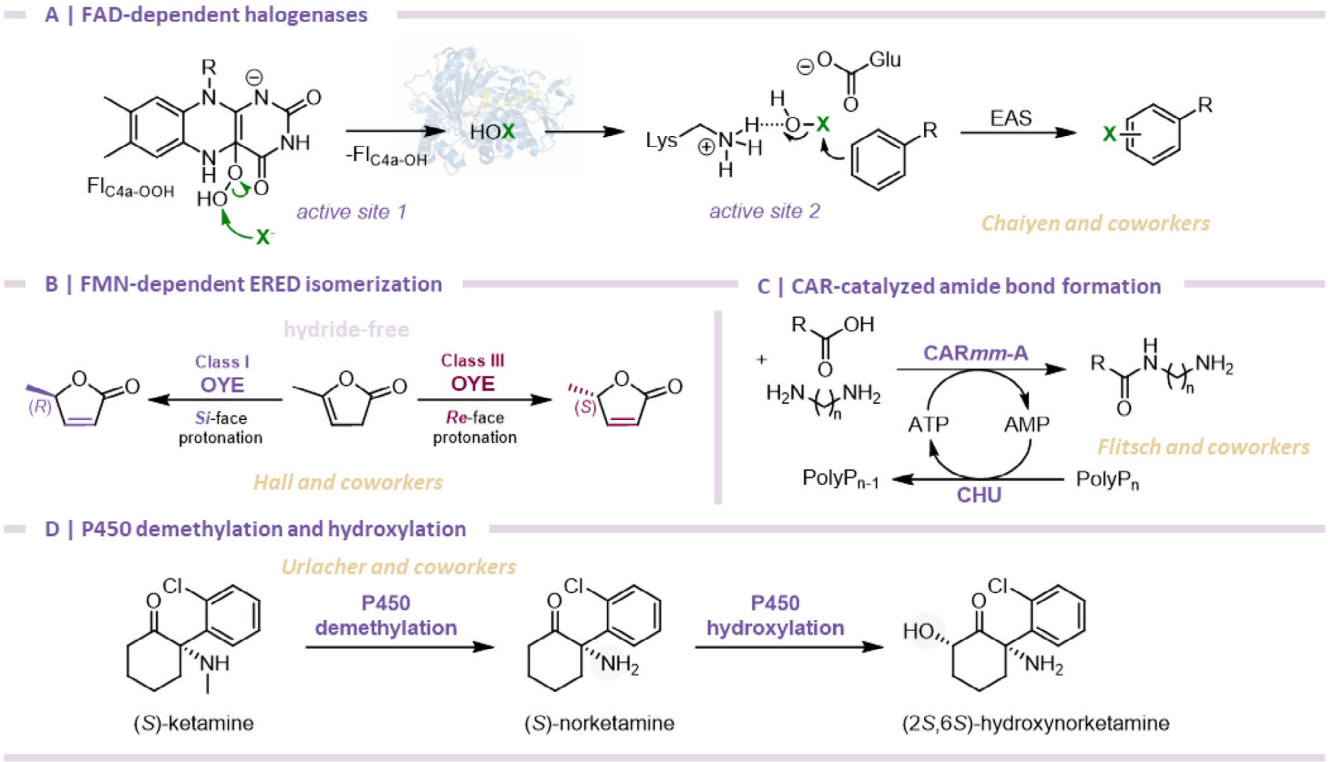


Figure 2. Selected examples of pioneering research on enzyme mechanism and characterization. (A) FAD-dependent halogenases (FDH) mechanism discovery to produce halogenated compounds, EAS = electrophilic aromatic substitution, (B) FMN-dependent ene reductase OYE isomerization elucidation to obtain chiral α,β-unsaturated lactones, (C) repurposing the mechanism of a CAR for amide bond formation with ATP cofactor recycling using a class III polyphosphate kinase (CHU), and (D) P450-catalyzed consecutive demethylation followed by hydroxylation.

(FPMOs) and their kinetic parameters. She especially elucidated the mechanism of flavin-dependent halogenases (FDHs), which are notoriously inefficient at catalyzing selective halogenation reactions. Chaiyen's group discovered that the hypohalous acid (HOX) formed in the active site causes this inefficiency. Her research on the mechanistic understanding of FDHs by exploring the intermediate transfer tunnel connecting the two active sites was crucial and opened the door to engineering with target hotspot locations to improve catalytic efficiency, thus enabling selective halogenation and opening the door to further applications (Figure 2A).[21] Her other landmark research on mechanistic insights of FPMOs includes her work on mono- and dioxygenases, such as tryptophan 2-monooxygenase and *p*–hydroxyphenylacetate 3–hydroxylase, among many others.[22]

Mélanie Hall (University of Graz, Austria) is renowned for her outstanding work on nicotinamide cofactor (NAD(P))-dependent oxidoreductases for asymmetric synthesis.[23] In particular, she has played an essential role in the characterization and development of flavin-dependent ene reductases (EREDs) and their mechanism. She established the systematic screening of EREDs, their substrate scope, and reactivity. Of note, her research group discovered an unprecedented isomerization activity in EREDs (Figure 2B).[24] This mechanistic insight, which was discovered not to require the NAD(P)H cofactor, therefore being a hydride-free redox neutral process, provides access to chiral α,β-unsaturated carbonyl compounds otherwise chemically difficult to obtain.[25] Further exploration of the mechanism of these enzymes offers promising prospects for obtaining valuable chiral products.

Helen Hailes (University College London, UK) has made a tremendous impact on the field with her group developing key biocatalytic synthetic methods by characterizing a wide variety of enzymes. Quite an impressive selection of these enzymes was discovered using functional metagenomics approaches and characterized for their use in organic synthesis, such as transketolases, transaminases, alcohol dehydrogenases, ene reductases, decarboxylases, tyrosinases, imine reductases, methyltransferases, and norcoclaurine synthases, in particular for the synthesis of benzylisoquinoline alkaloids.[26] She has exquisitely developed combinations of enzymatic steps into synthetic cascades and biological pathways.[27]

Sabine Flitsch (University of Manchester, UK) had an outstanding contribution in investigating several key biocatalysts, in particular with glycosyltransferases, which catalyze the stereo- and regioselective formation of glycosidic bonds. The development of these glycosyltransferase biocatalysts has been crucial to enable the synthesis of biologically active glycoconjugates and carbohydrates.[28] She also held a leading role in the retrosynthetic analysis of biocatalytic reactions, developing the valuable database named RetroBioCat, which is a planning tool to help find an enzymatic step for organic synthesis, and opens access to the biocatalytic tools available for chemists.[29] Recently, her team also impressively repurposed the mechanism of carboxylic acid reductases (CARs) to catalyze the formation of amide

bonds, which enabled the synthesis of pharmaceutically relevant products (Figure 2C).[30]

Véronique Alphand (Université Aix-Marseille, France) made seminal discoveries by investigating Baeyer–Villiger monooxygenases (BVMOs). Part of her group's excellent research work has been on the conversion of α,β-unsaturated ketones to chiral enol-lactones and ene-lactones via Baeyer–Villiger oxidation,[31] and the in vivo development of an enzymatic cascade combining BVMOs in a redox neutral process.[32] Her team also investigated the cofactor system of BVMOs type II.[33] These enzymes catalyze the formation of a lactone via a particular mechanism, wherein the monooxygenase component is only active with its externally reduced flavin cofactor. Thanks to these investigations, BVMOs type II were shown to be selective to produce bicyclic chiral lactones, yet remain challenging to be employed.

Vlada Urlacher (HHU Düsseldorf, Germany) provided exceptional insights into the ins and outs of cytochrome P450 monooxygenases.[34] Her research focus has been on the optimization of P450s and their biotechnological application. The impressive repertoire of her group's achievements includes the discovery and engineering of highly active and stable P450 enzyme variants for fast substrate conversion with excellent selectivities to produce chiral compounds.[35] One of those studies included constructing a triple mutant of the P450 154E1 from *Thermobifida fusca* YX to produce (2*S*,6*S*)-hydroxynorketamine from (*S*)-ketamine (Figure 2D), determining the mechanism to undergo a consecutive oxidative *N*-demethylation and regio- and stereoselective C6-hydroxylation. Her research group also actively studied immobilized enzymes and whole-cell catalysts, combining several enzymatic steps. Recently, she also made seminal contributions to the development of fungal aryl-alcohol oxidases.[36]

Kylie Vincent (University of Oxford, UK) has made outstanding contributions to the development of hydrogenases. Her pioneering work involves incredible mechanistic insights using various techniques on the Ni—Fe hydrogenase Hyd1 and its production in *E. coli*.[37] The hydrogenase's immobilization on carbon material enhances electron transfer to a co-immobilized NAD-reductase, thus enabling NADH cofactor recycling to couple to alcohol dehydrogenases, EREDs, imine reductases, and along with them, access to deuterated labeled products when starting from deuterium instead of hydrogen gas.[38] Her team also discovered the unprecedented nitro-to-amine reduction catalyzed by the hydrogenase Hyd1. These hydrogenase-based pioneering technologies led to the start-up company HydRegen with co-founders Holly Reeves and Sarah Cleary.

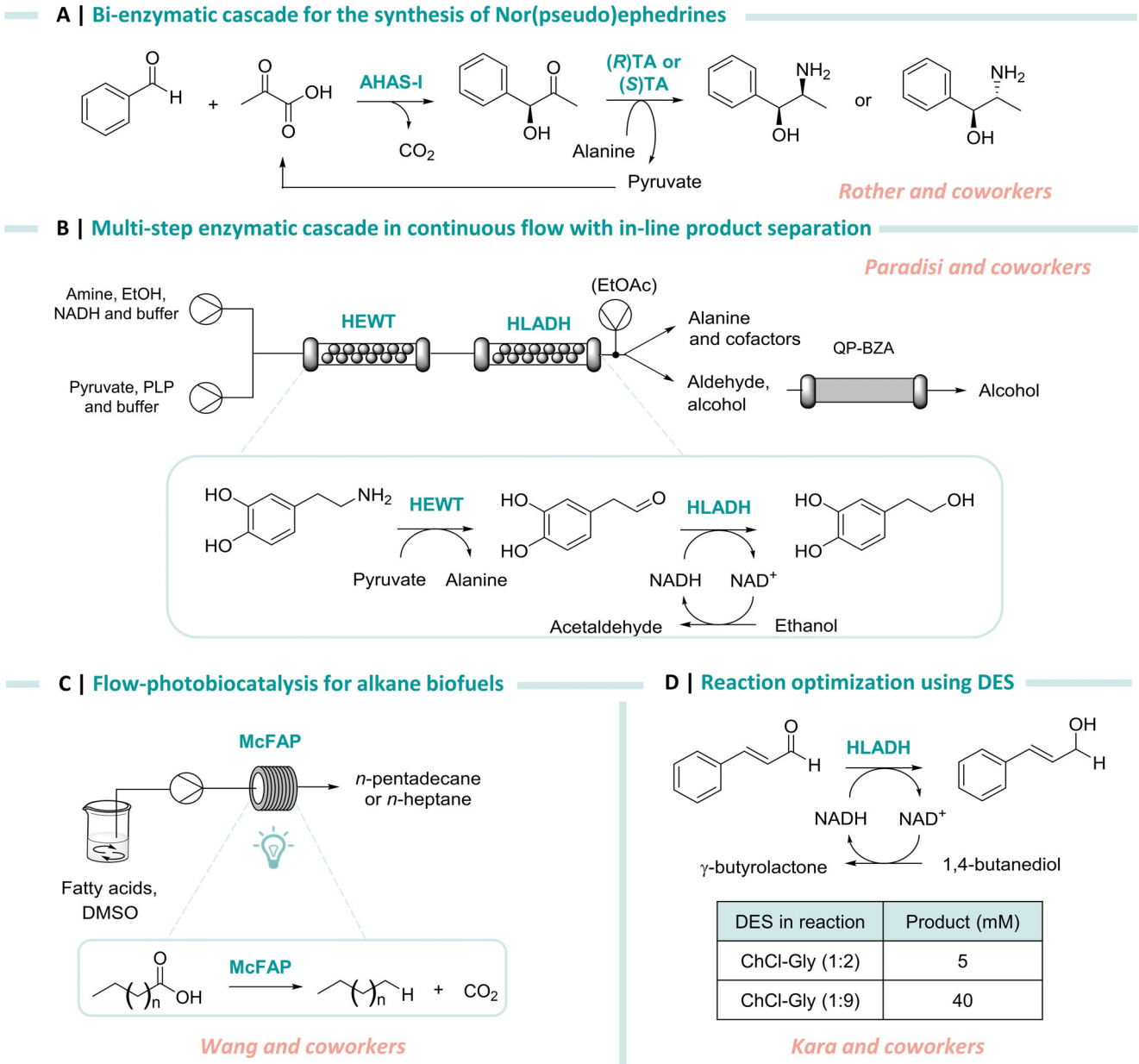
Rebecca Goss (University of St Andrews, UK) has performed incredible research in the biosynthesis of natural products that display relevant medicinal properties.[39] Her research focus has been on understanding how these medicinally bioactive compounds are assembled. To achieve this, each enzyme catalyzing these biosynthetic pathways has been characterized and used to establish large libraries of medicinally relevant compounds. Some of her most outstanding work has been on the discovery of halogenases and their mechanistic investigation.[40] Notably, her team characterized an uncommon flavin-dependent viral halogenase, revealed to catalyze the regioselective halogenation of a diverse range of substrates, with a previously unseen preference for forming aryl iodide species, adding on to the biocatalytic toolbox.

Martina Pohl (Forschungszentrum Jülich, Germany) is one of the pioneers to have developed the formate dehydrogenase from *Candida boidinii* (CbFDH) together with Maria-Regina Kula, a milestone in cofactor regeneration strategies for redox biocatalysis. Her work on lyases has significantly contributed to the advancement of enzymatic asymmetric synthesis, particularly through the engineering and application of carbon-carbon bond-forming enzymes. By systematically investigating enzyme mechanisms, substrate scopes, and stereoselectivity, she has expanded the toolbox of biocatalysts available for industrial applications. Among her most influential works, her studies on the stability and catalytic properties of *Cb*FDH,[41] provided essential insights into cofactor recycling systems, which are widely used in industrial biotransformations. Additionally, her research on thiamine diphosphate-dependent carboligases has laid the groundwork for stereoselective carbon-carbon bond formation in synthetic applications.[42]

4. Applied Biocatalysis

This section highlights key advancements in applied biocatalysis. Among the most widely studied strategies are enzyme immobilization techniques, which enhance enzyme stability and reusability, and multi-enzyme cascade reactions that replicate natural metabolic pathways, facilitating industrial implementation of biocatalysis. Flow biocatalysis also stands out as an enabling technology, offering sustainable and efficient chemical production by optimizing reaction conditions and improving scalability. Moreover, recent examples of biocatalysis integrated with electrocatalytic or photocatalytic approaches have emerged as promising methods for developing innovative new-to-nature reactions.

Dörthe Rother (Forschungszentrum Jülich and RWTH Aachen University, Germany) has made relevant scientific contributions to the development of multi-step enzyme cascades, chemo-enzymatic biotransformations, and the use of unconventional solvents for the synthesis of pharmaceuticals and fine chemicals with a focus on enantiomerically pure compounds.[43] One of her most outstanding works reported the coupling of (*R*)-selective acetohydroxy acid synthase (AHAS-I) and an (*R*)-selective transaminase (*R*-TA) for the synthesis of nor(pseudo)ephedrine (N(P)E) stereoisomers by using benzaldehyde and pyruvate as starting materials (Figure 3A).[44] The bi-enzymatic cascade achieved almost full conversion with high stereoisomeric purity (*de* > 98%, *ee* > 99%). This cascade could also be assembled by using an (*S*)-selective transaminase (*S*-TA), thus achieving the *S*-stereoisomer. The high selectivity is of utmost importance as these molecules serve as pharmaceutical building blocks and can also be used directly as active pharmaceutical ingredients (APIs). The sustainability of the process was enhanced through optimized atom economy and



DES in reaction	Product (mM)
ChCl-Gly (1:2)	5
ChCl-Gly (1:9)	40

Figure 3. Representative examples of significant advances toward the application of biocatalytic systems. (A) Assembly of a bi-enzymatic cascade for the (*R*)- or (*S*)- selective synthesis of nor(pseudo)ephedrines. The pyruvate generated as a by-product in the second enzymatic step (TA) could be reused by the first enzyme (AHAS-I), (B) integration of two immobilized enzymes (HEWT and HLADH) into two packed-bed reactors for the synthesis of high-value alcohols (hydroxytyrosol is exemplified) from biogenic amines. The ultra-efficiency of the system was achieved by product separation with EtOAc extraction and a QP-BZA column coupled in line, (C) immobilized photodecarboxylase (McFAP) on a membrane-reactor for the continuous photodecarboxylation of caprylic acid and palmitic acid, and (D) influence of DES (deep eutectic solvents) in the production of cinnamyl alcohol by HLADH. Different mixtures of ChCl (choline chloride) and Gly (glycerol) were used as DES in the biocatalytic reactions.

reduced waste generation, as the by-product (pyruvate) from the second reaction was reused by the first reaction.

Claudia Schmidt-Dannert (University of Minnesota, USA) is a pioneer in the compartmentalization and co-localization of multi-enzyme systems in vitro and also in vivo. Her approach to the co-immobilization of enzymatic cascades consists of genetically-programmable and self-assembling protein scaffolds. Inspired by nature, her group has used the bacterial microcompartment protein EutM from *Salmonella enterica* to engineer scaffolds for covalent linkage with biocatalysts using SpyTag–SpyCatcher covalent bond formation.[45] By an easy production in *E. coli*, followed by isolation, a bi-enzymatic system formed by an alcohol dehydrogenase (ADH) and an amine-dehydrogenase (AmDH) was self-assembled and tested for the conversion of alcohols to amines in a highly enantioselective manner. The co-immobilized system not only showed a faster reaction rate (~90% conversion in 24 h, compared to 48 h for the free system) but also enhanced biocatalyst stability (20% higher activity

after 24 h compared to the free system).[46] Schmidt-Dannert's research has shown the potential of biological systems toward the fabrication of functional biomaterials with a wide range of applications.

Francesca Paradisi (University of Bern, Switzerland) advances biocatalysis by integrating enzymes in flow reactors, making it a sustainable and highly productive approach.[47] One of her most representative works regards a flow cascade combining an ω-transaminase and an ADH for the transformation of aromatic amines into high-value alcohols such as hydroxytyrosol, tryptophol, and histaminol (Figure 3B).[48] While both enzymes were irreversibly immobilized onto solid supports and packed into two separate reactors, the NAD(P)H cofactors were in situ regenerated by simply adding a second substrate (EtOH) or an ancillary enzyme (GDH). A further leap forward was the collection of the partially purified waste waters containing the cofactors lost downstream the process of recirculation into the flow system. After 5 days of continuous operation, more than 80% of the alcohol was recovered, showing the ultra-efficiency of the system. With water recirculation she also succeeded in reducing the ratio of substrate/cofactors finally giving rise to self-sustaining enzyme-mediated processes. She is also the co-founder of a start-up company –inSEIT– which offers integral services for enzyme immobilization technologies.

Polona Žnidaršič-Plazl (University of Ljubljana, Slovenia) is known for her work on the integration of microprocessing engineering with biocatalysis.[49] Her research highlights the strategic advantages of microscale technology in bridging the gap between laboratory research and large-scale manufacturing while promoting sustainable production of high-value products through biocatalytic processes. One of her most recent works described the development and validation of a mathematical model for the continuous biocatalytic production of *L*-malic acid by fumaric acid hydration in a microreactor.[50] To this end, *Saccharomyces cerevisiae* whole cells were immobilized in hydrogel layers on the bottom and top of the microreactor. After continuous operation of the microbioreactor at different substrate concentrations and flow rates, the obtained results were in agreement with the predictions of the developed mathematical model comprising transport phenomena and reaction kinetics. This work demonstrated the importance of developing and validating mathematical models to identify optimal process conditions and enhance the performance of biocatalytic microreactors.

Yonghua Wang (University of Technology, South China) has pioneered the use of biocatalysis applied to food and biofuel science technology with particular focus on lipases as key catalysts for both the synthesis and transformation of food-related compounds[51] and biodiesel production.[52] In this context, she has recently succeeded in the evolution of a modified form of membrane-associated fatty acid photodecarboxylase from *Micractinium conductrix* (McFAP) to be used for photobiocatalytic alkane biofuel synthesis (Figure 3C). The innovative assembled photoenzyme-membrane was subsequently integrated into an illuminated flow system to achieve the continuous preparation of alkane biofuels. Under continuous conditions, the membrane-flow mesoscale reactor reached a space-time yield (STY) of 1.2 mmol L^{-1} h^{-1} providing stable catalytic performance across eight consecutive reaction cycles, culminating in a cumulative runtime of 8 h. These results set up novel benchmarks for the conversion of biomass into high-value products via enzyme-mediated processes.[53]

Selin Kara (Leibniz University Hannover, Germany; Aarhus University, Denmark) has had a major impact on the process intensification of biocatalytic reactions by using different approaches (i.e., flow biocatalysis, green solvents, multi-enzyme cascades).[54] Her research on non-conventional media, particularly deep eutectic solvents (DES), represents a significant contribution to the pursuit of greener alternatives to conventional organic co-solvents.[55] In 2022, her group reported a combined experimental and computational analysis of the effect of three DES on the behavior of a representative oxidoreductase, Horse Liver ADH (HLADH).[56] By performing the reduction of cinnamaldehyde to cinnamyl alcohol (a relevant compound for the food industry and cosmetics), HLADH demonstrated high activity and stability in DES, giving promising productivity results (15.3 g $L^{-1}d^{-1}$) (Figure 3D). Moreover, this work indicated that DES can be designed to be both substrate-solubilizer and enzyme-compatible, opening new research lines for more sustainable approaches in biocatalysis.

Lucia Gardossi (University of Trieste, Italy) devoted her research to the bioeconomy and enabling technologies for green chemistry. Pillars of Gardossi's research are the employment of immobilized enzymes[57] (she was the co-founder of a spin-off company –Sprin technology for sustainable chemistry– together with Mitsubishi Chemical Corporation) and the valorization of biomasses for the production of renewable and sustainable materials (e.g., biodegradable polymers).[58] In particular, one of her recent publications in this field highlights the importance of renewable monomers such as polyols and dicarboxylic acids obtainable via biotechnological production, to massively reduce the carbon footprint of the next generation plastics.[59] More specifically, polyesters, which are part of everyday life with applications in clothing, food packaging, car manufacturing, and biomedical devices, have been synthesized by exploiting cutinase 1 from *Thermobifida cellulosilytica* (Thc-cut1) under solvent-free and thin-film conditions. The biocatalyst was immobilized efficiently on a fully renewable cheap support based on milled rice husk. Experimental and computational investigations of Thc-cut1 revealed structural and functional features that make this protein efficient in polycondensation reactions. Bioinformatic analysis pointed out functional similarities with CaLB and provided guidelines for Thc-cut1 enzyme evolution.

5. Computational Approaches

Bioinformatics and computational tools are playing an increasingly vital role in biocatalysis by accelerating enzyme discovery, optimization, and design. Thus, reducing the time and cost of experimental trial-and-error methods. Techniques such as protein modeling, molecular dynamics simulations, and more recently, machine learning enable the prediction of enzyme-

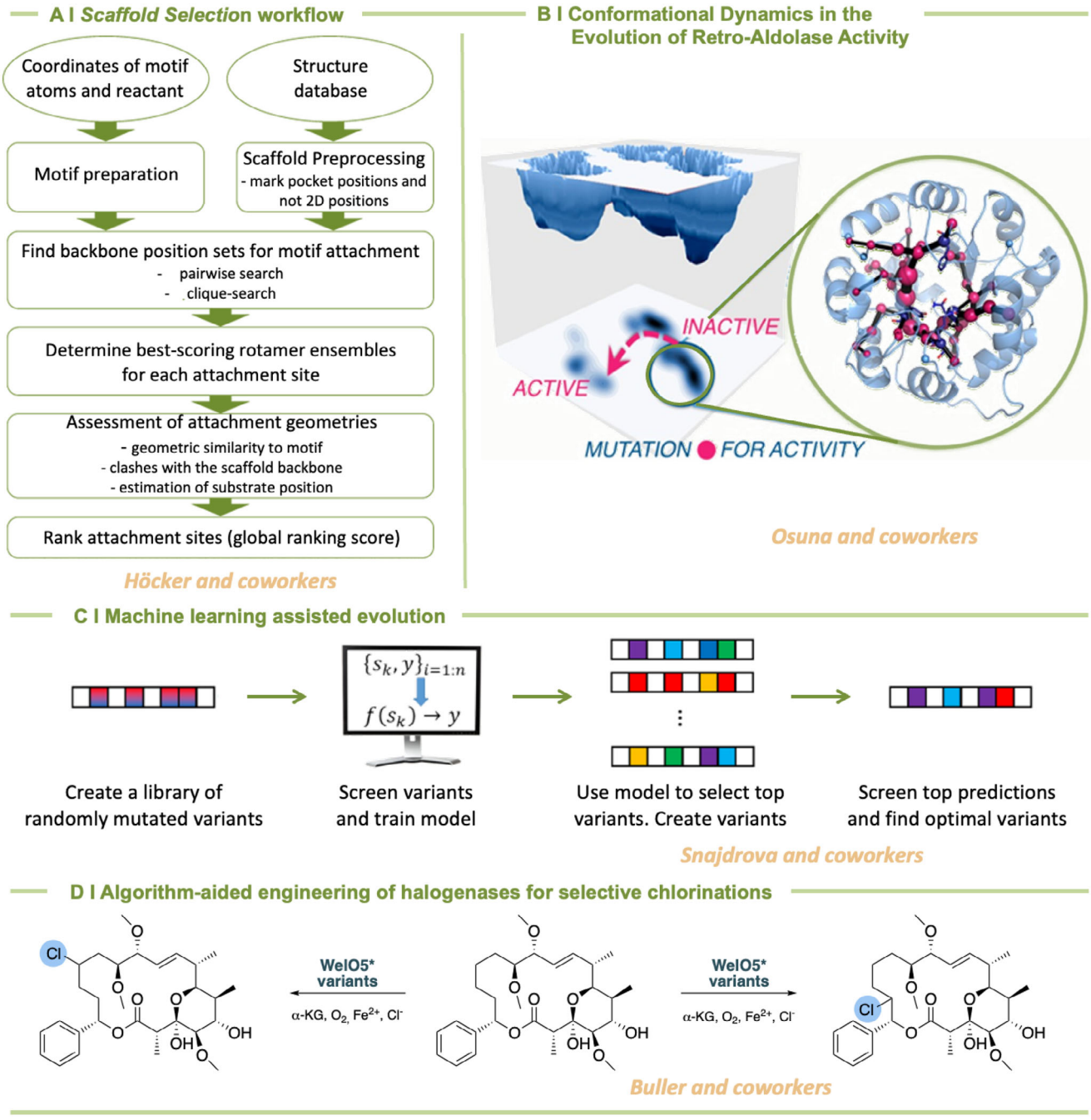


Figure 4. Computational tools in biocatalysis to increase the enzyme performance. (A) Workflow of *ScaffoldSelection* enabling the construction of novel proteins, (B) representation of the possible mutations to increase the activity of retro-aldolase enzymes via conformational dynamics, (C) workflow for machine learning-assisted enzyme evolution, and (D) regio-divergent halogenation of soraphen A catalyzed by variants of the non-heme iron-dependent halogenase WelO5* obtained by ML-guided enzyme engineering.

substrate interactions and the improvement of enzyme activity or stability.

Birte Höcker (University of Bayreuth, Germany) has provided important input to the field of protein design and its implications for the development of custom-made enzymes since early on.[60] One of her seminal works presented an algorithm that enables the construction of novel protein functions on existing protein scaffolds, which was named *ScaffoldSelection* (Figure 4A).[61] First, the method identifies pairs of backbone positions in pocket-like regions by using a representative set of structures from the Protein Data Bank. Then, it combines these to complete attachment sites using a graph theoretical approach. Finally, identified matches are assessed for their ability to accommodate the substrate or transition state. This method enabled the rapid preselection of protein scaffolds suited for a specific reaction type and the incorporation of a predefined amino acid motif in a faster fashion than other contemporary methods while maintaining the same accuracy. More recently, the computa-

tional studies of the Höcker group in the field of biocatalysis include the understanding of enzyme properties to efficiently degrade microplastics.[62]

Silvia Osuna (University of Girona, Spain) has made a significant leap forward in the understanding, design, and laboratory evolution of enzymes. In fact, while enzymes are the most efficient, specific, and selective catalysts, not all synthetic processes have a natural enzyme available to catalyze and speed up the reactions. Osuna's group has shown that by rationally introducing modifications at optimal positions – whether in the catalytic site or distant from it–considering the entire protein structure and its mechanism, both performance improvement and novel reactivities can be achieved.[63] Mutations involved in the shift to catalytically more favorable conformations have been demonstrated to be computationally predictable (Figure 4B).[64] This process, which is completed in a short period of time via simulation and computational analysis, dramatically reduces the costs compared to directed evolution. More recently, the Osuna group could also experimentally test in-house the computationally novel generated variants, thus providing experimental validations and consequently refining predictions for the obtainment of enzymes with increased stability, activity, and substrate selectivity.

Radka Snajdrova (Novartis, Switzerland) is recognized for her research on enzyme engineering and the application of biocatalytic processes to develop more sustainable and efficient pathways for drug synthesis. Her research has significantly contributed to integrating enzymatic approaches in the pharmaceutical industry. This includes the biocatalytic synthesis of chiral building blocks and the development of enzymatic reactions for pharmaceutical production. Recently, she has been part of advancing the integration of machine learning and AI-driven approaches in enzyme design to optimize biocatalytic reactions (Figure 4C).[65] In 2021, her group developed an enzyme engineering platform to compare machine-directed evolution to deep mutational scanning and error-prone PCR-based techniques, using imine reductases as a study case.[66] Their findings concluded that AI can provide a significant efficiency advantage over more traditional mutagenesis strategies if applied with a large enough training dataset. The platform is now being used to support early-stage drug discovery in industry.

Rebecca Buller (Zurich University of Applied Sciences, Switzerland) is one of the world's leading experts in biocatalysis and enzyme engineering guided by a computational approach. Her work has had a tremendous impact on the development and optimization of biocatalysts for the chemical and pharmaceutical industries. In particular, her research group pioneered the design and engineering of non-heme iron-dependent halogenases for the stereo- and regioselective halogenation of unactivated sp^3-hybridized carbon centers.[67] One of her outstanding studies, describes the algorithm-aided engineering of the aliphatic halogenase WelO5* for the late-stage functionalization of the macrolides soraphen A and C, potent antifungal agents (Figure 4D).[68] By using site-saturation mutagenesis in combination with ML to significantly reduce the screening effort, the inactive wild-type WelO5* was tailored into an effective halogenase for the functionalization of the tested macrolides with a more than 300-fold improved TTN. The ML-guided engineering approach was able to predict more active variants and allowed switching the regioselectivity of the halogenases. In addition, the Buller group is using its ML-guided engineering approaches to reduce the size of enzyme libraries for the improvement of other biocatalysts, for example, a ketoreductase for the synthesis of an ipatasertib precursor, thereby further expanding the enzyme toolbox for the development of industrial biocatalytic processes.[69]

6. Behind the Scenes: The Role of Female Mentorship

Mentors play a crucial yet often under-recognized role in fostering innovation, networking, and career development. Specifically, female mentorship not only helps to break down gender biases but also provides the next generation of women scientists with role models whose experiences often reflect unique challenges and pathways. Their mentorship contributes to a more inclusive research environment, encouraging diverse perspectives that drive scientific breakthroughs and expand the horizons of research in academia and industry.

Mentorship can take many forms, from training researchers through experimental challenges to providing career advice and fostering scientific networks. We would like to highlight the positive influence of female mentors who have contributed to advancing our careers beyond technical skills, providing essential support and guidance. In particular, we would like to specifically acknowledge Francesca Paradisi (University of Bern), Isabel Arends (Utrecht University), Kylie Vincent (University of Oxford), Martina Pohl (Forschungszentrum Jülich), and Dörte Rother (Forschungszentrum Jülich). Not only do they serve as role models for young women scientists, but they themselves have taken on roles beyond research, such as department heads or deans. As such, their experiences reflect the unique challenges of these roles and have been particularly inspiring on our leadership journey. In addition, their advice, ongoing support, and positive feedback have not only stimulated our own development to date but also serve as a role model for mentorship within the academic environment, demonstrating the importance of female support to further promote gender diversity and inclusion in science.

Founding and developing a company is no small feat, and we would especially like to highlight the inspiring initiatives of Maria Fátima Lucas, co-founder and CEO of Zymvol (Barcelona, Spain), accelerating enzyme discovery and design via bioinformatics and molecular modeling, and Holly Reeve, co-founder and CEO of HydRegen (Oxford, UK), developing hydrogenases for cofactor recycling and nitro to amine conversion. They have continuously shared their incredible journey to build their company.

In addition, young women leaders play a critical role in inspiring and mentoring the next generation of women scientists. They serve as tangible examples of success and help break down

societal perceptions that may discourage women from pursuing research or leadership roles. This exchange of experiences plays a crucial role in career development, particularly in securing professorships and navigating the academic path. Young women leaders, who have recently faced similar challenges, can offer valuable insights and strategies tailored to the specific hurdles as an emerging female scientist. This peer-to-peer exchange fosters a supportive network, enhances resilience, and contributes to a more informed and self-confident approach to securing leadership positions in academia. In this regard, Ana I. Benítez-Mateos would like to acknowledge the support of the co-authors of this perspective article, as well as Susana Velasco-Lozano (University of Zaragoza) and Cathleen Zeymer (TU Munich).

We would also like to emphasize that, in addition to the more personal and long-term mentoring relationships that we have experienced throughout our careers, positive influences on young women scientists can also be of a different nature. For example, the opportunity to interact with established women scientists in a more informal setting, such as at a conference, to build a network, or to seek advice on a scientific problem, can already have a positive influence that may stimulate women to pursue a scientific career or take on a leadership role.

7. Summary and Outlook

In this perspective article, we celebrate the remarkable accomplishments of female-led research groups in the field of biocatalysis, showcasing the work of women who are at the forefront of scientific advancement. This personal selection of researchers shows their significant contributions, pushing the boundaries of our understanding and expanding the possibilities of biocatalysis with innovative approaches and novel discoveries. Their efforts have not only enhanced the scientific knowledge in this domain but also shaped the future of the field by addressing critical challenges with creativity. By highlighting their achievements, we aim to draw greater attention to the diverse and impactful work being conducted by women, illustrating the breadth of their influence across various aspects of biocatalysis. Our goal goes beyond the recognition of individual excellence; we seek to promote the value of gender diversity and inclusion in science, particularly in (bio)catalysis, where representation and visibility can drive progress. Increased recognition of women's contributions is essential to foster an environment where diverse perspectives are embraced and celebrated. By amplifying the accomplishments of these excellent researchers, we hope to inspire a new generation of female scientists to embark on careers in biocatalysis and related fields, confident in their ability to contribute meaningfully to scientific progress. Through this initiative, we aim to play a part in building a more equitable and inclusive scientific community, where talent, innovation, and excellence are recognized and celebrated, regardless of gender. By encouraging diversity, we believe the field of biocatalysis — and science as a whole— will continue to benefit from a richer range of ideas, experiences, and expertise.

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Conflict of Interests

The authors declare no conflict of interest.

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