Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Editorial overview: New pieces in the redox puzzle: oxidative and reductive transformations in biotechnology

Kylie A Vincent and Bettina M Nestl

Current Opinion in Chemical Biology 2019, **49**:A1–A3

For a complete overview see the Issue

Available online 15th March 2019

https://doi.org/10.1016/j.cbpa.2019.02.008

Kylie A Vincent

Department of Chemistry, University of Oxford, Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, UK
e-mail: kylie.vincent@chem.ox.ac.uk

Kylie A Vincent is Professor of Inorganic Chemistry at the University of Oxford. She received her PhD from the University of Melbourne with Stephen Best in 2004 on chemistry of the iron–molybdenum cofactor of nitrogenase. She then moved to postdoctoral research on electrochemical studies of hydrogenases with Fraser Armstrong at the University of Oxford, before starting up her independent research group in 2007. Her research involves mechanistic studies of metalloenzymes, and development of biocatalytic hydrogenations.

Bettina M Nestl

Institute of Biochemistry and Technical Biochemistry, Department of Biochemistry, Universitaet Stuttgart, Allmandring 31, 70569 Stuttgart, Germany
e-mail: bettina.nestl@itb.uni-stuttgart.de

Bettina M Nestl is a junior research group leader within the Institute of Biochemistry and Technical Biochemistry at the Universitaet Stuttgart. She received her doctorate from the University of Graz, Austria, with Kurt Faber for her studies on enzymatic isomerizations. She continued to develop her interests in biocatalysis as a postdoc with Nicholas J. Turner at the University of Manchester, UK. Her research focuses on the development and engineering of novel enzyme catalysts for the synthesis of chiral amines and amino alcohols.

Chemists are increasingly looking to biocatalysis for selective oxidative and reductive transformations in the syntheses of bioactive natural products, chemicals, and pharmaceuticals. For reductive reactions, enzymes which mediate hydride transfer from nicotinamide cofactors for asymmetric ketone reductions are particularly well established for the generation of chiral alcohol functionalities in industry. These are now supplemented by further NAD(P)H-dependent enzymes including imine reductases and ene reductases. New biocatalytic reactivities are constantly being expanded via a combination of enzyme discovery and large-scale efforts in genetic engineering of enzymes.

The ubiquity of the nicotinamide cofactors as hydride donors has spurred development of various pathways for recycling NAD(P)H in cases where the enzymes are utilized in isolation from their cellular environment. Most of these recycling routes are themselves biocatalytic because of the difficulties in chemo-catalysis of the correct hydride addition to $NAD(P)^+$ to regenerate the reduced cofactor. Biocatalytic cofactor recycling using H_2 as a reductant is an attractive option because it avoids the carbon-based waste generated when simple alcohols or glucose are used as reductants. Photocatalytic cofactor recycling comes with additional challenges, but offers promise of harnessing energy from sunlight to couple NAD(P)H regeneration to the oxidation of water — the ultimate clean source of electrons. Biomimetic cofactors are tolerated to some extent (mainly by flavin-dependent oxidoreductases), and are being explored as cheaper, synthetic alternatives to NAD(P)H. In parallel, understanding is growing around enzymes that utilize other hydride transfer cofactors such as the reduced forms of pyrroloquinoline quinone (PQQ) or coenzyme F_{420} to mediate selective reductive catalytic transformations.

For oxidation reactions, the stage has been dominated by cytochrome P450 monooxygenases, which require NAD(P)H an electron donor for partial reduction of O_2, and typically insert one oxygen atom into a C–H bond. Many new reactivities are being discovered and developed for cytochrome P450s while further classes of enzymes are becoming established for C–H functionalization. Particularly exciting advances are arising from combining enzyme scaffolds with non-biological catalytic units to harness reactivity that is new to nature alongside the vast possibilities from genetic engineering of protein scaffolds.

In this issue of *Current Opinion in Chemical Biology*, we present a series of reviews that cover recent research advances in oxidation/reduction processes

using redox enzymes. The field of oxidoreductase-mediated organic synthesis has been an area of intensive investigation. As shown by these contributions, biologists, chemical biologists and chemists are developing efficient and novel redox enzymes and implementing them in new ways to carry out synthetic transformations of major interest for the industrial biotechnology sector.

The functionalization of inert carbon–hydrogen (C–H) bonds is of great importance in modern synthetic chemistry. In this light, the research area of C–H functionalization has received considerable attention and witnessed significant developments in recent years. Enzyme catalysts have proved effective for enabling selective transformations of C–H bonds, chemistry which has been applied in the total syntheses of natural products as well as molecules of chemical and pharmaceutical interest. The review by 2018 Nobel laureate in chemistry, Frances H. Arnold and co-workers offers stimulating insights into the engineering of cytochrome P450s and other heme proteins for novel, non-natural C–H functionalizations. Engineered heme proteins, including some which coordinate metals other than the native iron, have been generated to realize challenging but promising selective oxidations of C–H bonds. Notably, the chemistry of these engineered heme proteins has been expanded to C–H aminations as well as C–C bond formations. The formidable challenge of selective C–H functionalization also features in a review by the lab. of Hans Renata, which tackles transformations on C–H bonds in small-molecule building blocks as well as late-stage functionalization of natural products using both heme proteins and other enzymes. Examples include C–H functionalizations by oxygenase enzymes in natural product synthesis.

The discovery and engineering of enzyme catalysts with novel reactivities or with improved catalytic properties are of fundamental importance for the development of new biocatalytic applications. The review by Christopher Prier and Birgit Kosjek of Merck & Co., summarizes recent preparative-scale applications of redox enzymes. In their account, they describe entire processes from reaction execution and work-up to product isolation using redox enzymes with a particular focus on product preparations at scales of at least one gram. Further biotechnologically attractive enzymes are flavoenzyme amino acid oxidases, discussed by Yasuhisa Asano and Kazuyuki Yasukawa. Beyond the discovery and characterization by X-ray crystallography of novel amino acid oxidases from nature, the authors briefly introduce a novel oxidase with hydroxylating activity of non-activated C–C bonds and the generation of an amine oxidase by protein engineering. While the redox-chemistry performed by nicotinamide-dependent and flavin-containing enzymes is now well-established, in recent years pyrroloquinoline quinone (PQQ)-dependent redox enzymes have been presented. Nobuhumi Nakamura et al. introduce us to the world of fungal PQQ-dependent dehydrogenases and related enzymes. The discovery of a novel eukaryotic quinohemoprotein provided invaluable insight into the substrate specificity of this enzyme and provided a route into plant biomass degradation.

Considering that one-third of the oxidoreductases require one of the five adenine coenzymes for catalytic activity, it is easy to see why many practical enzyme applications must include considerations of the problems associated with coenzyme recycling. Exemplifying advances in the development of nicotinamide cofactor biomimetics, the contribution by Volker Sieber and co-workers reviews the current state-of-the-art in catalysis using these synthetic cofactor alternatives. The application of synthetic cofactors replacing NAD(P)H cofactors in chemical and enzymatic, as well as photobiochemical, systems is complemented by studies on regeneration processes for these biomimetic cofactors. Reporting on photoelectrochemical biotransformations, the review by Jinhyun Kim and Chan Beum Park concentrates on recent advances in the coupling of redox enzymes with photoelectrocatalysis. This technology addresses the transfer of photoexcited electrons to (or from) prosthetic groups of enzymes for photoelectrochemical biotransformations as well as cofactor regeneration. Continuing studies on the use of cleaner methods for cofactor recycling, Lars Lauterbach and Oliver Lenz present an up-to-date overview of H_2-driven biosyntheses and biotransformations. In addition to describing the use of the engineered hydrogen-oxidizing bacterium *Ralstonia eutropha* for the production of valuable chemical compounds, this review discusses how O_2-tolerant hydrogenases in *R. euthropa* can be employed for H_2-driven regeneration of nicotinamide cofactors.

General concepts for whole-cell or cell-free redox biocatalysis have been established. The piece by Florian Rudroff in this issue analyzes the successes and challenges in the development of efficient whole cell enzyme cascades using redox enzymes. Major challenges in terms of optimal carbon flux need to be solved in this area of biocatalysis to enable productive enzyme cascades. As an alternative to whole-cell systems, new multi-component synthetic routes using cell-free systems can be designed for the production of specialty compounds. These approaches are reviewed by Claudia Schmid-Dannert and Fernando Lopez-Gallego, who address the advances and opportunities for the design of self-sufficient and spatially organized cell-free biocatalytic systems. Here co-localization including enzyme attachment, clustering and encapsulation can be applied to increase the efficiency of multi-enzyme systems.

The final piece in this issue is presented by Todd Hyster and co-workers who take us in the area of radical

chemistry in nature by describing recent progress in diverting enzymes for biocatalytic hydrogen atom transfer reactions to generate products with impressive stereochemical control. While focusing on reactivities of *S*-adenosyl-L-methionine-dependent or α-ketoglutarate-dependent non-heme iron enzymes for initiating free-radical reactions, their account also contains examples of transformations with nicotinamide and flavin cofactors for terminating free-radical reactions in enzyme active sites.

In summary, the scientific advances in chemical biology discussed in this issue of *Current Opinion in Chemical Biology* highlight innovative and exciting new developments of enzymes catalyzing oxidation/reduction reactions. We would like to thank all authors and reviewers for their contributions and hope the readers find them enlightening and as enjoyable to read as we have found them during the preparation of this special issue.