

NATURE-INSPIRED CATALYSTS FOR CHALLENGING REACTIONS IN A GREENER FUTURE

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Abstract

Nature is capable of catalyzing and controlling some of the most challenging chemical reactions in the universe, such as the oxidation of methane and the recently discovered oxidative depolymerization of crystalline polysaccharides such as cellulose. While the mechanistic aspects of Nature's intricate tools are being unraveled, chemists are getting better at mimicking Nature by a combination of functionalized material synthesis, operando characterization and detailed mechanistic investigations. These increasing insights, along with better and more accessible tools for advanced structural modeling and theoretical chemistry, bring the fields of biological and chemical catalysis closer. We will discuss recent developments in both fields and show how these fields could and should merge to solve major future challenges.

Introduction

Alkanes, the main components of fossil fuels, and end products of anaerobic fermentation, are nonpolar molecules built up through single C-C and C-H bonds. In spite of their high energy content, they are kinetically rather inert. In industry, selective alkane functionalization relies on multi-step processes, where C-H bonds are broken in a first step to form H₂ and alkenes (C=C), alkynes (C≡C) or, in the case of methane, alcohols (C-O). Due to thermodynamic constraints, such processes rely on high temperatures (> 600° C) and, in the case of C₂₊ alkanes, catalysts which favour C-H bond cleavage

over C-C bond cleavage, in spite of the higher C-H bond strength.¹ The industrial production of C₂-C₄ alkenes amounts to more than 120 million metric tons per year,² and is the starting point of other major industrial processes, where alkenes are selectively converted to functionalized products under mild conditions. Due to the vast amount of energy that would be saved, the development of selective, one-step alkane partial oxidation processes would be a major step towards greener industrial production.

Methanol, the first oxidation product of methane, is a key industrial chemical, with a global production capacity of 100 million metric tons per year. It is produced from methane in a two-step process:



Due to thermodynamic constraints, the first step is typically carried out at a temperature of 800 °C and a pressure of 30 bar, while the second step is carried out at 270 °C and 50–100 bar. If a direct process from methane to methanol could be run in one step, it would be thermodynamically feasible at room temperature.



Shifting from the two-step to a one-step process would be a spectacular achievement and would lead to massive energy savings in both plant construction and operation. However, in spite of intense research efforts over the past 30 years, a selective process for direct methanol production from methane (often called the “Dream reaction”) has not yet been achieved.

The challenge lies in:

- (a) CO₂ and H₂O being the thermodynamically most stable products from methane and O₂ at moderate temperatures, and
- (b) methane being a completely symmetrical molecule with no dipole mo-

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2. Torres Galvis, H.M., *et al.*, Catalysts for Production of Lower Olefins from Synthesis Gas: A Review. *ACS Catal.* 2013, *3*, 2130.

ment. While catalytic methane activation is feasible even at room temperature, the replacement of a C-H bond with a C-OH bond inevitably breaks the symmetry and introduces a dipole moment to the molecule, thereby enhancing its ability to react further. The methanol yield obtained from reaction (3) is typically only 1–10 %.³

Interestingly, Nature is able to selectively produce methanol from methane, using methane monooxygenases (MMO) belonging to the sMMO and pMMO families.⁴ While these two enzyme types catalyse identical reactions, their structures and catalytic mechanisms are very different. Activity of the less abundant sMMO-type enzymes involves a di-iron center as well as NADH and FAD co-factors. The s in sMMO stands for soluble, which makes the enzyme easy to handle; however, the need for expensive co-factors limits the potential of this enzyme in industrial applications. The more abundant pMMO-type enzymes are integral membrane proteins, which are insoluble (p stands for particulate) and are thus of highly limited industrial potential, except when used in their natural context, i.e. the methanotrophic bacteria that are used to convert biomass to methane. Little is known about the catalytic mechanism of pMMO, which, however, is of major interest since pMMO only uses metal ions as co-factors. For long, the catalytic power of pMMO was ascribed to a di-copper centre, whereas some recent studies suggest that this center only contains a single catalytically important copper.⁵ The relatively simple catalytic architecture of pMMO is of major interest, since it could be mimicked in synthetic catalysts. Moreover, a similar catalytic architecture, with almost similar catalytic power has recently been discovered in enzymes that oxidatively break down glycoside bonds, the so-called lytic polysaccharide monooxygenases, or LPMOs.⁶

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6. (a) Vaaje-Kolstad, G., Westereng, B., Horn, S.J., Liu, Z., Zhai, H., Sørli, M. and Eijsink, V.G.H.; An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. *Science*, 2010, 330, 219, (b) Forsberg, Z., Mackenzie, A.K., Sørli, M., Røhr, Å.K., Helland, R., Arvai, A.S., Vaaje-Kolstad, G. and Eijsink, V.G.H.; Structural and functional characterization of a conserved pair of bacterial cellulose-oxidizing lytic polysaccharide monooxygenases. *Proc. Natl. Acad. Sci. USA*, 2014, 111, 8446, (c) Kracher, D., Scheiblbrandner, S., Felice, A.K.G., Breslmayr, E., Preims, M., Ludwicka, K., Haltrich, D., Eijsink, V.G.H. and Ludwig, R.; Extracellular electron transfer systems fuel oxidative cellulose degradation. *Science*, 2016, 352, 1098.

Alike the oxidation of methane by MMOs, oxidative cleavage of glycoside bonds involves controlled activation of a carbon-hydrogen bond (referred to as HAA, hydrogen atom abstraction), as illustrated in Figure 1.

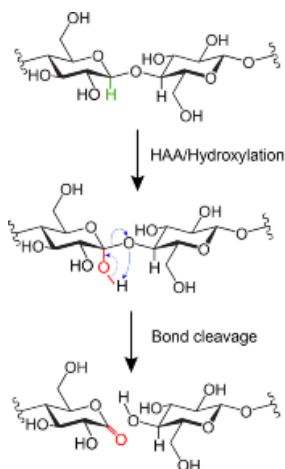


Figure 1. The reaction catalyzed by LPMOs. Hydrogen atom abstraction (HAA) by a copper-bound activated oxygen species is followed by hydroxylation of the substrate, which leads to spontaneous bond cleavage. See text for details. Picture made by Bastien Bissaro, NMBU.

Hydrogen abstraction is followed by hydroxylation, similar to what is expected to happen in MMOs. Phrased differently, in both enzyme types, catalysis involves insertion of electron-activated oxygen into the C-H bond to hydroxylate the substrate. The reaction scheme for this hydroxylation reaction is shown in Figure 2. In the case of LMPOs, the hydroxylation is followed by spontaneous bond cleavage.

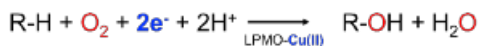


Figure 2. Reaction scheme for substrate hydroxylation by LPMOs as originally proposed by Vaaje-Kolstad et al. in 2010.

Nature has a wide arsenal of “common” hydrolytic enzymes to break glycosidic bonds, whose catalytic power depends in part on using binding energy to distort the substrate towards the transition state. However, when the substrate occurs in a rigid, “non-distortable”, or even crystalline structure,

as in cellulose, chitin, or the rigid co-polymeric structures of plant cell walls, such distortion cannot be achieved. It is assumed that the oxidative mechanism exerted by LPMOs has evolved to deal with substrates that are not amenable to the action of regular hydrolases. Both MMOs and LPMOs have high oxidative power where MMOs must be able to break a C-H bond of 105 kcal/mol while LPMOs can break bonds of at least 95 kcal/mol. The latter value is similar to the C-H bond strength of methylpropane (96 kcal/mol).

Enzyme-based C-H activation

The LPMOs and pMMO show that Nature has evolved Cu-dependent enzymes that use electron-activated oxygen to abstract a hydrogen atom from the substrate. Many questions remain as to the catalytic mechanism of these enzymes, including the nature of the most reactive oxygen species along the reaction pathway. The single copper in LPMOs and the one (of possibly two) catalytically most important copper in pMMO are coordinated by a unique histidine brace (Figure 3). The metal ion is coordinated by three nitrogens situated on two his-

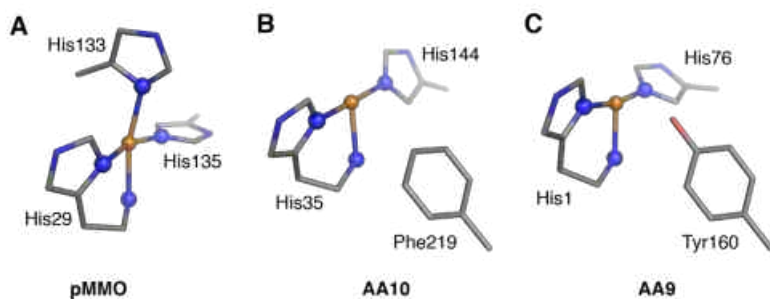


Figure 3. Catalytic centers in pMMO and two types of LPMOs (AA10 and AA9). The copper ion and coordinating nitrogen atoms appear as spheres and the connections between these spheres indicate the interactions. Note that His29 (A), His35 (B) and His1(C) are the N-terminal residues of the proteins and that the N-terminal amino group is involved in copper coordination. This picture was made by Åsmund Røhr Kjøndseth, NMBU.

tidines (two imidazole and one backbone nitrogen) in a T-shaped geometry termed the histidine brace. In pMMO, there is one additional protein ligand (His133; Figure 3). The perhaps most peculiar feature of this brace is the in-

volvement of the N-terminal amino group of the protein, which may play a special role in stabilizing reactive species emerging along the reaction pathway⁷.

Although details remain to be resolved, the general mechanism of the monooxygenase reaction catalyzed by pMMO and LPMO is that upon reduction the copper activates molecular oxygen to form a species that is sufficiently powerful to abstract a hydrogen atom from the substrate. The nature of this oxygen species remains unknown but for LPMOs rapidly accumulating data are converging towards the formation of a copper-oxyl intermediate⁸ (Figure 4). Importantly, completion of the reaction requires delivery of another electron and two protons (Figure 2) and it is currently not clear how and when this delivery happens. In particular, the delivery of the second electron is an enigma. Monooxygenases normally contain co-factors capable of storing both required electrons. While this may also be the case for pMMO, which possibly contains more than one catalytically important copper ion, this is most certainly not the case for LPMOs.

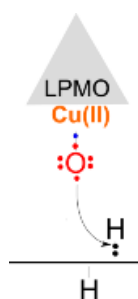


Figure 4. Schematic representation of a copper-oxyl species abstracting a hydrogen from a substrate. Picture made by Bastien Bissaro, NMBU.

Importantly, recent evidence suggests that LPMOs, and by analogy possibly also pMMO, may in fact not be monooxygenases but rather peroxygenases⁹.

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This implies that instead of using molecular oxygen to generate the reactive oxygen species, the enzymes use hydrogen peroxide (Figure 5). This very recent discovery requires further investigations, but is already supported by recent computational studies. This discovery may be a game-changer in understanding and exploiting LPMOs and MMOs, as well as in the design of nature-inspired synthetic copper catalysts, as discussed below.

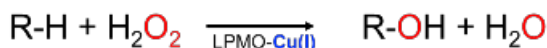


Figure 5. Alternative reaction scheme for substrate hydroxylation by LPMOs as proposed in 2017 by Bissaro et al.

Synthetic catalyst-based C-H bond activation

Advances in X-ray Diffraction (XRD) and Absorption Spectroscopy (XAS) enabled scientists to suggest that the active site of pMMO consists of a mono or di-copper-oxide entity, coordinated to aromatic and aliphatic amines.¹⁰ This finding has inspired a wave of synthetic catalysis studies aimed at selective methane oxidation. One branch of studies focuses on homogeneous Cu-complex catalysis in liquid phase. Decent activity has been reported at close to room temperature (see e.g.¹¹). However, methanol selectivity remains a challenge.¹² Another branch of studies aims to implement an additional feature of enzymatic catalysts, i.e. substrate confinement, by incorporating the mono-di- or tri-copper-oxide entities in a microporous structure, such as zeolites and zeotypes. Zeolites and zeotypes are crystalline materials with pore diameters in the 3-10 Å range. Cu-containing zeolites and zeotypes are active

monocopper enzymes depends on H₂O₂. *Nature Chem. Biol.*, 2017, 13, 1123, (b) Kuusk, S., Bissaro, B., Kuusk, P., Forsberg, Z., Eijsink, V.G.H., Sørli, M. and Våljamäe, P.; Kinetics of H₂O₂-driven degradation of chitin by a bacterial lytic polysaccharide monooxygenase. *J. Biol. Chem.*, 2018, 293, 523.

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methane oxidation catalysts. However, in order to avoid over-oxidation of methanol, methane oxidation must be carried out in a step-wise manner, where methane reacts with the catalyst to form surface methoxy groups in a first step, and where methanol is released by flushing with steam in a second step, before the catalyst active state is finally restored by high temperature treatment and contact with oxygen gas (see e.g. ¹³). It should be noted that the immediate surroundings of the di-copper oxide entity is different in zeolites and zeotypes than in Cu-based enzymes. In the enzyme, Cu is coordinated to aliphatic and aromatic nitrogen-containing ligands, while zeolites and zeotypes lattices are oxides; typically silicoaluminate and aluminophosphate, respectively, where Cu is coordinated to oxygen.

The catalysis group at the University of Oslo has gained international recognition for our work on zeolite- and zeotype-catalysed reactions, in particular the combination of material synthesis and mechanistic studies of the catalytic reactions, which enabled elucidation of structure-composition-function correlations based on single material parameter variation.¹⁴ Recently, we expanded those studies to methane partial oxidation to methanol over Cu-containing zeolites.¹⁵ Moreover, we have expanded the activity to another class of microporous materials; Metal-Organic Framework materials (MOFs). Like zeolites and zeotypes, MOFs are 3-D, crystalline, microporous structures. However, being composed of inorganic nodes and organic linkers, the MOF material class offers much wider opportunity of tailoring the active site and its surroundings, than the zeolite/zeotype material class. In 2008, our group published the first series of MOFs with a Zr-based node; UiO-66 and its isorecticular analogues UiO-67 and UiO-68.¹⁶ These materials had unprecedented chemical, thermal and mechanical stability compared to other

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MOFs, and are stable under catalysis process conditions up to at least 250 °C.¹⁷ The Oslo group has spent a major effort in elucidating correlations between synthesis conditions and material quality, and how changes in synthesis conditions affect e.g. the formation of defects in the materials.¹⁸

Synergies between catalyst and enzyme design

Inspired by recent progress in their respective fields and by the possible synergies that may emerge, the catalysis team in Oslo and the enzymology team in Ås are in the process of joining their forces to break new ground in both synthetic and enzymatic catalysis. The Oslo team is currently exploring functionalised ZrMOFs as catalysts for Cu-enzyme-inspired methane partial oxidation to methanol. The Ås team is digging deeper into catalytic features of LPMOs and is also starting to explore if and how LPMOs may be adapted to catalyse other selective C-H bond activations. Importantly, while pMMO is exceptionally difficult to handle experimentally, LPMOs are small, soluble and simple to produce. There is large variation among natural LPMOs, suggesting high evolvability. So, when working with LPMO-inspired synthetic catalysts, it is possible to test designs in both the synthetic catalyst and the enzyme, something that is not feasible when working with pMMO.

There are obvious similarities between the challenges and experimental tools connected to the design of new synthetic and enzyme catalysts. Proper substrate interactions and confinement are crucial in both catalyst types, as is the timely and spatially correct delivery of the co-substrates oxygen or hydrogen peroxide, and of electrons. Catalyst stability, i.e. protection from off-pathway oxidative damage, is also of crucial importance and requires a joint understanding of catalytic mechanisms in time and space.

We believe that a long term iterative effort in designing, optimizing and functionally testing both synthetic catalysts and engineered enzymes to selectively oxidize alkanes and other substrates will generate ground-breaking novel insights into enzyme selectivity and how such selectivity and enzyme efficiency may be transferred to simpler, synthetic analogues with industrial potential.

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