Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

# Polysaccharide degradation by lytic polysaccharide monooxygenases

Zarah Forsberg[1], Morten Sørlie[1], Dejan Petrović[1], Gaston Courtade[2], Finn L Aachmann[2], Gustav Vaaje-Kolstad[1], Bastien Bissaro[1], Åsmund K Røhr[1] and Vincent GH Eijsink[1]

The discovery of oxidative cleavage of glycosidic bonds by enzymes currently known as lytic polysaccharide monooxygenases (LPMOs) has had a major impact on our current understanding of the enzymatic conversion of recalcitrant polysaccharides such as chitin and cellulose. The number of LPMO sequence families keeps expanding and novel substrate specificities and biological functionalities are being discovered. The catalytic mechanism of these LPMOs remains somewhat enigmatic. Recently, novel insights have been obtained from studies of enzyme–substrate complexes by X-ray crystallography, EPR, NMR, and modeling. Furthermore, it has been shown that LPMOs may carry out peroxygenase reactions, at much higher rates than monooxygenase reactions, which affects our understanding and exploitation of these powerful enzymes.

**Addresses**
[1] Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), N-1432 Ås, Norway
[2] NOBIPOL, Department of Biotechnology and Food Science, Norwegian University of Science and Technology (NTNU), Sem Sælands vei 6/8, N-7491 Trondheim, Norway

Corresponding author: Eijsink, Vincent GH (vincent.eijsink@nmbu.no)

**Current Opinion in Structural Biology** 2019, **59**:54–64

This review comes from a themed issue on **Catalysis and regulation**

Edited by **Phil A Cole** and **Andrea Mattevi**

For a complete overview see the Issue and the Editorial

Available online 1st April 2019

**https://doi.org/10.1016/j.sbi.2019.02.015**



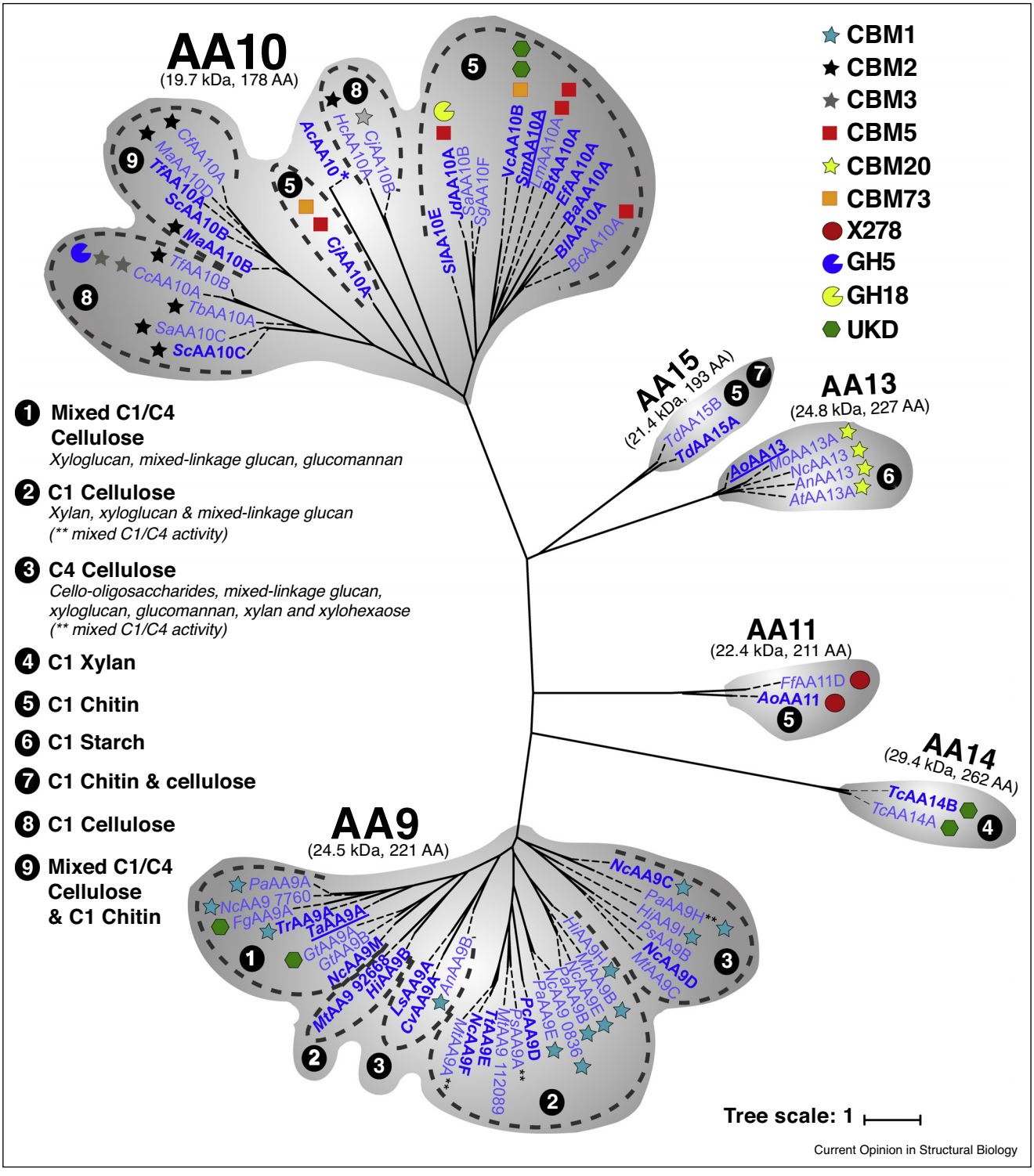
## Introduction

The role of redox enzymes in biomass conversion is gaining interest, as such enzymes may promote the conversion of recalcitrant polysaccharides [1,2,3,4•,5]. In comparison to canonical glycoside hydrolases, the role of redox enzymes (potentially) acting on plant cell wall polysaccharides has remained unclear. For example, fungal cellobiose dehydrogenase (CDH) has been studied for decades [6], without finding a clear role for this enzyme, although multiple biological roles have been proposed [7]. A major breakthrough came in 2010 when Vaaje-Kolstad *et al.* described oxidative cleavage of glycosidic bonds by enzymes known today as Lytic Polysaccharide MonoOxygenases (LPMOs). LPMOs are mono-copper enzymes that, in the presence of an external electron donor, catalyze hydroxylation of one of the carbons (C1 or C4) in the scissile glycosidic bond, which eventually leads to bond breakage by an elimination reaction [3,8,9]. In contrast to hydrolytic enzymes, which interact with single polysaccharide chains, LPMOs can act on polysaccharide chains that reside in a crystalline environment. This leads to disruption of the structure, making the cellulose more accessible for hydrolytic enzymes [10–12].

Since their discovery in 2010, LPMOs have been intensely studied, due to their great scientific and industrial interest. LPMOs are abundant in nature, in particular in fungi [13], and they catalyze a powerful oxidation reaction that involves multiple factors that may be hard to control. Major developments of recent years include the discovery of novel LPMO families [14•,15•] and increased insight into enzyme–substrate interactions from X-ray and neutron crystallographic, EPR, NMR and modeling studies [16••,17•,18••,19•,20–22,23•]. Furthermore, it has been discovered that next to, or perhaps even rather than, carrying out a monooxygenase reaction ($R\text{-}H + O_2 + 2e^- + 2H^+ \rightarrow R\text{-}OH + H_2O$), LPMOs carry out peroxygenation of their substrate ($R\text{-}H + H_2O_2 \rightarrow R\text{-}OH + H_2O$) [24••]. Both reaction mechanisms have been intensely studied using computational methods [25,26,27•,28•] and kinetics [29•,30••]. In a comprehensive review on oxidoreductases (potentially) involved in lignocellulose conversion, Bissaro *et al.* have discussed these recent discoveries in both a biological and applied perspective [4•].

All LPMOs described so far have a similar overall three-dimensional structure and a conserved, highly characteristic surface-located catalytic mono-copper site [31•]. As to date, the sequence-based classification system of the CAZy database [32] places LPMOs into six families of so-called 'auxiliary activities' (AA9-11 and AA13-15). The large sequence diversity and observed differences in substrate specificity (Figure 1), as well as the fact that LPMOs occur in several clades of the tree of life, indicate that LPMOs may be involved in biological processes

**Figure 1**




Phylogenetic tree of LPMOs.
The tree was built from 68 sequences, which represent the large majority of functionally characterized LPMOs. The underlying sequence alignment was based on catalytic domains only and on structural information from three selected LPMOs (*Ta*AA9A, PDB: 2YET; *Sm*AA10A, PDB: 2BEM; *Ao*AA13, PDB 4OPB; underlined in the figure). The 68 sequences were aligned using the T-Coffee Expresso online tool. The resulting MSA was employed as input to build the final phylogenetic tree using PhyML available via the online platform Phylogeny.fr. The names of LPMOs with a known three-dimensional structure are printed in bold face. The occurrence of additional domains, for example, CBMs, GHs, and unknown domains (UKD) is indicated by symbols. The dominating substrate specificity and oxidative regioselectivity (C1, C4, or mixed C1/C4) for each

other than biomass degradation, such as viral virulence [33] and bacterial pathogenicity [34,35]. It has been suggested that the recently discovered AA15s, found in arthropods, algae, oomycetes and complex animals, play a role in development and food digestion [14•]. It seems likely that LPMOs have multiple biological roles that remain to be discovered.

## The interaction of LPMOs with substrates

One of the major challenges in understanding LPMO catalysis lies in the insoluble nature of their substrates and the analytical problems this entails. Some AA9 LPMOs (i.e. *Group 3* in Figure 1) act on shorter soluble cello-oligosaccharides [36] and, while the biological relevance of this activity may be questioned, these LPMOs make good candidates for co-crystallization and soaking trials to generate enzyme–substrate complexes. In 2016, Frandsen *et al.* were the first to show a crystal structure of an AA9 LPMO in complex with cello-oligosaccharide ligands [18••]. Next to showing that ligand-binding is dominated by polar interactions (see also Courtade *et al.* [17•]), the data provided insight into how substrate-binding affects active site geometry, including the copper coordination sphere.

In the enzyme–substrate complex (Figure 2a), His1 stacks with the +1 sugar, and the space where the non-reduced LPMO (i.e. LPMO-Cu(II)) would bind an axial water (*black star* in Figure 2a) is filled by the C6-hydroxymethyl group of the +1 glycosyl unit. Frandsen *et al.* noted that a chloride ion, a potential mimic of superoxide or another activated oxygen species, occupied the fourth equatorial coordination position of the copper ion. Substrate-binding is associated with changes in the EPR spectrum [16••,18••,37], which could imply that the reactivity of the copper to some extent is controlled by the presence of substrate. Of note, various binding studies have shown that both the presence of potential superoxide mimics such as $Cl^-$ [18••] or $CN^-$ [17•] and reduction of the copper [38,39] promote substrate-binding. Together these studies suggest that the events leading to ternary complex formation are coupled, which is in line with conclusions derived from recent kinetic studies [30••,39].

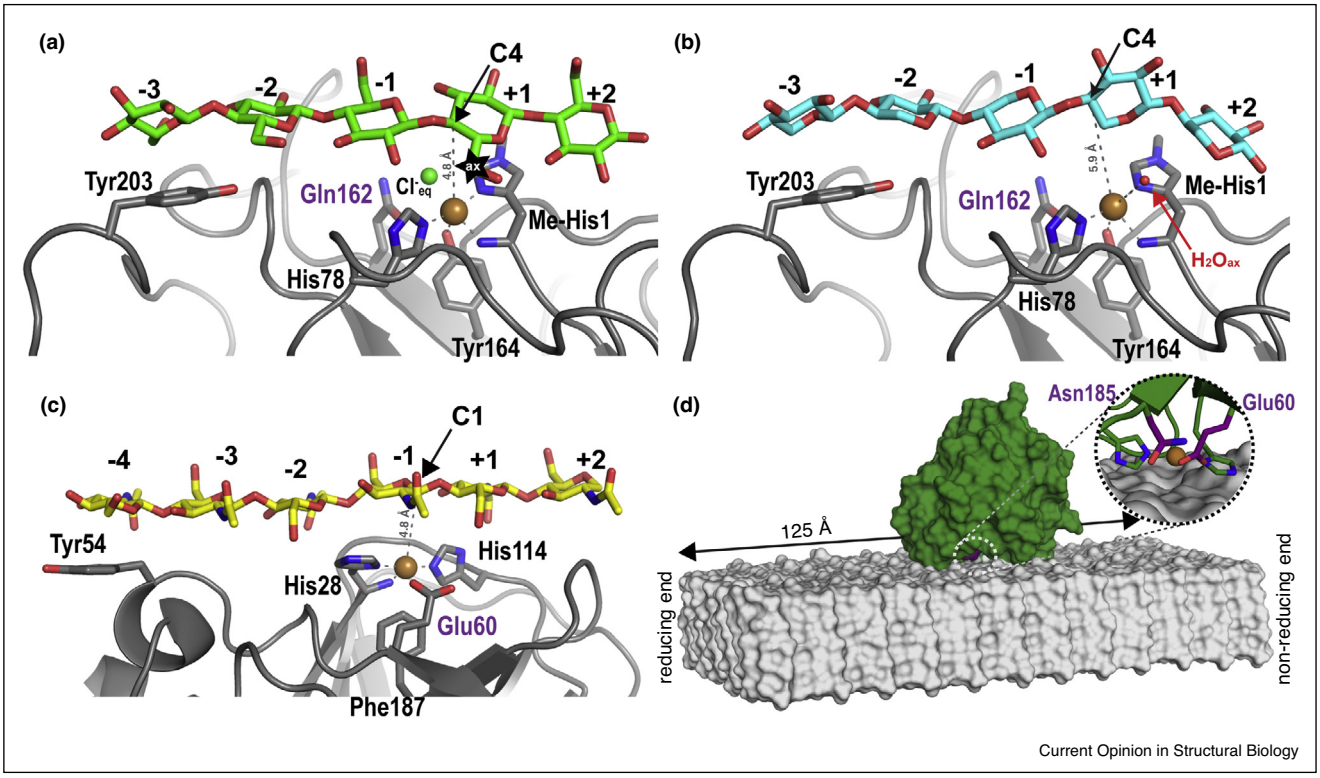
Simmons *et al.* reported crystal structures with xylo-oligosaccharide ligands using the same enzyme as Frandsen *et al.* The catalytic activity against xylohexaose was estimated to be 100-fold lower compared to cellohexaose, and the structural data showed that the interaction of the LPMO with xylopentaose was less distinct, compared to cellopentaose (Figure 2a,b). Stacking interactions with active site residues were not observed and the conformation of the copper site in the enzyme–substrate complex was clearly different (Figure 2a,b), as also confirmed by differences in the EPR signatures of the xylohexaose and cellohexaose complexes [19•].

In a combined biochemical, spectroscopic, and molecular modeling study, Bissaro *et al.* created an experiment-supported full-scale model of an LPMO, *Sm*AA10A, bound to a crystalline polysaccharide (chitin) surface [16••] (Figure 2c,d). Importantly, the model revealed a highly constrained active site geometry, with limited space near the copper site. The model also revealed a tunnel connecting the bulk solvent to the active site that seemed gated by a conserved second-shell glutamate, Glu60 (glutamine in some LPMOs, Figure 2). This tunnel is too narrow for bigger molecules, such as ascorbic acid and other reductants to pass, whereas smaller molecules such as $O_2$, $O_2^{\bullet-}$, $H_2O_2$ or $H_2O$, could enter or exit. It is worth noting that these observations add to 'the second electron conundrum', which entails that it is difficult to envisage how the second electron needed in a monooxygenase reaction (Figure 3) would be able to reach the catalytic complex [17•]. While the first electron can be recruited and stored by the non-substrate bound enzyme through reduction of Cu(II) to Cu(I), it is unclear how a second electron, which either has to be stored by the enzyme or timely supplied when required, can access the active site in the LPMO–substrate complex. It has been proposed that an electron transport chain or channel would allow delivery of a second electron [40], but this proposal is not supported by experimental evidence nor by conserved structural features across LPMO families.

Of note, a glutamate/glutamine is pointing toward the active site in all LPMOs, and mutational studies have shown that this residue is important for catalysis in both AA9 and AA10 LPMOs [41,42•] possibly because it helps in correctly positioning an oxygen species close to the active site [42•]. In the recent neutron structure of *Nc*AA9D, O'Dell *et al.* [22] showed evidence for an equatorially bound oxygen species interacting with His157 and Gln166 (where Gln166 would be analogous to Glu60 in *Sm*AA10A). Similar equatorial binding was proposed based on a neutron structure of *Jd*AA10A [21]. In a recent QM/MM study, Caldararu *et al.* [43] proposed that the second shell glutamate involved in this latter case (*Jd*AA10A-Glu65) plays an important role in $H_2O_2$ formation by the LPMO. Altogether, the above-mentioned studies support the idea that this conserved Glu/Gln, which, notably, occurs at quite different positions in

**(Figure 1 Legend Continued)** cluster are indicated by numbers 1–9. Known additional substrate specificities are shown below the major activity in smaller face. Note that most LPMOs have only been tested with a limited number of substrates, sometimes only one. Deviating oxidative regioselectivities are marked by **, as indicated in the figure. No activity has yet been shown for *Ac*AA10 (labelled with a blue asterisk), but the sequence was included as this is one of the few examples of a viral AA10. The average molecular weight (in kilo Dalton; kDa) and number of amino acids (AA) were calculated for each AA family using the primary AA sequences used to build the phylogenetic tree.

**Figure 2**




LPMO–substrate interactions.
The pictures show *Ls*AA9A-cellopentaose (PDB 5NLS; [19•]) **(a)**, *Ls*AA9A-xylopentaose (PDB 5NLO; [19•]) **(b)**, a model of *Sm*AA10A-chitohexaose **(c)**, and a model of *Sm*AA10A bound to β-chitin [16••] **(d)**. The structures shown in panels (a) and (b) were obtained from crystals soaked with oligosaccharides before X-ray diffraction [19•] and the models of panels (c) and (d) were obtained from experiment-guided MD simulations [16••]. The arrows indicate the oxidation sites; at the C4 carbon of the sugar bound in the +1 subsite for cellopentaose and xylopentaose bound to *Ls*AA9A and at the C1 carbon of the sugar bound to the −1 subsite for chitohexaose bound to *Sm*AA10A. Note the increased distance between the copper and the C4-carbon in panel (b) compared to panel (a). Axial ligands are labeled '*ax*' and are occupied by the C6-hydroxymethyl group (black star) in panel (a) and by a water in panel (b). The equatorial chloride ion shown in panel (a) is labeled '*eq*' and was obtained from the structure of *Ls*AA9A in complex with cellotriose [18••]. The white circle and insert in panel (d) show the entrance to the tunnel that connects the active site to the bulk solvent, including the gatekeeper residue Glu60 (corresponding to Gln162 in *Ls*AA9A) and Asn185.

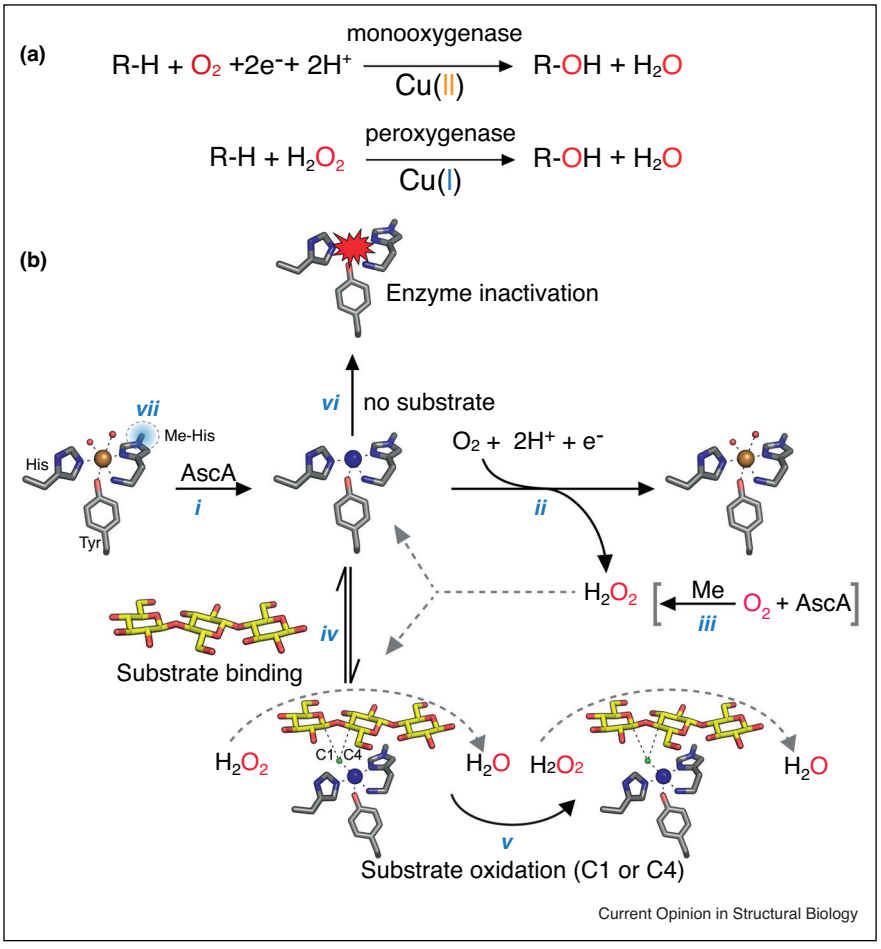
LPMO sequences, plays an important role in LPMO catalysis by positioning [16••,42•,44] and/or activating [27•,43] the oxygen co-substrate.

## The nature of the co-substrate and LPMO stability

In 2010, Vaaje-Kolstad *et al.* performed experiments with isotope-labeled dioxygen and water ($^{18}O_2$ and $H_2{}^{18}O$) leading to the conclusion that $O_2$ is essential for the enzyme reaction [3]. Apart from $O_2$, the monooxygenase mechanism (*top reaction* Figure 3a) requires two electrons from an external electron donor. It is well known that $H_2O_2$ is formed in LPMO reactions because of a two-electron reduction of $O_2$ by the electron donor (reductant) and because of the oxidase activity of a reduced LPMO [36,45]. Realizing this, noting that LPMOs tend to be co-expressed with $H_2O_2$ producing enzymes in fungal secretomes [2], and puzzled by light-activation of LPMOs [46] and the second-electron conundrum, Bissaro *et al.* assessed the possibility that $H_2O_2$ acts as a co-substrate of LPMOs [24••]. Such a peroxygenase reaction would only require a priming reduction of the LPMO, after which the enzyme could perform multiple catalytic cycles when supplied with $H_2O_2$ (Figure 3b).

Indeed, Bissaro *et al.* [24••] showed that $H_2O_2$ can drive LPMO reactions irrespective of the presence of $O_2$, and with consumption of only substoichiometric amounts of reductant. Importantly, experiments under aerobic conditions (i.e. 200–250 μM $O_2$ in the reaction mixture) with different concentrations of added isotope-labeled $H_2O_2$ ($H_2{}^{18}O_2$) showed that the incorporated oxygen came from $H_2O_2$ even when the $O_2$ concentration was 10-fold higher than the $H_2O_2$ concentration. Other evidence came from

**Figure 3**




Reactions involved in LPMO catalysis with focus on reactions involving $H_2O_2$.
Panel **(a)** shows an overview of the $O_2$-driven (monooxygenase) and the $H_2O_2$-driven (peroxygenase) reaction. Panel **(b)** shows an overview of key reactions in a typical LPMO reaction. Oxidized LPMO, where Cu(II) interacts with an axial and an equatorial water as shown here for *Ls*AA9A (PDB 5ACG), is reduced by a one electron reduction (***i***) to the LPMO-Cu(I) form, where the copper is coordinated by the three nitrogen ligands from the histidine brace (PDB 5ACF). $H_2O_2$ will be generated through the reaction of non-substrate-bound LPMO-Cu(I) with $O_2$ (oxidase activity, ***ii***) or from autoxidation of the electron donor, possibly catalyzed by trace metals (Me) in the solution (***iii***). The reduced enzyme binds to the (poly)saccharide substrate [38] and cleaves the glycosidic bonds using $H_2O_2$ (or $O_2$) as a co-substrate in the reaction (***iv***). Once primed (i.e. reduced), and when using $H_2O_2$, the LPMO can perform several catalytic events without the need of being reduced in between each catalytic cycles (***v***) [29•,49••]. The latter is less clear for the $O_2$-driven reaction (see [63] for possible reaction schemes). If a reduced LPMO reacts with $H_2O_2$ in the absence of substrate, or if the binding to the substrate is weak or unprecise (e.g. as a result of mutations on the binding surface or truncation of a CBM [23•,41,53]), the reaction may lead to oxidation of the active site and inactivation of the enzyme [24••,41] (***vi***). LPMOs produced in fungi tend to be methylated at the N-terminal histidine (***vii***), a post-translational modification that likely reduces inactivation at higher $H_2O_2$ concentrations [56]. In the lower panels (step ***v***), for illustration purposes, the green dot indicates the approximate position of the reactive oxygen species as derived from the structures determined by Frandsen *et al.* [18••].

the demonstration that ($H_2O_2$-consuming) horseradish peroxidase inhibits LPMO activity under standard conditions (1 mM ascorbic acid and atmospheric $O_2$, meaning that $H_2O_2$ is generated by the system itself) [24••]. Importantly, the catalytic rates obtained in $H_2O_2$ reactions were orders of magnitude higher that those typically observed in standard $O_2$ reactions, leading Bissaro *et al.* to propose that formation of $H_2O_2$ is rate limiting in standard $O_2$-driven reactions [4•,24••]. Of note, there is at least one example in the literature showing that an enzyme originally thought to be an oxidase in fact is a peroxidase [47].

Although the nature of the co-substrate of LPMOs, $O_2$ or $H_2O_2$, remains debated (e.g. [48•]; see below), the claim that LPMOs can carry out peroxygenase reactions at (unprecedented) high speed has been confirmed in several studies, by numerous research groups using multiple LPMOs from different AA families and with different

substrate specificities [29•,30••,48•,49••,50–52]. There are also recent studies showing correlations between the rate of the LPMO reaction and the rate of $H_2O_2$ production in the same reactions without LPMO substrate [53–55]. In this respect, Forsberg *et al.* have described a mutant of a cellulose-active AA10 LPMO that shows reduced cellulose-degrading activity in 'standard' reactions (i.e. with $O_2$ and ascorbic acid), and a reduced ability to activate $O_2$ (i.e. $H_2O_2$ production in the absence of substrate), but is as active as the wild-type enzyme in reactions driven by exogenously added $H_2O_2$ [53].

Bissaro *et al.* also showed that too high concentrations of $H_2O_2$ lead to oxidative damage of the active-site histidines, providing an explanation for the common observation that LPMOs are unstable under most reaction conditions (e.g. Refs. [49••,54]). Such inactivation is prevented by substrate binding [24••]. Indeed, high substrate-concentrations [23•] promote stability, whereas mutations that reduce substrate affinity of the LPMO domain itself [41,53] or that remove binding modules (i.e. CBMs) increase the sensitivity for autocatalytic inactivation [23•,53]. Of note, Courtade *et al.* [23•] described the first (NMR) structure of a complete CBM-containing LPMO and carried out an in-depth experimental assessment of how the CBM promotes LPMO activity and stability in a substrate-dependent manner. Experiments have shown that the methylation of the N-terminal histidine found in fungal LPMOs has little effect on enzyme functionality, but may provide higher resistance to oxidative damage [56].

## Recent insights from kinetic studies using $O_2$ or $H_2O_2$ as co-substrate

Reported catalytic rates for LPMOs under typical reaction conditions (atmospheric $O_2$, 1 mM reductant) tend to be in the order of 0.1 $s^{-1}$ or (much) lower, as recently reviewed by Bissaro *et al.* [4•]. Kinetic characterization of an AA9 LPMO acting on cellotetraose with $O_2$ as the co-substrate yielded a $k_{cat}$ of 0.11 $s^{-1}$, a $K_m$ of 43 μM with respect to the carbohydrate substrate and a $k_{cat}/K_m$ of $2.6 \times 10^3$ $M^{-1}$ $s^{-1}$ [18••]. The first comprehensive kinetic characterization of a (chitin-active) LPMO with $H_2O_2$ as the co-substrate yielded quite different values: a $k_{cat}$ of 6.7 $s^{-1}$ and $K_m$ values of 0.58 g $L^{-1}$ and 2.8 μM for chitin and $H_2O_2$, respectively [30••]. The resulting $k_{cat}/K_m$ is $2 \times 10^6$ $M^{-1}$ $s^{-1}$ for $H_2O_2$, which is in the same order of magnitude as reported $k_{cat}/K_m$ values for peroxygenases [57,58].

Hangasky *et al.* studied oxidation of cellohexaose by a fungal AA9 LPMO with $O_2$ or $H_2O_2$ as co-substrate and in the presence of 2 mM ascorbic acid [48•]. Reactions with $O_2$, at a concentration of 208 μM, and varying concentrations of cellohexaose, yielded an apparent $k_{cat}$ of 0.17 $s^{-1}$ and a $K_m$ of 32 μM with respect to cellohexaose. This yields a $k_{cat}/K_m$ of $5 \times 10^3$ $M^{-1}$ $s^{-1}$, which is similar to the value obtained by Frandsen *et al.* ([18••]; discussed above). Reactions with a constant cellohexaose concentration (1 mM) and varying $O_2$ concentrations (0–800 μM) yielded an apparent $k_{cat}$ of 0.28 $s^{-1}$ and a $K_m$ of 230 μM with respect to $O_2$, which corresponds to a $k_{cat}/K_m$ of $1 \times 10^3$ $M^{-1}$ $s^{-1}$. Of note, this value is about three orders of magnitude lower than the $k_{cat}/K_m$ determined by Kuusk *et al.* [30••] for $H_2O_2$-driven chitin conversion. Accordingly, reactions with $H_2O_2$ in the range from 12.5 to 100 μM by Hangasky *et al.* [48•] yielded rate constants between 4 to 15 $s^{-1}$. Although, the authors did not calculate a $K_m$ with respect to $H_2O_2$, this value can be estimated through a Michaelis–Menten analysis of data in Table S9 [48•], yielding a $K_m$ of 53 μM, which leads to an estimated $k_{cat}/K_m$ of $3 \times 10^5$ $M^{-1}$ $s^{-1}$.

A crucial difference between the $O_2$ and $H_2O_2$ mechanism concerns the need for a reductant. While in the $O_2$ mechanism, the reductant is consumed stoichiometrically with product formation, the $H_2O_2$ mechanism only requires a 'priming' reduction, and more reductant is only needed upon occasional re-oxidation of the LPMO (Figure 3). Still, the $H_2O_2$ mechanism does require reducing power, which indeed may become rate-limiting under certain conditions [24••]. It is well known that LPMO activity is reductant-dependent [2,59] but it is less clear why and how. Unravelling the role of the reductant is not straightforward because, next to LPMO reduction, the reductant will also affect the enzyme-dependent and enzyme-independent generation of $H_2O_2$. These complications have recently been unraveled in kinetic studies of a chitin-active LPMO by Kuusk *et al.* [29•] who showed that, once reduced, the LPMO carried out 18 oxidative cleavages using $H_2O_2$ as co-substrate.

Further illustrating the importance of $H_2O_2$, Bissaro *et al.* and Müller *et al.* showed that the LPMO activity in a commercial cellulase cocktail acting on Avicel could be increased by up to two orders of magnitude in anaerobic reactions with $H_2O_2$ feeding, compared to standard aerobic reactions utilizing $O_2$ and stoichiometric amounts of reductant [24••,49••]. Calculations showed that, under optimal conditions, each LPMO in the reaction mixture catalyzed at least 1500 peroxygenation reactions while the ratio between reactions catalyzed and reductant consumed was in the order of 15:1. This applied study also underpinned the risk of enzyme inactivation by 'overfeeding' $H_2O_2$ (Figure 3b).

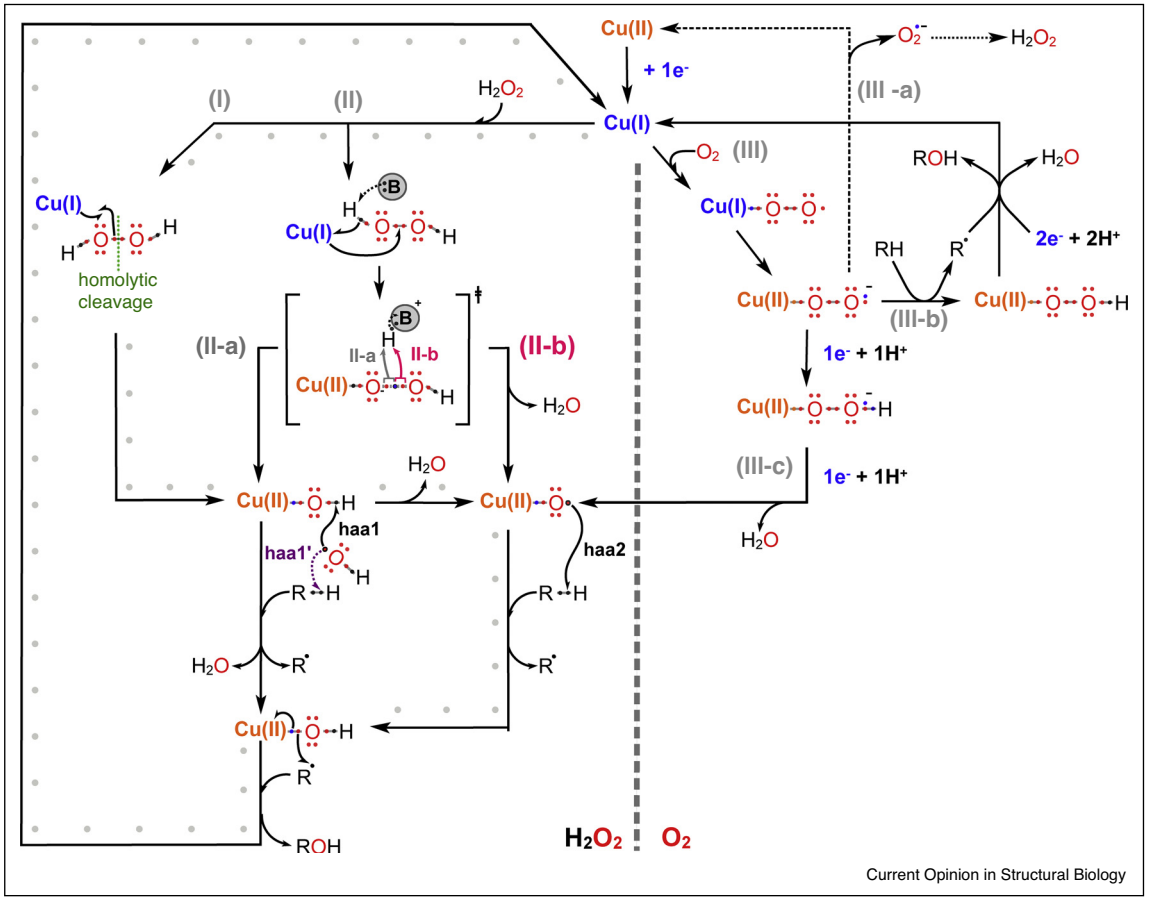
All in all, accumulating kinetic data indicates that $H_2O_2$ is the preferred co-substrate of LPMOs and that the peroxygenase reaction can reach much higher rates than the very low rates observed for $O_2$-driven reactions. Of course, as pointed out by Hangasky *et al.* [48•], and assuming that the monooxygenase reaction does occur at all (see below for discussion), what will happen in nature depends on the concentrations of $O_2$, $H_2O_2$, and reductant.

## Recent insights from modeling using $O_2$ or $H_2O_2$ as co-substrate

The discovery that LPMOs contain a single copper site without any apparent additional redox cofactor triggered the curiosity of the metalloenzyme community. Though several LPMOs have been subjected to spectroscopic methods, no reaction intermediates have yet been discovered. Thus, mechanistic insight at atomistic/molecular level has mainly been gained from computational efforts that have explored possible reaction pathways. In a combined spectroscopic and computational study, Kjaergaard *et al.* [60] demonstrated formation of a copper superoxide complex, $[CuOO]^+$, when a reduced LPMO interacts with $O_2$ in the absence of substrate. It seems, however, questionable whether superoxide is strong enough to abstract a hydrogen from a carbon in the scissile glycosidic bond, most studies conclude that a stronger oxidative species is needed [25,26,27•,28•,61,62•].

The first computational study to address the catalytic mechanism of LPMOs favored a copper oxyl, $[CuO]^+$, intermediate as the reactive species [61]. This hydrogen atom abstraction $[CuO]^+$ species appears in most computational studies and could also be a copper-oxo or protonated $[CuOH]^{2+}$ species. Importantly, while Kim *et al.* [61] considered axial binding of $O_2$, there is now abundant

**Figure 4**




Putative reaction mechanisms for polysaccharide oxidation by LPMOs using $H_2O_2$ (left side) or $O_2$ (right side) as co-substrate.
Reduced copper, and reducing equivalents are colored blue, oxidized copper and oxygen species are colored orange and red, respectively. $H_2O_2$ reacts with the Cu(I) center leading to the production of a hydroxyl radical via homolytic bond cleavage (**pathway I**) or via a base-assisted mechanism (**pathway II**). The proton that is held by the putative base can react either with the copper-bound oxygen atom (**pathway II-a**, grey) or with the leaving hydroxide group (**pathway II-b**, magenta), which leads to elimination of a water molecule and formation of a copper-oxyl intermediate. Pathways (I) and (II-a) both lead to the formation of a Cu(II)-hydroxide intermediate and a hydroxyl radical. This hydroxyl radical catalyzes hydrogen atom abstraction (haa) either from the Cu(II)-hydroxide (**haa1**) or from the substrate (**haa1'**). The former scenario leads to a Cu (II)-oxyl intermediate that can catalyze HAA on the substrate (**haa2**). In both cases (haa1+haa2 or haa1'), a water molecule is eliminated and a substrate radical ($R^{\bullet}$) and a common Cu(II)–OH intermediate are generated. The Cu(II)-associated hydroxide merges with the substrate radical through a rebound mechanism, leading to hydroxylation of the substrate and regeneration of the Cu(I) center, which can enter a new catalytic cycle. The mechanism analyzed in detail by Wang *et al.* is highlighted by grey dots [27•]. In the $O_2$-based mechanisms (right side of the figure), the pathways have been examined in the following computational studies; (III-a) [25,26,60], III-b [28•,61]. This figure is adapted from the supplementary information of Bissaro *et al.* [24••].

computational and structural evidence that the oxygen co-substrate binds the equatorial position (Figure 2a) [18••,21,22,44]. Bertini *et al.* [26] obtained a $[CuO]^+$ complex displaying a distorted tetrahedral symmetry, resulting in an in-between axial and equatorial oxygen atom position. The first computational report on a reaction mechanism involving $H_2O_2$ as a LPMO co-substrate supported one of the potential mechanisms put forward by Bissaro *et al.* [24••] that implies formation of a hydroxyl radical and a copper-associated hydroxide upon the $H_2O_2$ reaction with LPMO-Cu(I) (see Figure 4) [27•]. The calculations by Wang *et al.* showed that the highly reactive hydroxyl radical subsequently abstracts a hydrogen atom from the copper-associated hydroxide, resulting in a $[CuO]^+$ species. Finally, the $[CuO]^+$ species abstracts a hydrogen atom from the oligosaccharide substrate before it recombines with the substrate radical, in a rebound mechanism [61], yielding a hydroxylated product. Essentially similar conclusions were obtained by Hedegård and Ryde [28•]; however, the two reports do not agree on intermediate spin states, which is an interesting detail that deserves further attention.

Although the $[CuO]^+$ state regularly appears in the LPMO literature, it must be emphasized that several alternatives have been proposed for the oxygen species that abstracts the hydrogen from the substrate. The nature of the true intermediate reactive-oxygen species has not been experimentally determined and, despite recent modeling studies, multiple plausible mechanisms remain. Figure 4 shows prevalent possible mechanisms for both $O_2$ and $H_2O_2$-driven reactions. We refer to Walton and Davies [63] and Meier *et al.* [62•] for comprehensive reviews of mechanistic aspects of LPMOs.

Proper complex formation between enzyme and substrate is crucial for LPMO functionality and is of great importance in both experimental and computational assessment of LPMO reactivity. Simmons *et al.* [19•] have shown that variation in substrate positioning may occur (Figure 2a,b) and this observation, combined with (rather limited) studies of catalytic activity led these authors to suggest that the catalytic mechanism employed by LPMOs may vary, depending on the substrate. We believe that it is too early to conclude that one LPMO may have multiple legitimate catalytic mechanisms (see also below), but this cannot be excluded. More in general, there could be mechanistic variations among LPMOs.

## Conclusions

Despite years of intensive research, several LPMO secrets remain to be resolved. Research on these enzymes is complicated by the insolubility of what likely are their natural and industrially most relevant substrates. Experimental verification of the reactive oxygen intermediate is complicated by the fact that binding of the substrate helps in shaping the geometry and reactivity of the copper site, meaning that studies on enzymes in the absence of (appropriate) substrate can only tell part of the story. Another complication lies in the multitude of reactions that may occur in LPMO reactions, including autocatalytic inactivation of the LPMO, potential depletion of reductant, and both production and consumption of $H_2O_2$ through a variety of processes (Figure 3b; see also Bissaro *et al.* [4•] for further discussions). Indeed, kinetic data for LPMOs is scarce, and producing such data is experimentally challenging.

Lack of kinetic data may be the underlying reason to some of the current uncertainties in the field. We would argue that seemingly contradictory results may in part be due to the fact that some reports make quantitative statements about LPMO activity that are based on single time-point measurements. Because of the inactivation of LPMOs over time, to an extent this is dependent on the reaction conditions, including the (varying) substrate concentration, quantitative statements based on single time point measurements are risky at best and in most cases not valid. The reader is referred to Forsberg *et al.* [53] for an example illustrating this point. While the suggestion by Simmons *et al.* [19•] that LPMOs may use multiple reaction mechanisms may very well be true, this suggestion is based on single time point measurements of LPMO activity. Likewise, the suggestion by Hangasky *et al.* [48•] that LPMOs do use $O_2$ directly (next to $H_2O_2$) may very well be true, but, in our opinion, more detailed kinetic analysis is needed to substantiate this conclusion (see below).

The impact of substrate concentration on LPMO stability is of crucial importance [23•], not in the least in applied settings where LPMOs may become inactivated as the substrate becomes depleted [49••], that is at a time point during the process where they may be particularly needed for degrading the remaining, potentially most recalcitrant, material. Of note, the detailed kinetic studies by Kuusk *et al.* [30••] showed that the rate of auto-catalytic LPMO inactivation in the absence of substrate is three orders of magnitude lower that the rate of substrate cleavage (under substrate saturating conditions). So, as long as substrate concentrations are high, autocatalytic LPMO inactivation is largely prevented.

While the roles of $O_2$ and $H_2O_2$ as co-substrates remain somewhat controversial, it is now widely accepted that $H_2O_2$ is a bona fide co-substrate that yields high LPMO catalytic rates. It has been claimed that LPMOs become less specific and less stable when fueled by $H_2O_2$ [48•], but this claim is not substantiated by other studies, including our own published and unpublished data. Stability and specificity issues may occur, but these are affected by the reaction conditions (e.g. the LPMO–substrate-reductant-$O_2$/$H_2O_2$ ratio) and not by the nature of the co-substrate, $O_2$ or $H_2O_2$, as such.

Although it cannot be excluded that $O_2$ can be used directly as co-substrate, possibly via formation of a $H_2O_2$ molecule that never leaves the enzyme substrate complex [43,55], we would argue that current kinetic evidence is thin. For example, researchers have observed that under some conditions, neither catalase nor horseradish peroxidase inhibit LPMOs [48•,64], which may be taken to imply that free $H_2O_2$ does not play a role in catalysis. However, an equally plausible explanation would be that, under the conditions used, the LPMO effectively competes with these other $H_2O_2$-consuming enzymes, as discussed for catalase by Kuusk *et al.* [29•].

There is a great need for development of reliable, easy-to use assays of LPMO activity, which take into account all or most complexities discussed above and which, preferably, should address the formation of both soluble and insoluble oxidized products. Despite progress in recent years [51,52,65], more work is certainly needed.

The past years have shown massive progress and it seems likely that several open questions will be answered in the near future, while new LPMO functionalities may be discovered. One of the most important developments is the insight into the role of second shell residues, such as the Glu/Gln (discussed above), in positioning and/or activating the oxygen co-substrate [16••,21,22,28•,42•,43]. Perhaps existing LPMO mutants, or novel mutants designed based on the current insights, may eventually allow detection and characterization of relevant reaction intermediates. Generally, more extensive mutagenesis studies, including, most importantly, proper kinetic characterization of each enzyme variant, are essential for the field. More advanced and detailed studies of the interaction between LPMOs and their polymeric substrates (e.g. Ref. [10]), which, notably, include soluble polymers such as xyloglucan, are also greatly required.

## Conflict of interest statement

Nothing declared.

## Acknowledgements

We thank current and former team members and collaborators for their contributions to our LPMO research, which has been supported by the Research Council of Norway, most recently through grants 240967, 243663, 243950, 257622, 256766, 268002, 262853, 221576 and 226244. Additional support was received from the Marie-Curie FP7 COFUND People Programme, through the award of an AgreenSkills fellowship (under grant agreement n° 267196), and from the French Institut National de la Recherche Agronomique (INRA).

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Eriksson KE, Pettersson B, Westermark U: **Oxidation: an important enzyme reaction in fungal degradation of cellulose**. *FEBS Lett* 1974, **49**:282-285.

2. Kracher D, Scheiblbrandner S, Felice AK, Breslmayr E, Preims M, Ludwicka K, Haltrich D, Eijsink VGH, Ludwig R: **Extracellular electron transfer systems fuel cellulose oxidative degradation**. *Science* 2016, **352**:1098-1101.

3. Vaaje-Kolstad G, Westereng B, Horn SJ, Liu Z, Zhai H, Sørlie M, Eijsink VGH: **An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides**. *Science* 2010, **330**:219-222.

4. • Bissaro B, Várnai A, Røhr ÅK, Eijsink VGH: **Oxidoreductases and reactive oxygen species in conversion of lignocellulosic biomass**. *Microbiol Mol Biol Rev* 2018, **82** http://dx.doi.org/10.1128/MMBR.00029-00018.
Comprehensive review of oxidoreductases that are putatively involved in fungal biomass conversion, including a critical assessment of existing LPMO data in light of the newly discovered peroxygenase activity, and with an overview of available kinetic data.

5. Sützl L, Laurent C, Abrera AT, Schütz G, Ludwig R, Haltrich D: **Multiplicity of enzymatic functions in the CAZy AA3 family**. *Appl Microbiol Biotechnol* 2018, **102**:2477-2492.

6. Westermark U, Eriksson KE: **Cellobiose-quinone oxidoreductase, a new wood-degrading enzyme from white-rot fungi**. *Acta Chem Scand B* 1974, **B 28**:209-214.

7. Henriksson G, Johansson G, Pettersson G: **A critical review of cellobiose dehydrogenases**. *J Biotechnol* 2000, **78**:93-113.

8. Phillips CM, Beeson WT, Cate JH, Marletta MA: **Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by *Neurospora crassa***. *ACS Chem Biol* 2011, **6**:1399-1406.

9. Quinlan RJ, Sweeney MD, Lo Leggio L, Otten H, Poulsen JC, Johansen KS, Krogh KB, Jørgensen CI, Tovborg M, Anthonsen A *et al.*: **Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components**. *Proc Natl Acad Sci U S A* 2011, **108**:15079-15084.

10. Eibinger M, Sattelkow J, Ganner T, Plank H, Nidetzky B: **Single-molecule study of oxidative enzymatic deconstruction of cellulose**. *Nat Commun* 2017, **8**:894.

11. Vermaas JV, Crowley MF, Beckham GT, Payne CM: **Effects of lytic polysaccharide monooxygenase oxidation on cellulose structure and binding of oxidized cellulose oligomers to cellulases**. *J Phys Chem B* 2015, **119**:6129-6143.

12. Villares A, Moreau C, Bennati-Granier C, Garajova S, Foucat L, Falourd X, Saake B, Berrin JG, Cathala B: **Lytic polysaccharide monooxygenases disrupt the cellulose fibers structure**. *Sci Rep* 2017, **7**:40262.

13. Lenfant N, Hainaut M, Terrapon N, Drula E, Lombard V, Henrissat B: **A bioinformatics analysis of 3400 lytic polysaccharide oxidases from family AA9**. *Carbohydr Res* 2017, **448**:166-174.

14. • Sabbadin F, Hemsworth GR, Ciano L, Henrissat B, Dupree P, Tryfona T, Marques RDS, Sweeney ST, Besser K, Elias L *et al.*: **An ancient family of lytic polysaccharide monooxygenases with roles in arthropod development and biomass digestion**. *Nat Commun* 2018, **9**:756.
This paper describes the discovery of a new LPMO family (AA15) which expands the presence of LPMOs from mainly microbial to the animal kingdom.

15. • Couturier M, Ladeveze S, Sulzenbacher G, Ciano L, Fanuel M, Moreau C, Villares A, Cathala B, Chaspoul F, Frandsen KE *et al.*: **Lytic xylan oxidases from wood-decay fungi unlock biomass degradation**. *Nat Chem Biol* 2018, **14**:306-310.
This study describes a new LPMO family (AA14) acting specifically on co-polymeric assemblies of cellulose and xylan; this novel LPMO was shown to promote the efficiency of a cellulolytic enzyme cocktail in biomass conversion, likely by promoting the dissociation of hemicellulose from cellulose.

16. •• Bissaro B, Isaksen I, Vaaje-Kolstad G, Eijsink VGH, Røhr ÅK: **How a lytic polysaccharide monooxygenase binds crystalline chitin**. *Biochemistry* 2018, **57**:1893-1906.
Experiment-guided MD simulations showing how an AA10 binds to a crystalline substrate and the potential involvement of a conserved second shell Glu/Gln in transporting dioxygen species to the active site when bound to the substrate.

17. • Courtade G, Wimmer R, Røhr ÅK, Preims M, Felice AK, Dimarogona M, Vaaje-Kolstad G, Sørlie M, Sandgren M, Ludwig R *et al.*: **Interactions of a fungal lytic polysaccharide monooxygenase with beta-glucan substrates and cellobiose dehydrogenase**. *Proc Natl Acad Sci U S A* 2016, **113**:5922-5927.
NMR-based analysis of the interaction of a cellulose-active fungal LPMO with its (polymeric) substrate and with cellobiose dehydrogenase.

18. •• Frandsen KEH, Simmons TJ, Dupree P, Poulsen JCN, Hemsworth GR, Ciano L, Johnston EM, Tovborg M, Johansen KS, von Freiesleben P *et al.*: **The molecular basis of polysaccharide cleavage by lytic polysaccharide monooxygenases**. *Nat Chem Biol* 2016, **12**:298-303.
The first crystal structure of an LPMO in complex with an oligomeric substrate providing important insights into the effects of substrate binding on the catalytic copper site.

19. • Simmons TJ, Frandsen KEH, Ciano L, Tryfona T, Lenfant N, Poulsen JC, Wilson LFL, Tandrup T, Tovborg M, Schnorr K *et al.*: **Structural and electronic determinants of lytic polysaccharide monooxygenase reactivity on polysaccharide substrates**. *Nat Commun* 2017, **8**:1064.
Additional structures of AA9 LPMOs in complex with oligomeric substrates showing different binding modes for cellopentaose versus xylopentaose; the authors suggest that different catalytic mechanisms may occur depending on the substrate.

20. Tandrup T, Frandsen KEH, Johansen KS, Berrin JG, Lo Leggio L: **Recent insights into lytic polysaccharide monooxygenases (LPMOs)**. *Biochem Soc Trans* 2018, **46**:1431-1447.

21. Bacik JP, Mekasha S, Forsberg Z, Kovalevsky AY, Vaaje-Kolstad G, Eijsink VGH, Nix JC, Coates L, Cuneo MJ, Unkefer CJ *et al.*: **Neutron and atomic resolution X-ray structures of a lytic polysaccharide monooxygenase reveal copper-mediated dioxygen binding and evidence for N-terminal deprotonation**. *Biochemistry* 2017, **56**:2529-2532.

22. O'Dell WB, Agarwal PK, Meilleur F: **Oxygen activation at the active site of a fungal lytic polysaccharide monooxygenase**. *Angew Chem Int Ed* 2017, **56**:767-770.

23. • Courtade G, Forsberg Z, Heggset EB, Eijsink VGH, Aachmann FL: **The carbohydrate-binding module and linker of a modular lytic polysaccharide monooxygenase promote localized cellulose oxidation**. *J Biol Chem* 2018, **293**:13006-13015.
This paper presents an NMR investigation of the structure of a two domain LPMO, including its flexible linker, and shows how the CBM affects LPMO activity and stability and leads to localized oxidation of the substrate.

24. •• Bissaro B, Røhr ÅK, Müller G, Chylenski P, Skaugen M, Forsberg Z, Horn SJ, Vaaje-Kolstad G, Eijsink VGH: **Oxidative cleavage of polysaccharides by monocopper enzymes depends on $H_2O_2$**. *Nat Chem Biol* 2017, **13**:1123-1128.
Seminal study, originally published on biorxiv.org in 2016, revealing the peroxygenase activity of LPMOs and providing a rationale for the often-observed inactivation of LPMOs.

25. Hedegård ED, Ryde U: **Targeting the reactive intermediate in polysaccharide monooxygenases**. *J Biol Inorg Chem* 2017, **22**:1029-1037.

26. Bertini L, Breglia R, Lambrughi M, Fantucci P, De Gioia L, Borsari M, Sola M, Bortolotti CA, Bruschi M: **Catalytic mechanism of fungal lytic polysaccharide monooxygenases investigated by first-principles calculations**. *Inorg Chem* 2018, **57**:86-97.

27. • Wang BJ, Johnston EM, Li PF, Shaik S, Davies GJ, Walton PH, Rovira C: **QM/MM studies into the $H_2O_2$-dependent activity of lytic polysaccharide monooxygenases: evidence for the formation of a caged hydroxyl radical intermediate**. *ACS Catal* 2018, **8**:1346-1351.
QM/MM studies of $H_2O_2$-dependent LPMO catalysis using the crystal structure of an AA9 LPMO in complex with cellotriose as a starting point; the results show that $H_2O_2$ is a plausible co-substrate (or 'intermediate' in the words of the authors) and supports the notion that catalysis involves the consecutive formation of a hydroxyl radical and a Cu(II)-oxyl.

28. • Hedegård ED, Ryde U: **Molecular mechanism of lytic polysaccharide monooxygenases**. *Chem Sci* 2018, **9**:3866-3880.
Insightful QM/MM study showing that the peroxygenase reaction is possible from a theoretical point of view.

29. • Kuusk S, Kont R, Kuusk P, Heering A, Sørlie M, Bissaro B, Eijsink VGH, Väljamäe P: **Kinetic insights into the role of the reductant in $H_2O_2$-driven degradation of chitin by a bacterial lytic polysaccharide monooxygenase**. *J Biol Chem* 2018, **294**:1516-1528 http://dx.doi.org/10.1074/jbc.RA118.006196.
Elaborated kinetic study of $H_2O_2$-driven LPMO catalysis that unravels the complex (kinetic) relationships between multiple on-pathway and off-pathway reactions and provides insight into reductant functionality. In support of a 'priming' reduction, the authors show that a reduced LPMO can catalyze up to 18 oxidative cleavages.

30. •• Kuusk S, Bissaro B, Kuusk P, Forsberg Z, Eijsink VGH, Sørlie M, Väljamäe P: **Kinetics of $H_2O_2$-driven degradation of chitin by a bacterial lytic polysaccharide monooxygenase**. *J Biol Chem* 2018, **293**:523-531.
First comprehensive kinetic study showing that the use of $H_2O_2$ as co-substrate yields catalytic rates that are orders of magnitude higher than the rates observed in $O_2$-based reactions, and similar to rates reported for peroxygenases.

31. • Ciano L, Davies GJ, Tolman WB, Walton PH: **Bracing copper for the catalytic oxidation of C-H bonds**. *Nat Catal* 2018, **1**:571-577.
This perspective provides an in-depth description of copper binding by the histidine brace, in both LPMOs and particulate methane monooxygenase (pMMO), and discusses a variety of possible reaction intermediates, while comparing with results from studies with synthetic copper complexes.

32. Levasseur A, Drula E, Lombard V, Coutinho PM, Henrissat B: **Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes**. *Biotechnol Biofuels* 2013, **6**:41.

33. Chiu E, Hijnen M, Bunker RD, Boudes M, Rajendran C, Aizel K, Olieric V, Schulze-Briese C, Mitsuhashi W, Young V *et al.*: **Structural basis for the enhancement of virulence by viral spindles and their in vivo crystallization**. *Proc Natl Acad Sci U S A* 2015, **112**:3973-3978.

34. Paspaliari DK, Loose JSM, Larsen MH, Vaaje-Kolstad G: ***Listeria monocytogenes* has a functional chitinolytic system and an active lytic polysaccharide monooxygenase**. *FEBS J* 2015, **282**:921-936.

35. Agostoni M, Hangasky JA, Marletta MA: **Physiological and molecular understanding of bacterial polysaccharide monooxygenases**. *Microbiol Mol Biol Rev* 2017, **81**:e00015-00017.

36. Isaksen T, Westereng B, Aachmann FL, Agger JW, Kracher D, Kittl R, Ludwig R, Haltrich D, Eijsink VGH, Horn SJ: **A C4-oxidizing lytic polysaccharide monooxygenase cleaving both cellulose and cello-oligosaccharides**. *J Biol Chem* 2014, **289**:2632-2642.

37. Borisova AS, Isaksen T, Dimarogona M, Kognole AA, Mathiesen G, Várnai A, Røhr ÅK, Payne CM, Sørlie M, Sandgren M *et al.*: **Structural and functional characterization of a lytic polysaccharide monooxygenase with broad substrate specificity**. *J Biol Chem* 2015, **290**:22955-22969.

38. Kracher D, Andlar M, Furtmuller PG, Ludwig R: **Active-site copper reduction promotes substrate binding of fungal lytic polysaccharide monooxygenase and reduces stability**. *J Biol Chem* 2018, **293**:1676-1687.

39. Hangasky JA, Marletta MA: **A random-sequential kinetic mechanism for polysaccharide monooxygenases**. *Biochemistry* 2018, **57**:3191-3199.

40. Li X, Beeson WTt, Phillips CM, Marletta MA, Cate JH: **Structural basis for substrate targeting and catalysis by fungal polysaccharide monooxygenases**. *Structure* 2012, **20**:1051-1061.

41. Loose JSM, Arntzen MO, Bissaro B, Ludwig R, Eijsink VGH, Vaaje-Kolstad G: **Multi-point precision binding of substrate protects LPMOs from self-destructive off-pathway processes**. *Biochemistry* 2018, **57**:4114-4124.

42. • Span EA, Suess DLM, Deller MC, Britt RD, Marletta MA: **The role of the secondary coordination sphere in a fungal polysaccharide monooxygenase**. *ACS Chem Biol* 2017, **12**:1095-1103.

Mutational study shedding light on a hydrogen bonding network involving conserved residues in the secondary copper-coordination sphere and the potential role of this network in positioning an oxygen species close to the active site in AA9 LPMOs.

43. Caldararu O, Oksanen E, Ryde U, Hedegård ED: **Mechanism of hydrogen peroxide formation by lytic polysaccharide monooxygenase**. *Chem Sci* 2019, **10**:576-586.

44. Hedegård ED, Ryde U: **Multiscale modelling of lytic polysaccharide monooxygenases**. *ACS Omega* 2017, **2**: 536-545.

45. Kittl R, Kracher D, Burgstaller D, Haltrich D, Ludwig R: **Production of four *Neurospora crassa* lytic polysaccharide monooxygenases in *Pichia pastoris* monitored by a fluorimetric assay**. *Biotechnol Biofuels* 2012, **5**:79.

46. Cannella D, Möllers KB, Frigaard NU, Jensen PE, Bjerrum MJ, Johansen KS, Felby C: **Light-driven oxidation of polysaccharides by photosynthetic pigments and a metalloenzyme**. *Nat Commun* 2016, **7**:11134.

47. Wang C, Chang WC, Guo Y, Huang H, Peck SC, Pandelia ME, Lin GM, Liu HW, Krebs C, Bollinger JM Jr: **Evidence that the fosfomycin-producing epoxidase, HppE, is a non-heme-iron peroxidase**. *Science* 2013, **342**:991-995.

48. • Hangasky JA, Iavarone AT, Marletta MA: **Reactivity of $O_2$ versus $H_2O_2$ with polysaccharide monooxygenases**. *Proc Natl Acad Sci U S A* 2018, **115**:4915-4920.

This paper addresses the role of $H_2O_2$ and $O_2$ in LPMO catalysis, confirming the peroxygenase activity of LPMOs; the authors claim that $O_2$ is the true co-substrate of LPMOs.

49. •• Müller G, Chylenski P, Bissaro B, Eijsink VGH, Horn SJ: **The impact of hydrogen peroxide supply on LPMO activity and overall saccharification efficiency of a commercial cellulase cocktail**. *Biotechnol Biofuels* 2018, **11**.

This applied study shows a clear correlation between the rate of addition of $H_2O_2$, LPMO activity, and the overall saccharification efficiency of a commercial cellulase cocktail, Cellic CTec2; data in this paper were used by Bissaro *et al.* to calculate total turnover numbers per LPMO molecule ($\geq$1500) and per LPMO reduction ($\geq$15).

50. Mutahir Z, Mekasha S, Loose JSM, Abbas F, Vaaje-Kolstad G, Eijsink VGH, Forsberg Z: **Characterization and synergistic action of a tetra-modular lytic polysaccharide monooxygenase from *Bacillus cereus***. *FEBS Lett* 2018, **592**:2562-2571.

51. Breslmayr E, Hanzek M, Hanrahan A, Leitner C, Kittl R, Santek B, Oostenbrink C, Ludwig R: **A fast and sensitive activity assay for lytic polysaccharide monooxygenase**. *Biotechnol Biofuels* 2018, **11**:79.

52. Wang D, Li J, Wong ACY, Aachmann FL, Hsieh YSY: **A colorimetric assay to rapidly determine the activities of lytic polysaccharide monooxygenases**. *Biotechnol Biofuels* 2018, **11**:215.

53. Forsberg Z, Bissaro B, Gullesen J, Dalhus B, Vaaje-Kolstad G, Eijsink VGH: **Structural determinants of bacterial lytic polysaccharide monooxygenase functionality**. *J Biol Chem* 2018, **293**:1397-1412.

54. Loose JSM, Forsberg Z, Kracher D, Scheiblbrandner S, Ludwig R, Eijsink VGH, Vaaje-Kolstad G: **Activation of bacterial lytic polysaccharide monooxygenases with cellobiose dehydrogenase**. *Protein Sci* 2016, **25**:2175-2186.

55. Hegnar OA, Petrović DM, Bissaro B, Alfredsen G, Várnai A, Eijsink VGH: **Characterization of a lytic polysaccharide monooxygenase from *Gloeophyllum trabeum* shows a pH-dependent relationship between catalytic activity and hydrogen peroxide production**. *Appl Environ Microbiol* 2018, **85** http://dx.doi.org/10.1128/AEM.02612-02618 pii: e02612-18.

56. Petrović DM, Bissaro B, Chylenski P, Skaugen M, Sørlie M, Jensen MS, Aachmann FL, Courtade G, Várnai A, Eijsink VGH: **Methylation of the N-terminal histidine protects a lytic polysaccharide monooxygenase from auto-oxidative inactivation**. *Protein Sci* 2018, **27**:1636-1650.

57. Hofrichter M, Ullrich R: **Oxidations catalyzed by fungal peroxygenases**. *Curr Opin Chem Biol* 2014, **19**:116-125.

58. Matthews S, Belcher JD, Tee KL, Girvan HM, McLean KJ, Rigby SE, Levy CW, Leys D, Parker DA, Blankley RT *et al.*: **Catalytic determinants of alkene production by the cytochrome P450 peroxygenase OleTJE**. *J Biol Chem* 2017, **292**:5128-5143.

59. Frommhagen M, Westphal AH, van Berkel WJH, Kabel MA: **Distinct substrate specificities and electron-donating systems of fungal lytic polysaccharide monooxygenases**. *Front Microbiol* 2018, **9**:1080.

60. Kjaergaard CH, Qayyum MF, Wong SD, Xu F, Hemsworth GR, Walton DJ, Young NA, Davies GJ, Walton PH, Johansen KS *et al.*: **Spectroscopic and computational insight into the activation of $O_2$ by the mononuclear Cu center in polysaccharide monooxygenases**. *Proc Natl Acad Sci U S A* 2014, **111**:8797-8802.

61. Kim S, Ståhlberg J, Sandgren M, Paton RS, Beckham GT: **Quantum mechanical calculations suggest that lytic polysaccharide monooxygenases use a copper-oxyl, oxygen-rebound mechanism**. *Proc Natl Acad Sci U S A* 2014, **111**:149-154.

62. • Meier KK, Jones SM, Kaper T, Hansson H, Koetsier MJ, Karkehabadi S, Solomon EI, Sandgren M, Kelemen B: **Oxygen activation by Cu LPMOs in recalcitrant carbohydrate polysaccharide conversion to monomer sugars**. *Chem Rev* 2018, **118**:2593-2635.

Comprehensive review on LPMOs, addressing their occurrence, nomenclature, structure, industrial use, and catalytic mechanism, including insightful discussions of the reactive copper-oxygen species that may emerge during catalysis in reactions with $O_2$ or $H_2O_2$.

63. Walton PH, Davies GJ: **On the catalytic mechanisms of lytic polysaccharide monooxygenases**. *Curr Opin Chem Biol* 2016, **31**:195-207.

64. Möllers KB, Mikkelsen H, Simonsen TI, Cannella D, Johansen KS, Bjerrum MJ, Felby C: **On the formation and role of reactive oxygen species in light-driven LPMO oxidation of phosphoric acid swollen cellulose**. *Carbohydr Res* 2017, **448**:182-186.

65. Vuong TV, Liu B, Sandgren M, Master ER: **Microplate-based detection of lytic polysaccharide monooxygenase activity by fluorescence-labeling of insoluble oxidized products**. *Biomacromolecules* 2017, **18**:610-616.