Controlled depolymerization of cellulose by light-driven lytic polysaccharide oxygenases

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Lytic polysaccharide (mono)oxxygenases (LPMOs) perform oxidative cleavage of polysaccharides, and are key enzymes in biomass processing and the global carbon cycle. It has been shown that LPMO reactions may be driven by light, using photosynthetic pigments or photocatalysts, but the mechanism behind this highly attractive catalytic route remains unknown. Here, prompted by the discovery that LPMOs catalyze a peroxygenase reaction more efficiently than a monooxygenase reaction, we revisit these light-driven systems, using an LPMO from Streptomyces coelicolor (ScAA10C) as model cellulolytic enzyme. By using coupled enzymatic assays, we show that H2O2 is produced and necessary for efficient light-driven activity of ScAA10C. Importantly, this activity is achieved without addition of reducing agents and proportional to the light intensity. Overall, the results highlight the importance of controlling fluxes of reactive oxygen species in LPMO reactions and demonstrate the feasibility of light-driven, tunable enzymatic peroxygenation to degrade recalcitrant polysaccharides.
Environmetal threats and future shortage in fossil-based energy and chemicals call for the development of sustainable processes for converting renewable sources into carbon and energy. Plant biomass represents an abundant source of renewable material, mainly in the form of polysaccharides in plant cell walls. However, the co-polymeric and recalcitrant nature of these cell walls constitutes a major hurdle in the extraction and valorization of the carbohydrate building blocks. Chitin represents another abundant, but recalcitrant source of renewable material, found in e.g. the shells of insects and crustaceans. Facing the structural intricacies of these biomaterials, plant-degrading and chitin-degrading microorganisms have developed complex arsenals of chemical and enzymatic tools for their deconstruction. Among the enzymatic tools are enzymes today known as lytic polysaccharide monoxygenases (LPMOs), which play a major role in biomass conversion by oxidative cleavage and, thus, structural disruption of biopolymers such as chitin, cellulose, as well as co-polymeric structures made of cellulose and hemicelluloses. This disruptive action boosts the depolymerizing action of glycoside hydrolases. LPMOs classified today in families 9–11 and 13–16 of the auxiliary enzymes (AA) in the Carbohydrate Active enZymes database, are ubiquitous enzymes with key roles in biological conversion of biomass by fungi and bacteria, but also with suggested roles in microbial pathogenicity. LPMOs contribute to the efficiency of modern commercial cellulosic cocktails used at industrial scale.

The use of light as a cheap energy source represents a key pillar of the emerging bioeconomy. Although the field of photocatalysis has been explored for decades, the field of photobiocatalysis, i.e., catalysis at the cross roads between photocatalysis and enzymology has been emerging more recently. Harnessing the energy carried by visible light to drive biochemical processes, including enzymatic reactions, under eco-friendly conditions, constitutes a potentially valuable addition to currently available biotechnological tools. LPMO action requires energy in the form of reducing equivalents, and, in 2016, two studies were published that address the possibility of driving LPMO-catalyzed biomass reactions by visible light alone. These results suggest avenues towards sustainable exploitation of mono-copper catalysts.

Results

Properties of chlorophyllin. Chlorophyllin (Chl), a water-soluble derivative of chlorophyll, is composed of a porphyrin ring metallated with copper (Fig. 1; see Methods section). We verified that the used commercial Chl has the expected absorbance and fluorescence properties, in part by comparison with copper-deficient chlorin $e_6$ and Cu(II)-reconstituted chlorin $e_6$ (Supplementary Fig. 1). The UV–Vis absorbance spectrum of Chl was nearly identical to that of Cu(II)-reconstituted chlorin $e_6$, suggesting that the latter metallated species is the main constituent of the Chl powder (Supplementary Fig. 1a). Likewise, reconstituted Cu(II)-chlorin $e_6$ and Chl showed equivalent fluorescence spectra (Supplementary Fig. 1b), with (expected) sharp excitation ($\lambda_{\text{max}} = 343$ nm) and emission peaks ($\lambda_{\text{max}} = 685$ nm), corroborating the fact that the photoactive species present in the Chl powder is Cu(II)-chlorin $e_6$.

It has been shown that photo-excited chlorophyll has a very low reduction potential ($E_0 = -0.55$ V vs. SHE) and that saponified chlorophyll can catalyze the single electron reduction of $O_2$ into $O_2^-$, which requires a strong reductant ($E_0 = -0.33$ V vs. SHE). However, no similar data exist for Chl, which displays a molecular structure similar to saponified chlorophyll but binds copper instead of magnesium. To assess the redox properties of non-photo-excited Chl species, we performed square-wave voltammetry experiments (Supplementary Table 1 and Supplementary Fig. 2) and found that the reduction potentials, $E_{\text{p}0}$, of Chl, chlorin $e_6$, and metallated chlorin $e_6$ were all in the range of 0.6–0.7 V vs. SHE, in accordance with the reduction potentials for Chl and non-metallated chlorin $e_6$ reported by Novak and Komorsky-Lovric. The measured reduction potentials of non-photo-excited Chl species indicate that the LPMO in this study, ScAA10C-Cu(II) ($E_0 = -0.236 \pm 0.007$ V vs. SHE), cannot be reduced by these Chl species in absence of light.

Fueling LPMO reactions with light-activated chlorophyllin. We first repeated experiments initially described by Cannella et al., i.e., combining AscA and Chl/light, using ScAA10C and a light-nching system we previously successfully used for the V-TiO$_2$ system. Indeed, use of the Chl/light-AscA system gave very high initial catalytic rates, notably accompanied by almost immediate enzyme inactivation (Fig. 2a; orange curve, see the 25% curve in Supplementary Fig. 3a, c for more details concerning the initial phase of the reaction). In accordance with the work by Cannella...
et al., the initial rates obtained with Chl/light-AscA were much higher than the rates obtained in a standard reaction with only AscA (Fig. 2a, blue curve). Yet, the standard reaction with only AscA produced more oxidized products compared to the reaction with Chl/light-AscA, because the LPMO stayed active for a much longer time (Fig. 2a). Interestingly, at the light intensities used here, which are considerably higher than those used by Cannella et al., the Chl/light system could also fuel the LPMO reaction in the absence of AscA, yielding relatively stable progress curves spanning several hours (Fig. 2a, black curve). Most importantly, this result shows that LPMO catalysis can take place with visible light as the only energy source (i.e., in the absence of added reducing power). Control experiments, including experiments in which fresh reaction components (Avicel, AscA and/or LPMO) were added to a reaction with AscA + Chl/light after 60 min of incubation (i.e., long after product formation had ceased, Fig. 2a) showed that only addition of fresh LPMO led to the reinitiation of product formation, confirming the impact of enzyme inactivation (Supplementary Fig. 5).

We then assessed whether the ability of the Chl/light system to drive LPMO reactions could be linked to the expected production of O₂•− by this system35. Superoxide dismutase (SOD) enzymatically converts O₂•− to H₂O₂ (Supplementary Fig. 6) and screening of a range of SOD concentrations showed that low amounts of SOD (100 nM) increased the LPMO initial rate and that higher amounts of SOD (up to 1 µM) led to almost immediate inactivation of the LPMO (Fig. 2d; more data in Supplementary Fig. 7). Accordingly, we observed that addition of 100 nM SOD led to increased H₂O₂ production (Fig. 2f) while yield relatively low H₂O₂ accumulation in the complete system (Fig. 2e). On the other hand, addition of 1 µM SOD led to higher
**H$_2$O$_2$** levels (Fig. 2f) and accumulation of high levels of H$_2$O$_2$ in the reaction with the (rapidly inactivated) LPMO and substrate (Fig. 2e). These results indicate that the Chl/light system produces large amounts of O$_2$$^{**-}$ and that the degree of conversion O$_2$$^{**-}$ to H$_2$O$_2$, e.g., by SOD (Fig. 2d–f), determines both the catalytic rate and the stability of the LPMO.

Addition of catalase (katE), a H$_2$O$_2$ consuming enzyme (Supplementary Fig. 6), to the reaction containing a (too) high amount of SOD (1 mM) had a clear beneficial effect, leading to higher LPMO activity over a longer period (Fig. 2d) and little accumulation of H$_2$O$_2$ (Fig. 2f). Addition of catalase to the Chl/light system gave a stable LPMO reaction, with only a slight reduction in rate, no visible enzyme inactivation within the 12-h measuring period (Fig. 2d), and almost no accumulation of H$_2$O$_2$ (Fig. 2e).

In light of the idea that H$_2$O$_2$ drives LPMO action, it may seem surprising that, while catalase expectedly abolished accumulation of H$_2$O$_2$ (Fig. 2f), it hardly affected, or even had a seemingly positive effect on, LPMO activity. There are, however, straightforward explanations for this paradox. Firstly, the beneficial effect of catalase on the reaction with 1 mM SOD is due to catalase removing the surplus of H$_2$O$_2$ that otherwise would lead to LPMO inactivation. Secondly, kinetic data show that a reduced LPMO in the presence of substrate will easily compete with catalase for available H$_2$O$_2$; it is thus plausible that, while catalase consumes produced H$_2$O$_2$ in the absence of the LPMO (Fig. 2f), H$_2$O$_2$ will primarily be consumed by the LPMO in reactions containing both enzymes and an LPMO substrate (Fig. 2d).

If the ability of the Chl/light system to drive LPMO reactions indeed is due to the production of O$_2$$^{**-}$, which is subsequently converted to H$_2$O$_2$, the huge effect of adding AscA (Fig. 2a–c) suggests that AscA catalyzes the otherwise spontaneous conversion of O$_2$$^{**-}$ to H$_2$O$_2$, as has indeed been shown (see ref. 40, Supplementary Fig. 6). Accordingly, the initial rate of LPMO catalysis and the degree of enzyme inactivation could be modulated by varying the amount of AscA (Supplementary Fig. 8).

Likewise, there was a clear correlation between the light intensity, which determines the rate of O$_2$$^{**-}$ generation, and LPMO activity for both the Chl/light (Fig. 3 and Supplementary Fig. 3) and the Chl/light-AscA system (Supplementary Fig. 3). Figure 3 shows that the decrease in LPMO activity upon decreasing the light intensity applied to the Chl/light system (Fig. 3b) correlates with decreased production of H$_2$O$_2$ (measured in absence of the LPMO) (Fig. 3a). Importantly, in the absence of LPMO activity, H$_2$O$_2$ generated by Chl/light system will be further reduced, which implies that the measured apparent levels of H$_2$O$_2$ are likely an underestimation of the true levels of produced H$_2$O$_2$. On the other hand, when the LPMO and substrate are present, one could expect that the high affinity of the LPMO for H$_2$O$_2$ results in efficient integration of H$_2$O$_2$ in oxidized reaction products. Accordingly, while Fig. 3a shows an apparent retardation in the rate of H$_2$O$_2$ production in the absence of the LPMO, Fig. 3b shows a linear increase in LPMO products over time.

Taken together, the data presented above are compatible with a scenario in which the ability of the Chl/light and Chl/light-AscA systems to drive LPMO reactions is due to the light-driven generation of O$_2$$^{**-}$, which is converted to H$_2$O$_2$. As shown by Fig. 2 and Supplementary Figs. 3, 7, 8 and discussed above, and in...
agreement with several studies published since the discovery of the role of H$_2$O$_2$ in LPMO catalysis$^{22,25-29,41}$, H$_2$O$_2$, clearly is a double-edged sword: it can drive the LPMO reaction with high efficiency, but is also a potentially harmful entity if its levels are not controlled. Of note, while potentially harmful effects of H$_2$O$_2$ have been observed that exposure of Chl/AscA to light leads to consumption of O$_2$ and H$_2$O$_2$, as shown by increases in O$_2$ and H$_2$O$_2$ levels upon addition of SOD and catalase, respectively. On the other hand, Möllers et al. found that addition of catalase or SOD did not affect measured LPMO product levels, leading to the conclusion that the generated ROS do not affect LPMO catalysis. The absence of an effect of catalase on apparent LPMO activity is not surprising in light of kinetic considerations (see above). The absence of an effect of SOD could be due to the fact that Möllers et al. did not monitor LPMO activity over time and thus may have overlooked effects of LPMO inactivation. Perhaps, the authors encountered a situation similar to the one presented in Supplementary Fig. 9, where SOD effects are not visible because the LPMO becomes fully inactivated prior to the first sampling point. In our hands, SOD clearly has an effect on light-driven LPMO performance, as shown by Fig. 2d–f. Of course, one cannot exclude that TrAA9E, the family AA9 LPMO used by Möllers et al., employs a different mechanism compared to ScAA10C, in terms of both activation and inactivation.

**LPMO reduction by light-activated chlorophyll.** Hydrogen peroxide-driven LPMO activity requires a priming reduction of the LPMO from the Cu(II) to the Cu(I) state$^{22,39}$. The correlations between H$_2$O$_2$ availability and LPMO activity described above suggest that this priming reduction is not rate-limiting. This is supported by stopped-flow kinetics showing fast ($4.2 \times 10^3$ M$^{-1}$ s$^{-1}$) and full reduction of an AA10 LPMO when using as little as 5 µM AscA$^{25}$. The situation may be different when using the Chl/light system, without the added AscA. We have previously shown that superoxide, which is produced by the light-exposed Chl (Fig. 2), can serve as reductant$^{22}$. Another option would be the direct reduction of the LPMO by photo-excited Chl, as suggested by Cannella et al.$^{16}$. To probe this latter hypothesis, we initially attempted to monitor LPMO reduction by light-exposed Chl in anaerobic conditions using fluorescence$^{17}$, but we did not manage to establish conditions that allowed informative fluorescence measurements. In an alternative experiment, carried out under anaerobic conditions we first exposed the LPMO to Chl/light and then added substrate and H$_2$O$_2$ while switching off the light. Figure 4 shows that this approach led to only very low LPMO activity, compared to a control reaction with AscA, and that this activity was independent of the application of light. Thus, it would seem that light-induced reduction of the LPMO does not occur in anaerobic conditions, which supports the idea that, under aerobic conditions, the LPMO is mainly reduced by (oxygen-derived) superoxide and not by direct electron transfer from Chl to the LPMO.

Of note, however, this issue remains ambiguous. Given the expected low reduction potential of photo-excited chlorophyll, efficient reduction of the LPMO may occur. Thus, it is possible that in the two-phase experiment of Fig. 4, the LPMO gets reduced but is re-oxidized before being transferred to the substrate/H$_2$O$_2$ mixture. Indeed, measurement of reduction potentials of the ground state Chl (>0.6 V vs. SHE; see above), present in the first phase of the experiment, indicate that it can act as an oxidant of LPMO-Cu(I), which would regenerate LPMO-Cu(II). In a real (i.e., one-phase) experiment, LPMO-Cu(I) would have several possible fates: (i) re-oxidation in solution by either ground state Chl, by O$_2$ or by H$_2$O$_2$, or (ii) binding to the substrate and formation of a productive complex with the co-substrate, leading to substrate hydroxylation and cleavage. The distribution between these different pathways will depend on kinetics of the different reactions. Available kinetic studies predict that, in the presence of a suitable substrate, the productive substrate hydroxylation pathway will be favored$^{23,25}$.

**Metallation of Chl affects H$_2$O$_2$ production and LPMO activity.** The UV-Vis and fluorescence analyses of chlorin e$_6$ (Supplementary Fig. 1) showed that the photochemical properties of the
porphyrin ring are modulated by copper-binding. Measurements of \( \text{H}_2\text{O}_2 \) production rates by light-exposed chlorin \( e_6 \) revealed a drastic effect of copper binding on apparent \( \text{H}_2\text{O}_2 \) production (Fig. 5a). Compared to light-exposed Chl, light-exposed Cu(II)-depleted chlorin \( e_6 \) produced much more \( \text{H}_2\text{O}_2 \), whereas Cu(II)-reconstituted chlorin \( e_6 \) produced much less \( \text{H}_2\text{O}_2 \). The fact that Cu(II) reconstituted-chlorin \( e_6 \) yields less \( \text{H}_2\text{O}_2 \) than the commercial preparation of Chl suggests that the latter is likely not fully saturated with copper. Our results clearly show that metatlation of the pigment is an important parameter to take into account for future studies. Testing of these three compounds in LPMO reactions showed product formation patterns akin to what we describe above, where Cu(II)-depleted chlorin \( e_6 \) leads to very high LPMO activity and fast inactivation of the LPMO, while Chl and Cu(II)-chlorin \( e_6 \) give lower LPMO reaction rates and less inactivation (Fig. 5b). As discussed above, direct comparison of LPMO activity and the apparent ability of the corresponding LPMO-deficient system to generate (and accumulate) \( \text{H}_2\text{O}_2 \) is complicated by the many possible side reactions. For instance, Fig. 5b shows that reactions containing Chl or Cu(II)-chlorin \( e_6 \) yield equivalent LPMO activity (within the first 2 h) whereas the former system shows higher \( \text{H}_2\text{O}_2 \) accumulation in the absence of the LPMO (Fig. 5a). Still, Fig. 5 also points at a link between \( \text{H}_2\text{O}_2 \) generation and LPMO activity.

The AscA-driven reaction is complex and uncontrolled. For comparative purposes, and to further highlight the potential of the light-driven reactions described here, we also performed reactions with only AscA, a well-known and commonly used reductant to drive LPMO reactions. The reactions yielded less clear-cut results (Fig. 6a–c) compared to the studies with Chl/light (Fig. 2), which is likely due to the many possible redox reactions involving AscA, superoxide and \( \text{H}_2\text{O}_2 \) (see Supplementary Fig. 6). However, the same overall trend stood out: both higher LPMO activity and faster apparent enzyme inactivation were correlated with higher \( \text{H}_2\text{O}_2 \) levels. As above, addition of a small amount of SOD gave a higher initial rate and faster inactivation. The addition of catalase yielded stable LPMO product formation over time (Fig. 6a), likely because catalase lowers the steady-state \( \text{H}_2\text{O}_2 \) concentration. By keeping apparent \( \text{H}_2\text{O}_2 \) levels low, the LPMO becomes less active, but is also less prone to inactivation.

On a side note, Fig. 6a illustrates the risk of assessing LPMO activity by measuring single time points. Assessment of the effect of catalase in the experiment shown in Fig. 6a at e.g., 120 min vs. e.g., 480 min would lead to opposite conclusions as to the effect of catalase on LPMO catalysis.

Figure 6c shows another complexity of reactions with added reductant, in that production of \( \text{H}_2\text{O}_2 \) occurred when mixing 1 mM AscA with only the substrate, Avicel (Fig. 6c). Similar amounts of \( \text{H}_2\text{O}_2 \) were measured when Avicel was replaced by ScAA10C-Cu(II), where the latter is known to produce \( \text{H}_2\text{O}_2 \) when incubated with reductant and \( \text{O}_2 \) in the absence of substrate. These results indicate that in these AscA-driven reactions, the substrate contributes to the generation of \( \text{H}_2\text{O}_2 \), possibly because of the pro-oxidant properties of AscA that become apparent in the presence of free metals (see ref. 46 and below) that may be present in Avicel.

Digging further into these complexities, we then tested the effect of the addition of free transition metals on the LPMO-independent production rate of \( \text{H}_2\text{O}_2 \) by AscA and on LPMO-catalyzed degradation of Avicel. Figure 7a shows that the rate of \( \text{H}_2\text{O}_2 \) production in AscA solutions increased drastically upon addition of CuSO\(_4\). Due to technical limitations of the assay, only 50 \( \mu \)M AscA was present in the reactions displayed in Fig. 7a; at commonly used higher AscA concentrations (mM range), \( \text{H}_2\text{O}_2 \) production rates will be much higher. Figure 7b shows that the effect of CuSO\(_4\) on \( \text{H}_2\text{O}_2 \) production rates is reflected in LPMO activity, with similar trends as those seen in e.g., the progress curves of Fig. 2; increased amounts of CuSO\(_4\) led to higher initial catalytic rates (e.g., 0.13 \( \mu \)M \text{min}^{-1} vs. at least 19 \( \mu \)M \text{min}^{-1} in reactions with no added CuSO\(_4\), vs. 1 \( \mu \)M CuSO\(_4\), respectively) and to faster inactivation. At the higher CuSO\(_4\) concentrations, the enzyme was already inactivated at the first measuring point. A contribution of free transition metals to \( \text{H}_2\text{O}_2 \) production in standard (i.e., \( \text{O}_2 \)- and reductant-driven) LPMO reactions is an important parameter to consider since these metals may be present in significant amounts that vary between substrates and enzyme preparations. Such variations will inevitably lead to variations in observed LPMO activities.

Overall, it is clear that steady LPMO reactions require strict control of the delivery of reducing equivalents and \( \text{H}_2\text{O}_2 \), and of redox side reactions. Such control is likely not achieved in typical AscA-driven and \( \text{O}_2 \)-driven reactions reported in the literature. On the other hand, such control may be achieved by the photobiocatalytic systems described here or by using enzymatic
donors of both electrons and H$_2$O$_2$ such as cellobiose dehydrogenase$^{47,48}$. These latter set-ups allow the in situ and gradual production of both reducing equivalents and H$_2$O$_2$, offering thereby much greater control over the reaction.

**V-TiO$_2$-promoted photobiocatalytic oxidation of cellulose.**

Turning our attention to the previously described photobiocatalytic system using V-TiO$_2$ as photocatalyst and ScAA10C as model enzyme$^{17}$ (Fig. 1), we then investigated whether also this system could be based on the generation of ROS to fuel a peroxidase mechanism, rather than on the generation of electrons to fuel a monooxygenase mechanism. Irradiation of V-TiO$_2$ with visible light can lead to a variety of reactions, including production of H$_2$O$_2$ (Fig. 1; see ref. $^{49}$), but the V-TiO$_2$/light system was originally thought to work by providing the LPMO with electrons analogous to reductants such as AscA.

The possible involvement of ROS was investigated by analyzing whether the addition of a peroxidase to the system would influence V-TiO$_2$/light-driven LPMO-catalyzed oxidation of cellulose (Fig. 8). In accordance with previous observations for standard LPMO reactions with the use of AscA or cellobiose dehydrogenase as source of reducing equivalents$^{22}$, V-TiO$_2$/light-driven activity of ScAA10C was increasingly inhibited when increasing the quantity of peroxidase (Fig. 8a). This result indicates that H$_2$O$_2$ is indeed generated in this photobiocatalytic system and sustains LPMO catalysis.

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**Fig. 6 Characterization of AscA-driven cellulose oxidation by ScAA10C.** The graphs show time-courses for the release of aldonic acid products (a) and apparent H$_2$O$_2$ levels (b) upon incubating Avicel (10 g L$^{-1}$) with ScAA10C (0.5 mM) in presence of AscA (1 mM), in the dark. Reaction conditions varied in terms of the presence of SOD (0.1 mM) and katE (10 µg mL$^{-1}$). The legend code is indicated in each panel. c Apparent H$_2$O$_2$ levels during incubation of AscA (1 mM) with either ScAA10C (0.5 µM) or Avicel (10 g L$^{-1}$) or with no addition (buffer). All reactions were carried out in sodium phosphate buffer (50 mM, pH 7.0) at 40 °C, under magnetic stirring. Before product quantification, solubilized cello-oligosaccharides were hydrolyzed by $Tf$Cel5A, to convert the LPMO products to a mixture of only two oxidized products with a degree of polymerization of 2 and 3 [GlcGlc1A, (Glc)$_2$Glc1A], the amounts of which were summed up to yield the concentration of oxidized sites. Error bars show ± s.d. (n = 3, independent experiments). Source data are provided as a Source Data file.

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**Fig. 5 Effect of copper on H$_2$O$_2$ generation by light-exposed Chl and chlorin e6 and on light-driven LPMO activity.** a H$_2$O$_2$ production by 500 µM of light-exposed Chl, chlorin e6 or chlorin e6 supplemented with 0.9 molar equivalents of Cu(II). b Time-course release of soluble aldonic acid products released from Avicel by ScAA10C (1 µM) when fueled by light-exposed Chl, chlorin e6 or chlorin e6 complemented with 0.9 eq. Cu(II). All reactions were carried out with 500 µM pigment and exposed to visible light (f = 25% $I_{max}$, approx. 42 W cm$^{-2}$), in sodium phosphate buffer (50 mM, pH 7.0) at 40 °C, under magnetic stirring. Before product quantification, solubilized cello-oligosaccharides were hydrolyzed by $Tf$Cel5A, to convert the LPMO products to a mixture of only two oxidized products with a degree of polymerization of 2 and 3 [GlcGlc1A, (Glc)$_2$Glc1A], the amounts of which were summed up to yield the concentration of oxidized sites. Error bars show ± s.d. (n = 3, independent experiments). Source data are provided as a Source Data file.
In our initial study, we observed low apparent H$_2$O$_2$ levels in reactions with LPMO17. With hindsight, we can conclude that H$_2$O$_2$ generated in these reactions was consumed by ScAA10C. Given the importance of H$_2$O$_2$ in ScAA10C catalysis and the occurrence of competing H$_2$O$_2$-consuming side-reactions at the V-TiO$_2$ surface (e.g., photodecomposition\textsuperscript{50}), it is not certain that this photocatalyst is ideal for promoting LPMO activity. The photoreactivity of metal-doped TiO$_2$ depends on the nature and abundance of the metal used for doping\textsuperscript{51} and it is possible that other TiO$_2$-based catalysts could be more suitable for light-driven LPMO catalysis. On this note, a 2018 study showed that a gold-coated TiO$_2$ photocatalyst can fuel the (H$_2$O$_2$-consuming) unspecific peroxygenase from Agrocybe aegerita to hydroxylate aliphatic and aromatic compounds\textsuperscript{52}. The use of alternative...
tailor-made photocatalysts designed for efficient H$_2$O$_2$ generation$^{33,54}$ to drive LPMO reactions probably represents an interesting avenue of investigation.

Experiments with SOD added to the reaction (Fig. 8b) indicated that free superoxide likely is not generated in the V-TiO$_2$/light/LPMO system, suggesting that the LPMO is reduced by reducing equivalents generated at the V-TiO$_2$ surface. Accordingly, we have previously shown that light-exposed V-TiO$_2$ reduces ScaAA10C under anaerobic conditions$^{17}$. Of note, however, the rate-limiting step in this photobiocatalytic system likely resides in the thermodynamically challenging oxidation of water ($E^\circ_{0ox} = -1.23$ V vs. SHE for H$_2$O/O$_2$)$^{17,25}$. Thus, if an O$_2^-$ intermediate was formed, its accelerated conversion to H$_2$O$_2$ by SOD, may not be reflected in an increased LPMO rate, since none of the downstream reactions (relative to water oxidation) are rate-limiting. It is therefore difficult to conclude whether or not O$_2^-$ is produced, although the two-electron reduction of O$_2$ to H$_2$O$_2$ is thermodynamically much more likely than the single electron reduction to O$_2^{2-}$ (Supplementary Fig. 6).

**Light-driven activity of other LPMOs.** Earlier work has shown that the V-TiO$_2$/light system can drive multiple LPMOs, belonging to different families and acting on different substrates. The functionality of this system was demonstrated for a bacterial, C1-oxidizing, chitin-active AA10 LPMO (SmaAA10A)$^2$, a fungal, C1-oxidizing, cellulose-active AA9 LPMO (PcaAA9D)$^{56}$ and a bacterial, C1/C4 oxidizing, cellulose-active AA10 LPMO (ScaAA10B)$^{38}$ (Fig. S3 in Bissaro et al.$^{17}$).

To demonstrate the general applicability of the Chl/light system, we analyzed activity of SmaAA10 on chitin as well as the cellulose-oxidizing activity of a C1-oxidizing AA9 LPMO from the fungus *Neurospora crassa* (NcAA9F or NCU03328)$^{37}$. Supplementary Fig. 10 shows that both enzymes can be fueled by the Chl/light system, leading to chitin and cellulose oxidation, respectively. Of note, while showing the general applicability of the Chl/light system, these additional analyses showed that different LPMOs respond differently: while SmaAA10 performed better with Chl/light compared to AscA, NcAA9F performed relatively poorly when driven by Chl/light and was rapidly inactivated. Differences in the way LPMOs respond to reductants and H$_2$O$_2$ are commonly observed$^{8,22,58}$ and deserve further attention in future research.

The data presented in this study pinpoint several complications related to interpreting the outcome of LPMO reactions and provide insight into light-driven LPMO catalysis. One technical challenge concerns the measuring of actual H$_2$O$_2$ production rates by a given system in which H$_2$O$_2$ is the final product (i.e., not immediately used by an enzyme such as an LPMO). Our study also shows the intricacy of interpreting the effects of accessory enzymes such as catalase and SOD and demonstrates the absolute need for analyzing progress curves, rather than relying on single time point measurements of product formation. SOD accelerates the conversion of O$_2^{2-}$ into H$_2$O$_2$, which increases the LPMO initial rate but also leads to more rapid inactivation of the LPMO. On the other hand, catalase, by converting H$_2$O$_2$ into O$_2$ and H$_2$O, prevents accumulation of excess H$_2$O$_2$ and thus reduces LPMO inactivation. It is worth noting the linear progress curve that is obtained when using catalase in the light-driven reactions displayed in Fig. 2d. All in all, the present data clearly show that LPMOs use H$_2$O$_2$ and that controlling H$_2$O$_2$ levels is key to optimizing LPMO catalysis.

The set of experiments presented here demonstrates that when exposed to light, Chl can reduce O$_2$ to O$_2^{2-}$ leading to H$_2$O$_2$ production, via either spontaneous disproportionation or chemical reduction (e.g., by AscA). H$_2$O$_2$ production can be regulated by light intensity but also by metatlation of the porphyrin ring, which tunes the photochemical properties of the pigment. Although photo-excitation of the pigment is clearly needed to fuel LPMO activity, we were not able to discriminate whether reduction of the LPMO occurs via direct electron transfer from photo-excited Chl or via superoxide. Importantly, studies of a completely different system for light-driven driven LPMO catalysis, based on using V-TiO$_2$ particles, showed that also in this case light-driven oxidation of cellulose by the LPMO entails a peroxygenation reaction. Of note, the earlier study on driving LPMO reactions with the V-TiO$_2$/light system$^{17}$ showed that LPMO activity can be controlled by switching the light on or off and that the system also works when using regular sunlight.

Most importantly, our data show that these LPMO-catalyzed peroxygenation reactions can be fueled by light only and that other sources of energy, such as reducing equivalents used in standard LPMO reactions and in earlier work on light-driven LPMO catalysis, are not required. The use of light allows tunable in situ generation of H$_2$O$_2$, which then fuels polysaccharide oxidation at rates that are higher than those reported when LPMOs were still believed to act as monooxygenases only. Considering the large current interest in copper catalysts (e.g., Snyder et al.$^{59}$), we expect that the present findings will have implications beyond processing of biomass.

**Methods**

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**Materials.** Chemicals and enzymes were purchased from Sigma-Aldrich unless otherwise indicated. The crystalline cellulose used was Avicel® PH-101 (50 µM particles). The superoxide dismutase (SOD, recombinantly expressed in E. coli) was stored (100 µM, eq. 1.63 mg mL$^{-1}$) in sodium phosphate buffer (100 mM, pH 7.5) at 4 °C. The peroxydisulfate from horseradish (HRP, type II) was stored (0.5 mg mL$^{-1}$, eq. 0.95 µM) in sodium phosphate buffer (50 mM, pH 6.0) at 4 °C. The catalase katE from *Streptomyces sierox* (recombinantly expressed in E. coli) was produced in-house and stored (1.8 mg mL$^{-1}$) in Tris-HCl buffer (50 mM, pH 8.0) at −20 °C. Ascorbic acid (100 mM) and Amplex red (10 mM) stock solutions were prepared in water and DMSO respectively, aliquoted, stored at −20 °C, and thawed in the dark for 10 min just before use. The V-TiO$_2$ powder was kindly provided by Dr. Frank Hollmann (Delft University, Netherlands) and prepared according to a previously described protocol$^{40}$. According to literature, the Chl purchased from Sigma consists of about 72% Cu(II)-cholorin e$_2$ and 10% Cu(II)-isochlorin e$_4$.$^{57}$ Chlorin e$_2$ was purchased from Frontier Scientific (Logan, Utah, USA). Copper-reconstituted chlorin e$_6$ was prepared by incubating chlorin e$_6$ with 0.9 molar equivalent of CuCl$_2$ for 30 min at 4 °C in deionized Milli-Q water.

**Production and purification of recombinant LPMOs.** The recombinant AA10 LPMO from *Streptomyces coelicolor* (ScaAA10C) was produced and purified according to previously described protocols$^{138}$. Note that ScaAA10C refers to the wild-type full-length enzyme, which comprises an AA10 domain connected via a linker to a CBM2 domain (UniProt Q9RJY2). ScaAA10C was stored in sodium phosphate (50 mM, pH 6.0), copper-saturated with Cu(II)SO$_4$ and desalted (PD MidiTrap G-25, GE Healthcare) before use$^{10}$. LPMOs from *Serratia marcescens* (SmaAA10A) and *Neurospora crassa* (NcAA9F or NCU03328) were produced and purified as previously described$^{37,57}$ and copper saturated in the same way as for ScaAA10C, except that the buffer was Bis-Tris pH 6.5 and pH 6.0, respectively.

**Standard photobiocatalytic reaction conditions.** The reactor was a cylindrical glass vial (1.1 mL with conical bottom (Thermo Scientific) and the reaction volume was 500 µL. Typical reactions were carried out as follows: the enzyme (0.5 µM) and Avicel (10 g mL$^{-1}$) were mixed in sodium phosphate buffer (50 mM final concentration after all additions; pH 7.0 or 6.0 for Chl and V-TiO$_2$ studies, respectively) followed by incubation at 40 °C under magnetic stirring during 20 min. Photobiocatalytic reactions contained either chlorophyll or chlorin e$_6$ (500 µM, unless stated otherwise) or V-TiO$_2$ (5 mg mL$^{-1}$) as a light harvester. The reaction was initiated by adding ascorbic acid (to a final concentration of 1 mM, unless stated otherwise), and/or turning on the light (I = 25% I$_{max}$, eq. to 42 W cm$^{-2}$, unless stated otherwise). At regular intervals, 55 µL samples were taken from the reaction mixtures and soluble fractions were immediately separated from the insoluble substrate by filtration using a 96-well filter plate (Millipore) operated with a vacuum manifold. When it was needed to also measure H$_2$O$_2$ in the reaction mixture, the 55 µL sample was mixed with 55 µL of NaOAc buffer (50 mM, pH 4.5) before filtration (see below). By separating soluble and insoluble fractions, LPMO activity is stopped, as the LPMO used in this study does not oxidize soluble cello-oligosaccharides. Filtered samples were frozen (−20 °C) prior to further analysis. Prior to product quantification, 30 µL of sample was mixed with...
30 µL of a solution of endoglucanase Cel5A from Thermobifida fusca (100 µg/mL) or endoglucanase Cel5A (2 µM in the premix) prepared in Bis-Tris buffer (25 mM, pH 6.0), followed by incubation immersed overnight at 50 °C to convert the substrate to a mixture of glucose, cellulose and C1-oxidized products with a degree of polymerization of 2 and 3 ([GlcGlc]1A, [GlcGlc]1A). For chlorin e6, the 50 µL reaction mixture was mixed with 50 µL 0.2 M CH3COONa pH 4.0 prior to filtration to ensure chlorin e6 precipitation on the filters. TCEsA for treatment of samples containing chlorin e6 was prepared in 0.2 M CH3COONa pH 6.5 to increase pH for efficient solubilization of cello-oligosaccharides and added to the filtrate as described above.

Analysis of reaction products. For qualitative analysis, samples were analysed by MALDI-TOF MS, as previously described. For quantitative analysis, cello-oligosaccharides (native and oxidized) were separated by high performance anion exchange chromatography (HPAEC) and monitored by pulsed amperometric detection (PAD) using a Dionex Bio-LC equipped with a Carbopac PA1 column as previously described. Chromatograms were recorded and analyzed using Chromelon 7.0 software. Oxidized dimers and trimers were quantified using GlcGlcGlc1A (and GlcGlc1A standards obtained by incubating (40 °C, 1000 rpm) cellulose (2 mM) or cellotriose (2 mM) with the cellulose dehydrogenase from Myrocoecum thermophilum (MCHDH, 2 µM, 3 successive additions every 24 h to obtain maximum conversion of 95%). Chito-oligosaccharides resulting from the action of cello-oligosaccharides dehydrogenase on a chitin were also analysed using a Dionex Ultimate 3000 UHPLC system equipped with a Rezex RFQ-Fast acid H+ (8%) 7.8 × 100 mm column as previously described. The elution of chito-oligosaccharides was monitored using a UV detector (194 nm). Prior to analysis of solubilized mixtures of chito-oligosaccharides, these chito-oligosaccharides were hydrolyzed with a chitobiase, SmGH20, from S. marcescens (1 µM final concentration) yielding chitobionic acid as the only oxidized product.

H2O2 production measurements. The method is adapted from a previously reported protocol with some modifications explained hereafter. For each reaction (carried out as described above), 55 µL were sampled at regular intervals and mixed with 55 µL of NaOAc buffer (50 mM, pH 4.5) before filtration as described above. Notably, the decrease in pH obtained by the addition of NaOAc makes chlorophyllin insoluble, meaning that this compound (if present) was removed from the solution during the filtration step, leading to a transparent and stable filtrate usable for colorimetric analysis. Thirty milliliters of each filtrate usable for colorimetric analysis. Thirty microliter of each filtrate was analyzed by HPLC using a Dionex Bio-LC equipped with a Carbopac PA1 column as previously described. The elution of chito-oligosaccharides was monitored using a UV detector (194 nm). Prior to analysis of solubilized mixtures of chito-oligosaccharides, these chito-oligosaccharides were hydrolyzed with a chitobiase, SmGH20, from S. marcescens (1 µM final concentration) yielding chitobionic acid as the only oxidized product.

Verification of superoxide dismutase (SOD) activity. SOD activity was verified according to a published protocol. In brief, a stock solution of pyrogallol (15 mM) was prepared in 10 mM HCl; the tube was wrapped in aluminum foil and kept on ice. Prior to each measurement, the background absorbance (A650 nm) of 50 mM Tris-HCl pH 8.0 was monitored and allowed to stabilize. For the reference reaction, pyrogallol was added to a final concentration of 200 µM and the A650 nm was measured every 10 s for 5 min. For the superoxide dismutase (SOD) reactions, SOD was quickly added after the pyrogallol to obtain maximum conversion of 95%). Chito-oligosaccharides resulting from the action of cello-oligosaccharides dehydrogenase on a chitin were also analysed using a Dionex Ultimate 3000 UHPLC system equipped with a Rezex RFQ-Fast acid H+ (8%) 7.8 × 100 mm column as previously described. The elution of chito-oligosaccharides was monitored using a UV detector (194 nm). Prior to analysis of solubilized mixtures of chito-oligosaccharides, these chito-oligosaccharides were hydrolyzed with a chitobiase, SmGH20, from S. marcescens (1 µM final concentration) yielding chitobionic acid as the only oxidized product.

Fluorescence measurements. Fluorescence signals were recorded using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies) using a 2 ml quartz cuvette (Hellma Analytics, 101-QS). Data acquisition was performed at room temperature with a PMT detector voltage of 600 V. Excitation and emission spectra were acquired at respective maximum emitting and exciting wavelengths, determined iteratively and shown in Supplementary Fig. 1.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Data sharing. Details of the data sharing policies for this journal are available at https://www.nature.com/naturecommunications. This article was published open access; therefore, the data that support the findings of this study are available within the paper and its supplementary information files and from the corresponding author upon reasonable request. The data that support the findings of this study are available from the corresponding author upon reasonable request. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Volatility measurements. Stock solutions of chlorophyllin and chlorin e6 (5 mM) were prepared fresh each day in deionized Milli-Q water. These were protected from light in aluminum foil and kept on ice. Prior to volatility measurements, more dilute in a 1:1 ratio with sodium acetate (200 mM, pH 4.0).

To determine the H2O2 concentration (in all reactions devoid of pigment and for those containing C1-oxidation) containing reaction were pooled. 50 µL of this 54 µL pool (or a diluted in a 1:1 ratio with sodium acetate (200 mM, pH 4.0).

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Author contributions
B.B. and V.G.H.E. conceived the study. B.B. and E.K. performed experiments. B.B., E.K., A.K.R., and V.G.H.E. interpreted data. B.B. and V.G.H.E wrote the initial draft. All authors contributed in writing the current version of the manuscript.

Competing interests
The authors declare no competing interests.

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