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Light-driven oxidation of polysaccharides by photosynthetic pigments and a metalloenzyme

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Oxidative processes are essential for the degradation of plant biomass. A class of powerful and widely distributed oxidative enzymes, the lytic polysaccharide monooxygenases (LPMOs), oxidize the most recalcitrant polysaccharides and require extracellular electron donors. Here we investigated the effect of using excited photosynthetic pigments as electron donors. LPMOs combined with pigments and reducing agents were exposed to light, which resulted in a never before seen 100-fold increase in catalytic activity. In addition, LPMO substrate specificity was broadened to include both cellulose and hemicellulose. LPMO enzymes and pigment derivatives common in the environment of plant-degrading organisms thus form a highly reactive and stable light-driven system increasing the turnover rate and versatility of LPMOs. This light-driven system may find applications in biotechnology and chemical processing.
The oxidation of plant biomass by enzyme catalysed reactions is a basic mechanism in the global carbon cycle and of increasing importance and value for industrial applications. The major plant cell wall-derived polymers cellulose, hemicellulose and lignin are oxidized by enzymes, which often are metalloenzymes.

One class of powerful oxidative enzymes is the extracellular copper-based lytic polysaccharide monooxygenases (LPMOs), which are monooxygenases capable of oxidizing the most recalcitrant polysaccharides. When combined with hydrolases there is a strong synergistic effect on biomass degradation. LPMOs are found in fungi, bacteria and viruses, spanning a number of terrestrial and aquatic ecosystems and are classified in the carbohydrate active enzyme database as auxiliary activity (AA) enzymes class 9, 10, 11 and 13. The enzymes play an essential role, yet not well-understood, in the turnover of organic matter. Some organisms have multiple genes for LPMO enzymes, and especially in plant cell wall-degrading fungi, the AA9 family is highly present with some species containing >30 AA9-encoding genes. All identified substrates are naturally abundant polysaccharides including cellulose, hemicellulose, starch and chitin, in which the glycosidic bonds are oxidized. The products are oxidized oligosaccharides and their non-oxidized counterparts. Specifically for glucans the oxidation of the pyranose ring can take place at either the C1 position producing aldonic acids, or at the C4 position producing 4-ketoadolase (gemiols). Independently from the C1 and/or C4 type of oxidation, one molecule of dioxygen is needed for the LPMO catalytic cycle: one oxygen atom is inserted in the carbohydrate chain for breaking the glycosidic bond, and the second atom is released from the reaction as part of a water molecule.

The LPMOs have a mononuclear type 2 copper active site, with a t-shaped coordination sphere termed the histidine brace, where two histidines provide three nitrogen ligands, two from N-His and one from the terminal amine. Moreover, LPMOs require an extracellular electron donor to complete their catalytic cycle, and both proteins and plant-derived molecules have been found to serve as donors. However, the specific types of electron donors, as well as the mechanism of electron transfer are still unresolved. Ascorbic acid has often been used as electron donor experimentally, and is also present in non-lignified plant biomass, but other donors such as lignin have been described.

In addition, the enzyme cellulose dehydrogenase (CDH) functions as a donor with the electron transfer presumably taking place from the haem b-containing cytochrome domain of CDH. Independently from the C1 and/or C4 type of oxidation, one molecule of dioxygen is needed for the LPMO catalytic cycle: one oxygen atom is inserted in the carbohydrate chain for breaking the glycosidic bond, and the second atom is released from the reaction as part of a water molecule.

As LPMOs require dioxygen, we wanted to rule out that dioxygen produced by the thylakoid membranes was the cause of the observed activity enhancement, and therefore the water-soluble chlorophyll derivative chlorophyllin was tested in the same assay. This resulted in an equally pronounced light-driven accumulation of oxidized products (Fig. 1b), showing that chlorophyll pigments on light excitation transfer electrons to the LPMO, regardless of whether the chlorophyll pigment is membrane-bound (thylakoids) or soluble (chlorophyllin).

The light-driven oxidation was expanded to experiments with two LPMOs from Thermobifida fusca and Thermosascus aurantiacus (Supplementary Figs 2 and 3). For all enzymes tested, both C1 and C4 oxidation increased in the light-driven system. Not only the swollen PASC cellulose but also crystalline cellulose in the form of Avicel was tested as a substrate, and it was similarly oxidized by the light-driven system (Supplementary Fig. 4).

To investigate the light response of the combined pigments and LPMO-catalysed cellulose oxidation, experiments were performed using blue, red, green and sunlight with the same range of intensity. In accordance with the absorption spectra of chlorophyll pigments: sunlight, blue and red light induced the highest levels of cellulose oxidation (Fig. 1c). A light response experiment with alternating darkness and sunlight further proved that light activated the oxidation of cellulose (Fig. 1d).

The level of oxidized products from a 3-h light-induced reaction (Fig. 1c) measured by the amount of glucose units oxidized to gluconic acid, shows that ~10% of the cellulose was oxidized using photo-excited pigments and sunlight, compared with 0.5% without pigments and only ascorbic acid and sunlight.

Results

Light boosted LPMO activity: oligosaccharide products. The activity of the LPMOs was detected in an assay using phosphoric acid-swollen cellulose (PASC) as substrate, and the released oligosaccharide products were detected and quantified by high-performance anion exchange chromatography (HPAEC) after incubation. The gluconic acid on the C1 oxidized oligosaccharides was quantified and used as a measure of enzyme activity. The assay for quantification was based on a complete hydrolysis of the oxidized PASC with a cellulase mixture and subsequent detection of gluconic acid by HPAEC.

Initially, PASC was exposed to LPMO from Thielavia terrestris and suspended thylakoid membranes from a cyanobacterium (Synechococcus sp. PCC 7002) in the presence of ascorbic acid and sunlight. The light response resulted in a 20-fold increase in release of oligosaccharides as compared with incubation in darkness or incubation under conventional conditions with ascorbic acid and light (without the thylakoids, Fig. 1a). Replacing the cyanobacterial thylakoids with membranes prepared from a higher plant (Arabidopsis thaliana) produced similar results (Supplementary Fig. 1).

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No products were detected without the enzyme (Fig. 1c,d). The levels of cellulose oxidation catalysed by light-induced electron transfer and LPMOs are to the best of our knowledge higher than any previously reported result\(^1,9\).

Control experiments were performed to investigate the light response of thylakoids and chlorophyllin when incubated with LPMO and without any reductant: the pigments alone were able to donate electrons to LPMO but were subject to photobleaching and degradation (Supplementary Figs 5 and 6 and Supplementary Discussion). Non-enzymatic reactions from light-induced reactive oxygen species, which potentially can break down cellulose, were also investigated. This was done by combining the pigment preparations and ascorbic acid, but without LPMO enzyme added. These experiments did not result in oxidation of cellulose and confirmed that the applied pigments or ascorbic acid caused cellulose oxidation only when combined with LPMO (Fig. 1c,d, and Supplementary Fig. 7).

**Oxygen consumption.** As LPMOs require oxygen for their catalytic cycle, oxygen measurements were used to monitor the reaction catalysed by the *T. terrestris* LPMO. The results show that oxygen is consumed by the photosystem in a light-dependent manner. In Fig. 2a, the oxygen consumption is shown during incubation in light or darkness: from 0 to 900 s the reactions were carried out in darkness, while from 900 to 2400 s white light was applied. Without the enzyme (blue line) there was no oxygen consumption observed. Without chlorophyllin but with LPMO and ascorbic acid (magenta line) the oxygen consumption was very low, and there was no response to light. For the complete photosystem composed of reductant, pigment and enzyme (turquoise line) there was the same oxygen consumption in darkness as seen for the incubation without chlorophyllin (magenta line), but when the light was switched on (at 900 s) a rapid consumption of oxygen was observed. The oxygen consumption correlated with the release of gluconic acid. At the end of the reaction after 2,400 s, 0.7% of the PASC cellulose was oxidized at the C1 positions to gluconic acid, corresponding to a stoichiometric ratio of 1:0.8 between dioxygen consumption and C1 product formation. As the C4 oxidation products are not included, it is reasonable to assume a 1:1 ratio between dioxygen and oxidation products. Without the pigment

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**Figure 1 | Cellulose oxidation by T.tLPMO9E combined with light-induced electron transfer.** The released oligosaccharides from PASC were measured by HPAEC showing non-oxidized (blue) and oxidized (magenta) reaction products. Each chromatogram is average of three replicate experiments. (a) Thy + Asc + LPMO + PASC + Sun: Thylakoids, ascorbic acid and LPMO with PASC in sunlight for 3 h (150–200 \(\mu\)mol photons s\(^{-1}\) m\(^{-1}\)), yellow line. Control 1 Thy + Asc + LPMO + PASC + Dark: same conditions but in darkness, green line. Control 2: Asc + LPMO + PASC + Sun: same conditions but without thylakoids, black line. (b) Same experiments as in a but with chlorophyllin (Chl) as pigment. Peak annotations of native oligosaccharides (blue) were done using the pure compounds as standard: Glc\(_2\), cellobiose; Glc\(_3\), cellotriose; Glc\(_4\), cellotetraose; Glc\(_5\), cellopentaose; Glc\(_6\), cellohexaose; Glc\(_7\), celloheptaooside; oxidized oligosaccharides (magenta) were assigned by comparing with literature chromatograms performed with identical separation conditions as done by Westereng\(^37\): Glc\(_2\)Glc1A, cellobionic acid; Glc\(_3\)Glc1A, cellotrionic acid; Glc\(_4\)Glc1A, cellopentaionic acid; Glc\(_5\)Glc1A, cellohexaoxonic acid; Glc\(_6\)Glc1A, cellohexaenoic acid; Glc\(_7\)Glc1A, celloheptaoxoic acid. On the y-axis is reported the intensity of the signal in nC (nano Coulomb) without further adjustments. (c) PASC oxidation with LPMO (T.tAA9E), chlorophyllin and ascorbic acid in response to blue, green, red light (150 \(\mu\)mol photons s\(^{-1}\) m\(^{-1}\)) and sunlight (150–200 \(\mu\)mol photons s\(^{-1}\) m\(^{-1}\)). Oxidation was measured with HPAEC quantifying the gluconic acid (oxidation of the C1 position at the pyranose ring). Asc: PASC and ascorbic acid in sunlight (black bar). No LPMO: PASC, chlorophyllin and ascorbic acid in sunlight. (d) Chlorophyllin, ascorbic acid, LPMO and PASC incubation was run for two cycles of darkness for 2 h and sunlight for 5 min, or always in darkness (4 h 10 min), black line with diamonds and green light with square, respectively. Negative control experiment missing LPMO was run in parallel with the darkness/sunlight cycles. The percentage of oxidized cellulose was measured by quantification of gluconic acid formed (C1 oxidation).
then chlorophyllin is added after 700 s. The incubation only
occurs at 900 s. (chlorophyllin, ascorbic acid,
ascorbic acid and PASC; the turquoise line shows the whole photosystem with
4 electrons in de-localized and overlapping
π-orbitals).

The photosystem reactivity could be controlled by switching
the light on and off (Fig. 2b). At the start of the experiment, only
LPMO, ascorbic acid and PASC were present. After five cycles of
light and darkness there was no sign of oxygen consumption.
After 700 s, chlorophyllin was added, followed by a clear oxygen
consumption in light and no oxygen consumption in darkness
(Fig. 2b).

Non-cellulosic substrate and lignin as reductants. Ascorbic acid
is a naturally available reductant for pigments only in non-lig-
nified plant tissue. To look for alternative natural reductants, we
replaced ascorbic acid with extracted lignin. Indeed, insoluble
lignin extracted from wheat straw could replace ascorbic acid in
the light-driven assay with chlorophyllin (Fig. 3a). This points to
how light-induced electron transfer may also be functional in
degradation of lignified biomass. The functionality of lignin as a
multiple electron donor to the pigments may be ascribed to its
electrons in de-localized and overlapping π-orbitals.

To our surprise, the specificity of the T. terrestris LPMO
changed when used in the light-induced electron-transfer assays.
This particular LPMO is thought to be strictly cellulose specific,
but when combined with light-induced electron transfer from
pigments, the enzyme also oxidized the hemicellulose xyloglucan
(Fig. 3b). Oxidation of both cellulose and hemicellulose has been
reported for LPMOs from Neurospora crassa20 and from
Podospora anserine20. The degradation pattern of xyloglucan in
Fig. 3b is similar to a xyloglucan degradation pattern previously
reported20.

Discussion
The light-induced boosting of PASC oxidation presented here
clearly shows the pivotal role of the LPMO enzyme for the light-
induced oxidation of carbohydrates. We suggest a model in which
light excitation of the pigments generates a strong reductant that
efficiently reduces the copper ion in the LPMO enzyme by
donating an electron as shown in Fig. 4. Subsequently the

Figure 2 | Oxygen consumption during cellulose oxidation with light or in
darkness. (a) Oxygen consumption with different combinations of the
photosystem: blue line shows chlorophyllin, ascorbic acid and PASC
(without T. terrestris LPMO); magenta line shows T. terrestris LPMO, ascorbic
acid and PASC; the turquoise line shows the whole photosystem with
chlorophyllin, ascorbic acid, T. terrestris LPMO and PASC. Light is turned on
at 900 s. (b) Alternating cycles of light and darkness: at the beginning of
the incubation only T. terrestris LPMO, ascorbic acid and PASC are present,
then chlorophyllin is added after 700 s.

Figure 3 | HPAEC analysis of light-induced polysaccharide oxidation with
TtLPMO9E. (a) Lignin as reductant: LPMO oxidative activity on cellulose
(PASC) when incubated with chlorophyllin and organosolv lignin as
reductant in 3 h of sunlight. Controls with only chlorophyllin or organosolv
lignin in sunlight are included. Each chromatogram is average of three
replicate experiments. For details on peak annotation see Fig. 1 or
Supplementary Fig. 1. (b) Xyloglucan as substrate: LPMO oxidative activity
on xyloglucan (1% w/w) when incubated with chlorophyllin and ascorbic
acid and exposed to 3 h of sunlight. Pronounced is the presence of oxidized
species detectable from minute 17 to 24. A control experiment with LPMO
and ascorbic acid shows no degradation of the xyloglucan. The xyloglucan
is a heteropolymer of xylose, glucose, galactose and arabinose. The division
of peaks in oxidized and non-oxidized is derived from the model heptamer
xylooligosaccharide XXXG7H in its reduced form made of four units of
glucose of which three are substituted with one xylose. While XXXG is the
same, but oxidized in the reducing end of the last subunit in the glucan
backbone.

(b)
consumption when incubated with the photosystem at 25°C (Fig. 2a) and 0.55 s (Fig. 1d). Previously published turnover values for a oxidized product after two cycles of 5 min light exposure LPMO by measuring reducing ends of oxidized PASC at 50°C were 0.01–0.04 s

potentially are powerful oxidizers. However, the extensive zinc, could by themselves generate hydroxyl radicals, that thylakoid preparations, which contain magnesium, copper and oxidation raised the question if non-enzymatic Fenton-reactions were induced by light. Exposure of chlorophyllin or oxidized pigment is reduced by acquisition of an electron from a reductant such as ascorbic acid or lignin, thus now available for a new cycle of excitation. During the monooxygenase reaction of LPMO, dioxygen is split, and one oxygen atom is incorporated into the substrate, and the other is reduced to water.

The oxidation of a PASC cellulose chain is shown as a red monomer of glucose oxidized in the C1 position to an aldonic acid oligosaccharide, whereas the blue monomer will be released as a non-oxidized oligosaccharide.

The results presented support the proposed mechanism and synergy of light-induced electron donation for the LPMOs (Fig. 4). But how are the electrons donated to the active site, directly or via long-range transfer through the protein? Tan et al. suggested a direct electron transfer to the LPMO-active site from CDH via a haem-binding cytochrome complex. Another possibility is through protein long-range electron transfer. This allows the enzyme to be adsorbed to its substrate during the electron transfer, and a possible pathway for electron transfer from CDH has been suggested. In the LPMO AA9 family we found, using molecular modelling, other possible electron-transfer pathways from the surface to the active site, potentially capable of transferring electrons from the excited pigments. Specifically for the TaLPMO9A a putative pathway goes from a histidine (His87) surface ligand through 12 covalent bonds to the Cu(II), equivalent to a tunnelling length of 16.8 Å with a calculated pathway coupling decay value of 2.2 x 10⁻³. The histidine is situated on the side of LPMO and thus free of cellulose that is binding to the LPMO during catalysis. Similar pathways were located in other AA9 LPMOs all located very much in the same position but with different ligands at the surface (Supplementary Fig. 9). The exact mechanism for the suggested electron transfer is so far unresolved, and the existence of parallel paths cannot be ruled out.

As the light-induced electron transfer reported caused a tremendous increase in products, as well as a change in enzyme specificity, the energy of the transferred electrons seems to alter the cycle or state of the active site. As suggested above, the high-energy photoelectron from pigments could be

oxidized form of the pigments is reduced by a reductant with lower redox potential, in this case ascorbic acid or lignin.

The initial observation of light-induced boosting of PASC oxidation raised the question if non-enzymatic Fenton-reactions were induced by light. Exposure of chlorophyllin or thylakoid preparations, which contain magnesium, copper and zinc, could by themselves generate hydroxyl radicals, that potentially are powerful oxidizers. However, the extensive control experiments examining all the system components revealed no formation of oxidation products from such reactions (Supplementary Fig. 7), also there was no detectable oxygen consumption with an enzyme-free control (Fig. 2a).

Not only the level of oxidized products, but also the reactivity increased with light-induced electron transfer. Using ascorbic acid or CDH as electron donors, an end-level of ~2% oxidized cellulose is reached after 24–36 h (refs 9,20). With the light-induced electron-transfer system, the level of LPMO-oxidized cellulose was 2.3% after two cycles of 5 min (Fig. 1d, Supplementary Fig. 8 and Supplementary Discussion). It is thus possible to achieve the same level of oxidation in less than a hundredth of the time required without light-induced electron transfer. These data are also supported by the LPMO turnover frequency determined to be 0.25 s⁻¹ based on the oxygen consumption when incubated with the photosystem at 25°C (Fig. 2a) and 0.55 s⁻¹ at 50°C based on the accumulation of oxidized product after two cycles of 5 min light exposure (Fig. 1d). Previously published turnover values for a N. crassa LPMO by measuring reducing ends of oxidized PASC at 50°C were 0.01–0.04 s⁻¹ (ref. 22).

Based on our results, we conclude that the catalytic activity for LPMOs combined with the photosystem for light-induced electron transfer results in up to 2 orders of magnitude higher level of catalytic activity than previously reported.
Enzymes are produced by expression in a host organism and subsequently purified. This results in a protein content of 127 mg g\(^{-1}\) of dry material. Commercial cellulase mixtures Celluclast 1.5l and Novozym 188 were obtained from Novozymes A/S. The Celluclast 1.5l mixture was purchased from Nzytech Ltd (Portugal). All LPMOs were free of any residual T. fusca enzymes.

The observed light-driven enzymatic oxidation performed by LPMO consumes oxygen, oxidizes carbon and produces water. Biocatalysts are used in biotechnology and environmental applications to convert organic materials into useful products.

Methods

### Enzymes

**T. terrestris** LPMO (TLPm09E, previously TGH61E) and **T. aurantiacus** (TalPm09A) were donated from Novozymes A/S (Denmark). The enzymes are produced by expression in a host organism and subsequently purified. **T. fusca** AA10 (TLPm010A) cloned and expressed in Escherichia coli was purchased from Nytelych Ltd (Portugal). All LPMOs were free of any residual crystalline cellulase or hemicellulose activities. Commercial cellulase mixtures Celluclast 1.5l and Novozym 188 were obtained from Novozymes A/S. The Celluclast 1.5l mixture had a protein content of 127 mg g\(^{-1}\), containing 62 filter paper units (FPU) per g, and a \(\beta\)-glucosidase activity of 15 U per g. Novozym 188 had a protein content of 220 mg g\(^{-1}\), containing a \(\beta\)-glucosidase activity of 231 U per g.

### Chemicals, materials and substrates

Ascorbic acid was obtained from Sigma-Aldrich. Crystalline cellulose, Sigma-Aldrich PH101) was swollen with phosphoric acid to a concentration of 3.57 mg Chl per ml (Chl a/b ratio = 4.06 mg Chl per ml. Putative contaminations derived from cytoplasmic membranes in the thylakoid suspension have no impact on light-induced electron transfer to LPMOs, which was tested with disrupted E. coli K-12 cells.

### Preparation of PASC microcrystalline cellulose substrate

Avicel microcrystalline cellulose was obtained from Sigma-Aldrich. Xylo-glucan product code P-XYGLN was obtained from Megazyme Ltd, Ireland. Chlorophyllin product code C-100.000-WS-P produced by extraction of Synechococcus sp. PCC 7002 (referred to as Synechoecoccus) grown in medium A containing 2 g NaNO\(_3\), per l as previously described. Synechococcus cells were harvested in 50 ml volumes by centrifugation (5,000 g for 5 min) and subsequently resuspended in 1 ml thylakoid washing buffer (pH 6.35) as described. The resuspended thylakoid was transferred to a microtube containing 500 \(\mu\)l glass beads (glass beads for cell disruption, 0.1–0.25 mm diameter, Retsch Technology GmbH, Haan, Germany) followed by cell disruptive sonication (Amplitude 50, 3 min processing time, 5 s on/off cycle).

The observed light-driven enzymatic oxidation performed by LPMO consumes oxygen, oxidizes carbon and produces water. Looking ahead, this process may find applications in technology areas for chemical processing such as conversion of recyclable biomass to fuels and chemicals. We speculate that the light-induced oxidation can be applied in a broader application scheme for oxidation of C–H bonds.

### Preparation of plant thylakoid membranes

Plant thylakoid membranes were extracted from A. hibulana (L.) Heynh. Ecotype Columbia. Plants were grown in compost in a controlled environment (Percival AR-60, Lom, Boone, IA) at a photosynthetic flux of 130–150 \(\mu\)mol of photons per m\(^2\) per s, 20 °C and 70% humidity. Leaves from 50–25 plants were pooled and homogenized using a blender fitted with razor blades in ice cold buffer containing 20 mM Tricine (pH 7.5), 10 mM NaCl, 5 mM MgCl\(_2\), 0.4 M sucrose, 5 mg ml\(^{-1}\) bovine serum albumin (BSA) and 100 mM sodium ascorbate. The homogenate was immediately filtered through two layers of nylon mesh (31 \(\mu\)m pore size), after which the filtrate was centrifuged (6,000 \(g\), 10 min). The pellet was resuspended in a small volume of homogenization buffer without ascorbate and BSA with 20% glycerol (w/v). Total chlorophyll (Chl) and Chl a/b ratio were determined in 80% acetone according to the method of Lichtenthaler. The final concentration of the thylakoid membranes applied in the light-induced electron-transfer experiments was 3.57 mg Chl per ml (Chl a/b ratio = 3.0).

### Elemental analysis inductively coupled plasma (ICP)

Chlorophyllin and thylakoid suspensions were analysed for the presence of metals on an Aurora Elite ICP-MS system from Bruker. Samples of chlorophyllin 12% (w/w) and extracted thylakoid suspensions 2% (w/w) were diluted to 500–1,000 p.p.b. dry matter in 1% nitric acid. The samples were analysed for \(\text{Mg}^{2+}\), \(\text{Mg}^{2+}\), \(\text{Cu}^{2+}\), \(\text{Zn}^{2+}\) and \(\text{Zn}^{2+}\). Chlorophyllin contained 0.41% Mg, 2.0% Cu and 0.05% Zn relative to total dry matter. Extracted thylakoid suspensions contained 1.8% Mg, 0.007% Cu and 0.0007% Zn relative to total dry matter.

### Quantification of glucose and cellobiose by HPLC

The quantification of glucose and cellobiose was done using an Ultimate 3000 high-performance liquid chromatography (HPLC, Dionex, Germering, Germany) equipped with refractive index detector (Shodex, Japan) and ultraviolet detector at 210 nm (Dionex). The separation was performed in a Phenomenex Rexa ROA column at 80 °C with 5 mM H\(_2\)SO\(_4\) as eluent at a flow rate of 0.8 ml min\(^{-1}\).

### Measurement of oligosaccharide and gluconic acid by HPAEC

The measurement of oligosaccharide and gluconic acid by HPAEC was performed as follows: 200 ml were centrifuged at 14,000 g for 2 min and 100 ml of the supernatant was inserted in the HPLC conical vial, without any further light exposure (wrapped in aluminum foil). HPAEC was run on an ICSS5000 system (two different machines available in the laboratory), equipped with a PAD detector.

Organosolv lignin extraction. The lignin fraction was prepared from wheat straw (Triticum aestivum L.). The straw was ball milled for 20 min and the cellulose and hemicellulose fractions were removed by hydrolytic enzymes (Cellulact 1.5L and, Novozym 188 in a 5:1 v/v) at a final dosage of 75 FPU per g of dry lignocellulose substrate for 144 h at 50 °C followed by washing with MilliQ water at a ratio of 11:1 of dry material. The amount of residual carbohydrates was <2% in the final material. Finally, the residual material was suspended in an aqueous ethanol solution (50:50 water/ethanol) at a 5:1 liquid/solid ratio and heated at 220 °C in a 1 l Parr reactor for 80 min. After heating, the lignin residue was filtered at 75 °C. Solubilized lignin was precipitated by adding water at three times the original volume and recovered by filtration. This insoluble lignin fraction was dried at 40 °C and ground with a pestle and mortar.

### Preparation of chlorophyllin

A stock solution of 12% w/v (166 mM) chlorophyllin was prepared by dissolving the powder in water (MilliQ-quality). The chlorophyllin was kept in darkness while stored at 4 °C. Prior to each experiment, an aliquot of the stock solution was incubated in darkness for 2 h at room temperature.

### Preparation of cyanobacterial thylakoid suspensions

Thylakoid suspensions, containing light-harvesting antennae (phycobilisomes) and thylakoid membranes, were prepared from the cyanobacterium Synechococcus sp. PCC 7002 (referred to as Synechoecoccus) grown in medium A containing 2 g NaNO\(_3\), per l as previously described. Synechococcus cells were harvested in 50 ml volumes by centrifugation (5,000 g for 5 min) and subsequently resuspended in 1 ml thylakoid washing buffer (pH 6.35) as described. The resuspended thylakoid was transferred to a microtube containing 500 \(\mu\)l glass beads (glass beads for cell disruption, 0.1–0.25 mm diameter, Retsch Technology GmbH, Haan, Germany) followed by cell disruptive sonication (Amplitude 50, 3 min processing time, 5 s on/off cycle). The cell extract was then centrifuged (12,000 g, 4 °C, 20 min) and the pellet, containing unbroken cells and cell walls, was discarded. An additional centrifugation (16,000 g, 30 min) separated the light-harvesting antennae (phycobilisomes, supernatant) from the thylakoid membranes (pellet). Absorption spectra of the thylakoid suspensions in the supernatant were performed with a UV1800 spectrophotometer (Shimadzu, Kyoto, Japan) and the chlorophyll a content was calculated as described.

### The thylakoid suspension was subjected to light-induced electron-transfer experiments with an average concentration of 0.21 ± 0.06 mg Chl per ml. Putative contaminations derived from cytoplasmic membranes in the thylakoid suspension have no impact on light-induced electron transfer to LPMOs, which was tested with disrupted E. coli K-12 cells.

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Quantification of cellulose oxidation for TfLPMO9E. To quantify the total oxidation of cellulose (as shown in Fig. 1c), the residual PASC together with the contaminant containing oligosaccharides were digested with a commercial cellulase mixture (Cellic CTec2), stand-still incubated with both enzymes for 1 h under a magnet at room temperature, and then centrifuged at 30,000 g for 10 min. The supernatant was then collected and chromatographed (HPAEC) to measure the release of oligosaccharides and additionally treated with cellulase to quantify the monomeric glucose and gluconic acid (see above). An LPMO activity control, applying the same LPMO dosage was incubated for 24 h with cellulose to quantify the monomeric glucose and gluconic acid (see above).

Oxygen consumption measurements. The oxygen consumption was measured with a Chlororab 2 System (Hansatech, England), with an oxygen sensor mounted at the bottom of a sealed reaction chamber. The ml reaction chamber was stirred by a magnet and equipped with a window for light exposure. Reactants could be added to the acceptor side of the capillary, but no oxygen exchange with the external environment was observed. The measurements are given as oxygen concentration in µmol per ml. White LED light (4100 K) at 150 µmol photons per m² per s was applied. The reactions were started at the beginning according to the dosages explained in the standard conditions, but at a lower temperature of 25 °C. Note that there is no headspace in the reaction chamber, why much less oxygen is available compared with the conditions described in standard conditions for enzymatic reactions. The combinations tested were: complete photosystem: LPMO (TfLPMO9E) + chlorophyllin + Asc + PASC; the negative control experiments: chlorophyllin + Asc + PASC (no enzyme present); and the LPMO + Asc + PASC (no pigment present). From 0 to 900 s these reactions were carried out in darkness, while from 900 to 2400 s white light was supplied (results shown in Fig. 2a).

The complete photosystem (LPMO + chlorophyllin + Asc + PASC) was measured with alternating cycles of light/darkness without or with pigment. The conditions were identical to standard (see above) with the difference that LPMO was dosed at 0.01 µmol g⁻¹ to lower the rate of oxygen consumption, and a temperature of 25 °C. TfLPMO9E, ascorbic acid and PASC were first added. After five cycles of light and darkness (2 min each), chlorophyllin was added through the cannula, and three cycles of light and darkness were alternated (results shown in Fig. 2b).

Lignin as reductant. Ascorbic acid, generally used as a reductant in the standard conditions for the enzymatic hydrolysis, was replaced with organosolv-extracted lignin to investigate its putative reducing capacity. The organosolv lignin was suspended in citrate-phosphate buffer (pH 6.3) and was added to a final concentration of 5 mg ml⁻¹ (equal to a molar concentration of 25 mM based on lignin monomers) to the reaction mixture and incubated under standard conditions (see above) with TfLPMO9E. Control experiments were performed as described above, replacing ascorbic acid with lignin.

Light-induced oxidation of xyloglucans. Xyloglucan 1% w/w was used as a substrate, replacing PASC and incubated with chlorophyllin, ascorbic acid and TfLPMO9E, the others parameters were according to standard conditions (see above). A release of oligosaccharides was detected using HPAEC. The observed pattern was a mixture of cello-oligosaccharides and xylooligo-oligosaccharides, which could not be fully separated, as seen for other published works, therefore the peaks are separated into non-oxidized and oxidized products only.

Light-induced oxidation of crystalline cellulose. Avicel was used as a cellulose substrate at 1% w/w instead of PASC and all other conditions were same as standard conditions (see above).

Protein structure modelling. The PDB structures from the RCSB PDB Protein data bank were adapted for the HARLEM Molecular Modelling Package program (Kurnikov et al, available at http://harlem.chem.cmu.edu/). The PDB structures were (after adding hydrogens) and used in HARLEM for possible LRET pathways from the surface to the metal ion.
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Authors contributions

D.C., K.R.M. and C.F. planned and participated in the experiments; P.E.I. and N.U.F. participated in planning of the experiments; M.J.B. planned and performed the modelled experiments. K.S.J together with all authors analysed the data and wrote the manuscript.

Additional information

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