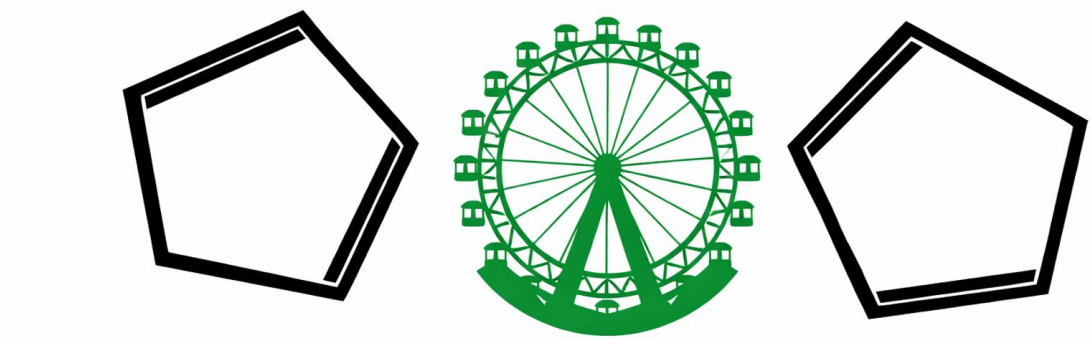
5th LPMO Symposium

Vienna, 19-20 November 2026



Program

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Book of Abstracts

FWF Österreichischer Wissenschaftsfonds excellent = austria

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About the Symposium

Dear Colleagues and Friends,

It is our great pleasure to welcome you to the 5th LPMO Symposium in Vienna. Ten years on from the first meeting in Copenhagen in 2016, we are delighted to bring the international LPMO community together once again. Over the past decade, our understanding of LPMOs has advanced considerably, yet many fundamental questions remain. Their remarkable oxidative chemistry, complex catalytic mechanism, minimalist yet intricate architecture, and still emerging biological functions continue to challenge researchers across disciplines. At the same time, their potential for biomass conversion and polysaccharide modification is generating interest in their biotechnological application.

The diverse scientific program reflects the widespread research interests and expertise of the participants. During the two-day meeting, we will discuss the following topics: (i) catalytic mechanisms and functional characterization, (ii) biotechnological applications and polysaccharide modification, (iii) theoretical and computational insights, (iv) structural aspects of LPMOs, (v) enzyme modularity and carbohydrate-binding modules, and (vi) their roles in plant-pathogen interactions and plant cell wall degradation. We are particularly pleased to welcome our distinguished colleagues who will open each of the six thematic sessions, as well as all other speakers contributing to the scientific program. Together with the poster presentations, these contributions promise two days of stimulating scientific discussions and exchange.

Beyond the scientific sessions, we hope that this symposium provides opportunities to connect with colleagues, meet new members of the LPMO community, exchange ideas, and establish collaborations. As the field continues to evolve, it is essential to bring together different perspectives and areas of expertise to address the questions that lie ahead. We are very happy to host you in Vienna and are grateful to all who have contributed to this meeting. We wish you a symposium that is enjoyable, inspiring, and scientifically rewarding!

Your Organizing Committee

Gaurav Patil, Florian Csarman and Roland Ludwig

Practical Information

Venue and Travel

The 5th LPMO Symposium will take place at **CAPE 10**, a modern and accessible event venue located close to Vienna's main railway station (Wien Hauptbahnhof).

Venue address:

CAPE 10
Alfred-Adler-Straße 1
1100 Vienna

The venue is conveniently accessible by public transport:

- **Train:** Wien Hauptbahnhof – approximately a 6-minute walk
- **Underground:** U1, station Südtiroler Platz – approximately 10-minute walk
- **Tram:** Line D, stop Alfred-Adler-Straße – approximately a 1-minute walk
- **Bus:** Line 69A, stop Alfred-Adler-Straße – approximately a 1-minute walk

From Vienna International Airport (VIE):

The most convenient option is to take a direct Railjet (RJ/RJX) or Intercity (IC) train to Wien Hauptbahnhof. From there, CAPE 10 is just a 6-minute walk away.

Alternatively, take the S7 or City Airport Train (CAT) to Wien Mitte and continue by underground (U1) towards Oberlaa, getting off at Wien Hauptbahnhof.

Taxis and ride services are also available directly from the airport: The journey approximately takes 20–30 minutes, depending on traffic.

For further information, please visit the LPMO Symposium website or the CAPE 10 website.

Oral Presentations

Members of the organizing committee will be available during registration and throughout the breaks to assist presenters with technical questions and presentation setup.

We kindly ask all speakers to transfer their PowerPoint presentations (.pptx) to the conference computer during registration or one of the breaks, preferably well before their scheduled session.

To ensure a smooth program and avoid technical delays, presenters are encouraged to check their slides on the conference computer in advance.

Poster Session

Posters will remain on display throughout the symposium, giving participants the opportunity to view the contributions and discuss them with the presenters during the scheduled poster sessions and breaks.

We kindly ask all poster presenters to mount their posters shortly after registration, preferably before the start of the scientific program.

Posters should be in A0 portrait format (84.1 × 118.9 cm). Each poster board will be assigned a number corresponding to the poster number listed in this Book of Abstracts. The numbers will also be displayed at the venue.

Lunch and Coffee Breaks

Lunch and refreshments will be provided for all participants at the conference venue during the scheduled breaks. These breaks offer excellent opportunities to exchange ideas, meet colleagues, and continue scientific discussions in a relaxed and informal atmosphere.

Conference Dinner

The conference dinner will take place on Thursday, November 19, at 19:00, at the restaurant Der Ringsmuth, located at Johannitergasse 1, 1100 Vienna, in walking distance from the conference venue (a 10-min walk, approximately 700 meters).

The dinner will feature traditional Austrian goose dishes, a seasonal specialty typically enjoyed in November. Vegetarian and vegan alternatives will also be available for participants who prefer a different option.

Conference Photograph

A group photograph of all symposium participants is scheduled for Thursday, November 19, from 12:30 to 12:45 p.m., following the poster session.

Weather permitting, the photograph will be taken outdoors on the rooftop terrace of CAPE 10.

We warmly invite all participants to join us and capture a lasting memory of the 5th LPMO Symposium!

Program

Thursday, 19 November 2026

08:30 - 09:00	**Registration and refreshments**
09:00 - 09:10	**Welcome and organizational remarks**
09:10 - 11:20	**SESSION 1: Catalytic mechanisms and functional characterization**
09:10 - 09:50	Keynote lecture: **On the functional characterization of LPMOs** Vincent G.H. Eijsink (Norwegian University of Life Sciences, Ås, Norway)
09:50 - 10:20	**Catalase-like reaction by LPMOs enables reductant independent degradation of cellulose** Priit Väljamäe (Tartu University, Estonia)
10:20 - 10:40	**Early stages of the peroxidase reaction of the NcAA9C LPMO studied by stopped-flow and rapid freeze-quench** Gabriele Deplano (Max Planck Institute, Mühlheim an der Ruhr, Germany)
10:40 - 11:00	**Remote but ruling: LPMO reactivity under the control of second sphere residues** Nathan Montmailler (Norwegian University of Life Sciences, Ås, Norway)
11:00 - 11:20	**Insights into how LPMOs protect themselves from self-destruction: hole hopping routes and histidine methylation** Tom Z. Emrich-Mills (Norwegian University of Life Sciences, Ås, Norway)
11:20 - 12:30	**Coffee break and poster session**
12:30 - 12:45	**Group photo**
12:45 - 13:45	**Lunch break**
13:45–15:45	**SESSION 2: Biotechnological applications and polysaccharide modification**
13:45 - 14:15	Session opening lecture: **Advancing the use of LPMOs in biomass valorisation: leveraging redox control and substrate specificity** Aniko Varnai (Norwegian University of Life Sciences, Ås, Norway)
14:15 - 14:45	**AA17 lytic polysaccharide monooxygenases: oxidative cleavage of methyl-esterified homogalacturonan** Mirjam Kabel (Wageningen University, The Netherlands)
14:45 - 15:15	**Characterizing the oxidative repertoire of the model green alga *Chlamydomonas reinhardtii*** Aidan Salter (University of York, United Kingdom)
15:15 – 15:45	**Integrating electrochemistry into the LPMO enzyme discovery toolkit** Connor Miles (University of York, United Kingdom)

15:45 - 16:15 **Coffee break**

16:15 - 17:45 **SESSION 3: Theoretical and computational insights into LPMO catalysis**

16:15 - 16:45 Session opening lecture: **Computational exploration of LPMO polysaccharide oxidation: pitfalls and conclusions so far**
Erik D. Hedegård (University of Southern Denmark, Odense, Denmark)

16:45 - 17:15 **Unravelling the LPMO reaction mechanism using QM MM simulations**
Dominik Hörtnagel (BOKU University, Vienna, Austria)

17:15 - 17:45 **Computational approaches to characterize LPMOs: from family-wide overview to active site details.**
Katarzyna Szleper-Shendy (Silesian University of Technology, Gliwice, Poland)

19:00 **Conference dinner and poster awards**

Friday, 20 November 2026

08:30 - 09:00	**Refreshments**
09:00 - 11:10	**SESSION 4: Structural insights into LPMOs**
09:00 - 09:40	Session opening lecture: **Not just for biomass: structure and function of the *Cryptococcus neoformans* virulence factor Cel1, a lytic polysaccharide monooxygenase** Leila Lo Leggio (University of Copenhagen)
09:40 - 10:10	**Structure-guided deep learning reveals five novel LPMO-like families and the first cellulose-active LPMO in algae** Daniel Leadbeater (University of York, United Kingdom)
10:10 - 10:40	**Exploring the structural mechanism of the lytic polysaccharide monooxygenase reaction: reaction priming and substrate binding** Christos Kosinas (University of Copenhagen, Denmark)
10:40 - 11:10	***De novo* designed β-hairpin peptides as LPMO mimics** Luisa Ciano (University of Nottingham, United Kingdom)
11:10 - 11:40	**Coffee break**
11:40–12:40	**SESSION 5: LPMO Architecture: Modularity, CBMs and non-catalytic domains**
11:40 - 12:10	Session opening lecture: **Insight into the function of disordered C-terminal regions in LPMOs** Jean-Guy Berrin (INRAE, Aix-Marseille Universtiy, France)
12:10 - 12:40	**Copper beyond the active site: discovery of metal-binding domains in LPMOs** Zarah Forsberg (Norwegian University of Life Sciences, Ås, Norway)
12:40 - 13:40	**Lunch break**

13:40 - 15:40 **SESSION 6: LPMOs in plant–pathogen interactions and plant cell wall degradation**

13:40 - 14:10	Session opening lecture: **Exploring the role of AA10 LPMOs in fungal plant pathogens** Bastien Bissaro (INRAE, Aix-Marseille Universtiy, France)
14:10 - 14:40	**From AA17 LPMOs to oxidative glycan editing: exploring the interplay of *Phytophthora* enzymes** Federico Sabbadin (University of York, United Kingdom)
14:40 - 15:10	**Not all LPMOs are made equal: secretome adaptation to scavenge ROS in *N. crassa* grown under photoredox stress** Constance Padoan (Université Libre de Bruxelles, Belgium)
15:10 - 15:40	**Functional diversification of AA17 LPMOs reveals multiple polysaccharide-oxidising activities in plan-pathogenic oomycetes** Sune Fjermedal (The Technical University of Denmark, Lyngby, Denmark)

15:40 **Closing remarks**

SESSION 1

Catalytic mechanisms and functional characterization

S1.1. On the functional characterization of LPMOs

Vincent G.H. Eijsink, Anton A. Stepnov, Ole Golten, Tom Z. Emrich-Mills, Aniko Várnai, Gustav Vaaje-Kolstad, Zarah Forsberg

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Keywords: oxygen, hydrogen peroxide, copper, kinetics, lignocellulose, chitin

Functional studies of lytic polysaccharide monooxygenases (LPMOs) are challenging due to the combined presence of reductants, oxygen, hydrogen peroxide, transition metals and an insoluble, slowly diffusing substrate that may contain redox-active lignin [1-8]. Autocatalytic enzyme inactivation [9,10] adds another complexity, as does the fact that the nature of the insoluble and potentially heterogeneous substrate may change as an LPMO reaction proceeds. It has taken us and others active in the LPMO field many years to get to grips with these challenges, and mistakes have been made along the way. The LPMO literature is riddled with errors, partly because the field has been on a learning curve and partly because papers lacking essential control reactions are not subjected to rigorous peer review. For a long time, reliable kinetic studies of LPMOs were lacking. A breakthrough came in 2024, when Schwaiger et al. described a hydrogen peroxide sensor that, for the first time, and at reasonable cost, allowed straightforward measurement of LPMO kinetics with a wide variety of substrates [11,12]. This sensor also allows quantitative assessment of redox robustness, which is a topic of great current academic and industrial interest [13-16]. In my presentation, I will discuss opportunities and pitfalls in the experimental characterization of LPMOs, pointing at commonly made mistakes and at how these can be avoided. I will also try pointing at some open questions that current LPMO research and methodology have not yet been able to answer.

[1] Eijsink VGH et al., 2019, Biotechnol Biofuels 12, 58; doi: 10.1186/s13068-019-1392-0.
[2] Stepnov AA et al., 2021, Biotechnol Biofuels 14, 28; doi: 10.1186/s13068-021-01879-0.
[3] Stepnov AA et al., 2022, Sci Reports 12, 6129; doi: 10.1038/s41598-022-10096-0.
[4] Kommedal EG et al., 2023, Nature Comms 14, 1063; doi: 10.1038/s41467-023-36660-4.
[5] Golten O et al., 2023, FEBS Lett 597, 1363–1374; doi:10.1002/1873-3468.14629.
[6] Golten O et al., 2025, FEBS Lett 599, 1317-1336; doi: 10.1002/1873-3468.15105.
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[9] Bissaro B et al., 2017, Nature Chem Biol 13, 1123-1128; doi: 10.1038/nchembio.2470.
[10] Loose J et al., 2018, Biochem 57, 4114-4124; doi: 10.1021/acs.biochem.8b00484.
[11] Schwaiger L et al., 2024, ACS Catal 14, 1205-1219; doi: 10.1021/acscatal.3c05194.
[12] Carletti A et al., 2024, ACS Catal 14, 14586–14594; doi: 10.1021/acscatal.4c04510.
[13] Kuusk S et al., 2023, J Biol Chem 299, 105094; doi: 10.1016/j.jbc.2023.105094.
[14] Zhao J et al., 2023, J Am Chem Soc 145, 20672-20682; doi: 10.1021/jacs.3c06607.
[15] Ayuso-Fernández et al., 2024, Nature Comms 15, 3975; doi: 10.1038/s41467-024-48245-w.
[16] Ayuso-Fernández I, Emrich-Mills TZ et al., 2026, Proc Natl Acad Sci USA 123, e2521617123; doi: 10.1073/pnas.2521617123.

S1.2. Catalase-like reaction by lytic polysaccharide monooxygenases (LPMOs) enables reductant independent degradation of cellulose

Priit Väljamäe

Department of Molecular and Cell Biology, University of Tartu. Tartu, Estonia.

LPMOs are monocopper enzymes that catalyze the cleavage of glycosidic bonds in polysaccharides by hydroxylating C1 and/or C4 carbons using hydrogen peroxide as a cosubstrate. Besides the sugar substrate and the H_2O_2 cosubstrate, LPMO catalysis crucially depends on the external reductant, which is needed for the "priming" reduction of LPMO-Cu(II) resting state into catalytically competent LPMO-Cu(I). LPMOs remain in their Cu(I) form after polysaccharide peroxygenase reaction, but they can be re-oxidized to the Cu(II) form in reductant oxidase and peroxidase side reactions. Here I introduce a novel family AA10 LPMO (*Ci*LPMO10A) from the alkali tolerant bacterium *Cellulomonas iranensis*. At pH 9.0 and above *Ci*LPMO10A was able to release O_2 from H_2O_2 in the catalase-like reaction, suggesting its ability to abstract electron from H_2O_2. Consistent with this, H_2O_2 is sufficient for the cellulose peroxygenase reaction by *Ci*LPMO10A without the addition of external reductant. In this talk I will discuss the kinetics of this catalase-like reaction and the cellulose peroxygenase reaction by *Ci*LPMO10A. I will also discuss the role of the second sphere amino acid residues of *Ci*LPMO10A in these processes.

S1.3. Early stages of the peroxidase reaction of the *Nc*AA9C LPMO studied by stopped-flow and rapid freeze-quench

Gabriele Deplano[1], Oliwia S. Rakoczy[1], Tom Z. Emrich-Mills[2], Synnøve E. Rønnekleiv[2], Kushal Sengupta[1], Vincent G. H. Eijsink[2] and Serena DeBeer[1]

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Keywords: *Nc*9AA9C, stopped-flow UV-Vis, rapid freeze-quench, EPR, XAS

Significant effort has been put in elucidating the actual catalytic mechanism at play in the reaction between LPMOs and H_2O_2, but discussed reaction steps still almost entirely rely on results from computations. [1, 2] DFT calculations mostly support the reaction of the Cu(I) active site with H_2O_2 to form a Cu(II)-OH species coupled with an OH radical, based on energy arguments; following H_2O_2 splitting, the latter may react again with the Cu(II)-OH to form H_2O and a highly reactive Cu(II)-oxyl species that has been connected with catalytic activity on the substrate. Although there is still debate on whether this active Cu oxyl species should form if no substrate is bound to the protein, the reaction step immediately after its formation is often regarded as the rate-determining step of the reaction, and it should thus be observable experimentally. In this study, we investigate the early stages (> 7 ms) of the peroxidase reaction on the *Nc*AA9C LPMO from *Neurospora crassa*, using stopped-flow UV-Vis spectroscopy, to test this mechanistic hypothesis. We find that in excess of H_2O_2 at basic pH an unknown species forms before the hole hopping mechanism kicks in to protect the active site from overoxidation (Figure 1a). We subsequently use rapid freeze-quenching to trap this early intermediate and characterize its electronic structures with EPR and X-ray spectroscopies (Figure 1b).

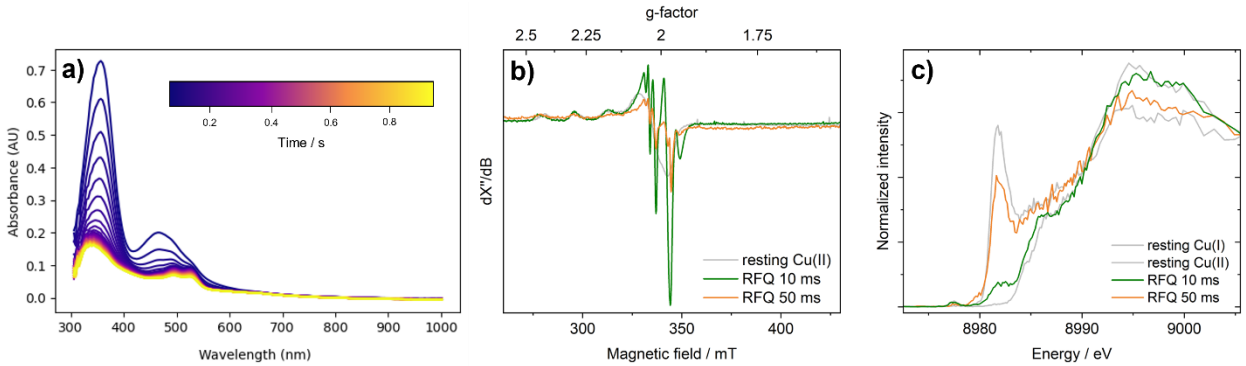

Figure 1. Early stages of the *Nc*AA9C peroxidase reaction studied after reaction with H_2O_2. Stopped-flow UV-Vis (a) in solution between 7 ms and 1 s; EPR (b) and high-resolution XAS (c) spectra of reference states and early time points.

[1] A. E. Batka *et al.* (2026), *Chem. Rev.*, 126, 14, 8016–8033. 10.1021/acs.chemrev.6c00006
[2] B. Bissaro *et al.* (2020), PNAS, 117 (3), 1504-1513. 10.1073/pnas.1904889117

S1.4. Remote but Ruling: LPMO Reactivity Under the Control of Second Sphere Residues

Nathan Montmailler[1], Sergio A. V. Jannuzzi[2,†] Synnøve E. Rønnekleiv[1], Tom Z. Emrich-Mills[1], Gustavo A. Santos[1], Morten Sørlie[1], Serena DeBeer[2], Åsmund K. Røhr[1], Vincent G. H. Eijsink[1].

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Keywords: LPMO, second sphere, peroxidase, inactivation, inhibition, spectroscopy

Lytic polysaccharide monooxygenases (LPMOs) are mono-copper enzymes that activate the strong C-H bonds of recalcitrant polysaccharides using powerful radical chemistry. These enzymes are able to perform valuable reactions at high rates and under mild conditions. This remarkable catalytic performance justifies their increasing use in industry and as blueprints for designing effective synthetic catalysts. All LPMOs bind their copper in a histidine brace, yet spectroscopy reveals strikingly diverse coordination environments which are visible by eye as a span of the colors of concentrated enzyme solutions. This variation affects copper reactivity and interactions with inhibitors. In two complementary studies we have investigated a range of wild-type and mutant LPMOs from three families, asking what controls this spectral and functional variation, and what its chemical handles would look like. Combining reaction kinetics, and X-band EPR spectroscopy, supported by DFT, we tracked a direct link from the second sphere to reactivity, which can be exploited in the design of catalysts for C-H bond activation.

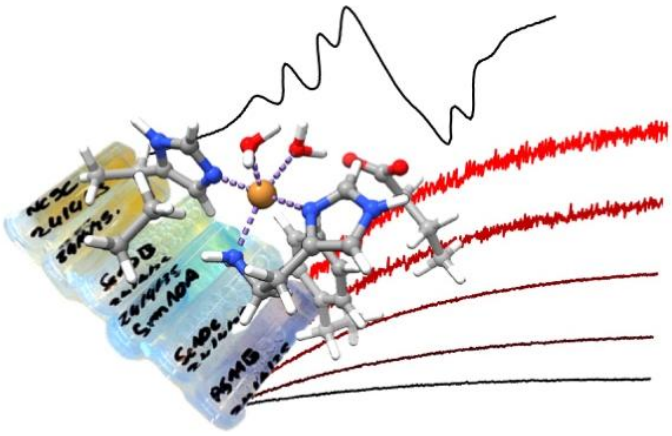

Figure 1. Illustration of the question asked in this study: How are reaction rates, spectroscopic features and the extended structures of LPMO active sites connected?

S1.5. Insights into how LPMOs protect themselves from self-destruction: hole hopping routes and histidine methylation

Tom Z Emrich-Mills[1], Iván Ayuso-Fernández[1], Rannei Skaali[1], Zarah Forsberg[1], Gustavo Santos[1], Lukas Rieder[2], Morten Sørlie[1], Åsmund K Røhr[1], Vincent GH Eijsink[1]

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Keywords: Oxidative damage, hole hopping, histidine methylation

Oxidative cleavage of glycosidic bonds by LPMOs entails the generation of powerful oxidizing species that can also damage the enzyme. Recent studies have revealed mechanisms that LPMOs use to minimise damage. LPMOs contain hole hopping routes consisting of chains of aromatic residues that help to dissipate damaging radicals away from the catalytic centre. Using ancestral sequence reconstruction and enzyme resurrection, we have recently discovered that oxidative stability mediated by hole hopping routes has been a major driver of LPMO evolution [1]. Follow-up studies have shown that hole hopping is a general phenomenon in LPMOs, but with variation between the enzymes. Many fungal LPMOs carry a methylation of the catalytic N-terminal histidine that may affect oxidative stability. In an ongoing study, we have compared variants of *Nc*AA9C with no detectable differences except for His1-methylation. We find that methylation raises the number of turnovers before inactivation in the absence and, even more so, in the presence of cellulose substrate. EPR, stopped flow spectroscopy and real-time activity measurements using an electrochemical H_2O_2 sensor [2] indicate that methylation has no effect on the redox properties of the copper and its reaction with co-substrate. Instead, methylation likely acts after formation of the Cu-oxyl intermediate, raising the energy barrier for damaging reactions (Fig. 1). Together, these studies suggest that methylation and hole hopping may act in concert to protect LPMOs from their own oxidative power.

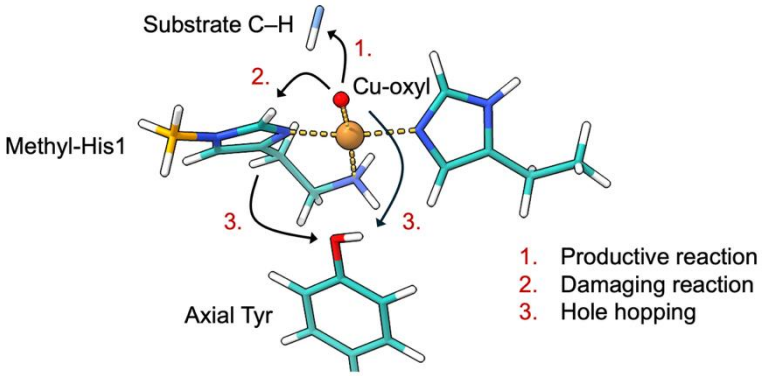

Figure 1. Hypothetical fates of a Cu-oxyl intermediate in His1-methlyated *Nc*AA9C.

[1] Ayuso-Fernández I, Emrich-Mills T, et al. (2026) Redox robustness drives LPMO evolution. *PNAS*, 123(3): e2521617123. DOI: 10.1073/pnas.2521617123
[2] Schwaiger L, et al. (2024) Electrochemical monitoring of heterogenous peroxygenase reactions unravel LPMO kinetics. *ACS Catal.*, 14(2): 1205-1219. DOI: 10.1021/acscatal.3c05194

SESSION 2

Biotechnological applications and polysaccharide modification

S2.1. Advancing the use of LPMOs in biomass valorisation: leveraging redox control and substrate specificity

Anikó Várnai[1], Heidi Østby[1], Camilla F. Angeltveit[1], Fredrik G. Støpamo[1], Zarah Forsberg[1], Peter Kidibule[1], Svein J. Horn[1], Vincent G. H. Eijsink[1]

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Keywords: biomass valorisation, controlled processing, substrate specificity

The discovery of lytic polysaccharide monooxygenases (LPMOs) has transformed the enzymatic processing of recalcitrant insoluble polysaccharides such as cellulose and chitin, with applications ranging from total saccharification [1,2] and production of oligosaccharides [3], to fibre engineering [4]. A better understanding of the H_2O_2-dependent peroxygenase reaction mechanism, and the possibility of dosing liquid H_2O_2 rather than relying on aeration with air or O_2, has made it possible to control the extent of oxidation in clean feedstocks. Achieving the same control on complex substrates, including plant biomass containing lignin and fungal or insect biomass containing other redox-active compounds, remains a challenge.

Furthermore, plant and fungal cell walls are co-polymeric structures in which access to cellulose and chitin is restricted by other polysaccharides. Nature has evolved two main strategies to overcome this: interplay between LPMOs and hemicellulolytic CAZymes [5], and gene multiplicity, with LPMO repertoires that act on structural elements beyond cellulose and chitin microfibrils. Targeted hemicelluloses include xylan, which forms a protective sheath around cellulose microfibrils in plant cell walls [6], and β-glucans, which are interwoven with and overlay the chitin scaffold in fungal walls.

Here, we discuss recent advances and perspectives on gaining full control over LPMO-driven biomass processing, focusing on two key challenges: redox control, with its impact on operational stability, and substrate specificity.

References

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[2] Costa THF et al. (2020) Demonstration-scale enzymatic saccharification of sulfite-pulped spruce with addition of hydrogen peroxide for LPMO activation. *Biofuels Bioprod Bioref*, 14(4): 734-745. DOI: 10.1002/bbb.2103

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[4] Marjamaa K et al. (2025) LPMO-catalyzed oxidation of cellulosic fibers with controlled addition of a reductant and H_2O_2. *ACS Sustain Chem Eng*, 13(1): 220-231. DOI: 10.1021/acssuschemeng.4c06802

[5] Forsberg Z et al. (2025) A modular enzyme with combined hemicellulose-removing and LPMO activity increases cellulose accessibility in softwood. *FEBS J*, 292: 75-93. DOI: 10.1111/febs.17250

[6] Deralia PK et al. (2026) Preserving native cellulose–xylan architecture enables structure–property control in holocellulose nanofibrils and high-performance sustainable materials. *bioRxiv* 2026.2006.2007.730583. DOI: 10.64898/2026.06.07.730583

S2.2. AA17 lytic polysaccharide monooxygenases: oxidative cleavage of methyl-esterified homogalacturonan

Peicheng Sun[1,2], Florian Csarman[3], Mao Peng[2], Astrid Müller[2], Nan Zhang[1], Henk. A. Schols[1], Willem J.H. van Berkel[1], Ronald P. de Vries[2], Roland Ludwig[3], Mirjam A. Kabel[1]

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Keywords: pectin, methyl esterification, LPMO, substrate specificity, Oomycetes

The oomycete AA17 family, suggested to play a critical role in plant-pathogen interactions, has so far characterized *Phytophthora infestans* AA17 (*Pi*AA17) LPMOs showing pectic homogalacturonan (HG) degradation. Here we show that beyond HG, randomly distributed methyl-esterified HG is a better substrate for these *Pi*AA17 LPMOs, in line with methylated HG present in living plant cell walls [1]. In addition, we established the phylogenetic distribution of the AA17 family, which diverged into six clades [1]. We demonstrated that *Pi*AA17 LPMOs generated C4-oxidized and decarboxylated C4-oxidized oligogalacturonides, by subjecting digests to advanced liquid chromatographic and mass spectrometric analysis. Product characterization suggested that *Pi*AA17C preferably cleaved HG between two non-methylated GalA residues, followed by cleavages involving one GalA and one MeGalA residue. Oxidative cleavage between two MeGalA residues was the least preferred [1]. Non-methylated oligogalacturonide DP6 was the shortest substrate cleaved by *Pi*AA17C. We furthermore explored the catalytic performance of two *Pi*AA17 LPMOs using real-time monitoring of H_2O_2 consumption, and by applying various pectic substrates [2]. Overall, different affinities, operational stabilities, and catalytic efficiencies were observed for the AA17 LPMOs for different pectic substrates. Both PiAA17s showed a clear preference for a moderate, and random methyl-esterified HG [2]. Of interest was that these AA17 LPMOs revealed not yet discovered C1-oxidative activity on β-(1→4)-galactan side chains of rhamnogalacturonan-I, contrasting their known C4-oxidation of (methylated) HG. Nevertheless, besides high turnover rates on galactan (kcat ≈ 11.8 and 7.9 s-1, respectively), rather high Km values (≈ 4.8 and 5.0 mg mL^{-1}, respectively) were found, and a low operational stability, hence (methylated) HG is seen as better substrate [2].

[1] Sun P, Peng M, Csarman F, Müller A, Zhang N, van Berkel WJH, Ludwig R, de Vries RP, Kabel MA (2025) Oxidative cleavage of methyl-esterified pectic homogalacturonan by an AA17 lytic polysaccharide monooxygenase from *Phytophthora infestans*. *Carbohydr. Polym.*, 368: 124112
[2] Sun P, Csarman F, Peng M, Schols HA, van Berkel WJH, de Vries RP, Ludwig R, Kabel MA (submitted) Distinct catalytic performance of AA17 Lytic Polysaccharide Monooxygenases toward pectic galactan and methyl-esterified homogalacturonan.

S2.3. Characterizing the oxidative repertoire of the model green alga *Chlamydomonas reinhardtii*

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Keywords: LPMO, multimodular, hydroxyproline-rich glycoprotein, recalcitrance

The discovery of the AA15 lytic polysaccharide monooxygenase (LPMO) family revealed the broad presence of these unique oxidative enzymes across the tree of life, including in microalgae. Despite this, efforts to characterize algal LPMOs remain limited. To address this gap, *Chlamydomonas reinhardtii* serves as an ideal model organism: it possesses a fully sequenced genome, a near-saturating mutant library targeting its three AA15 LPMOs, and an established precedent for mixotrophic growth on recalcitrant biomass (Blifernez-Klassen et al.). In this work, we recombinantly expressed individual domains belonging to the multimodular *C. reinhardtii* LPMOs in *Pichia pastoris*. Signal peptide engineering enabled the recovery of functional LPMO for two of the three targets. Biochemical characterization confirmed that all three enzymes are stabilized by Cu(II), with CrAA15b and CrAA15c exhibiting oxidative activity toward cellulose and chitin, respectively. Because *Chlamydomonas* lacks a cellulosic or chitinaceous cell wall—relying instead on a hydroxyproline-rich glycoprotein wall—the physiological role of these enzymes has remained obscure. We show that the predominant activity of CrAA15c is the generation of chitobiose and chitobionic acid, and we provide *in vivo* evidence demonstrating that *Chlamydomonas* utilizes chitobiose to support mixotrophic growth.

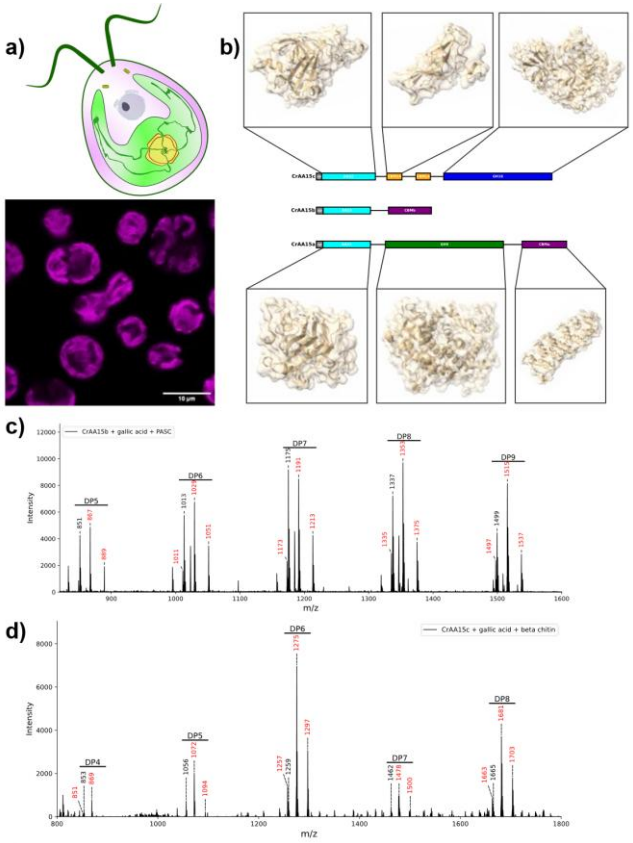

Figure 1. (A) Diagram of *Chlamydomonas reinhardtii* (top) and confocal microscopy images of *Chlamydomonas* with chlorophyll autofluorescence. (B) Schematic diagram of *Chlamydomonas* LPMO enzyme modular architectures, with AlphaFold3 models of respective domains indicated. (C) MALDI-TOF MS plot demonstrating CrAA15b activity on cellulosic substrates. (D) MALDI-TOF MS plot demonstrating CrAA15c activity toward β-chitin from squid pen.

S2.4. Integrating Electrochemistry into the LPMO Enzyme Discovery Toolkit

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Keywords: Enzyme Discovery, Electrochemistry, Assays

Lytic polysaccharide monooxygenases (LPMOs) are a widespread family of copper metalloenzymes that play an essential role in the industrial production of bioethanol from agricultural waste. The cleavage of polysaccharides via oxidation, catalysed by a resident copper cofactor coordinated by the LPMOs histidine brace, is key to biological and industrial utilisation of recalcitrant biological waste feedstocks. Despite the keen industrial and academic interest in these enzymes rapid screening of putative LPMOs still remains a challenge.

Protein film electrochemistry presents several clear advantages in this area, allowing for specific quantitative analysis of metalloenzymes in a crude form that would otherwise be incompatible with solution-based dye assays. Adsorption of LPMOs to a pyrolytic graphite edge rotating disk electrode, as previously described in the Parkin group, allows for kinetic and thermodynamic characterisation that is relatively insensitive to most biological contaminants [1]. The inclusion of chronoamperometric hydrogen peroxide reduction assays allows for fast quantitative data to be collected, with minimal requirements for concentration or purity, allowing for initial comparison of reaction kinetics between different LPMOs. Complimentary to this is the use of qualitative matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) based substrate assays, to fingerprint the substrate specificity of these enzymes. This combined screening approach allows more rapid selection of promising candidates for further study and potential industrial application. We demonstrate this by identifying and characterizing LPMOs from an industrial partners metagenome library, from which we identified novel LPMOs including interesting candidates for more detailed examination.

[1] Reid E., Miles C., Lloyd-Laney H., Nairn A., Branch J., Garland N., Yates N., Ascham A., Walton P., Hemsworth G. and Parkin A. (2026) Electrochemical Insight into the Copper Redox Chemistry and H2O2 and O2 Reducing Capability of Two AA10 Lytic Polysaccharide Monooxygenases. *ACS Electrochemistry*, 2: 239-257. DOI: 10.1021/acselectrochem.5c00266

SESSION 3

Theoretical and computational insights into LPMO catalysis

S3.1. Computational exploration of LPMO polysaccharide oxidation: pitfalls and conclusions so far

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Keywords: LPMO Mechanism, QM/MM, DFT

Although it is now 15 years since the discovery of lytic polysaccharide monooxygenases, a complete picture of their substrate oxidation mechanism has remained elusive. In particular, the energetics of the actual oxidation event have been predicted rather differently (see e.g. ref. [1]). With outset in recent investigations from my group, I will demonstrate which intermediates are most important for the substrate oxidation mechanism – and how a correct setup of the employed QM/MM methods is critical.
First, I will show why sufficiently large QM regions are required to obtain the result that only H_2O_2 is a viable co-substrate for the *Ls*AA9 LPMO [2] – as indeed observed experimentally. Next, I will show how molecular dynamics simulations [3] are essential to identify the relevant conformer of the Cu(II)-oxyl species that oxidizes the polysaccharide. This explains the large variation in activation energies calculated over the years [1]. In fact, we show the barrier for the key oxidation is rather low, suggesting that the rate is not determined by substrate oxidation, but rather is controlled by another step in the mechanism. Finally, I will present a recent investigation elucidating how a rare mutation of a second-sphere glutamine does not alter the core mechanism. [4]

[1] M. M. Hagemann, E. D. Hedegård (2023) Molecular mechanism of substrate oxidation in lytic polysaccharide monooxygenases: insight from theoretical investigations. *Chem. Eur. J.*, 29(7):e202202379.
DOI: 10.1002/chem.202202379

[2] M. M. Hagemann, E. K. Wieduwilt, U. Ryde, E. D. Hedegård (2024) Investigating the Substrate Oxidation Mechanism in Lytic Polysaccharide Monooxygenase: H_2O_2- versus O_2-Activation. *Inorg. Chem.*, 63(46), 21929–21940.
DOI: 10.1021/acs.inorgchem.4c03221

[3] M. M. Hagemann, U. Ryde, E. D. Hedegård (2025), How structural dynamics influence the substrate oxidation energetics in lytic polysaccharide monooxygenases. *Inorg. Chem. Front.* 12(24), 8352–8362.
DOI: 10.1039/d5qi01590a

[4] Huang, E. K. Wieduwilt, Hedegård, L. Lo Leggio *et al.* manuscript in preparation

S3.2. Unravelling the LPMO reaction mechanism using QM MM simulations

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Keywords: QM MM simulations, periodic cellulose fibril, H_2O_2-driven catalysis

Most hybrid quantum mechanical / molecular mechanical (QM/MM) studies of LPMO catalysis represent cellulose using short oligomers of one to three glucosyl units, which cannot capture the collective dynamics or crystalline constraints of an extended fibril. To address this limitation, we developed a fully periodic QM/MM model of LPMO9c bound to an effectively infinite cellulose fibril. We employ this model to reproduce and critically evaluate the H_2O_2-driven mechanism proposed by Ryde and co-workers [1] at an explicit periodic cellulose-water interface. The pathway is followed from H_2O_2 binding and O-O bond cleavage through formation of the reactive copper-oxygen species, hydrogen-atom abstraction, hydroxyl rebound, and glycosidic-bond cleavage (Figure 1; adapted from [1]). The QM region comprises the copper histidine brace, H_2O_2-derived intermediates, key second-sphere residues, and the reacting glucose residues, while the remaining protein, solvent, and cellulose fibril are treated classically. Comparison with reduced oligomer models will assess how the extended fibril affects reaction structures and energetics, providing a computational test of the experimentally supported H_2O_2 mechanism [1,2].

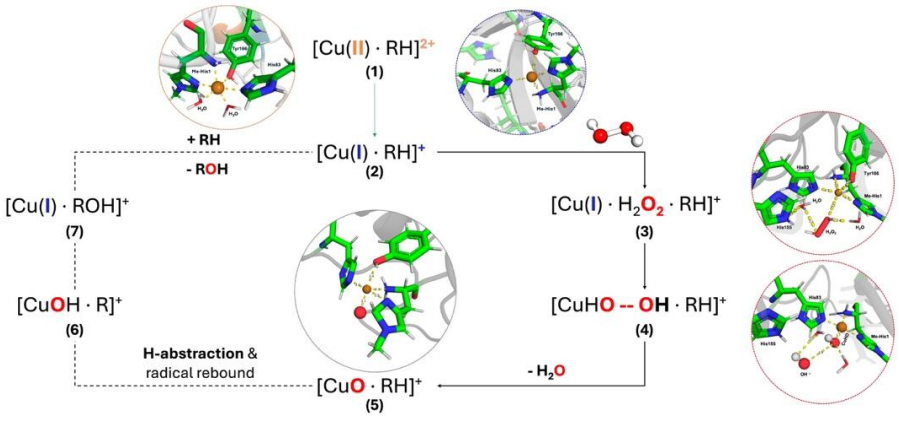

Figure 1. H_2O_2-driven reaction mechanism investigated using the periodic LPMO9c–cellulose QM/MM model. Adapted from [1].

[1] Hagemann MM, Wieduwilt EK, Ryde U, and Hedegård ED (2024) Investigating the substrate oxidation mechanism in lytic polysaccharide monooxygenase: H_2O_2- versus O_2-activation. Inorg. Chem., 63: 21929–21940. DOI: 10.1021/acs.inorgchem.4c03221
[2] Bissaro B, Røhr ÅK, Müller G, Chylenski P, Skaugen M, Forsberg Z, Horn SJ, Vaaje-Kolstad G, and Eijsink VGH (2017) Oxidative cleavage of polysaccharides by monocopper enzymes depends on H_2O_2. Nat. Chem. Biol., 13(10): 1123–1128. DOI: 10.1038/nchembio.2470

S3.3. Computational approaches to characterize LPMOs: from family-wide overview to active site details.

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Keywords: structural bioinformatics, multiscale modelling, protein-surface interactions

LPMOs are diverse in substrate specificity, oxidative regioselectivity and stability, yet the origins of these differences are not fully understood. They may arise from individual structural features, the way the enzyme binds to the polysaccharide surface, or subtle differences in the active-site vicinity. Here we present various computational approaches that address each of these aspects.
First, to compare structural features across the family, we developed LPMOcat (https://lpmo-cat.polsl.pl), a web resource of 160 characterised LPMOs with uniform annotation of features such as substrate-binding regions and second-sphere residues, pre-computed properties including hydrophobicity and flexibility, and predicted hole-hopping pathways.
Next, to probe substrate recognition, we built a Brownian dynamics pipeline involving generation of encounter complexes between LPMOs and polysaccharide surfaces and the subsequent analysis with Prot2Surf [1]. By quantifying protein–surface encounters by combining distance- and angle-based clustering as well as a residue-level contact analysis, Prot2Surf rapidly distinguishes productive binding modes from non-productive, thus it enables the fast prediction and characterization of protein association on surfaces.
Finally, to investigate differences in the active-site vicinity, we combined MD simulations, QM/MM optimization and EPR parameter calculations for two LPMOs, NcAA9C and BaAA10A [2]. The response to the change in copper oxidation state is carried almost entirely by the exogenous ligands rather than the histidine brace and the computed EPR parameters showed that the position of the equatorial water, not the axial residue, distinguishes the two enzymes.

This work was supported by the NewCat project under the European Commission EIC Pathfinder program (Grant No. 101046815) and NAWA STER Program, within the framework of the NIH4DS - New International Horizons for Doctoral School project. This work was realized with the collaboration of Silesian University of Technology and Otto-von-Guericke University Magdeburg under the Cotutelle Agreement. We gratefully acknowledge Polish high-performance computing infrastructure PLGrid (HPC Centers: ACK Cyfronet AGH) for providing computer facilities and support within computational Grant Nos. PLG/2022/015696, PLG/2024/017171, and PLG/2025/018297.

[1] Muñiz-Chicharro A, Tanriver G, Góra A (2026) Prot2Surf: fast analysis of protein–surface binding modes. *bioRxiv* https://doi.org/10.64898/2026.08.26.747352
[2] Szleper-Shendy K, Escorcia AM, Kumar R, Góra A, Stein M (2026) Multiscale Modeling of Lytic Polysaccharide Monooxygenases in Oxidized and Reduced Redox States. *J. Phys. Chem. B* https://doi.org/10.1021/acs.jpcb.6c04364

SESSION 4

Structural insights into LPMOs

S4.1. Not just for biomass: structure and function of the *Cryptococcus neoformans* virulence factor Cel1, a lytic polysaccharide monooxygenase

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Keywords: fungal meningitis, saccharide binding, virulence

In recent years, LPMOs and other LPMO-like proteins sharing the His-brace motif have emerged as important proteins in human pathogens, besides their applications in bioconversion. Cryptococcus neoformans (Cn) is the causative agent of fungal meningitis, a severe and deadly disease especially for immunocompromised patients. Several genes known or likely to encode LPMO and LPMO-like proteins are present in the cryptococcal genome, including the LPMO-like Bim1/Cbi1 [1], and members of AA9, AA11 and AA14 LPMO families. The CNAG_00601 (or CEL1) gene product (hereon CnAA9) possesses an AA9 catalytic domain and an intrinsically disordered C-terminus, also characteristic of some LPMOs [2]. CnAA9 is now established as a cryptococcal virulence factor, based on evidence including transcriptomics [3] and a murine inhalational model [4], but the exact activity has been elusive.
Here we combine evidence coming from experimental structure determination of the catalytic domain, showing a remarkably extensive aromatic surface able to bind glycans and saccharides in the crystal, with functional characterization including solid state NMR analysis of cryptococcal cell wall. The emerging view, supported by various pieces of evidence, is that CnAA9 is essential in remodeling the cryptococcal cell wall during infection, most likely by targeting the chitosan within it. The talk will highlight the challenges (as well as successes) in characterizing LPMO action in complex systems, both structurally and functionally.

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[1] Garcia-Santamarina et al (2020) A lytic polysaccharide monooxygenase-like protein functions in fungal copper import and meningitis *Nature Chemical Biology* 16, 337-344.
[2] Tamburrini, K. C. et al (2021) Bioinformatic Analysis of Lytic Polysaccharide Monooxygenases Reveals the Pan-Families Occurrence of Intrinsically Disordered C-Terminal Extensions *Biomolecules* 11.
[3] Chen et al (2014) The *Cryptococcus neoformans* transcriptome at the site of human meningitis. *mBio* 5, e01087
[4] Probst et al (2023) A fungal lytic polysaccharide monooxygenase is required for cell wall integrity, thermotolerance, and virulence of the fungal human pathogen *Cryptococcus neoformans PLoS Pathog* 19, e1010946.

S4.2. Structure-Guided Deep Learning Reveals Five Novel LPMO-like Families and the First Cellulose-Active LPMO in Algae

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Keywords: Structure guided deep learning, Algae carbohydrate metabolism

LPMOs play diverse biological roles in development, pathogen-host interactions and carbohydrate metabolism. However, extreme sequence divergence conceals remote homologs in genomic databases, leaving vast regions of oxidative carbohydrate biology hidden. To uncover this diversity and bypass sequence-based limitations, we developed CONFERS (Conserved Feature Encoding for Recognition of Structures), a structure-guided deep learning framework that identifies LPMO-like proteins from conserved 3D catalytic and structural features rather than sequence similarity. Applying CONFERS across predicted algal structural proteomes revealed five previously unrecognised LPMO-like families (A to E) spanning diverse algal lineages and displaying striking variation in substrate-binding surfaces and domain architectures. Experimental characterisation confirmed that these predictions extend beyond structural similarity. A family A representative from the bloom-forming haptophyte *Emiliania huxleyi* is a copper-dependent, cellulose-active LPMO and the first biochemically characterised LPMO from algae. By contrast, a family E member from *Nannochloropsis oceanica* retains copper-dependent oxidase and peroxidase chemistry but lacks detectable activity on canonical LPMO substrates, revealing substantial functional diversification. Together, these findings uncover an unexpectedly rich landscape of oxidative carbohydrate-active enzymes in algae and demonstrate how structure-guided deep learning can illuminate the functional "dark matter" that remains invisible to sequence-based annotation.

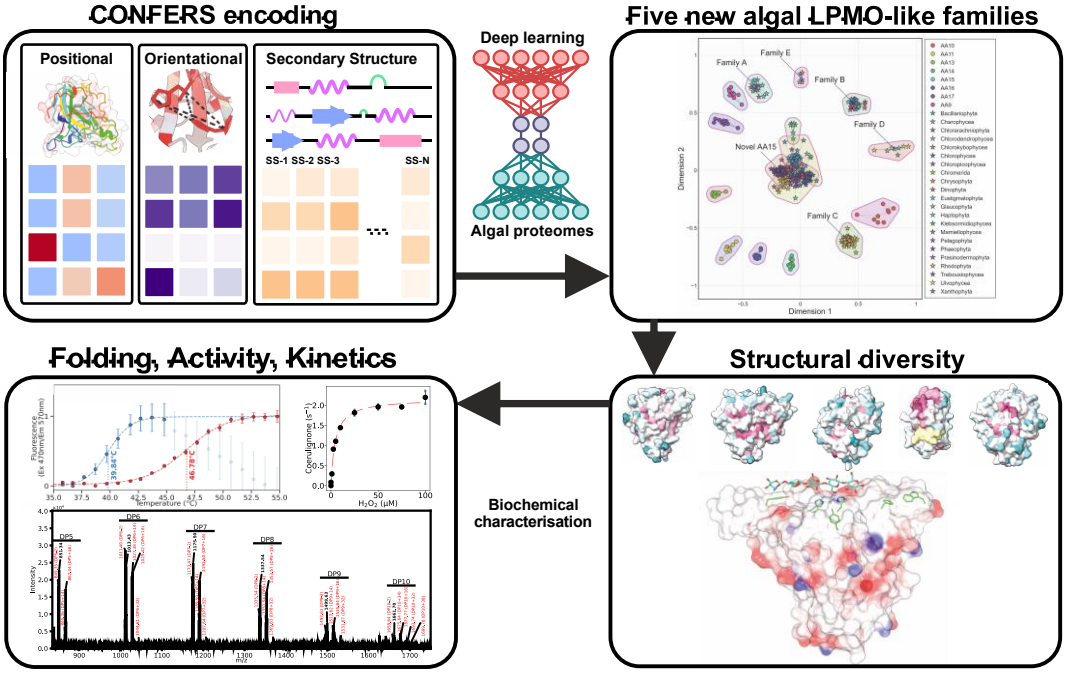

Figure 1. CONFERS discovery pipeline.

S4.3. Exploring the structural mechanism of the Lytic Polysaccharide Monooxygenase reaction: reaction priming and substrate binding

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Keywords: *Ls*AA9A, substrate binding, copper reduction

Lytic polysaccharide monooxygenases (LPMOs) are enzymes that oxidatively cleave glycosidic bonds in recalcitrant polysaccharides deploying a simple but unique copper-centered geometry [1-3]. Catalysis requires reduction of the active-site copper from the resting Cu(II) state to Cu(I) state ("priming reduction"), and although biochemical and spectroscopic studies [4,5] suggest that several LPMOs bind saccharides with higher affinity once reduced, direct structural evidence has been missing. Focusing on a structure-centered approach in exploring mechanistic aspects, we employ advanced X-ray crystallography on a model AA9 from *Lentinus similis* (*Ls*AA9A) [6] and report the first *in crystallo* visualization of Cu(I)-state-dependent binding of cellotriose(Cell3) to *Ls*AA9A at room temperature. Copper reduction was triggered either progressively, via X-ray-induced photoreduction during dose-resolved, multi-crystal data collection, or directly, by chemical reduction of *Ls*AA9A crystals with ascorbate. Structural changes upon progressive Cell3 binding to *Ls*AA9A as Cu(II) is reduced to Cu(I) will be presented and discussed. These results provide the first direct structural link between copper redox state and saccharide affinity in an LPMO and establish room-temperature, dose-resolved crystallography as a route to further capture intermediates along the catalytic cycle.

[1] Quinlan RJ, et al. (2011) Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. *Proc. Natl. Acad. Sci. USA*, 108(37): 15079-15084.https://doi.org/10.1073/pnas.1105776108
[2] Aachmann FL, et al. (2012) NMR structure of a lytic polysaccharide monooxygenase provides insight into copper binding, protein dynamics, and substrate interactions. *Proc. Natl. Acad. Sci. USA*, 109(46): 18779-18784.https://doi.org/10.1073/pnas.1208822109
[3] Hemsworth GR, et al. (2013) The copper active site of CBM33 polysaccharide oxygenases. *J. Am. Chem. Soc.*, 135(16): 6069-6077. https://doi.org/10.1021/ja402106e
[4] Kracher D, et al. (2018) Active-site copper reduction promotes substrate binding of fungal lytic polysaccharide monooxygenase and reduces stability. *J. Biol. Chem.*, 293(5): 1676-1687.
https://doi.org/10.1074/jbc.RA117.000109
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[6] Frandsen KEH, et al. (2016) The molecular basis of polysaccharide cleavage by lytic polysaccharide monooxygenases. *Nat. Chem. Biol.*, 12(4): 298-303.
https://doi.org/10.1038/nchembio.2029

S4.4. *De novo* designed β-hairpin peptides as LPMO mimics

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Keywords: LPMO mimics, His brace, Cu-binding peptides

Among carbohydrate-active enzymes, Lytic Polysaccharide MonoOxygenases (LPMOs) stand out for their unique active site and their selective oxidative activity on glycosidic bonds in polysaccharides.

The active site of LPMOs contains a copper ion coordinated in a 3N T-shaped site by the so-called histidine brace (His brace) motif, which includes the bidentate N-terminal His, bound *via* both amine and imidazole groups, and the side chain of another His. The development of LPMO mimics could be beneficial to understand the catalytic mechanism, modulate substrate specificity and allow a controlled catalyst immobilisation.

Hence, we designed *de novo* peptides folding into β-hairpins and binding Cu in an LPMO-like His-brace motif. ATR-FTIR and CD spectroscopy showed that the peptides secondary structure is dominated by antiparallel β-strands and turns, commensurate with the formation of β-hairpins. EPR spectroscopy revealed the presence of a single axial Cu^{II} species with parameters comparable to those of LPMOs. Lastly, catalytic assays using p-nitrophenyl-β-D-glucopyranoside as a model substrate demonstrated that the Cu-hairpins can perform LPMO-like activity. Furthermore, the Cu-hairpins are able to perform light-driven oxidation of PASC in the presence of melanin, similarly to some LPMOs, an activity that is unreported for any LPMO mimic so far. This work is the first example of a β-hairpin LPMO-mimic, and these data will be presented highlighting their implication in LPMO-mimic development.[1]

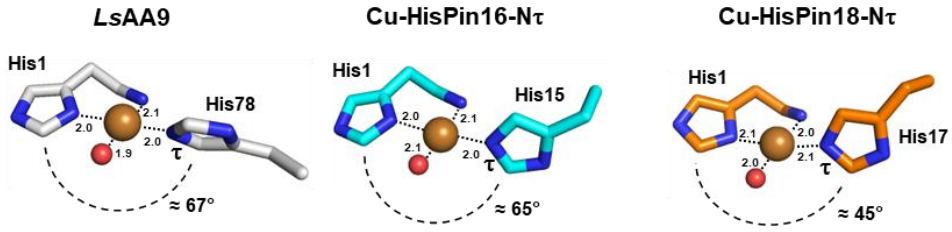

Figure 1. Structure and key metric parameters for Cu-binding sites in *Ls*AA9 (7PYL), Cu-HisPin16 and Cu-HisPin18

[1] Falcone E., Tomey R., Turley E., Robinson D., Cannella D., and Ciano L. (2025) *De novo* designed β-hairpins mimicking the copper-binding histidine brace motif of lytic polysaccharide monooxygenases. *Angew. Chem. Int. Ed*., e202513990. DOI: 10.1002/anie.202513990

SESSION 5

LPMO Architecture: Modularity, CBMs and non-catalytic domains

S5.1. Insight into the function of disordered C-terminal regions in LPMOs

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The structure–function paradigm has long guided the biochemical characterization of enzymes, with protein function generally linked to a well-defined three-dimensional structure. However, many proteins contain intrinsically disordered regions that lack a stable 3D structure and can fulfil important biological functions. We recently revealed the widespread occurrence of intrinsically disordered C-terminal regions (C-tails) in fungal lytic polysaccharide monooxygenases (LPMOs) from different families [1], raising the question of their functional significance. In this presentation, I will discuss our recent efforts to decipher the biochemical and biological functions of these non-canonical regions in fungal saprotrophs and phytopathogens [2,3]. Using *in vitro* and *in vivo* approaches, we have investigated the properties of fungal LPMO C-tails and revealed that they are not merely passive extensions of the catalytic domain, but can either influence the properties and behaviour of LPMOs or have functions of their own [2,3].

[1] Tamburrini KC, Terrapon N, Lombard V, Bissaro B, Longhi S, Berrin JG (2021) Bioinformatic Analysis of Lytic Polysaccharide Monooxygenases Reveals the Pan-Families Occurrence of Intrinsically Disordered C-Terminal Extensions. *Biomolecules*, 11(11):1632. doi:10.3390/biom11111632.
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S5.2. Copper beyond the active site: discovery of metal-binding domains in LPMOs

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Keywords: Non-catalytic copper sites, redox regulation, copper homeostasis

Recent discoveries indicate that some modular LPMOs possess additional copper-binding sites beyond the catalytic histidine brace, suggesting a previously unrecognized layer of regulation in LPMO biology. We recently identified a copper-binding subgroup of CBM2 domains that is exclusively associated with cellulose-active LPMOs and demonstrated that the auxiliary copper site in these domains protects the enzyme from oxidative inactivation [1]. Building on these findings, we have now identified an auxiliary copper-binding site in a distinct LPMO-associated domain from intestinal pathogens [2]. These enzymes operate in host-associated environments where copper availability is shaped by nutritional immunity, microbial competition, and copper-mediated antimicrobial stress. Structural, biochemical, and copper-binding studies reveal striking similarities between the various LPMO-appended copper-binding modules despite their distinct architectures and biological contexts (Figure 1), pointing to shared principles for copper acquisition and sequestration, as well as regulation of LPMO activity. Together, these findings suggest that copper-binding domains beyond the catalytic center constitute an important functional feature of modular LPMOs, expanding the roles of copper in these enzymes from catalysis to homeostasis, protection, and environmental adaptation.

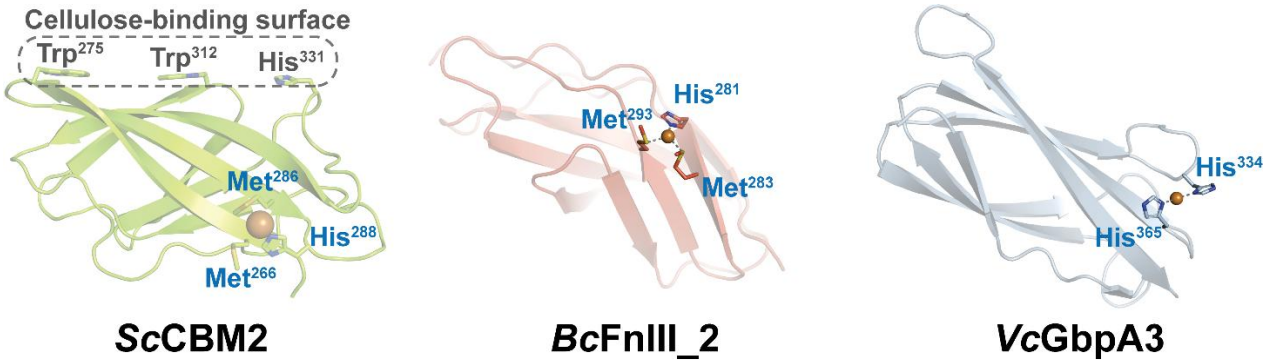

Figure 1. Copper sites in *S. coelicolor* CBM2 (*Sc*LPMO10C), *B. cereus* FnIII_2 (*Bc*LPMO10A) and *V. cholerae* GbpA3 (*Vc*GbpA).

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SESSION 6

LPMOs in plant–pathogen interactions and plant cell wall degradation

S6.1 Exploring the role of AA10 LPMOs in fungal plant pathogens

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Keywords: Fungal Cell Wall, Pathogenesis, CAZymes, Oxidases, Disordered proteins

Lytic polysaccharide monooxygenases (LPMOs) are monocopper enzymes found across all kingdoms of life. LPMOs oxidatively degrade major polysaccharides, such as cellulose and chitin. Although there is now a broad corpus of data on the *in vitro* biochemistry of LPMOs, their *in vivo* mechanism of action and biological roles are still largely unexplored [1, 2]. Here, we investigated the biological role of *Um*AA10 [3], the unique and chitin-active LPMO of the corn smut fungus *Ustilago maydis*, which analysis of publicly available transcriptomic data identified as highly up-regulated during late stages of maize infection. Using *in planta* infection assays, we showed that mutants overexpressing *Um*AA10 are hyper-virulent. We also employed *in vitro* assays to show that this phenotypic trait is associated with strongly enhanced resistance to osmotic and cell wall stresses, and is accompanied by alterations in the composition of the fungal cell wall. Using a combination of biophysical methods, we demonstrate that *Um*AA10 is anchored to fungal cell wall branched β-glucans through an intrinsically disordered C-terminal tail, unveiling a new type of carbohydrate-protein interaction that we hypothesize contributes to the correct cellular localization of the enzyme. Phylogenetic analyses revealed that this protein has probably been acquired from bacteria by horizontal gene transfer and that such an event has occurred multiple times in the fungal kingdom. We anticipate that the function of the *Um*AA10 LPMO might be shared with other fungi, and possibly bacteria, opening new avenues for the understanding and control of microbial cell wall properties.

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S6.2. From AA17 LPMOs to oxidative glycan editing: exploring the interplay of *Phytophthora* enzymes

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Keywords: LPMOs, oxidative enzymes, plant immunity

The oomycete genus *Phytophthora* includes some of the most destructive plant pathogens, notably *P. infestans*, responsible for potato late blight. During infection, degradation of the plant cell wall releases carbohydrate fragments that act as danger signals, triggering plant immune responses. Our research has uncovered a remarkable diversity of oxidative enzymes secreted by *Phytophthora*, raising important questions about their roles in plant cell-wall degradation and immune regulation.

We first identified AA17 LPMOs in *P. infestans*, demonstrating that they catalyse C4-oxidative cleavage of pectin and contribute to pathogen virulence [1]. Our recent collaborative biochemical and structural studies have revealed additional AA17 subfamilies capable of C4-oxidative cleavage of cellulose and xylan, substantially expanding the known substrate range of these enzymes. In parallel, we discovered a family of secreted *Phytophthora* berberine bridge enzyme-like (BBE) oxidases that C1-oxidise pectin-derived oligogalacturonides, suppressing their ability to trigger plant immunity [2]. We have now identified *Phytophthora* BBEs that extend this chemistry to cello- and xylo-oligosaccharides.

Together, our findings reveal an unexpectedly versatile repertoire of oxidative enzymes in *Phytophthora* species, targeting pectin, cellulose, xylan and their derived oligosaccharides through distinct C4 and C1 chemistries. The breadth of these activities, combined with the co-localisation of AA17 LPMOs and BBEs at the host-pathogen interface, suggests that these enzymes may act in concert to reshape cell-wall-derived danger signals, suppress host immunity and promote infection.

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S6.3. Not all LPMOs are made equal: secretome adaptation to scavenge ROS in *N. crassa* grown under photoredox stress.

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Keywords: LPMO, *Neurospora crassa*, secretomics, ROS-stress resistance.

For a decade after their discovery, LPMOs were predominantly used as drop-in enzymes in cellulolytic cocktails to improve biomass degradation at industrial scale. However, in recent years, the community has started to investigate their biological functions, including their involvement in pathogenic lifestyles and their interaction dynamics with the environment.

In nature, LPMOs are not only present in organisms having very different lifestyles and tropisms, but they are also present in multiple copies within the same genome, legitimizing speculations about their possible contribution to diverse physiological mechanisms rather than merely supplying carbon sources. Owing to the unique multiple activities within the same enzymatic pocket, their ability to use H_2O_2 as a co-substrate may indicate a possible role in coupling polysaccharide oxidation with ROS scavenging *in-vivo*, particularly in environments where organisms struggle with high redox stress.

To address this, we investigated *Neurospora crassa*, a light-responsive fungus grown in excess of photo-redox stress, a condition which lead to a steady and sublethal production of H_2O_2 in the growth medium in presence of raw bagasse or Avicel cellulose as carbon stock. Hypothesizing that the fungus prioritizes proteins more resistant to oxidation or capable of scavenging ROS, the secretomes were analyzed in MS/MS and compared to a control grown in optimal conditions. Interesting it was observed a global reprogramming with deep changes in the CBHs and LPMOs families, among the top 20 secreted enzymes. Clearly two LPMOs, both carrying a CBM domain, were over-secreted during the photo-redox stress compared to the canonical LPMOs, mostly used during growth in optimal conditions.

After heterologous expression and MS investigations, it was found that both LPMOs carried a heavier weight than predicted due to a peculiar glycosylation profile at the CBM and linker peptide domains. The truncated versions, containing only the catalytic sites showed a higher sensitivity to oxidative stress. The full enzymes, besides showing a good resistance to H_2O_2 in presence of a polysaccharide substrate, were degrading H_2O_2 much more efficiently compared to the canonical LPMO used in optimal conditions, upon investigation with ultra-sensible electrochemical sensor.

Overall, these results hint at a more protective function of these LPMOs which withstand and detoxify hydrogen peroxide while still being active on relevant substrates. They also point out the difficulty in understanding *in vivo* mechanisms by doing *in vitro* studies as well as the struggles to develop a proper way to quantify enzyme resistance to H_2O_2.

S6.4. Functional diversification of AA17 LPMOs reveals multiple polysaccharide-oxidising activities in plan-pathogenic oomycetes

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Keywords: Oomycete, LPMO, AA17, Substrate diversity

Oomycetes such as *Phytophthora* and *Saprolegnia* are major pathogens of crops and farmed fish, causing diseases including potato late blight, soybean root rot and saprolegniosis in salmonids. Penetrating the host tissues is a key step in infection. LPMOs of auxiliary activities family 17 (AA17) are part of the early enzyme cocktail secreted during host invasion by *Phytophthora infestans* and *Phytophthora sojae*, yet to date they have been characterised exclusively as polygalacturonate-active enzymes [1, 2]. To explore the remarkable sequence diversity within this enzyme family, we heterologously expressed, purified and characterised multiple AA17 candidates from diverse oomycete species, including *Phytophthora* and *Saprolegnia*, and screened them against a broad range of polysaccharide substrates. In addition to confirming polygalacturonic acid activity for several members closely related to the previously characterised enzymes, we have uncovered activities not previously reported for AA17, including on various β-glucans and xylan. Using NMR and mass spectrometry, we detected ascorbate-dependent release of degradation products compatible with C4 oxidative cleavage.

We further present a crystal structure of the *Phytophthora nicotianae* P1569 LPMO, *Pn*AA17D, in complex with xylotetraose, providing structural insight into how an AA17 LPMO engages a hemicellulosic substrate. Bioinformatic analysis of the family, combined with these newly identified substrate specificities, shows that AA17 divides into distinct clusters that correlate with structural features apparently specialised for the observed substrate preferences. Together, these findings redefine AA17 as a far more functionally diverse LPMO family than previously recognised.

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POSTER SESSION

Nr.	Presenting participant	Affiliation
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P2	Razan Daoud	University of Duisburg-Essen
P3	M Pilar Escribano	Norwegian University of Life Sciences (NMBU)
P4	Benjamin Grace	University of York
P5	Senta Heiss-Blanquet	IFP Energies nouvelles
P6	Maximilian Huemer	Wood K Plus
P7	Jesús Jiménez Ascencio	University of São Paulo (EEL-USP)
P8	Qunliang Li	Guangxi University
P9	Hedda Lilleås	Norwegian University of Life Sciences (NMBU)
P10	Maarja Lipp	Tallinn University of Technology
P11	Daniel Marke	University of Duisburg-Essen
P12	Sophia Mihalyi	BOKU University
P13	Maja Grønlund Mollatt	Norwegian University of Life Sciences (NMBU)
P14	Christian Pétré	Université Libre de Bruxelles
P15	Oliwia Rakoczy	Max Planck Institute for Chemical Energy Conversion
P16	Tonči Rezić	University of Zagreb
P17	Synnøve Elisa Rønnekleiv	Norwegian University of Life Sciences (NMBU)
P18	Ariane Jalila Simaan	CNRS Aix Marseille Université
P19	Martha Inés Vélez Mercado	Universidade São Paulo
P20	Hanna Vovk	BOKU University
P21	Meit Vyas	BOKU University
P22	Adonis Yanos	BOKU University
P23	Anikó Várnai	Norwegian University of Life Sciences (NMBU)

P1. Yeast Surface Display for Activity-Based Screening and Engineering of LPMOs

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Keywords: ultra-high-throughput screening, yeast surface display, biotin phenol, protein engineering, FACS

The efficient engineering and directed evolution of LPMOs are often hindered by the lack of scalable methods to quantify their catalytic activity, particularly given their complex interactions with heterogeneous and insoluble substrates. This analytical bottleneck limits the application of high-throughput screening techniques for the engineering of LPMOs with improved catalytic performance, stability during turnover, and altered substrate specificity [1,2].
Here, we present a modular yeast surface display platform for the engineering of LPMOs that enables the functional evaluation of catalytic domains both independently and in combination with carbohydrate-binding modules (CBMs). To directly link catalytic activity to a detectable signal, an activity-based labeling strategy using biotin-phenol as a reporter substrate was established [3,4]. Its oxidation by active LPMOs generates reactive phenoxyl radicals that covalently label nearby residues, enabling activity-dependent biotinylation of surface-displayed enzyme variants. This labeling translates catalytic turnover into a fluorescent signal detectable at the single-cell level using fluorescently labeled streptavidin conjugates. Activity-dependent labeling was validated by gel-shift assays and flow cytometry, supporting the link between LPMO activity and cell-associated fluorescence. The strategy was subsequently applied to library screenings using magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS), enabling the enrichment of variants based on activity-dependent labeling together with selection based on expression levels of the protein of interest.
By combining modular enzyme display with covalent activity labeling, this platform establishes a strategy for activity-driven screening and enrichment of LPMO variants, providing a foundation for ultra-high-throughput directed evolution. Its modular architecture also enables the evaluation of catalytic domains in combination with different CBMs, creating opportunities to investigate and engineer substrate recognition alongside catalytic performance. Ultimately, this approach aims to accelerate the development of LPMOs with improved stability, tailored substrate specificity, and enhanced activity for biopolymer modification and sustainable biomass conversion.

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P2. Reaction Mechanism of the H_2O_2-Dependent Cellulose Oxidative Cleaving Enzyme CelOCE: A Computational Investigation

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Keywords: CelOCE, QM/MM, Peroxygenase

Lytic polysaccharide monooxygenases (LPMOs) utilize copper to catalyze the oxidative cleavage of recalcitrant polysaccharides [1]. Recently, a cellulose oxidative cleaving enzyme (CelOCE) emerged as a novel, C1-regioselective copper enzyme lacking the canonical LPMOs' histidine brace [2]. While the mechanistic pathway of CelOCE remains undefined, a LPMO-like mechanism is the first that comes to mind. Employing molecular dynamics and QM/MM calculations, we here investigate the classical Cu-oxyl pathways and controlled Fenton-type homolysis to elucidate how CelOCE tames highly reactive radical species for hydrogen atom abstraction at the substrate's C1 position. The resulting catalytic cycle is unlike LPMOs', establishing a new paradigm in redox enzymology [3].

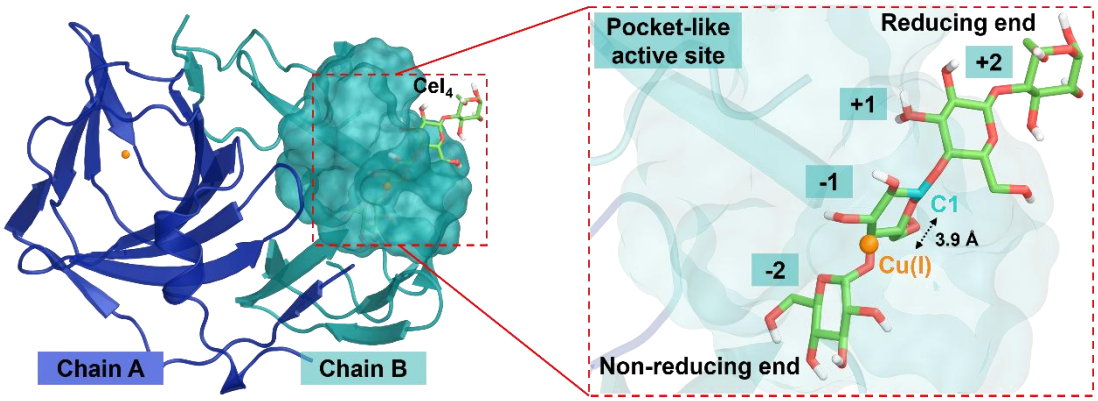

Figure 1. Homodimeric CelOCE structure highlighting the pocket-like active site and the productively accommodated cellotetraose substrate positioned for oxidative attack.

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P3. Beyond chitin degradation: Exploring the role of a multidomain AA10 LPMO in the fish pathogen *Moritella viscosa*

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Keywords: Multidomain LPMO, *Moritella viscosa*, virulence.

Moritella viscosa is a major bacterial pathogen associated with winter ulcer disease in Atlantic salmon, causing significant economic losses in aquaculture. Despite its relevance, the molecular mechanisms underlying host colonization and virulence remain poorly understood. Interestingly, the *M. viscosa* genome encodes a four-domain auxiliary activity family 10 (AA10) lytic polysaccharide monooxygenase (LPMO), designated MvLPMO10A. Although AA10 LPMOs are primarily known for their role in chitin degradation, recent studies have demonstrated their involvement in bacterial virulence, including the fish pathogen *Aliivibrio salmonicida* and the opportunistic human pathogen *Pseudomonas aeruginosa* [1,2]. Moreover, the structurally related GbpA protein from *Vibrio cholerae* has been shown to mediate bacterial adhesion to mucins and host colonization [3]. Here, we investigate the biochemical properties and potential role of MvLPMO10A in *M. viscosa* virulence. Recombinant MvLPMO10A was produced and characterized through substrate-binding and oxidative activity assays. Preliminary results revealed weak binding to α-chitin and no detectable oxidative activity towards α- or β-chitin under the conditions tested, raising questions about its physiological substrate. Furthermore, proteomic analyses of *M. viscosa* exposed to Atlantic salmon mucus revealed increased production of outer membrane vesicles (OMVs) containing putative virulence-associated proteins, including MvLPMO10A. Together with the established roles of related LPMOs in bacterial pathogenesis, these observations motivate further investigation of MvLPMO10A as a potential virulence factor and vaccine candidate. Future studies will explore its substrate specificity and interactions with host-derived components, particularly mucins, to elucidate its biological function during host–pathogen interactions.

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P4. KESTREL: A ligand-field program to probe the spectroscopy of 3d transition metals and metalloproteins in real-time

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Keywords: Ligand-field theory, software, spectroscopy

KESTREL is an easy-to-use computational package for calculating d-d transition energies, paramagnetic susceptibilities and EPR parameters in real-time. Taking the geometry of any 3d metalloprotein or complex as input, the ligand-field is parameterised using chemically meaningful e_σ and e_π parameters which reflect the type and strength of metal-ligand bond [1]. This approach permits real-time calculations and exploration of how the chemical environment affects the spectroscopy.

Assuming minimal metal-ligand spatial overlap, KESTREL evaluates the two-electron integrals for dynamical correlation between d-orbital configurations brought about by the ligand field. Unlike DFT, this multiconfigurational approach simultaneously calculates the energies, configurations and spin of all states. Following successful applications of KESTREL to biomimetic Fe(IV)-oxido species [2] and Fe(II) spin-crossover complexes [3], we demonstrate how KESTREL reproduces the spectroscopy of various LPMOs and can provide new insights into the key metal-ligand bonding interactions and structure of the active site.

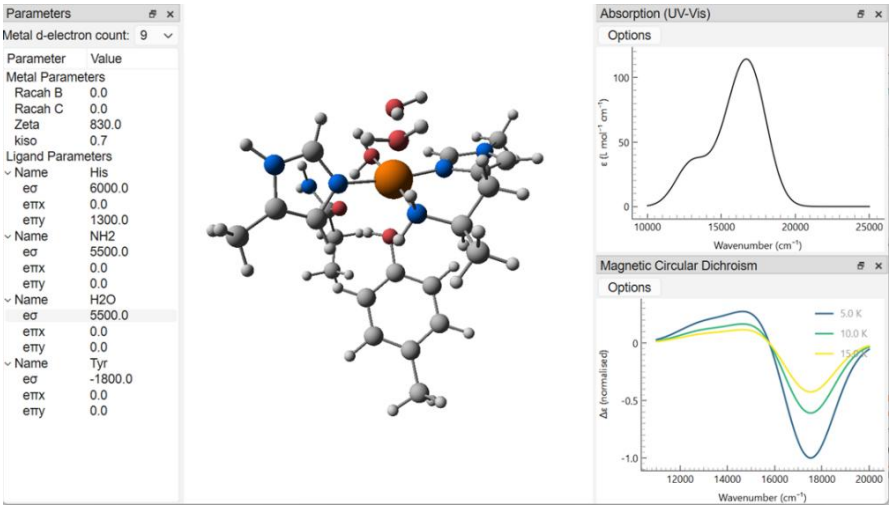

Figure 1. KESTREL's GUI showing calculations for the active site of *Ls*AA9. Parameter values are entered (left panel) with results obtained in real-time (right panel).

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P5. Change of regioselectivity by mutation of potential O-glycosylation sites in a C1-oxidizing AA9 enzyme

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Keywords: AA9, glycosylation, site-directed mutagenesis, regioselectivity

Cellulose-active LPMO enzymes show variable regioselectivity, generating either lactones /aldonic acids (C1-oxidation), ketoaldoses/geminal diols (C4-oxidation), or catalyze a double C1/C4 oxidation. Several structural features have been identified which determine this property : aromatic amino acids situated on the substrate binding surface and certain residues near the catalytic site were found to impact regioselectivity by influencing the substrate positioning of the enzyme. Mutation of N-glycosylated residues on the enzyme-substrate interface was also shown to modulate the C1/C4-oxidation ratio [1,2]. In order to study the role of glycosylations on regioselectivity and other enzymatic properties of a C1-oxidizing AA9 enzyme, we mutated its predicted N- and O-glycosylation sites. Mutation of three serine and threonine residues situated between the L2 and L3 loops (mutant S1) did not impact the amount of oxidated oligomers, but led to lower levels of reduced cello-oligosaccharides. Mutant S3 which had two other potentially glycosylated serine residues replaced by alanins displayed no significant difference compared to wild type activity. However, when both sites were simultaneously mutated, a shift in regioselectivity from C1 to C4 oxidation profile was observed, although neither of these sites are supposed to interact directly with the active site or participate in substrate binding.
Mass spectrometry analysis did not reveal any differences in the glycosylation profiles, suggesting that the S/T -> A changes are responsible for the observed phenotype. Future structural and modeling studies are underway to understand the molecular mechanism behind the findings.

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P6. LPMO-enabled surface carboxylation of lyocell for enhanced cationic dye uptake

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Keywords: *Dc*LPMO9A, Lyocell fabric, Cationic dye, Dye uptake improvement

Regenerated cellulosic fibers such as lyocell are bio-based, biodegradable, and widely used in textile applications. Their surface chemistry can be selectively tailored to improve downstream processing steps, including dyeing. Conventional reactive dyes are widely used for cellulose because they exhibit high affinity and form covalent bonds with the fiber. However, these stable bonds can complicate color removal during textile recycling [1]. Cationic dyes could offer an alternative, but their uptake by unmodified cellulose is limited by the lack of anionic binding sites [2].
Here, we demonstrate an enzymatic surface-functionalization strategy for lyocell using the C1-oxidising lytic polysaccharide monooxygenase (LPMO) *Dc*LPMO9A from *Delitschia confertaspora*. LPMO treatment introduces carboxyl groups into accessible cellulose regions, thereby creating anionic binding sites for cationic dyes. The extent of C1 oxidation was quantified by carboxyl-selective fluorescent labelling and correlated with dye uptake. Using rhodamine 6G (R6G) as a model cationic dye, enzymatically treated lyocell showed up to 75% higher dye uptake at enzyme loadings below 1% (w/w), while maintaining fabric yield and fiber integrity.
These results demonstrate that LPMO-catalyzed oxidation can serve as a mild and selective route to functionalize lyocell surfaces for improved cationic dyeing. Beyond demonstrating a new application for LPMOs in textile processing, this approach may reduce dependence on reactive dyes and support the development of more recycling-compatible coloration strategies for cellulosic textiles.

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P7. Low levels of AfLPMO9E enhance saccharification at reduced cellulase cocktail loadings

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Keywords: AA9 LPMO, cellulose oxidation, lignocellulose biomass, reducing-sugars.

Lytic polysaccharide monooxygenases (LPMOs) promote oxidative cellulose cleavage, although their contribution to lignocellulose saccharification depends on reaction conditions, substrate characteristics, and enzyme dosage [1,2]. Here, we investigated the oxidative activity of recombinant *Af*LPMO9E from *Aspergillus fumigatus* var. *niveus* and its effects on enzymatic saccharification. HPAEC-PAD analysis of phosphoric acid-swollen cellulose (PASC) showed pH-dependent product release (pH 4–10), with prominent enzyme-associated signals at pH 4 and 7, consistent with changes in oxidation-associated products. Saccharification was evaluated through an 11-run face-centered central composite design was conducted using defibrillated eucalyptus kraft pulp (10% solids, 72 h), varying APEX cellulase cocktail loading (5–15 mg protein g^{-1} dry biomass) and AfLPMO9E supplementation (0.5–5% relative to APEX protein), without exogenous reductant. The greatest improvement occurred at 5 mg g^{-1} APEX and 0.5% AfLPMO9E, increasing reducing-sugar concentrations from 20.71 to 28.46 g L^{-1} (+37.4%). At 15 mg g^{-1} APEX, supplementation improved sugar release by only 5.8%, although the highest absolute concentration was observed (41.54 g L^{-1}). Higher AfLPMO9E proportions did not provide additional improvement under the tested conditions, highlighting the importance of balancing oxidative and hydrolytic enzyme loadings. Subsequent kinetic experiments confirmed a time-dependent benefit, with AfLPMO9E increasing sugar release by 36.1% after 72 h (29.01 versus 21.32 g L^{-1}), while the improvement maintained after 96 h. Notably, LPMO-supplemented hydrolysis achieved within 72 h the same sugar concentration reached by APEX alone after 96 h, suggesting accelerated saccharification rather than a sustained increase in final sugar release. Overall, these findings demonstrate that *Af*LPMO9E supplementation can improve saccharification efficiency, particularly at lower cellulase loadings and intermediate hydrolysis times.

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P8. Complete genome sequencing and analysis of thermophilic fungus *Aspergillus fumigatus* GXU-8 from composts

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Keywords: *Aspergillus fumigatus*, Metagenomics, Lytic polysaccharide monooxygenases, Lignocellulose degradation, Composts

To explore enzyme and gene resources related to lignocellulose degradation and realize the efficient utilization of straw resources, *Aspergillus fumigatus* GXU-8, a thermophilic fungi, was isolated from composting habitats in this study. Metagenomics analysis showed that the strain GXU-8 harbors 429 carbohydrate-active enzyme (CAZy) genes, including 225 glycoside hydrolases (GHs), 67 glycosyltransferases (GTs), and 54 auxiliary activity enzymes (AAs). Within the genome, 39 cellulase genes (including 8 AA9 family lytic polysaccharide monooxygenases), 92 hemicellulase genes, and 36 ligninase genes were primarily identified, constituting an "oxidation-hydrolysis" synergistic lignocellulose degradation network. Thus, *Aspergillus fumigatus* GXU-8 possesses a comprehensive and efficient lignocellulose-degrading enzyme system, provides a candidate microbial resource for value-added valorization of straw wastes.

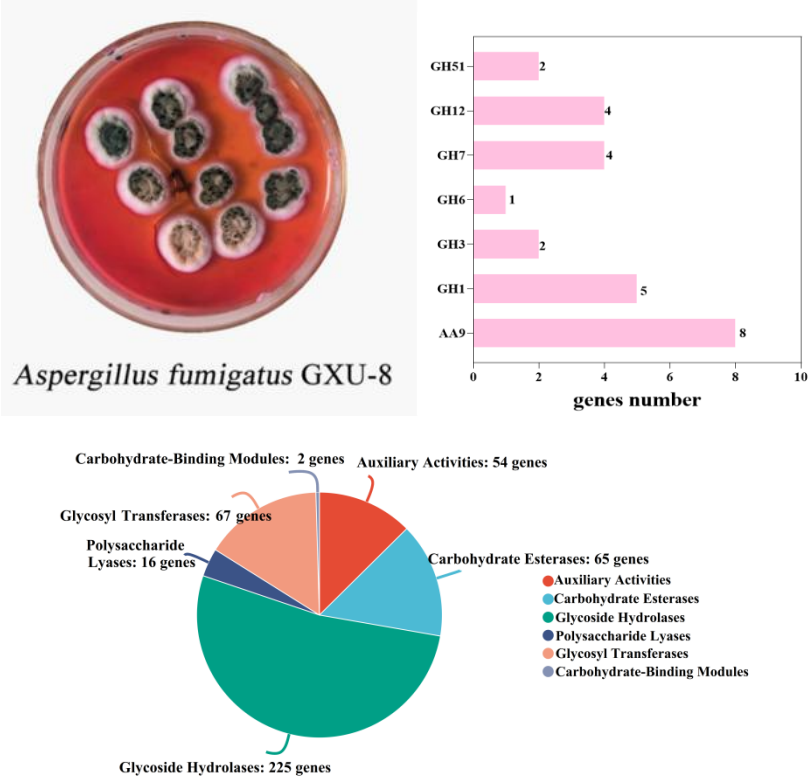

Figure 1. CAZy annotation results of *Aspergillus fumigatus* GXU-8.

P9. Structure–Function Relationships in Family AA11 LPMOs: A Comparative Study of AfAA11A and AfAA11B

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Keywords: Lytic polysaccharide monooxygenase (LPMO), AA11, Chito-oligosaccharides, Peroxygenase, Structure-function relationship, Enzyme co-evolution

Fungal family lytic polysaccharide monooxygenases (LPMOs) of family AA11 exhibit functional diversity despite sharing high structural similarity [1]. Phylogenetic analysis divides the catalytic domains of fungal AA11 LPMOs into two distinct clades: Clade I, containing *Aspergillus fumigatus Af*AA11A, which efficiently degrades insoluble crystalline α- and β – chitin [2]; and Clade II, containing *Af*AA11B, which, next to being active on chitin, displays a specialized ability to efficiently cleave soluble chito-oligosaccharides [1, 3]. This functional divergence raises intriguing questions regarding their true physiological roles in nature.

Here, we present a systematic comparative analysis of *Af*AA11A and *Af*AA11B across their three key reaction pathways: oxidase activity (H_2O_2 production), peroxidase activity (H_2O_2 consumption without substrate), and productive peroxygenase activity screened across various chitinous substrates. The reaction pathways were studied using well-established methods such as the Amplex Red/HRP assay and product quantification via HPLC. Additionally, real-time activity measurements using an electrochemical H_2O_2 sensor allowed deeper insight into the peroxidase and peroxygenase pathways [4]. Despite sharing identical active-site second-coordination sphere residues, *Af*AA11A and *Af*AA11B exhibit distinct kinetic profiles, H_2O_2 tolerance and activity on crystalline chitin and oligosaccharides. In addition, a broader co-evolution study was performed, mapping key residues that distinguish these enzymes from each other and serve important structural roles. Together, these results provide new insights into the evolutionary diversity and structure–function relationships in family AA11 LPMOs.

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P10. Catalase-like reaction by lytic polysaccharide monooxygenases (LPMOs) enables reductant independent degradation of cellulose

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Keywords: bacterial LPMO, alkaline, reductant-independent, hydrogen peroxide

LPMOs are monocopper enzymes that catalyze the hydroxylation of C1 and/or C4 carbons of glycosidic bonds in polysaccharides using hydrogen peroxide as a cosubstrate. Besides the sugar substrate and H_2O_2 cosubstrate, LPMO catalysis also depends on the external reductant that is needed for the "priming" reduction of LPMO-Cu(II) resting state into catalytically competent LPMO-Cu(I). LPMOs remain in their Cu(I) form after polysaccharide peroxygenase reaction but can be re-oxidized to the Cu(II) form in reductant oxidase and peroxidase side reactions. Dependency on external reductants complicates the application of LPMOs especially under alkaline conditions which often promote abiotic oxidation of reductants.

Here we describe an alkalitolerant, family AA10 LPMO (*Ci*LPMO10A) from the bacterium *Cellulomonas iranensis*. *Ci*LPMO10A was stable over a broad pH range of 3.0 – 11.5. We found that at pH 9.0 and above, *Ci*LPMO10A was able to release O_2 from H_2O_2 in the catalase-like reaction suggesting its ability to abstract electron from H_2O_2. Consistent with this, H_2O_2 suffices for cellulose peroxygenase reaction by *Ci*LPMO10A without the addition of an external reductant. The product profile and cellulose peroxygenase activity of *Ci*LPMO10A measured under alkaline conditions with H_2O_2 but without the reductant was similar to that measured at pH 6.0 in the presence of H_2O_2 and ascorbate as a reductant.

P11. A Quantum of Doubt: How Crystal-Clear Are LPMO Structures?

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Keywords: Quantum Refinement, Oxygen Species, LPMO active sites

Understanding how LPMOs form H_2O_2 requires knowing which intermediates form at the active site [1]. Several published LPMO crystal structures have been interpreted as containing oxygen species bound, or pre-bound, to the catalytic Cu site [2, 3]. However, at typical macromolecular resolutions, these species can be difficult to distinguish from partially occupied or disordered water molecules [4]. X-ray-induced photoreduction of Cu further complicates their identification by displacing the water ligands, therefore smearing their electron density [4]. Moreover, protonation and oxidation states are difficult to determine from X-ray diffraction data alone [1].
Quantum refinement aims to address these limitations by incorporating quantum mechanical (QM) calculations into crystallographic refinement [5]. For a selected region, the empirical restraints of conventional crystallographic refinement are replaced by QM restraints [5]. The refined model is thus constrained by both the experimental data and the theoretically obtained electronic structure of that region [5]. Alternative protonation and oxidation states can then be evaluated based on their agreement with diffraction data and the strain in their QM description.
Here, we present preliminary work applying the quantum refinement program QRef [5] to an LPMO structure containing a proposed oxygen species. We compare alternative bound species, active site protonation states, and Cu oxidation states. This case study illustrates how quantum refinement can complement conventional crystallographic refinement when the interpretation of such an active site is ambiguous.

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P12. *Neurospora crassa* LPMO gene diversity and substrate specificity

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Keywords: substrate specificity, *Neurospora crassa*, LPMO gene diversity

The environmentally friendly hydrolysis and valorization of agricultural and food processing residues play a crucial role in development of circular bioprocesses that are currently advanced in the Cluster of Excellence Circular Bioengineering. The filamentous fungus *N. crassa* encodes for more than 10 different LPMOs mainly in the auxiliary activity family AA9 (Figure 1) that have not all been characterized yet. Interestingly, the well-described NcLPMO9C NCU02916 exhibits a broad substrate specificity as well as high activity [1, 2], which raises the question why *N. crassa* possesses so many additional LPMO genes and what are their specific functions. Four genes (NCU 00836, 07974, 07760, 08746) were chosen for recombinant production by *P. pastoris* in a bioreactor, purified with Strep-tag and characterized on a rotating disc electrode (RDE) for substrate specificity and kinetic parameters in comparison to previously described NcLPMOs (NCU 02916, 07898) [3].

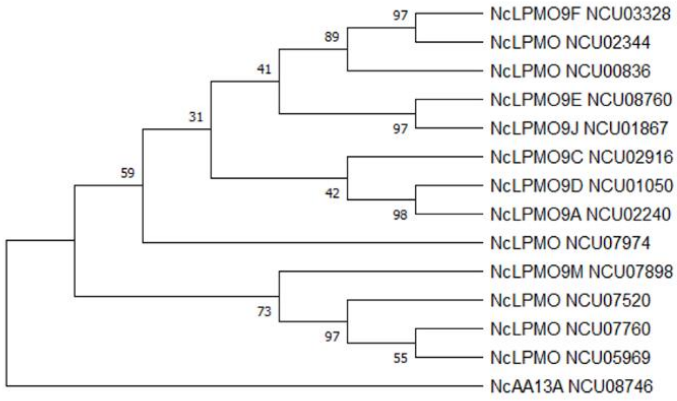

Figure 1. Bootstrap consensus tree of Nc LPMOs rooted at NcAA13A.

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P13. Lytic Polysaccharide Monooxygenases in Organic Solvents: Structural and Functional Characterization of *Sm*AA10A

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Keywords: Organic solvents, *Sm*AA10A, chitin

Enzyme performance in organic solvents has been studied for decades due to the industrial importance of efficient biocatalysis and solvent-based processes [1-3]. However, the effects of organic solvents on lytic polysaccharide monooxygenases (LPMOs), key enzymes in biomass degradation, remain unexplored. In this work, we investigate the structural integrity of the copper active site and the catalytic activity of the chitin-active LPMO *Sm*AA10A in water-organic solvent systems containing acetonitrile (ACN) or ethanol (EtOH). Electron paramagnetic resonance (EPR) and fluorescent spectroscopy show that the copper active site remains structurally intact in solutions containing up to 90% ACN or EtOH. Interestingly, the enzyme is active in reductant-driven reactions with β-chitin at solvent concentrations up to 90%, albeit at a lower rate than in the absence of organic solvents. The progress curves show that operational stability of the enzyme [4] remains relatively unchanged under most tested conditions. Remarkably, while *Sm*AA10A exhibits only minimal activity toward shorter chitooligosaccharides in aqueous solutions, the presence of organic solvents significantly enhances the catalytic activity towards chitopentaose and chitotetraose. These results demonstrate that *Sm*AA10A is a robust LPMO that can tolerate and perform catalysis in organic solvent-containing reaction mixtures. Our findings provide a foundation for exploring LPMO-catalyzed reactions in non-conventional solvent systems and may expand the application area for these powerful biocatalysts.

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P14. Scaling up AA9-based cello-oligosaccharides production for plant protection

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Keywords: Plant vaccine, LPMOs (AA9), Cello-oligosaccharides

The Zelda European project aims to develop a practical alternative to conventional pesticides for field applications. This approach relies on the enzymatic breakdown of biomass to produce cello-oligosaccharides (COS), which are applied as foliar sprays to stimulate plant immunity against phytopathogens. The ultimate goal is to maintain sufficient crop yields under field conditions while eliminating the need for pesticides.

COS production relies on the activity of AA9 enzymes derived from *Thermothelomyces terrestris*. These enzymes catalyse the oxidative cleavage of cellulose, generating cellooligosaccharides with different degrees of polymerization (DP), in both native and oxidized forms. When applied to plant leaves, these molecules act as damage-associated molecular patterns (DAMPs), triggering plant immune responses.

One of the main challenges of the Zelda project is the scale-up of the entire production process, from enzyme expression to COS generation. Particular attention is given to the use of AA9 enzymes to process lignocellulosic biomass derived from birchwood, a promising renewable feedstock for the production of these bioactive molecules.

Zero-waste Lignocellulose-Derived biorefinery products for smArt plant protection | ZELDA | Project | Fact Sheet | HORIZON | CORDIS | European Commission. (s. d.). https://cordis.europa.eu/project/id/101214981

P15 Spectroelectrochemical studies of surface-immobilized LPMOs

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Keywords: spectroelectrochemistry, direct electron transfer, protein immobilization

Lytic polysaccharide monooxygenases (LPMOs) are copper-containing enzymes capable of cleaving recalcitrant polysaccharides through activation of C-H bonds. The catalytic cycle is initiated by reduction of active site Cu(II) to Cu(I). In nature, this electron transfer can be mediated by variety of donors, including phenolic compounds and redox partner proteins [1]. In research, LPMOs are typically reduced with ascorbic acid [2], although other small molecule reductants such as gallic acid and plant-derived phenolics are used as well [3]. This strategy is widely used, although it might introduce uncertainty regarding the stoichiometry of reductant to copper. Here, LPMOs are immobilized on a modified electrode surface, enabling direct electron transfer between the electrode and the copper active site. The applied potential defines the redox state of the copper centre, while spectroscopic measurements performed in parallel report on its structural and electronic properties. The modified working electrode therefore serves as a platform for studying LPMOs in the absence and presence of substrates under well-defined conditions.

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P16. Unravelling the Impact of Lignocellulosic Phenolic and Quinones on Lytic Polysaccharide Monooxygenase (LPMO) Activity Using a Structure Causal Model

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Keywords: Phenolics, quinones, lytic-polysaccharide monooxygenase (LPMO), molecular descriptor structural causality model (SCM), directed acyclic graph (DAG)

Lignocellulosic biomass is the primary feedstock for second-generation biorefineries, but its hydrothermal pretreatment generates phenolic by-products that interfere with enzymatic hydrolysis. While classical cellulases are generally inhibited by these compounds, Lytic Polysaccharide Monooxygenases (LPMOs) rely on external electron donors, creating a complex interplay where some phenolics and quinones inhibit while others activate the enzyme [1]. To decode this dichotomy, an SCM based on a directed acyclic graph (DAG) was developed using 454 molecular descriptors [2, 3]. Five key descriptors accurately mapped to LPMO modulation: Hydrogen bond donors (nHBDon) and electronic contribution of hydroxyl H (khs.sOH) were dominant activators facilitating proton-coupled electron transfer, whereas the size of the conjugated π-system (nAtomP) and terminal oxygen distribution (MDEO.11) drove deactivation through steric hindrance and unproductive electron distribution. This integrated approach provides a powerful predictive tool to tailor pretreatment strategies and maximize LPMO efficiency in commercial cellulolytic cocktails.

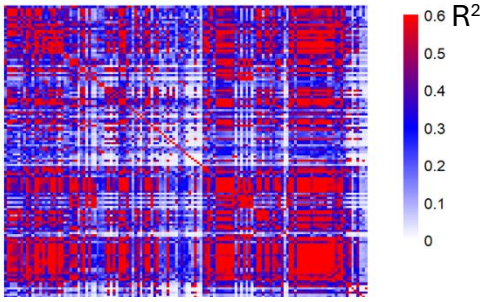

Fig. 1. Correlation R^2 of LPMO molecular descriptors mapped into Hilbert space (HSIC).

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P17. Exploring the Role of a Pectin-Active AA17 LPMO During *Phytophthora cactorum* Infection

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Keywords: AA17, oomycete, pectin

The discovery of the AA17 family of lytic polysaccharide monooxygenases (LPMOs) in 2021 [1] expanded the known substrate range of LPMOs to include pectin. Subsequent studies have revealed broader substrate specificity within this family [2], raising questions about the physiological functions of the AA17 LPMOs and their potential roles in plant cell wall modification. AA17 LPMOs have so far only been identified in oomycetes [1,2], a group that includes several economically important plant pathogens. Despite increasing knowledge of the biochemical properties of these enzymes, their biological functions remain poorly understood, including their roles during host infection.

Here, we investigate an AA17 LPMO from *Phytophthora cactorum*, an oomycete responsible for diseases in a wide range of plants, including strawberry [3]. *P. cactorum* expresses multiple AA17 LPMOs [4], and expression analysis during strawberry infection revealed that *Pc19407* was expressed at the early stages of infection, with expression peaking at 48 hours post-inoculation. The catalytic domain of Pc19407 was recombinantly expressed in *Pichia pastoris*. Structural characterization using AlphaFold and EPR spectroscopy suggests an octahedral copper coordination environment at the active site, consistent with previously characterized AA17 LPMOs. Polysaccharide substrate specificity was investigated using an electrochemical hydrogen peroxide sensor [5], revealing Pc19407 activity on pectin and strawberry fruit extract. Together, these findings contribute to our understanding of the biochemical diversity of AA17 LPMOs and provide a foundation for investigating their physiological roles during oomycete-plant interactions.

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P18. Structure-function relationships of LPMO bioinspired model complexes

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Keywords: bioinspired catalysis, mechanistic investigation, copper complex

The development of Lytic Polysaccharide Monooxygenase (LPMO) mimics is of importance for advancing fundamental mechanistic understanding and for designing functional catalysts with applications in energy and environmental fields [1]. Several groups have prepared and characterized low molecular weight copper complexes their activity was tested on various substrates under different reaction conditions rendering the establishment of structure-function relationships rather difficult [2].

A few years ago, we have established robust screening assays on soluble substrates using our reference complexes [3] and obtained preliminary data on extended polysaccharides [4]. We used these assays to study peptide-based LPMO complexes [5]. In the present work, we introduced chemical diversity by modifying the donor sets, the flexibility, the steric hindrance, and the electronic properties of the complexes [6]. The new complexes were first evaluated on soluble substrates to provide structure-function information. Mechanistic investigations were then carried-out to rationalize the observed reactivities. Assays on cellulose with selected catalysts reveals that the reactivity trends remain consistent when transitioning from soluble to extended substrates. These results have the potential to guide the future design of more efficient LPMO-inspired catalysts for applications in biomass conversion.

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P19. Comparative analysis of seven LPMOs of the AA9 family from *Thermothelomyces thermophilus*

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Keywords: AA9s, EPR, Redox potential, Activity assays

The genome of T. thermophilus harbors the genes of a total of 31 LPMOs, of which 23 belong to the AA9 family [1]. AA9 LPMOs exhibit broad substrate specificity and regioselectivity compared to other LPMO families [2], indicating functional diversification and substrate specialization. The prevalence and abundance of the AA9 family in microorganisms underscore the pivotal role that AA9 LPMOs play in the deconstruction of complex plant biomass. In this work, seven different AA9 LPMOs from T. thermophilus were successfully expressed recombinantly in Aspergillus nidulans A773 [3] and purified to homogeneity. We determined substrate specificity and regioselectivity under apparent monooxygenase conditions. EPR spectroscopy was utilized to characterize the electronic environment of the copper center, and non-turnover peaks in cyclic voltammograms recorded at different pH values were analyzed to determine the redox potential of the copper center. Finally, we evaluated off-pathway reactions, including reductant peroxidase activity (using a H2O2 sensor or 2,6-DMP assay) and reductant oxidase activity using the Amplex Red assay. Together, these approaches enable comparisons of biochemical and electrochemical properties among the seven enzymes. Overall, this comparative analysis highlights the biochemical and electrochemical diversity of AA9 LPMOs, providing insights into their distinct catalytic properties and potential roles in lignocellulose degradation.

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P20. *Neurospora crassa* AA9C is active on starch

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Keywords: starch active AA9, prebiotic effect, LPMO-treated hemicelluloses, oligosaccharides, *Neurospora crassa*

Lytic polysaccharide monooxygenases (LPMOs) are capable of depolymerizing lignocellulosic biomass. LPMOs from CAZy auxiliary activity family AA9 demonstrate catalytic activity for a broad range of hemicelluloses, such as LPMO from *Neurospora crassa* (*Nc*AA9C) that can depolymerize xyloglucans, β-glucans, glucomannans, etc. *Nc*AA9C was used to generate hemicellulose-derived oligosaccharides from food processing residues to evaluate their prebiotic potential. To this purpose, oat bran, pumpkin seed cake and rapeseed cake were fractionated and subsequently treated with *Nc*AA9C. The products of the depolymerization treatment were then used as the carbon sources to evaluate the growth of the two probiotic bacteria *Lacticaseibacillus rhamnosus* LGG and *Bifidobacterium adolescentis* ATCC 15703. Only the LPMO hydrolysates of raw oat bran, oat bran alkaline extract ethanol precipitant and oat bran alkaline extract acid neutralized precipitant promoted the growth of the probiotic bacteria. Based on the heterogeneous composition of oat bran, it was supposed, that the growth inducing factor in the experiments could be starch. To verify if starch can be a source of the malto-oligosaccharides identified by HPAEC-PAD in the LPMO-treated oat bran fraction, HPAEC-PAD was also performed for two commercial starches treated with LPMO indicating the malto-oligosaccharides as the products of degradation. A rotating disk electrode-based activity assay revealed an activity of *Nc*AA9C on native potato starch with a turnover number of 6.15 s^{-1}, similarly to nanocrystalline cellulose (7.90 s^{-1}). The oat bran alkaline extract acid neutralized precipitant, which is rich in starch had an activity slightly above starch (7.92 s^{-1}), whereas preparations expected to be rich in hemicellulose demonstrated a much higher activity (ethanol precipitated alkaline extracts of oat bran, pumpkin seed cake and rapeseed cake with turnover numbers of 42.85, 35.33 and 24.55 s^{-1}, respectively. In any case, this study found for the first time that an LPMO from CAZyfamily AA9 is active on starch. The results demonstrate that LPMO can generate oligosaccharides in food processing residues, which however show very little or no prebiotic potential. The observed starch-depolymerizing activity of *Nc*AA9C is a new finding that requests further studies of its mechanism in comparison to AA13 family LPMO.

P21. LPMO interactions on cell wall polymers

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Keywords: LPMO, CBM, linker, Sabatier principle, cellulose

Neurospora crassa AA9C lytic polysaccharide monooxygenase (*Nc*AA9C) degrades constituents of plant biomass like cellulose and multiple hemicellulosic substrates of industrial interest. This study investigates how *Nc*AA9C's substrate binding, catalytic efficiency and product spectrum is influenced by the presence/absence of a CBM and the length of the linker between LPMO and CBM. The Sabatier principle has been proposed as a tool to estimate kinetic constants and engineer enzymes, especially for for enzymes converting insoluble substrates [1]. For LPMOs a Sabatier-like behavior has been discussed in the community, which puts the function of the CBM to target a specific substrate and localize the LPMO in its close vicinity in focus. As a working hypothesis of this project we postulate that a maximal turnover is the result of a "Goldilocks" binding affinity that keeps the LPMO close to the substrate polymer for several catalytic events before allowing the relocation of the enzyme to a fresh part of the polymer. The linker length is regulating the attacked area. A shorter linker results in smaller oligosaccharides and necessitate a more frequent change of position. This hypothesis will be tested by the production of NCAA9Z variants with CBMs of different binding affinities and different linker lengths based on computational comparisons. The analysis of degradation-products will be used to explore how differently designed variants modulate their catalytic behaviors and to find the optimum balance between substrate affinity and catalytic efficiency.

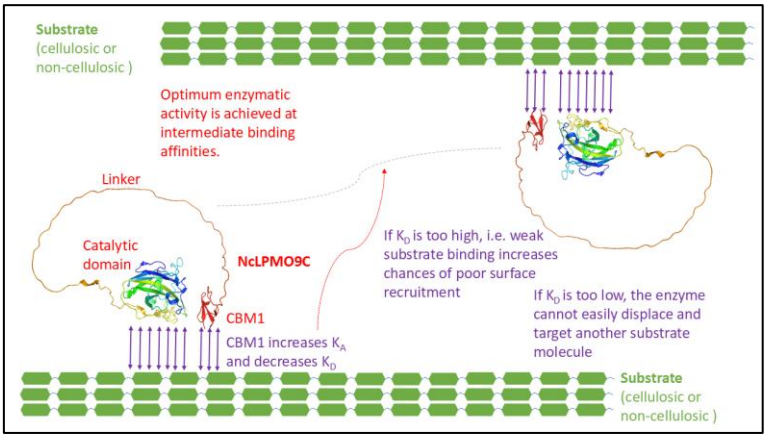

Figure 1. Investigating association and dissociation of *Nc*LPMO9C in regard to CBM and linker length.

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P22. Simplifying Recombinant Production of LPMOs: One-Step Affinity Capture with On-Column Tag Cleavage

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Keywords: recombinant protein expression, affinity purification, on-column tag cleavage, Protein Select™ technology

Engineering cleavable N-terminal tags is a common strategy for generating recombinant proteins with the correct N-terminus, often using specific proteases [1]. For lytic polysaccharide monooxygenases (LPMOs), precise N-terminal processing is essential because the conserved N-terminal histidine coordinates copper through the histidine brace, which is critical for catalytic activity [2]. This study evaluated Protein Select™ (Cytiva) technology as an integrated approach combining affinity purification with N-terminal processing to produce functional LPMOs.

A key challenge in producing recombinant LPMOs is achieving high yields of soluble protein in *E. coli*. To optimize soluble protein production, different expression strains and conditions were evaluated, including BL21(DE3), T7 Express, and SHuffle. Among the tested conditions, SHuffle yielded the highest amount of soluble protein. Four CAZy family AA9 enzymes from *Neurospora crassa* — *Nc*AA9C, *Nc*AA9F, *Pc*AA9D, and *Sm*AA10A—were recombinantly expressed in *E. coli* and subsequently purified using the Protein Select™ platform. Purity was assessed by SDS-PAGE, while protein identity and N-terminal processing were characterized by mass spectrometry using intact protein mass analysis and peptide mapping. Catalytic activity was evaluated using a rotating disk electrode (RDE)-based assay [3]. All four enzymes were successfully purified, their native N-termini were confirmed by mass spectrometry, and their catalytic activity was retained. These results demonstrate the potential of Protein Select™ technology as a streamlined workflow for producing correctly processed, functional LPMOs for downstream biochemical applications.

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P23. Xylan-active AA9 LPMOs and their role in cleaving xylan coating cellulose microfibrils in natural plant cell walls

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Keywords: xylan–cellulose interactions, LPMO multiplicity, LPMO promiscuity

Xylan–cellulose interactions are central to plant cell wall architecture, with xylan forming a protective sheath around cellulose microfibrils that can impede enzymatic degradation by pathogenic and decomposing microorganisms [1]. Understanding how xylanolytic enzymes give cellulose-active enzymes access to the microfibril surface is therefore key to understanding biomass decomposition. Lytic polysaccharide monooxygenases (LPMOs) that oxidatively cleave a range of plant polysaccharides are recognised as important contributors to biomass deconstruction and plant pathogenicity. Within the AA9 family, a subgroup of otherwise cellulose-active LPMOs has been shown to also cleave cellulose-bound xylan [2,3].

Since the discovery of xylan-active AA9s, differences in their preference for xylan versus cellulose and in their sensitivity to xylan substitution patterns have become increasingly evident and resolvable [2-4]. Here, we discuss these differences and show how the activity of xylan-active AA9 LPMOs towards cellulose and xylan can be simultaneously assessed quantitatively by quantifying the cellulose- and xylan-derived oligosaccharides these enzymes release [3]. Building on this, we investigate the capability of xylan-active AA9s to cleave acetylated xylan that is naturally associated with cellulose fibrils in plant cell walls using well-defined, individualised holocellulose nanofibrils (hCNFs) prepared from native plant cell wall material as a physiologically relevant substrate. Using this approach, we show that xylan-active AA9s enhance cellulose depolymerisation by cellulases and cellulose-active LPMOs and discuss the potential biological significance of their ability to increase cellulose accessibility.

Our findings deepen our understanding of the biological role of fungal AA9 LPMOs and lend support to the hypothesis that the multiplicity of AA9 genes in these organisms may be a consequence of their evolution to be able to degrade the various elements of the plant cell wall, a complex, multi-faceted and highly recalcitrant natural substrate.

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