Supplementary information

Continuous and sensitive monitoring of LPMO reactions using an optical H_2O_2 sensor

Leonor Vieira Carneiro[1,2], Anders Ørts Tjell[3], María Dolores Fernandez-Ramos[4], Torsten Mayr[3#], Daniel Kracher[1,2#]

[1] Institute of Molecular Biotechnology, Graz University of Technology, 8010 Graz, Austria

[2] BioTechMed-Graz, Graz, Austria

[3] Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, 8010 Graz, Austria

[4] Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain

[#]Corresponding authors

Torsten Mayr, torsten.mayr@tugraz.at; +43 316 873 32504; Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, 8010 Graz, Stremayrgasse 9/II, Austria

Daniel Kracher, daniel.kracher@tugraz.at; +43 316 873 4084; Institute of Molecular Biotechnology, Graz University of Technology, 8010 Graz, Petersgasse 14, Austria

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Table S1. The sequence of the pET28a_LsAA9A wild-type construct for periplasmic expression (the pelB sequence is highlighted in blue and the C-terminal twin strep tag is highlighted in orange and green).

protein sequence	MKYLLPTAAAGLLLLAAQPAMA**H**TLVWGVWVNGVDQGDGRNIYIRS PPNNNPVKNLTSPDMTCNVDNRVVPKSVPVNAGDTLTFEWYHNTR DDDIIASS**H**HGPIAVYIAPAASNGQGNVWVKLFEDAYNVTNSTWAVD RLITAHGQHSVVVPHVAPGDYLFRAEIIALHEADSLYSQNPIRGAQFYI SCAQITINSSDDSTPLPAGVPFPGAYTDSTPGIQFNIYTTPATSYVAP PPSVWSGALGGSIAQVGDASLEGGSGWSHPQFEKGGGSGGGSGG SAWSHPQFEK
DNA sequence	ATGAAGTATCTGCTACCTACAGCTGCTGCCGGTCTCTTGTTGTTA GCCGCACAACCTGCAATGGCACACACTCTAGTATGGGGTGTCTG GGTTAATGGAGTTGATCAAGGGGATGGAAGAAATATCTACATTCG ATCACCGCCAAACAACAACCCTGTAAAGAACCTCACGTCTCCTGA TATGACTTGTAACGTTGATAATAGAGTTGTTCCTAAAAGCGTACCG GTGAATGCAGGTGATACTCTTACGTTCGAGTGGTACCACAATACG CGAGATGACGACATAATAGCTAGCAGCCACCATGGTCCCATAGC AGTGTATATTGCTCCAGCTGCTTCTAATGGCCAAGGCAATGTTTG GGTTAAGTTGTTTGAAGATGCTTATAACGTCACCAACTCAACCTG GGCAGTTGATAGATTGATTACTGCACACGGCCAACATTCTGTCGT CGTTCCTCACGTCGCACCAGGTGACTATCTCTTCCGTGCCGAGAT TATTGCTCTACACGAGGCAGATTCATTGTATAGCCAAAATCCAATC AGAGGTGCTCAGTTTTACATCTCTTGTGCTCAGATTACTATTAACT CTTCGGATGATTCTACGCCCCTTCCAGCTGGAGTTCCTTTCCCAG GTGCTTATACTGACAGCACACCAGGTATCCAATTTAACATATACAC CACTCCGGCTACATCCTACGTTGCTCCTCCTCCAAGTGTCTGGTC AGGAGCTTTGGGTGGATCAATTGCTCAGGTGGGAGACGCTTCAC TAGAGGGTGGCTCGGGATGGTCCCATCCACAATTCGAGAAAGGT GGAGGTTCCGGAGGTGGATCGGGAGGTTCGGCGTGGAGCCACC CGCAGTTCGAAAAA

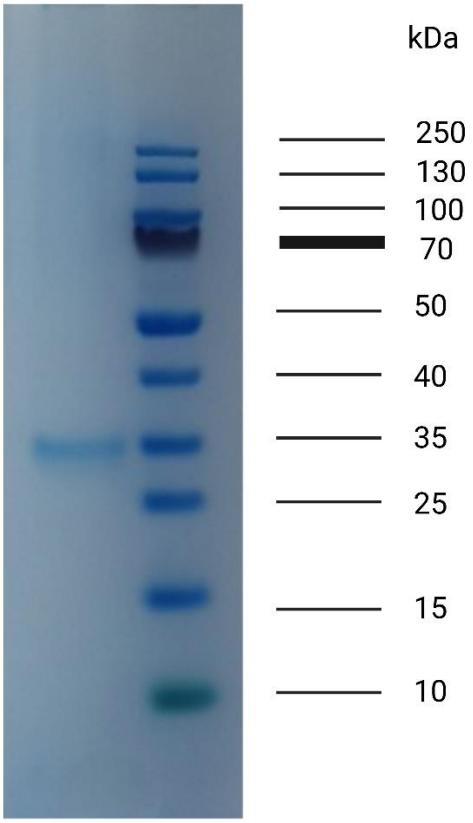

Figure S1. *Ls*AA9A sequence cloned in a pET28a vector. SDS gel showing band corresponding to the protein purified and copper regenerated (purity ≥95%; determined by densitometry using Image J, National Institutes of Health, Wayne Rasband).

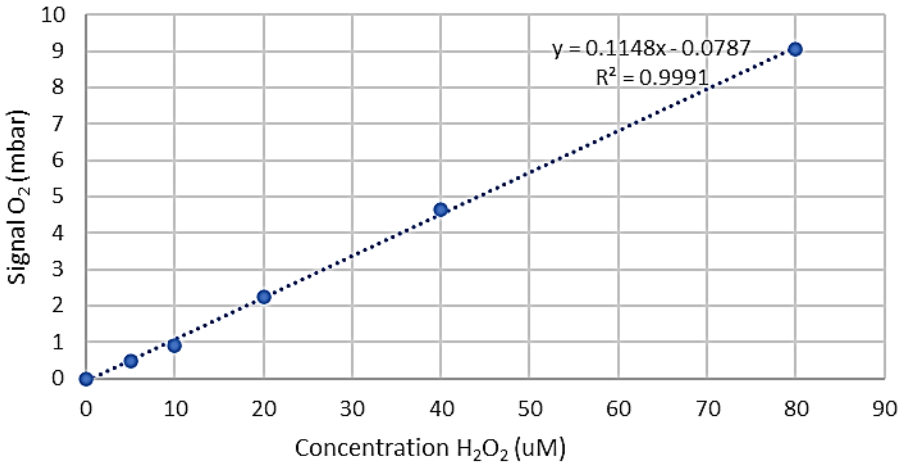

Figure S2. Calibration curve used for the calculation of H_2O_2 concentrations using the H_2O_2 sensor.

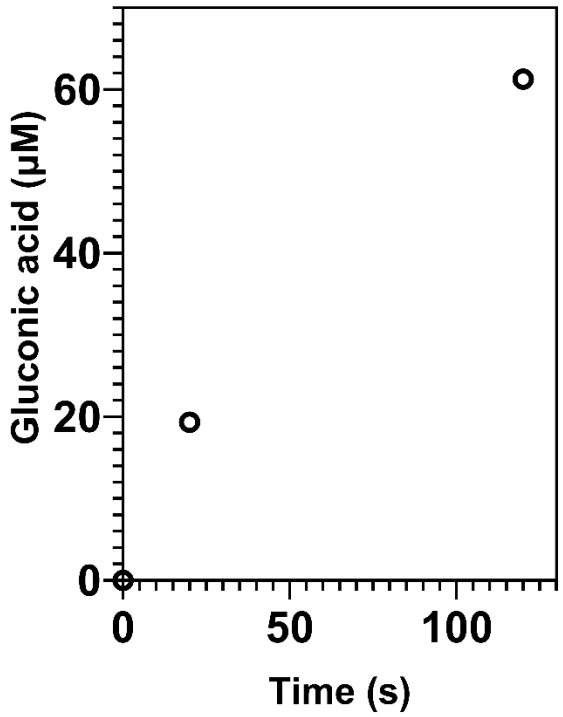

Figure S3. Product formation of the reaction of *Ls*AA9A with cellotetraose at pH 6. Gluconic acid is quantified with the D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit from Megazyme, after hydrolysis of the reaction mixture with β-glucosidase and adjustment with NaOH to pH 11.

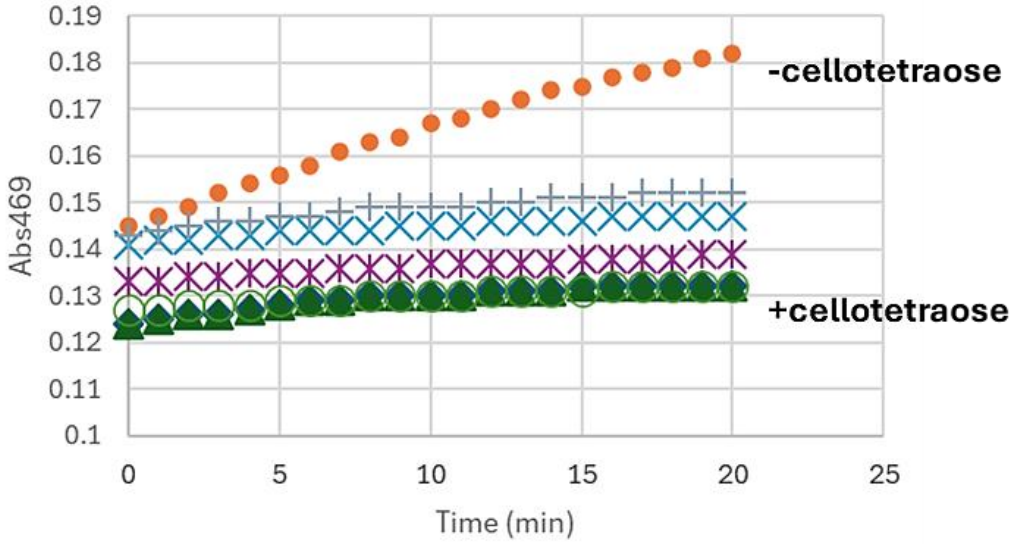

Figure S4. DMP assay using *Ls*AA9A (1 µM) in the absence and presence of 50 µM cellotetraose in 50 mM phosphate buffer, pH 6. Reactions were carried out in the presence of 10 mM DMP and 50 µM H_2O_2.

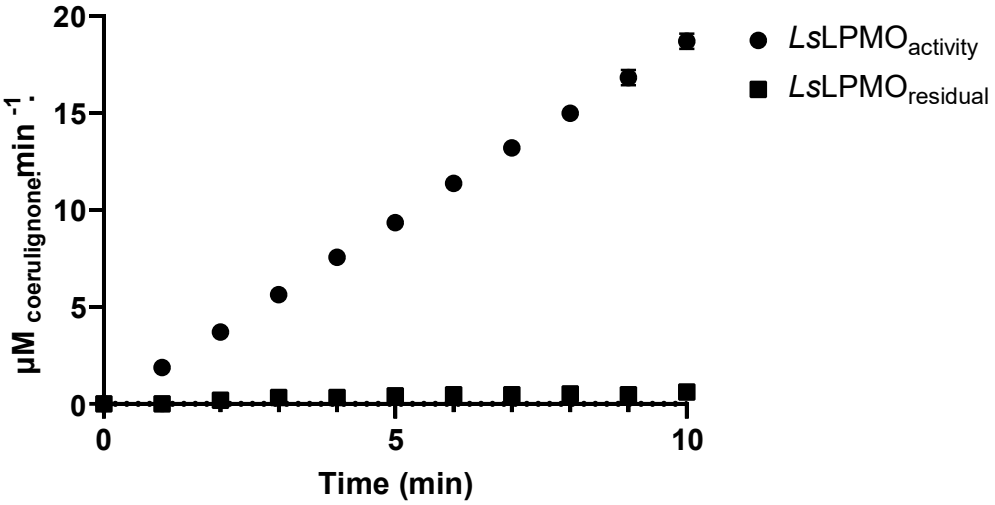

Figure S5. DMP assay using *Ls*AA9A before and after reaction with cellotetraose (0.1mM). *Ls*AA9A shows a residual activity of 0.16 µM.min^{-1} in comparison with the original activity of 1.87 µM.min^{-1}, corresponding to a decrease in activity to 11%.

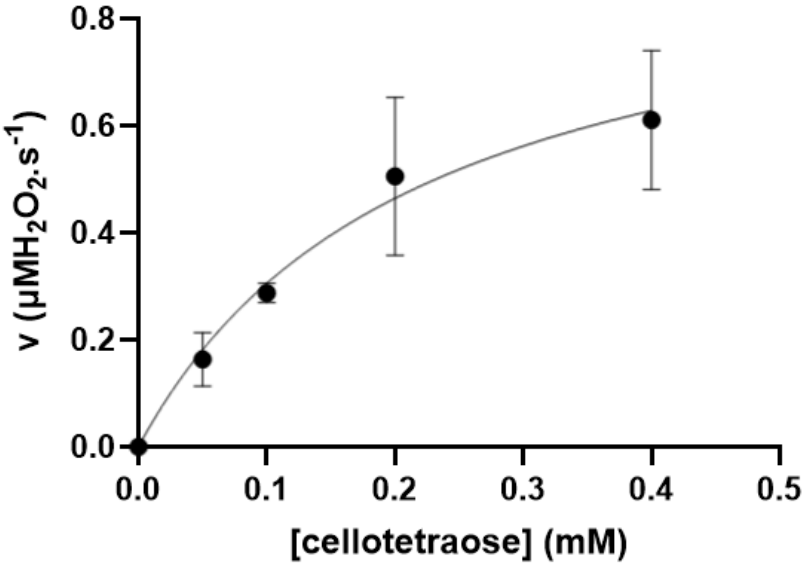

Figure S6. Michaelis-Menten kinetics of *Ls*AA9A. Kinetic parameters were determined using initial rates and exclude substrate inhibition. Data were fitted to the Michaelis Menten equation using Graph Pad Prism (10.4.1, Dotmatics).

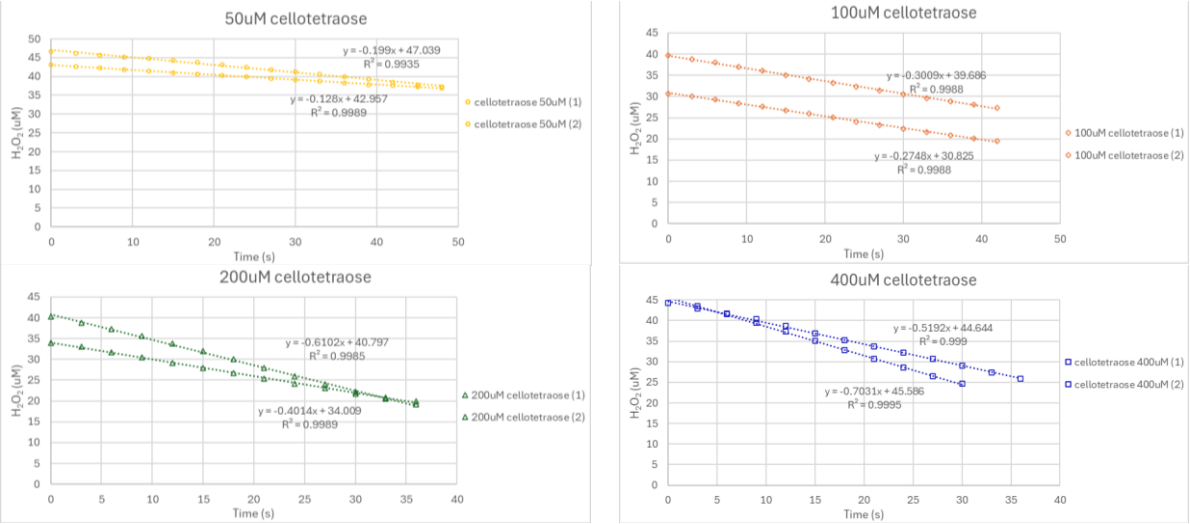

Figure S7. Initial rates of H_2O_2 consumption by *Ls*AA9A with different cellotetraose concentrations obtained with the H_2O_2 sensor.