Table 1. Overview of domain truncation, CBM substitution and linker studies in LPMOs. An updated version of this table is available online at https://github.com/gcourtade/papers/tree/master/2022/LPMO-modularity-review

Enzyme (regioselectivity, substrate specificity)	**Modular organization** of wild-type (WT) and truncation variants	**Key findings** [(a)]	**References**
***Vc*LPMO10B (GbpA)** (C1-oxidizing, chitin)	AA10-GbpA2-GbpA3-CBM73 (WT) AA10 AA10-GbpA2-GbpA3 GbpA2-GbpA3 CBM73	Chitin-binding is mainly aided by the CBM and to a lesser extent by the LPMO domain. The LPMO domain is required for mucin binding and GbpA2 and GbpA3 in combination with the LPMO domain are important for intestinal colonization in a cholera mouse model.	Wong *et al* 2012 https://doi.org/10.1371/journal.ppat.1002373
***Sc*LPMO10C** (C1-oxidizing, cellulose)	AA10-CBM2 (WT) AA10	Loss of cellulose binding affinity for the CBM truncated enzyme. Reduced cellulose activity of CBM truncated enzyme. NMR spectroscopy study showing structural and dynamic features of a modular LPMO. The substrate-binding affinity resides with the CBM. Comparison of the catalytic performance of full-length and truncated LPMO revealed that the CBM is beneficial for LPMO activity and operational stability at lower substrate concentrations and promotes localized and repeated oxidation of the substrate. Truncation of the CBM leads to elevated H_2O_2 production and decreased enzyme stability (both in absence and presence of cellulose). Release of copper by damaged enzymes promote H_2O_2 production, which increases LPMO catalytic rate and inactivation. Observed synergistic effects when combining the two enzyme forms are due to a combination of high oxidase activity (i.e., increased LPMO-dependent H_2O_2 production) by the truncated enzyme and efficient productive use of H_2O_2 (i.e., peroxygenase activity) by the full-length enzyme.	Forsberg *et al* 2014 https://doi.org/10.1021/bi5000433 Forsberg *et al* 2014 https://doi.org/10.1073/pnas.1402771111 Courtade *et al* 2018 https://doi.org/10.1074/jbc.ra118.004269 Stepnov *et al* 2022 https://doi.org/10.1038/s41598-022-10096-0
***Nc*LPMO9C** (C4-oxidizing cellulose, cello-oligosaccharides, xyloglucan)	AA9-CBM1 (WT) AA9	K_d measured for phosphoric acid swollen cellulose (PASC) and xyloglucan showed weaker binding for CBM truncated *Nc*LPMO9C. No CBM truncation effect on activity on PASC but a 2-fold reduction in the rate of xyloglucan degradation. Truncation of the CBM reduced the binding affinity and LPMO activity but did not affect regioselectivity. The linker is important for thermal stability.	Borisova *et al* 2015 https://doi.org/10.1074/jbc.M115.660183 Laurent *et al* 2019 https://doi.org/10.3390/ijms20246219

***Cf*LPMO10** (C1/C4- oxidizing, cellulose and C1-oxidizing, chitin)	AA10-CBM2 (WT) AA10 AA10-CBM2^{Tb} AA10-CBM3 AA10-CBM10	Study on deleting and replacing CBMs in two cellulose-oxidizing LPMOs. Introduction of other types of cellulose binding CBMs both potentiated and inhibited the LPMO activity. Such effects were both enzyme and substrate specific. Changed ratios between non-oxidized and oxidized products when replacing the CBM2 by a CBM10 –> CBMs can modulate the mode of action of LPMOs.	Crouch *et al* 2016 https://doi.org/10.1074/jbc.M115.702365
***Tb*LPMO10** (C1-oxidizing, cellulose)	AA10-CBM2 (WT) AA10 AA10-CBM2^{Cf} AA10-CBM3 AA10-CBM10	See above (*Cf*LPMO10).	Crouch *et al* 2016 https://doi.org/10.1074/jbc.M115.702365
***Cj*LPMO10A** (C1-oxidizing, chitin)	AA10-CBM5-CBM73 (WT) AA10 AA10-CBM5	Removal of both CBMs reduced LPMO activity toward α-chitin compared with the full-length enzyme, but in synergistic reactions with an *endo*-chitinase equal levels of solubilized products were observed. Structural analysis of two similar chitin-binding CBMs with different affinity for crystalline chitin and soluble chitohexaose. The effect of CBMs on chitin oxidation is substrate concentration dependent; at low concentrations, the CBM-containing variants performed better, whereas at high concentrations the differences were less apparent.	Forsberg *et al* 2016 https://doi.org/10.1074/jbc.M115.700161 Madland *et al* 2021 https://doi.org/10.1016/j.jbc.2021.101084
***Hj*LPMO9A** (C1/C4-oxidizing cellulose)	AA9-CBM1 (WT) AA9-21 amino acid linker fragment AA9 *(including three mutated variants; Y24A, Y211A & Y24A_Y211A)*	Removal of the CBM, post-translationally by papain hydrolysis, led to a truncated variant with 21 remaining residues of the predicted linker which exhibited reduced binding and activity towards cellulose compared to the full-length enzyme. The X-ray structure revealed that the glycosylated linker forms an integral part covering a hydrophobic patch on the catalytic LPMO domain. Removing the CBM resulted in reduced binding but did not alter the oxidative regioselectivity. However, the effects of point mutations in the catalytic domain on oxidative regioselectivity became more apparent in the absence of the CBM.	Hansson *et al* 2017 https://doi.org/10.1074/jbc.m117.799767 Danneels *et al* 2019 https://doi.org/10.1002/biot.201800211
***Tf*LPMO10B** (C1-oxidizing, cellulose)	AA10-FnIII-CBM2 (WT) AA10 AA10-FnIII AA10-CBM2	Binding is mediated mainly by the CBM and to some extent by the LPMO domain. Removal of the FnIII-like domain (called X1) had no effect on binding nor on activity.	Kruer-Zerhusen *et al* 2017 https://doi.org/10.1186/s13068-017-0925-7

***Ma*LPMO10B** (C1/C4- oxidizing, cellulose and C1-oxidizing, chitin)	AA10-CBM2 (WT) AA10	Deletion of the CBM affected the operational stability of the LPMO but did not affect the ratio of regioselective C1:C4 oxidation.	Forsberg *et al* 2018 https://doi.org/10.1074/jbc.M117.817130
***Bc*LPMO10A** (C1- oxidizing, chitin)	AA10-FnIII-FnIII-CBM5 (WT) AA10 AA10-FnIII AA10-FnIII-FnIII	Enzyme functionality was strongly dependent on the CBM that is responsible for substrate binding and protects the enzyme from autocatalytic inactivation. Truncation of one or two of the FnIIIs (both in combination with the CBM) resulted in essentially the same effect as when only the CBM was removed.	Mutahir *et al* 2018 https://doi.org/10.1002/1873-3468.13189
***Bt*LPMO10A** (C1-oxidizing, chitin)	AA10-FnIII-FnIII-CBM5 (WT) AA10 CBM5 AA10-FnIII-CBM5 AA10-CBM5 FnIII-FnIII	The CBM is essential for binding to α- and β-chitin. The FnIII-like domains do not have a role in chitin-binding.	Manjeet *et al* 2019 https://doi.org/10.1016/j.ijbiomac.2019.01.183
***Pa*LPMO9H** (C1/C4-oxidizing cellulose, cello-oligosaccharides, xyloglucan)	AA9-CBM1 (WT) AA9	Truncation of the CBM weakened substrate binding and affected the catalytic performance on nanofibrils, amorphous and crystalline cellulosic substrates, but there was no effect on the activity on cellohexaose. Increasing the substrate concentration reduces the need for a CBM. The truncated variant showed a modified regioselectivity with increased C1-oxidation. Optical and atomic force microscopy of the insoluble fraction revealed that both variants can promote disruption of the cellulose network and that the CBM is not essential.	Chalak *et al* 2019 https://doi.org/10.1186/s13068-019-1548-y
***Jd*LPMO10A** (C1-oxidizing, chitin)	AA10-CBM5-GH18 (WT) AA10 AA10-CBM5 CBM5-GH18 GH18	Synergy study that showed intramolecular synergy between the LPMO domain and the chitinase (GH18) domain. Comparison of the chitinolytic efficiency of the full-length enzyme and combinations of truncated variants showed that the full-length enzyme is more efficient compared to any combination of its separately produced domains.	Mekasha *et al* 2020 https://doi.org/10.1074/jbc.RA120.013040
***Mt*LPMO9B** (C1-oxidizing, cellulose)	AA9-CBM1 (WT) AA9	The CBM promotes cellulose degradation in the full-length enzyme but does not affect oxidative regioselectivity.	Sun *et al* 2021 https://doi.org/10.1021/acssuschemeng.1c04100
***Bc*LPMO9C** (Unknown regioselectivity, cellulose,)	AA9-CBM1 ^{55} AA linker (WT) AA9-CBM1 ^{44} AA linker AA9-CBM1 ^{18} AA linker AA9-CBM1 ^{7} AA linker AA9	Studies on linker truncation showed that shortening the linker or removing the CBM reduces substrate binding.	Srivastava *et al* 2022 https://doi.org/10.1128/spectrum.02697-21

[a] Note that the more quantitative statements in this Table need to be read with caution, because the quality of the underlying kinetic analyses varies. For example, especially in early studies, the impact of autocatalytic LPMO inactivation, leading to non-linear progress curves, was not always considered (Eijsink *et al* 2019 https://doi.org/10.1186/s13068-019-1392-0).