**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>

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| --- | --- | --- | --- |
| **Enzyme**  (regioselectivity, substrate specificity) | **Modular organization**  of wild-type (WT) and truncation variants | **Key findings** | **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 at lower substrate concentrations and promotes localized and repeated oxidation of the substrate.   Truncation of the CBM leads to elevated H2O2 production and decreased enzyme stability (both in absence and presence of cellulose).  Release of copper by damaged enzymes promote H2O2 production, which increased LPMO catalytic rate followed by inactivation.  Observed synergistic effects when combining the two enzyme forms, that are due to a combination of high oxidase activity (i.e., increased LPMO-dependent H2O2 production) by the truncated enzyme and efficient productive use of H2O2 (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 | *Kd* measured for phosphoric acid swollen cellulose (PASC) and xyloglucan showed weaker binding for CBM truncated *Nc*LPMO9C.  No CBM truncation effect on activity comparison for PASC but a 2-fold reduction in catalytic rate against xyloglucan.  Truncation of the CBM reduced the binding affinity and activity but did not affect regioselectivity.  The linker is important for the 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.  Introducing other types of cellulose binding CBMs both potentiated and inhibited the LPMO activity. Such effects were both enzyme and substrate specific.  Changed ratio between native and oxidized products when replacing the CBM2 to 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 regioselectivity. However, the effects of point mutations in the catalytic domain 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. Although, 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 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 | The enzyme functionality was strongly dependent on the CBM that is responsible for substrate binding and protects the enzyme from 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 | CBM truncation weakened the binding and affected the catalytic performance on nanofibrils, amorphous and crystalline cellulosic substrates, although the isolated catalytic domain retained 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 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 an LPMO and a chitinase.  Comparison of the chitinolytic efficiency of the full-length enzyme and combinations of truncated variants showed that the full-length enzyme was 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 promote cellulose degradation in the full-length enzyme but did not affect regioselectivity in the truncated variant. | 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 | Study on linker truncation showed that shortening the linker or removing the CBM reduced substrate binding. | Srivastava *et al* 2022 <https://doi.org/10.1128/spectrum.02697-21> |