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Supporting information for article:

**The crystal structure of CbpD clarifies substrate-specificity motifs
in chitin-active lytic polysaccharide monooxygenases**

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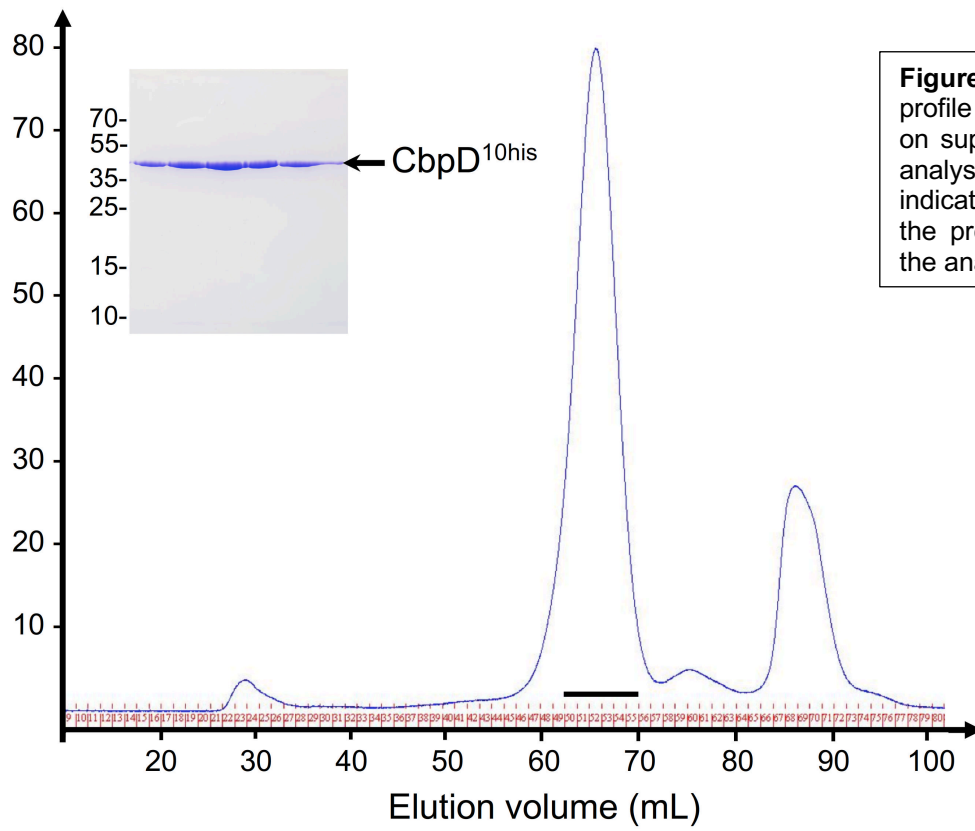


Figure S1 CbpD purification: SEC profile of CbpDHis purified by IMAC on superose 200 16/60. SDS-PAGE analysis of the SEC fractions indicated by the black line revealed the presence of full-length CbpD in the analyzed peak (43kDa).

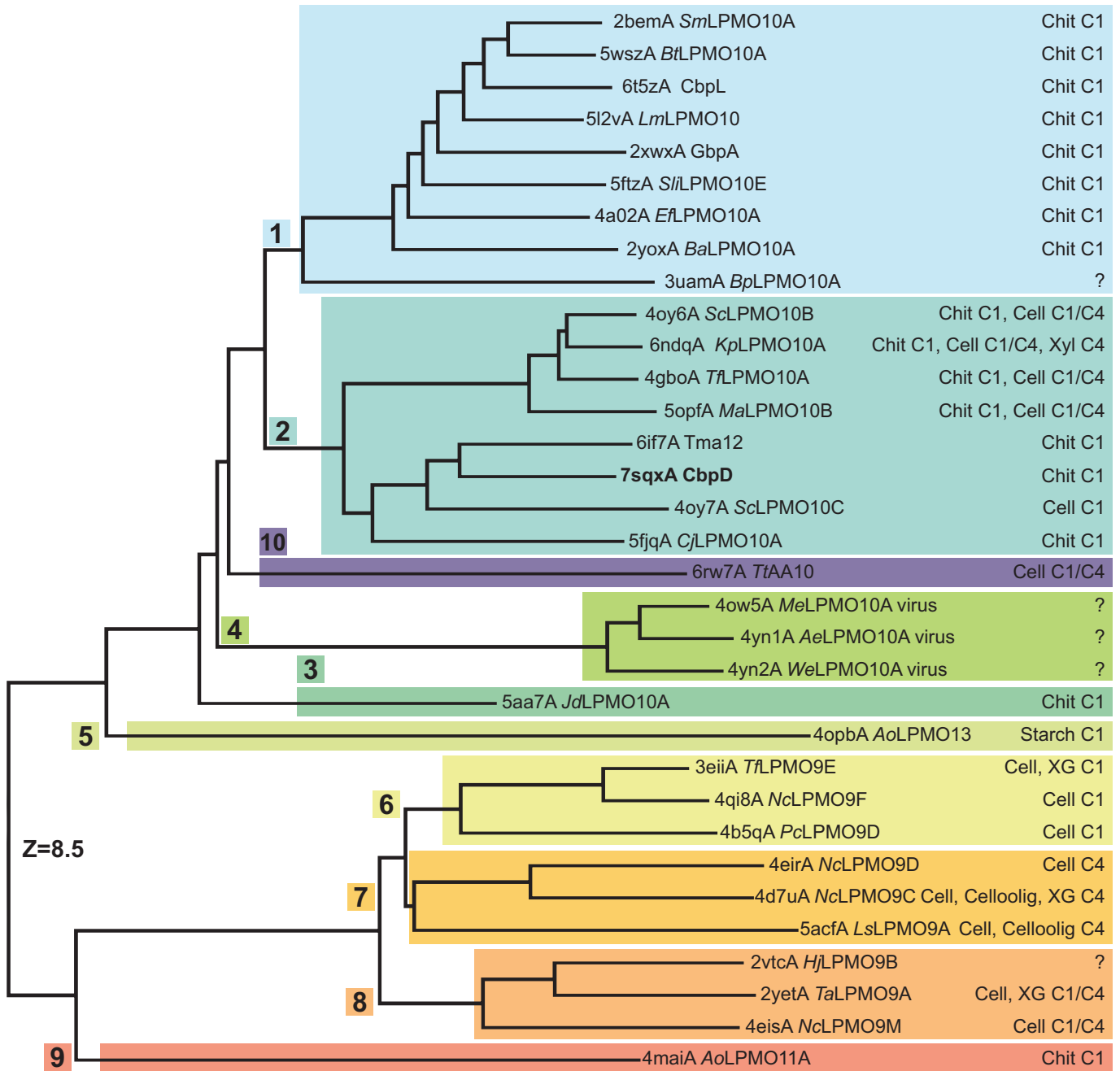


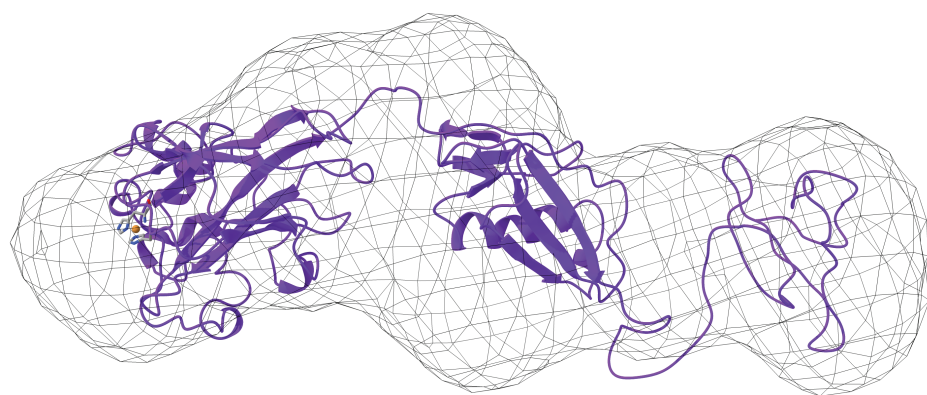
Figure S2 The structural diversity of LPMOs: The expanded dendrogram of unique LPMO crystal structures from Vaaje-Kolstad *et al.* 2017 in addition to CbpD (bold) and subsequent LMPOAA10 structures determined and listed in the CAZy AA10 database. CbpD is a member of structural cluster 2, and *TtAA10* may represent a possible 10th structural cluster. Structures are identified by their PDB identifier and the chain ID, followed by their known substrate(s). The scale indicates the DALI Z-score. Some LPMOs have been referred to by other names in the literature, which are indicated in parenthesis; *Sm*LPMO10A (CBP21), CbpL (*Pi*LPMO10A), GbpA (*Vc*LPMO10B, *Vc*AA10B), *Ef*LPMO10A (*Ef*CBM33A, *Efa*CBM33), *Ba*LPMO10A (*Ba*AA10A, ChbB, *Ba*CBM33), *Tf*LPMO10A (E7), *Sc*LPMO10C (*Ce*S2, *Sc*AA10C), *Ao*LPMO13 (*Ao*(AA13)), *Tf*LPMO9E (*Tt*GH61E), *Pc*LPMO9D (*Pc*GH61D), *Nc*LPMO9D (PMO-2, NCU01050), *Nc*LPMO9C (NCU02916), *Ls*LPMO9A (*Ls*(AA9)A), *Hj*LPMO9B (EG7, *Ce*l61B), *Ta*LPMO9A (*Ta*GH61A), *Nc*LPMO9M (PMO-3, NCU07898), *Ao*LPMO11 (*Ao*(AA11)). Following the naming convention of subsequently discovered and characterized LPMOs, CbpD may also be referred to as *Pa*LPMO10A, *Tma*12 may also be named *Tm*LPMO10A, and *Tt*AA10 may be called *Tf*LPMO10A.

The organism of each LPMO is:

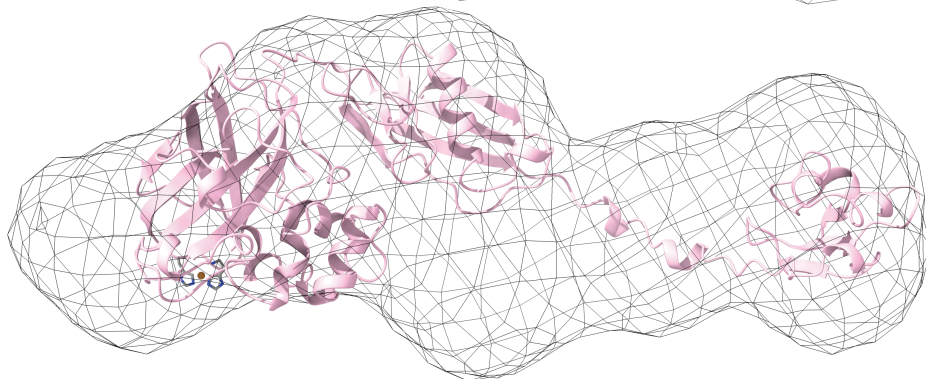
Serratia marcescens (*Sm*LPMO10A); *Bacillus thuringiensis* Serovar *kurstaki* (*Bt*LPMO10A); *Photorhabdus luminescens* (CbpL); *Listeria monocytogenes* (*Lm*LPMO10); *Vibrio cholera* (GpbA); *Streptomyces lividans* (*Sl*iLPMO10E); *Enterococcus faecalis* (*Ef*AA10A); *Bacillus amyloliquefaciens* (*Ba*AA10A); *Burkholderia pseudomallei* (*Bp*AA10A); *Streptomyces coelicolor* (*Sc*LPMO10B, *Sc*LPMO10C); *Kitasatospora papulose* (*Kp*LPMO10A); *Thermobifida fusca* (*Tf*LPMO10A); *Micromonospora aurantiaca* (*Ma*LPMO10B); *Tectaria macrodonta* (*Tma*12); *Pseudomonas aeruginosa* (CbpD); *Cellvibrio japonicus* (*Cj*LPMO10A); *Teredinibacter turnerae* (*Tt*AA10A); unidentified entomopoxvirus (*Me*LPMO10A, *We*LPMO10A); *Anomala cuprea* entomopoxvirus (*Ae*LPMO10A); *Jonesia denitrificans* (*Jd*LPMO10A); *Aspergillus oryzae* (*Ao*LPMO13, *Ao*LPMO11A); *Thermothielavioides terrestris* (*Tf*LPMO9E); *Neurospora crassa* (*Nc*LPMO9F, *Nc*LPMO9D, *Nc*LPMO9C, *Nc*LPMO9M); *Phanerodontia chrysosporium* (*Pc*LPMO9D); *Lentinus similis* (*Ls*LPMO9A); *Trichoderma reesei* (*Hj*LPMO9B); *Thermoascus aurantiacus* (*Ta*LPMO9A)

	160	169	
<i>Pseudomonas aeruginosa</i> CbpD	T G K H V I Y N V W Q R	R	Chitin
<i>Bacillus amyloliquefaciens</i> BaLPMO10A	S G Y H I I L G V W D V		Chitin
<i>Tectaria macrodonta</i> Tma12	S G S H L I Y V I W Q R	R	Chitin
<i>Photorhabdus laumondii</i> CbpL	Q G Y H V I L G V W T I		Chitin
<i>Cellvibrio japonicus</i> CjLPMO10A	T G R H I I Y S I W Q R	R	Chitin
<i>Enterococcus faecalis</i> EfLPMO10A	K G Y H V I Y A V W G I		Chitin
<i>Jonesia denitrificans</i> JdLPMO10A	T G E H T I L A R W N V		Chitin
<i>Listeria monocytogenes</i> LmLPMO10	S G Y Y L I L G V W N I		Chitin
<i>Serratia marcescens</i> BJL200 SmLPMO10A	S G S H V I L A V W D I		Chitin
<i>Streptomyces ambofaciens</i> SamLPMO10B	D G R Q K V L A V W N V		Chitin
<i>Streptomyces griseus</i> SgLPMO10F	T G K Q K V L A V W N V		Chitin
<i>Streptomyces lividans</i> SliLPMO10E	S G H H V I L A V W T V		Chitin
<i>Vibrio cholerae</i> GbpA	E G Y Q V I L A V W D V		Chitin
<i>Bacillus thuringiensis</i> ACCC10066 BtLPMO10A	S G Y H L I L A V W E I		Chitin
<i>Bacillus licheniformis</i> BIAA10A	L G Y H V I L A V W D V		Chitin
<i>Bacillus thuringiensis</i> ATCC33679 BtLPMO10A-FL	S G Y H V I L A V W D V		Chitin
<i>Bacillus cereus</i> BcLPMO10A	S G Y H V I L A V W D V		Chitin
<i>Serratia marcescens</i> KCTC2172 22kDa Protein	A V R S D P L P C G D I		Chitin
<i>Streptomyces coelicolor</i> ScLPMO10B	T G R H V V Y T I W Q A		Chitin, Cellulose
<i>Kitasatospora papulosa</i> KpLPMO10A	S G R H V V Y T I W Q A		Chitin, Cellulose, Xylose
<i>Thermobifida fusca</i> TfLPMO10A	S G R H V V F T I W K A		Chitin, Cellulose
<i>Micromonospora aurantiaca</i> MaLPMO10B	T G R H V V Y T I W Q A		Chitin, Cellulose
<i>Streptomyces coelicolor</i> ScLPMO10C	S G D A L I F M Q W V R	R	Cellulose

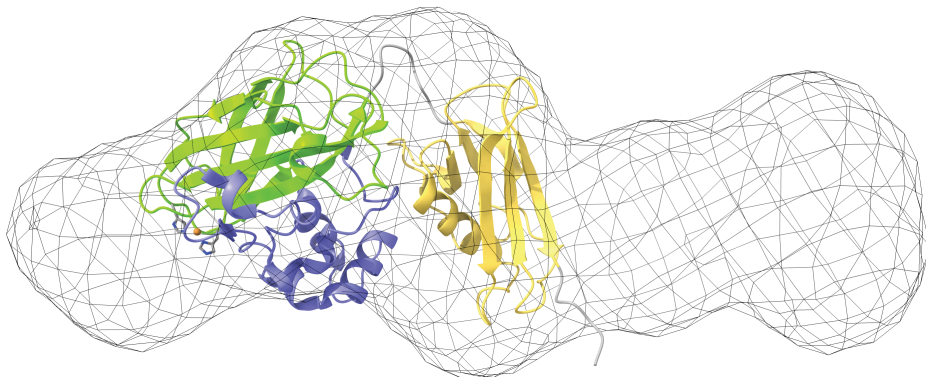
Figure S3 Position 169 in chitin-active sequences: While previous studies have identified that a short, aliphatic residue is conserved in chitin-active LPMOAA10s (residue 169 in CbpD), creating a shallow cavity on the substrate binding surface that could either accommodate the oxygen species cosubstrate or the N-acetyl group of chitin, the sequence alignment of the chitin-active chitin-active LPMOAA10s listed as characterized in the CAZy database, in addition to CbpD and the other members of the LPMO structural cluster 2 shows Arg (red boxes) is present in 16.7% of chitin-active characterized LPMOAA10s. Position numbering is based on the sequence of CbpD. Boxed LPMO names are members of the LPMO structural cluster 2 (Figure S4). The characterized substrate for each LPMOAA10 is provided to the right of the sequence alignment.



Average map value: 0.07



Average map value: 0.08



Average map value: 0.09

Figure S4: The crystal structure of CbpD adopts a more compact conformation than either observed in solution or predicted by AI: Pepsi-SAXS model (Askarian *et al.*, 2021) (SASBDB ID: SASDK42) (top, purple); RoseTTAFold model (middle, pink); and crystal structure (bottom, colored as in Figure2A) of CbpD fit into the 15 Å resolution volume map of the CbpD SAXS model envelope generated by Askarian *et al.* (2021). Average map values after fitting each structure into the density map are provided (right). His brace (sticks) and a Cu ion (sphere) placed in the active site are shown for orientation.