Supporting Information

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SI Materials and Methods

Cloning. All of the recombinant polysaccharide monooxygenases (PMOs) were cloned by using a two-step restriction-free (RF) procedure (1). Briefly, the desired sequence was amplified by PCR from a DNA template. The resulting amplicons were purified and inserted in the target plasmid in a secondary PCR. E7 and E8 coding sequences were amplified from *Thermobifida fusca* genomic DNA and cloned into the pET27b(+) vector (Novagen). E7-*a*, E7lnk-*a*, E8-*a*, E8Δ-*a*, and E8ΔΔ-*a* were cloned by inserting a sequence coding for the DocA dockerin module, amplified from the vector pET21-11A-*a* (2), at the desired position of the receiver vector pET27-E7 or pET27-E8. All PCRs were performed in a TProfessional Basic Thermocycler (Biometra) by using Phusion High-Fidelity DNA Polymerase (New England Biolabs). All enzyme constructs were designed to contain a His tag for subsequent purification. The plasmids and primers used for the restriction-free cloning are available in Table S1. The plasmid encoding the wild-type and chimeric glycoside hydrolases and the recombinant scaffoldins were obtained from previous studies (3, 4). Plasmid were maintained and propagated in *Escherichia coli* DH5α.

Expression and Purification. All recombinant proteins were expressed in *E. coli* BL21(DE3), grown in autoinduction media (5), and supplemented with the appropriate antibiotics (kanamycin: 100 $\mu g \cdot mL^{-1}$, ampicillin: 100 $\mu g \cdot mL^{-1}$) at 20 °C in UltraYield flasks (Thomson Instrument Company). Cells were harvested by centrifugation and resuspended in TBS buffer (140 mM NaCl, 3 mM KCl, and 25 mM Trisa at pH 7.4) with 10 mM imidazole. For proteins expressed in the cytosol, cell lysis was achieved by sonication and the lysate was subsequently clarified by centrifugation and filtration (0.45 μm). For proteins targeted to the periplasmic space, the periplasmic fraction was collected by cold osmotic shock (6) and then clarified by filtration (0.45 μm). A protease inhibitor mix was added to both the clarified lysate and the periplasmic fraction. Protein purification was performed by immobilized metal-ion affinity chromatography (IMAC) on a nickel-NTA column (Qiagen) by using an AKTA-prime system (GE Healthcare). Bound proteins were eluted with a gradient of imidazole in TBS buffer, and fractions were collected and analyzed by SDS polyacrylamide gel electrophoresis (SDS/PAGE) before pooling. The proper maturation of the signal peptide was checked by SDS/PAGE by comparing the size of the $(His)_6$-tagged protein located in the perisplasm (processed) and the cytosol (unprocessed). The trifunctional scaffoldin Scaf-ABT was further purified by an additional affinity-purification step: The elution fractions were incubated with 0.75 $mg \cdot mL^{-1}$ phosphoric acid swollen cellulose (PASC) (7) for 1 h at 4 °C to allow binding of the carbohydrate-binding modules (CBM) to the cellulose. The PASC matrix was washed successively with TBS buffer/1 M NaCl, and then with TBS buffer without additives. The scaffoldin was eluted with 1% triethylamine and neutralized with 1 M 2-(*N*-morpholino) ethanesulfonic acid (Mes) buffer at pH 5. The purified proteins were concentrated by ultrafiltration (Vivaspin, 10 kDa molecular mass cutoff, PES membrane; Sartorius) and buffer exchanged against TBS buffer to achieve a 10^4-fold dilution. Protein concentration was determined spectrophotometerically (OD_{280}) with a NanoDrop (ThermoScientific), using the extinction coefficient of each protein, computed by using the Protparam tool (http://web.expasy.org/protparam). Purified proteins were stored in 50% (vol/vol) glycerol at −20 °C.

Cohesin–Dockerin Interaction. The cohesin–dockerin interaction was analyzed by using an ELISA. The dockerin-bearing enzyme was coated on a MaxiSorp plate well (Nunc) by incubating 0.1 μg of protein in 0.1 M Na_2CO_3 overnight. The plate was then blocked by incubating 1 h with a solution of 2% (wt/vol) BSA, 0.05% Tween 20, and 1 mM $CaCl_2$ in TBS buffer (blocking buffer). Excess blocking solution was removed by washing three times with a wash buffer (blocking buffer without BSA). A CBM-bearing cohesin was then incubated for 1 h with the coated dockerin-bearing enzyme in wash buffer. After washing, the CBM cohesins bound to the plate were detected by using a primary rabbit α-CBM antibody and a secondary goat α-rabbit antibody labeled with a horseradish peroxidase. Antibody detection was performed by using 3,3′,5,5′-tetramethylbenzidine (ThermoScientific) and quantified spectrophotometrically (OD_{450}).

Designer Cellulosome Assembly. An equimolar mixture of the different proteins constitutive of the designer cellulosome was prepared in a solution of 50 mM sodium acetate at pH 5.5, 1 mM $CaCl_2$, 0.05% Tween 20, 2 mM ascorbic acid, 1 μM $CuSO_4$, and incubated for 2 h at 37 °C. The mixture was then split in two equal fractions, combined with either native or denaturing (1% SDS) sample buffer. The electrophoretic mobility of the proteins was analyzed by PAGE under native and denaturing conditions. The native gel was comprised of a 4.3% stacking gel and a 9% separation gel, whereas the denaturing gel was comprised of a 5% stacking gel and a 10% separation gel. Migration was carried out at 100 V. The gels were stained by using InstantBlue Coomassie-based staining (Expedeon).

Cellulose Degradation Assays. Cellulose degradation was assayed by using 10 $mg \cdot mL^{-1}$ of microcrystalline cellulose (Avicel PH-101; Sigma Aldrich) as substrate, in 50 mM sodium acetate at pH 5.5, 1 mM $CaCl_2$, 2 mM ascorbic acid, 1 μM $CuSO_4$, and 0.05% Tween 20 at 50 °C in a vertical shaker incubator for 72 h. Enzyme concentrations were 1 μM for the wild-type and chimeric PMOs activity assay, 1 μM for the CBM-restoration assays (coupled to 1 μM Scaf-A), and 0.5 μM for the designer cellulosomes assays (coupled to 1 μM of each cellulase and 1 μM Scaf-ABT). In the case of the designer cellulosomes, an equimolar mixture of the different components was first allowed to interact for 2 h at 37 °C in the reaction buffer without ascorbic acid, before addition of the missing reagent and substrate. The reactions were stopped after 72 h by addition of 50 mM NaOH. The substrate was removed by centrifugation, and the supernatant stored at −20 °C before analysis of the soluble sugars.

Soluble Sugar Analysis. Soluble sugars released during the enzymatic degradation of cellulose were analyzed by high-performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) by using an Dionex ICS3000 system equipped with a CarboPac PA1 column coupled to a CarboPac PA1 guard column (both from Dionex). The amperometric detector was equipped with a disposable gold electrode (Dionex), and the standard carbohydrate waveform recommended by the manufacturer was applied. Injection volume was set at 20 μL for all samples. Flow was set at 1 $mL \cdot min^{-1}$. Oxidized and native oligosaccharides were eluted by using a method derived from Westereng et al. (8): Initial conditions were set to 0.1 M NaOH (eluent A), followed by a linear gradient toward an increasing proportion of eluent B (1 M NaOAc in 0.1 M NaOH). The gradient reached 10% B at 10 min after

injection and 30% B at 25 min; these steps were followed by a 5-min linear gradient to 100% B to flush the column. Reconditioning was achieved by running initial conditions for 9 min. Because this protocol did not allow for an efficient separation of glucose and gluconic acid, these monosaccharides were quantified separately by using an isocratic elution at 20 mM NaOH (9). Peak assignment was based on the analysis of individual standards. Cellobiose, cellotriose, cellotetraose, and cellopentaose were purchased from Megazyme. Gluconic acid and glucose were purchased from Sigma Aldrich. Oxidized oligosaccharides were obtained via enzymatic oxidation of native standards by recombinant cellobiose dehydrogenase (rCDH) from *Pycnoporus cinnabarinus*. The rCDH was prepared according to Bey et al. (10) and incubated at 0.2 $\mu g \cdot \mu L^{-1}$ with 500 $\mu g \cdot mL^{-1}$ of native oligosaccharide in 80 mM citrate buffer pH 5 at 50 °C for 24 h.

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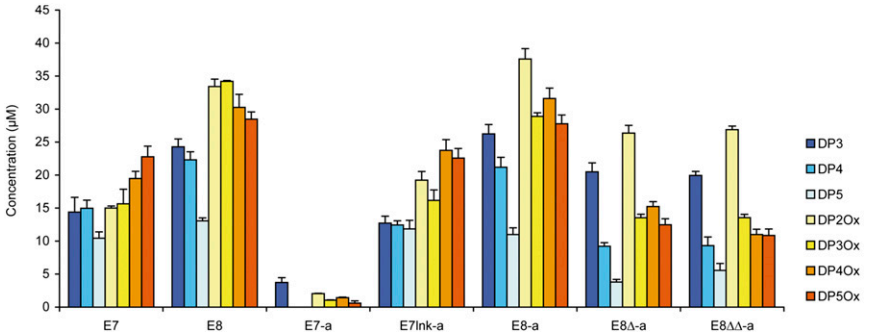

Fig. S1. Oxidative cleavage of cellulose by E7, E8, and their dockerin variants. Quantification was by HPAEC analysis of the soluble sugar was released from the cleavage of microcrystalline cellulose (Avicel, 10 mg/mL) by 1 μM PMO. The concentration (in micromolars) of each sugar was determined by integration of the peak area, and comparison was with a standard curve. Values are the mean of three biological replicates ($n = 3$). Error bars correspond to one SD (error bar = ±SD).

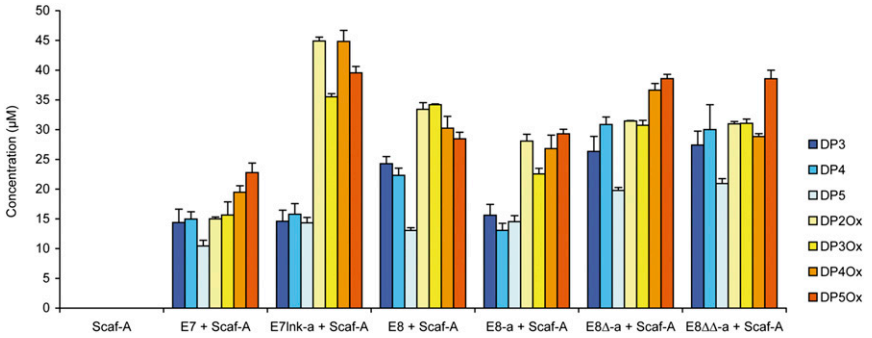

Fig. S2. Restoration of the substrate targeting by scaffoldin-borne CBM. Quantification was by HPAEC analysis of the soluble sugar released from the cleavage of microcrystalline cellulose (Avicel, 10 mg/mL) by 1 μM PMO + 1 μM Scaf-A. The concentration (in micromolars) of each sugar was determined by integration of the peak area, and comparison was with a standard curve. Values are the mean of three biological replicates ($n = 3$). Error bars correspond to one SD (error bar = ±SD).

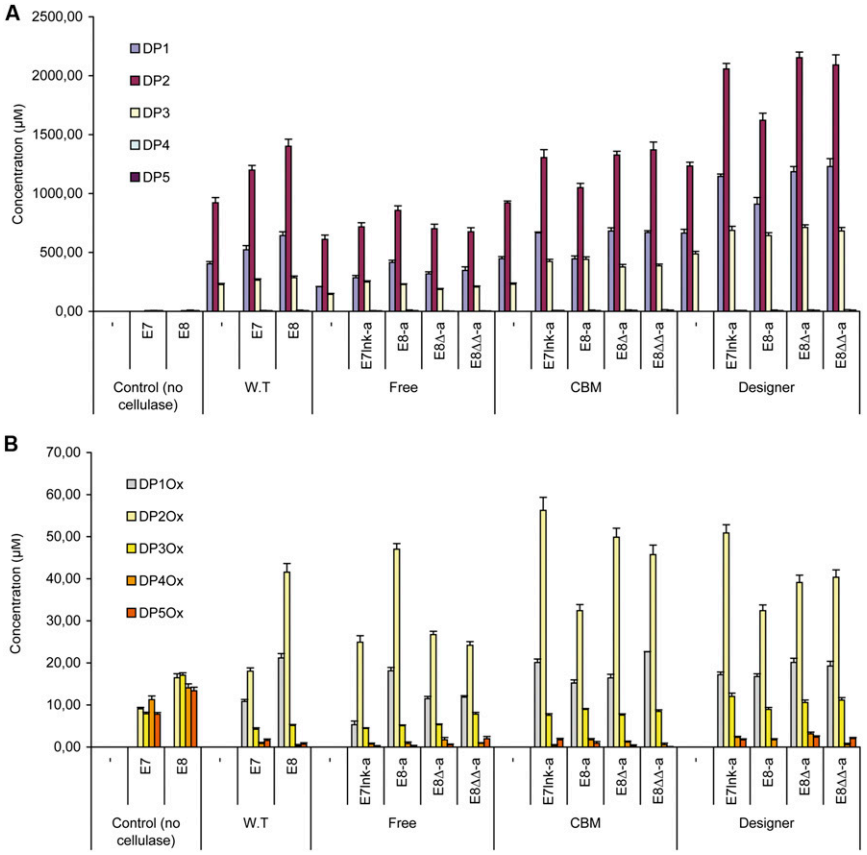

Fig. S3. Degradation of cellulose by PMO-containing designer cellulosomes. Quantification was by HPAEC analysis of the soluble sugars released from the cleavage of microcrystalline cellulose (Avicel, 10 mg/mL). Different architectures were assayed, such as the following: free enzymes (Control, W.T. and Free), enzymes bound to monovalent scaffoldins ("CBM"), and enzymes bound to trivalent scaffoldin (Designer). Each protein was added at a final concentration of 0.5 μM. Concentrations of reducing sugars (*A*) and oxidized sugars (*B*) are shown. Values are the mean of three biological replicates ($n = 3$). Error bars correspond to one SD (error bar = ±SD).

Table S1. Plasmids and primers used for the restriction-free cloning of the recombinant enzymes

Vector name	Forward primer* (5′ – 3′)	Reverse primer* (5′ – 3′)	Primary PCR template	Secondary PCR template
pET27-E7	TGCTGGTCTGCTGCTCCTCGCTGCCCAGCCGG-CGATGGCCcacgggtcggtcatcaac	ATCTCAGTGGTGGTGGTGGTGGTGCTCGAC-gacgaagttcacgtcgctg	*T. fusca* genomic DNA	pET27b(+)
pET27-E7-*a*	TACTACCTGTGCAGCGACGTGAACTTCGTCga-gctcactacaacaccaac	ATCTCAGTGGTGGTGGTGGTGGTGCTCGAC-ttcttctttctcttcaacag	pET21a-11A-*a*	pET27-E7
pET27-E7lnk- *a*	TACTACCTGTGCAGCGACGTGAACTTCGTCgg-cggcggcgacgacggcgg	ATCTCAGTGGTGGTGGTGGTGGTGCTCGAC-ttcttctttctcttcaaca	pET27-E8ΔΔ-DocA	pET27-E7
pET27-E8	CTGCTCCTCGCTGCCCAGCCGGCGATGGCCca-cggcgcgatgacctac	ATCTCAGTGGTGGTGGTGGTGGTGCTCGAC-ggccacggagcagaggctg	*T. fusca* genomic DNA	pET27b(+)
pET27-E8- *a*	CTGCTCCTCGCTGCCCAGCCGGCGATGGCCca-cggcgcgatgacctac	ATCTCAGTGGTGGTGGTGGTGGTGCTCGAC-ttcttctttctcttcaacag	pET21a-11A-*a*	pET27-E8
pET27-E8Δ- *a*	TTCAACTCGGGTGACGCTCCCGCGCTCGCCga-gctcactacaacacca	ATCTCAGTGGTGGTGGTGGTGGTGCTCGAC-ttcttctttctcttcaacag	pET21a-11A-*a*	pET27-E8
pET27-E8ΔΔ- *a*	GACGACGGCGGTTCGGGCGGTCCTCAGCCGga-gctcactacaacaccaa	ATCTCAGTGGTGGTGGTGGTGGTGCTCGAC-ttcttctttctcttcaaca	pET21a-11A-*a*	pET27-E8

*Lowercase letters correspond to the sequence complementary to the primary PCR template. Uppercase letters correspond to the sequence complementary to the secondary PCR template.