Supplementary Information

Experimental Procedures

Construction of expression vectors for recombinant proteins: The gene encoding CD_{CelD} and CBM3a were synthesized from several oligonucleotides and external primers by means of overlap extension polymerase chain reaction (PCR) with LA-taq DNA polymerase, and the gene encoding CBM4 was amplified from the vector obtained from Toyota Central R&D Laboratories (Nagakute, Japan) by means of PCR with KOD+ polymerase and two external primers. Each of the CD_{CelD}, CBM3a and CBM4 fragments produced was inserted into the *Nco*I–*Sac*II site of pRA2b vectors containing an IgA hinge linker (SPSTPPTPSPSTPP), a biotin acceptor peptide (AviTag; GGLNDIFEAQKIEWH), and a poly-histidine tag (HHHHHH) in that order at the C-terminus, to produce the plasmids for CD_{CelD}, CBM3a, and CBM4 with an AviTag at the C-terminus (pRA2b-bioCelD, pRA2b-bioCipA, and pRA2b-bioCBD2endc).

The gene encoding intact CelD with a poly-histidine tag at the N-terminus (polyHis–CD_{CelD}–dockerin) and CBM3a with a poly-histidine tag at the N-terminus and the seventh cohesin of CipA (from *Clostridium thermocellum*) at the C-terminus (polyHis–CBM3a–cohesin) was also synthesized from several oligonucleotides and external primers by means of overlap extension PCR with KOD+ polymerase. Each of the gene fragments produced was inserted into the *Nco*I–*Spe*I site of pRA2b vectors (IgA hinge linker, AviTag, and poly-histidine tag were eliminate from the pRA2b vector by the digestion with *Nco*I and *Spe*I) to generate the plasmids for intact CelD and cohesion-fused CBM3a (pRA2b-CelD and pRA2b-CipAcohesin).

Preparation of recombinant proteins: To prepare intact CelD and cohesin-fused CBM3a, *E. coli* BL21 Star (DE3) star cells were transformed by the plasmid of pRA2b-CelD and pRA2b-CipAcohesin, and the transformed *E. coli* cells were incubated in 2 × YT medium containing 100 μg/ml ampicillin at 28 °C. Each protein was induced by adding 1 mM

isopropylthiogalactoside, and then the transformed *E. coli* cells were incubated at 20 °C. The harvested cells were centrifuged, and the pellet was suspended in a Tris-HCl solution (50 mM, pH 8.0) with 200 mM NaCl. After sonication, the suspension was centrifuged at 6300×*g* for 30 min, and the supernatant was purified on a metal–chelate chromatography column. For intact CelD, gel filtration chromatography (Hi-Load 26/60 Superdex 200 size exclusion column, GE Healthcare, Little Chalfont, UK) was further performed.

For CD_{CelD}, CBM3a, and CBM4 with a biotinylated tag, we first transformed *E. coli* BL21 Star (DE3) by the plasmid of pBIRAcm encoding biotin ligase (Avidity Inc., Aurora, CO) and then transformed the same cells by the plasmids of pRA2b-bioCelD, pRA2b-bioCipA, and pRA-bioCBD2endc, respectively. The transformed *E. coli* cells were incubated in 2 × YT medium containing 100 μg/ml ampicillin and 34 μg/ml chloramphenicol at 28 °C, and each protein and biotin ligase were induced by adding 1 mM isopropylthiogalactoside in the presence of 50 μM of D-biotin (Sigma, St. Louis, MO). After the induction, transformed *E. coli* cells were incubated at 20 °C. The harvested cells were centrifuged, and the pellet was suspended in a Tris-HCl solution (50 mM, pH 8.0) with 200 mM NaCl. After sonication, the suspension was centrifuged at 6300×*g* for 30 min, and the supernatant was purified on a metal–chelate chromatography column. For CD_{CelD}, gel filtration chromatography was also performed. The fractions containing proteins with biotinylated tags were collected and stored after the presence of biotin was confirmed by means of western-blotting analysis using streptavidin–horseradish peroxidase (GE Healthcare).

Clustering of biotinylated CD_{CelD} and CBM: The biotinylated CD_{CelD} and CBM were mixed with streptavidin at various concentration (nM) ratios (CD_{CelD}:CBM:streptavidin = 40:0:0, 40:120:0, 40:0:10, 40:13:13, 40:40:20, 40:120:40) in a 50 mM MES buffer solution (pH 6.0) with 10 mM $CaCl_2$ at 4 °C for 24 h. For the clustering of CD_{CelD} and CBM on nanoparticle, commercially obtained streptavidin-conjugated CdSe nanoparticles (particle size: 20 nm, Invitrogen, Carlsbad, CA) were mixed with biotinylated CD_{CelD} and CBM at various concentration (nM) ratios (CD_{CelD}:CBM:CdSe = 40:0:1.3, 40:13:1.8, 40:40:2.7, 40:120:5.3) in the sodium acetate solution at

4 °C for 24 h. In the case of the preparation of the CD-CBM clusters for avicel degradation, the biotinylated CD_{CelD} and CBM were mixed with streptavidin and nanoparticle at the concentration (μM) ratios of CD_{CelD}:CBM:streptavidin = 2.5:7.5:2.5 and CD_{CelD}:CBM:nanoparticle = 2.5:7.5:0.33, respectively.

Preparation of PSC: Phosphoric-acid swollen cellulose (PSC) was prepared from Avicel (Fluka, PH-101) according to a previously published method (Walseth, TAPPI, 1952, 35, 228–233). Avicel (4 g) was dissolved in 100 ml of phosphoric acid. After stirring for 1 h at 4 °C, the solution was diluted with 1900 ml of cold water. After stirring for 1 h at 4 °C, the extracted amorphous cellulose was collected by filtration with filter paper. The amorphous cellulose was washed 4 times with ultrapure water, 2 times with 1% $NaHCO_3$ (neutralization), and then 3 more times with ultrapure water. The cellulose paste was homogenized (2 min × 3) with a Multi-brander mill BLA-501 (Nihonseiki Kaisha Ltd., Japan). The resultant slurry was resuspended with 50 mM sodium acetate buffer (pH 5.0) for enzyme assay and stored at 4 °C.

Degradation activity assays: Clustered CD_{CelD}–CBM complexes were added to a 50 mM MES buffer solution (pH 6.0, 10 mM $CaCl_2$) containing 1 mg/ml PSC or 10 mg/ml avicel at 45 °C. All the final concentrations of CD_{CelD} in the reaction solution were adjusted to 40 nM and 2.5 μM for degradation activity assays against PSC and avicel, respectively. After incubation intervals (10 min–96 h), 5 μl of the supernatant was mixed with 195 μl of tetrazolium blue chloride (TZ) assay buffer (1 mg/ml tetrazolium blue chloride, 0.5 M sodium tartrate, 200 mM NaOH, pH 5.0) at 100 °C for 3 min. The reacted solutions were rapidly cooled in an ice bath, and the absorbance of the solutions at 655 nm was measured. The concentrations of produced reducing sugars were estimated from the absorbance by normalizing with that of glucose reacted in TZ-assay buffer.

Analysis of Binding Affinities of CBM to PSC and avicel: Substrates (1 mg) were added to 1 ml of 50 mM MES buffer solution (pH 6.0, 10 mM $CaCl_2$) containing clustered CD-CBM complexes, and

the mixture was incubated for 30 min at 4 °C. After centrifugation, the absorbance at 280 nm in the supernatant was measured to quantify the residual proteins in the supernatant.