Electronic Supplementary Information

Enzymatic Synthesis and Post-Functionalization of Two-Dimensional Crystalline Cellulose Oligomers with Surface-Reactive Groups

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Experimental details

Materials. α-D-Glucose 1-phosphate (αG1P) disodium salt n-hydrate was purchased from Wako Pure Chemical Industries. 1-Azide-1-deoxy-β-D-glucopyranoside was purchased from Sigma-Aldrich. 1-Ethynyl pyrene was purchased from Tokyo Chemical Industry Corporation. EM stainer was purchased from Nissin EM Corporation. All other reagents were purchased from Nacalai Tesque. Ultrapure water with more than 18.2 MΩ·cm was supplied by a Milli-Q system (Merck Millipore) and was used throughout all of the experiments.

Preparation and activity assay of cellodextrin phosphorylase (CDP). Preparation and purification of CDP from *Clostridium thermocellum* YM4 and determination of the enzymatic activity were performed according to the established method.[1] CDP was prepared using an *Escherichia coli* BL21-Gold(DE3) strain containing a plasmid including *cdp* gene and was purified by Ni-NTA column (GE Healthcare) using fused His-tag. Then, the buffer of the CDP solutions was exchanged to 20 mM 3-morpholinopropane-1-sulfonic acid (MOPS)-sodium buffer (pH 7.5) containing 0.02% sodium azide. The enzymatic activity was determined by quantification of the amount of inorganic phosphate produced from 10 mM αG1P in the presence of 10 mM cellobiose in 50 mM MOPS-sodium buffer (pH 7.5) at 37 °C. One unit of the activity was defined as the amount of enzyme that could produce 1 μmol of inorganic phosphate per minute from αG1P at 37 °C, as previously described.[1]

CDP-catalyzed synthesis of cellulose oligomers. Cellulose oligomers as control samples were synthesized using CDP according to the previous study.[1] Purified CDP (0.2 U/mL) was incubated with αG1P (200 mM) as a monomer and β-D-glucose (50 mM) as a primer in 500 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.5) for 3 days at 60 °C. After incubation, the water-insoluble products were washed by ultrapure water through centrifugation/redispersion cycles (20,400 rpm) more than 5 times and were then heated at 100 °C for 10 minutes to inactivate CDP. The resultant products were stored at 4 °C until use.

CDP-catalyzed synthesis of azide-containing cellulose oligomers. Except for using 1-azide-1-deoxy-β-D-glucopyranoside as a primer, all of the procedures were the same as the synthesis of cellulose oligomers.

Post-functionalization of surface-azidated two-dimensional (2D) crystalline cellulose oligomers. Surface-azidated 2D crystalline cellulose oligomers (0.45% (w/v)) were incubated with 1-ethynyl pyrene (4.5 mM), copper(II) sulfide (0.45 mM), and ascorbic acid (1.1 mM) in DMF at ambient temperature for 1 day. After incubation, the orangish products were washed by DMF through centrifugation/redispersion cycles (20,400 rpm) more than 5 times. The resultant products were stored at 4 °C under light shielding conditions.

Attenuated total reflection-Fourier transform infrared (ATR-FTIR) absorption spectroscopy. Products dispersed in ultrapure water were lyophilized for 1 day. Spectra were obtained with the cumulative number of 100 under ambient conditions in the wavelength between 350-7800 cm^{-1} with a resolution of 2.0 cm^{-1} on a JASCO FT/IR-4100 spectrometer.

^{1}H nuclear magnetic resonance (NMR) spectroscopy. Lyophilized products were dissolved in 4% NaOD/D_2O. ^{1}H NMR spectra of product solutions (3% (w/v)) were recorded on a Bruker DPX-300 spectrometer operated at 300 MHz. The chemical shifts were recorded relative to D_2O (δ 4.79).

Transmission electron microscopy (TEM) observations. A collodion-coated copper EM grid was placed on a droplet of 2D crystalline cellulose oligomers (0.2% (w/v)) for 1 h and was then placed on a droplet of ultrapure water. The sample was negatively stained with EM stain. The prepared grid was dried in a desiccator for at least 12 h. TEM images were taken with a Hitachi H-7650 Zero A microscope operated at 100 kV.

Ultraviolet-visible (UV-Vis) absorption spectroscopy. UV-Vis absorption spectra of pyrene-conjugated 2D crystalline cellulose oligomers (0.0034% (w/v)) dispersed in DMF were measured using a JASCO V-550 or V-670. The measurements were conducted under ambient conditions in the wavelength range between 200-850 nm with a resolution of 0.5 nm and a scanning speed of 400 nm/min.

Fluorescence spectroscopy measurements. Fluorescence spectra of pyrene-conjugated 2D crystalline cellulose oligomers (0.0034% (w/v)) dispersed in DMF were obtained using a JASCO FP-6500. The measurements were conducted by excitation at 386 or 440 nm. 1-Ethynyl pyrene (6 μM) dissolved in DMF as a reference was excited at 300 nm. All measurements were performed at 25 °C in the wavelength range between 200-700 nm with a resolution of 1 nm and a scanning speed of 1000 nm/min.

Circular dichroism (CD) spectroscopy. CD spectra of pyrene-conjugated 2D crystalline cellulose oligomers (0.034% (w/v)) dispersed in DMF were recorded on a JASCO J-820 under N_2 atmosphere at 25 °C using a quartz cell with a thickness of 0.2 cm. The data represent the average of 4 scans in the wavelength range of 190-850 nm with a resolution of 0.5 nm and a scanning speed of 100 nm/min.

Elemental analysis. Elemental analysis of lyophilized samples was conducted using a YANACO CHNcorder MT-6 using antipyrine or caffeine as standards.

Wide-angle X-ray diffraction (WAXD) measurements. Dispersions of 2D crystalline cellulose oligomers were cast on a plastic plate in a desiccator for 3 days. WAXD measurements of the resulting film were performed under ambient condtions using a Rigaku Nanoviewer with Cu Kα radiation (λ = 0.154 nm).

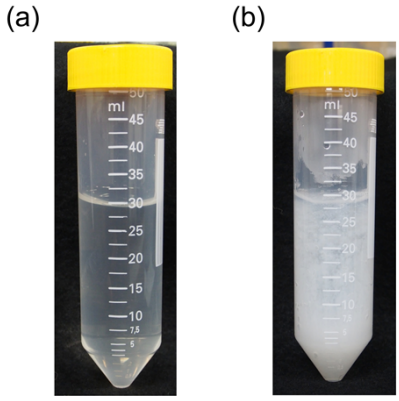

Fig. S1 Photographic images of the reaction solutions (a) before and (b) after the incubation for the enzymatic synthesis of the 2D crystalline cellulose oligomers using αG1P monomers and β-glucosyl azide primers by CDP.

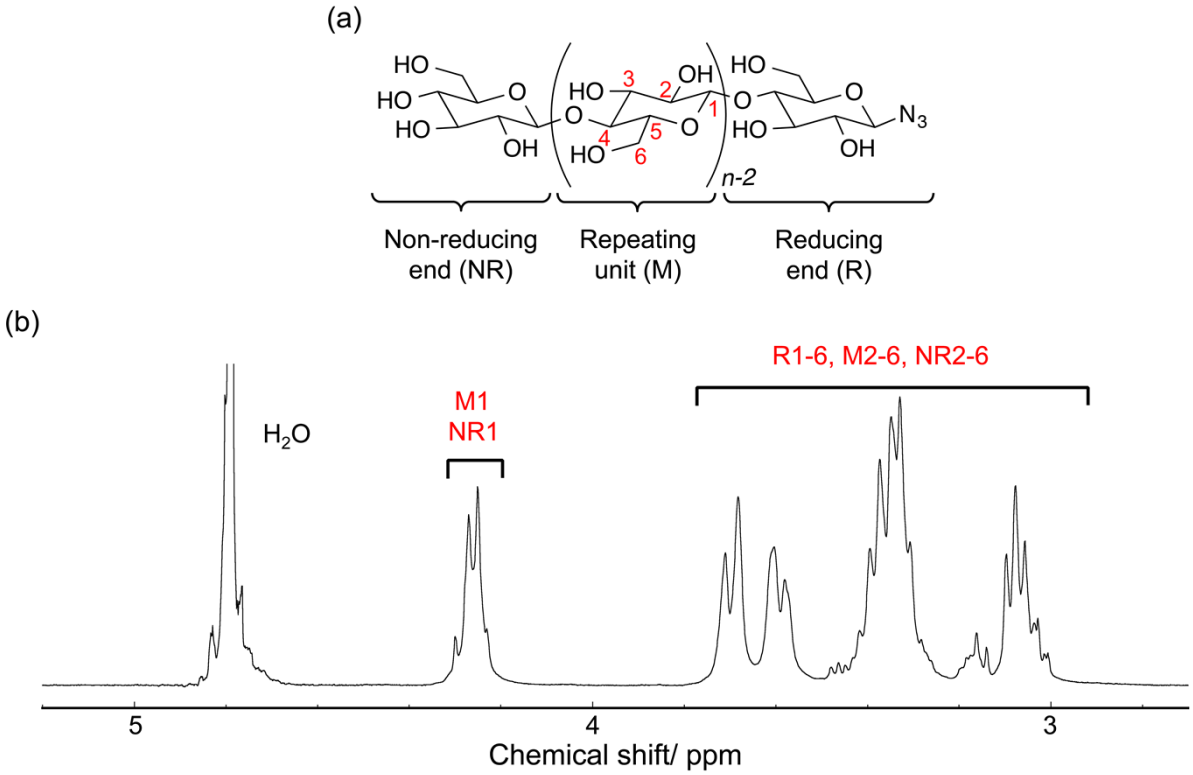

Fig. S2 (a) Chemical structure and (b) ^{1}H NMR spectra of azide-containing cellulose oligomers prepared by CDP-catalyzed enzymatic reactions using αG1P monomers and β-glucosyl azide primers.

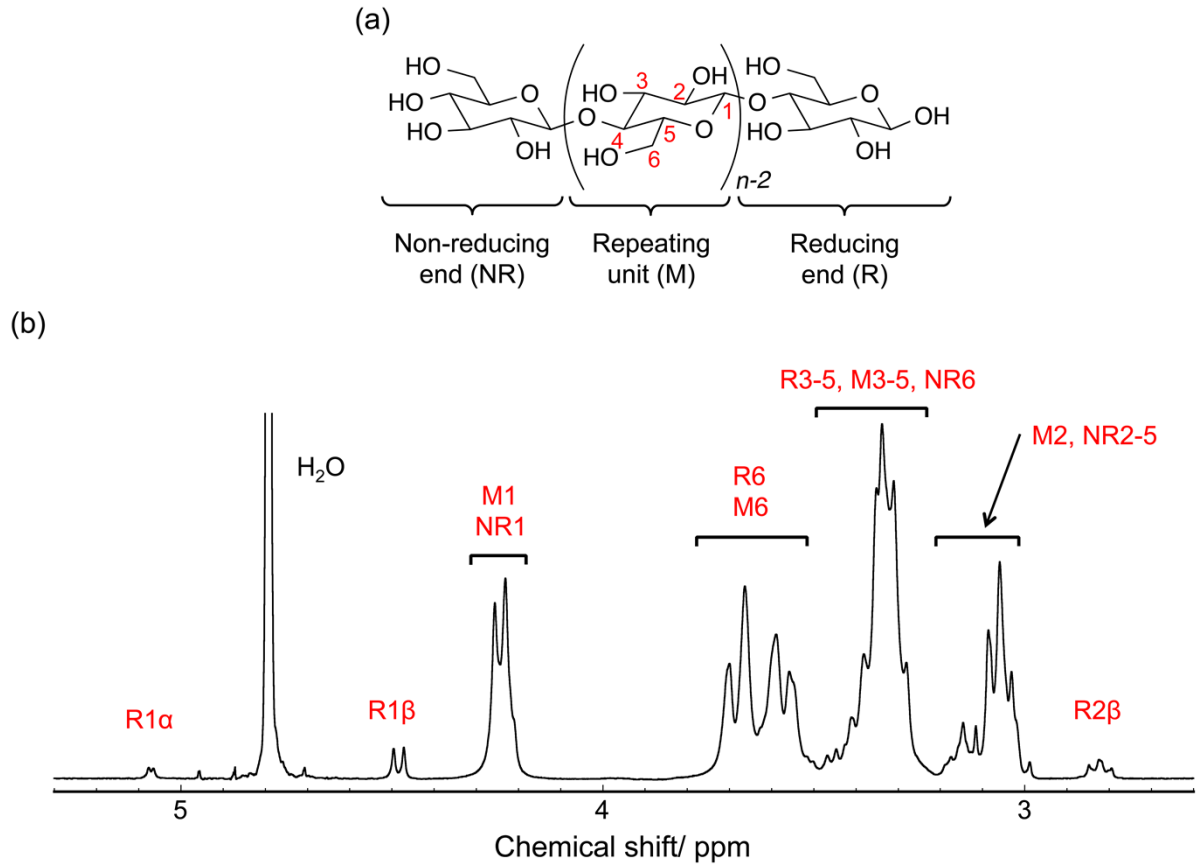

Fig. S3 (a) Chemical structure and (b) ^{1}H NMR spectra of cellulose oligomers prepared by CDP-catalyzed enzymatic reactions using αG1P monomers and β-D-glucose primers.

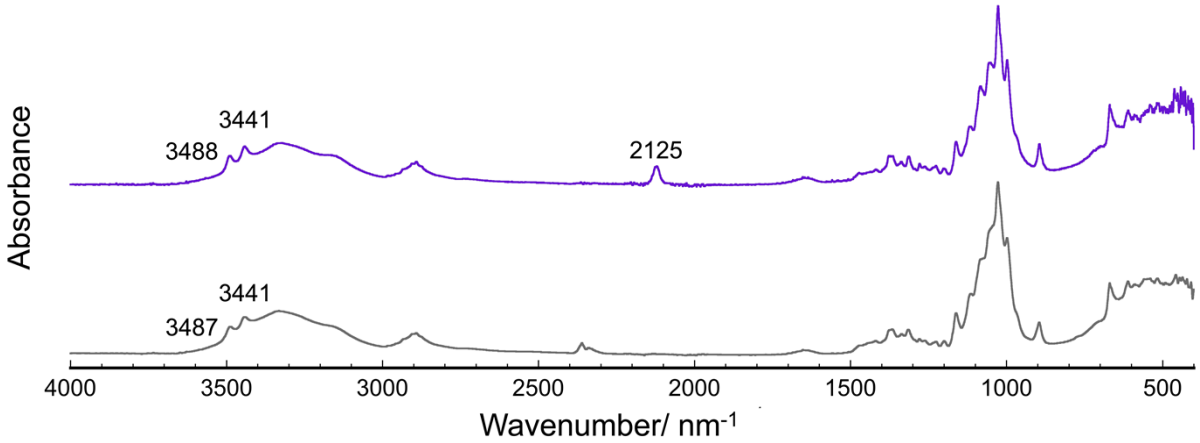

Fig. S4 ATR-FTIR spectra of 2D crystalline cellulose oligomers with surface-reactive azide groups (purple) and 2D crystalline cellulose oligomers (gray).

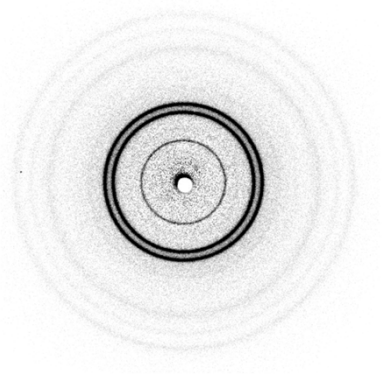

Fig. S5 WAXD diagrams of films composed of 2D crystalline cellulose oligomers with surface-reactive azide groups.

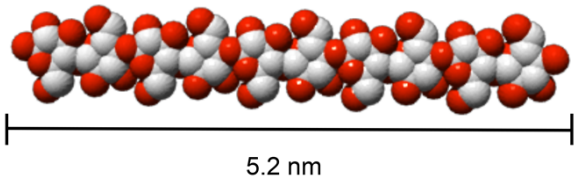

Fig. S6 A CPK model of cellodecaose in the cellulose II allomorph.

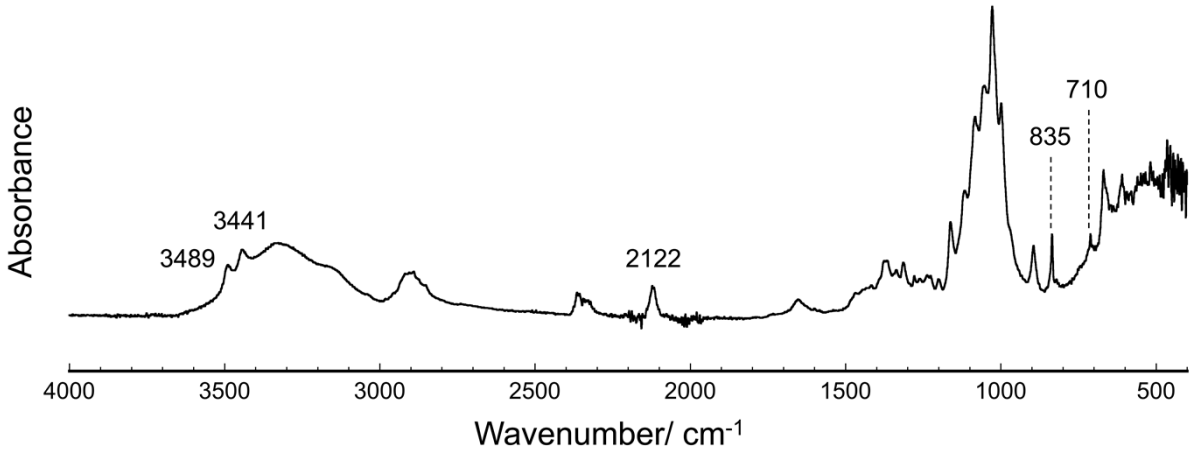

Fig. S7 ATR-FTIR spectra of pyrene-conjugated 2D crystalline cellulose oligomers.

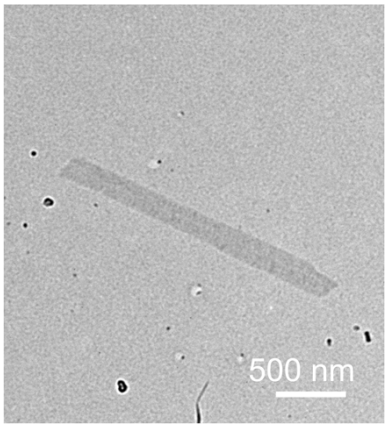

Fig. S8 TEM images of pyrene-conjugated 2D crystalline cellulose oligomers.

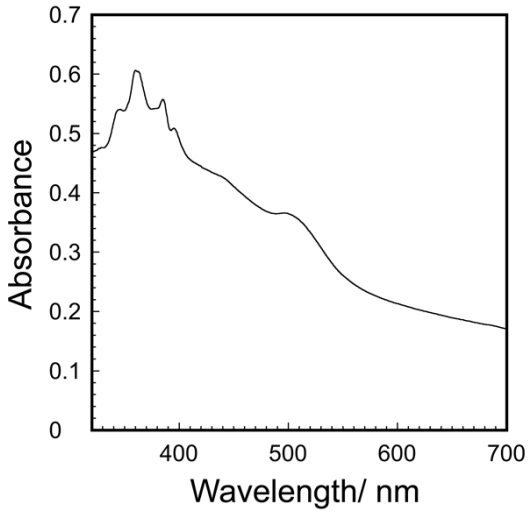

Fig. S9 UV-Vis absorption spectra of pyrene-conjugated 2D crystalline cellulose oligomers dispersed in DMF (0.0034% (w/v)).

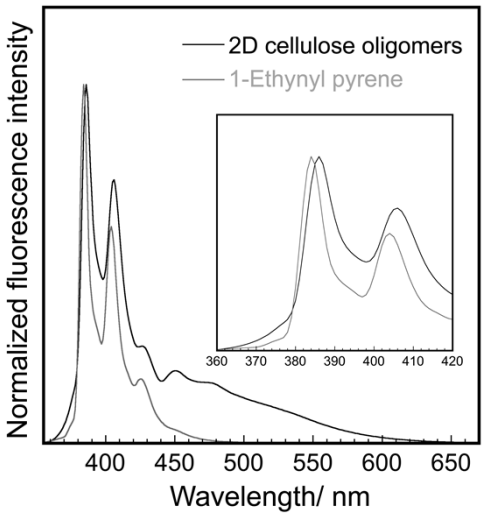

Fig. S10 Fluorescence spectra of pyrene-conjugated 2D crystalline cellulose oligomers (0.0034% (w/v), black) and 1-ethynyl pyrene (6 μM, gray) in DMF. The spectra were normalized against the main peak at 385 nm. The inset highlighted the region where the main peaks were located.

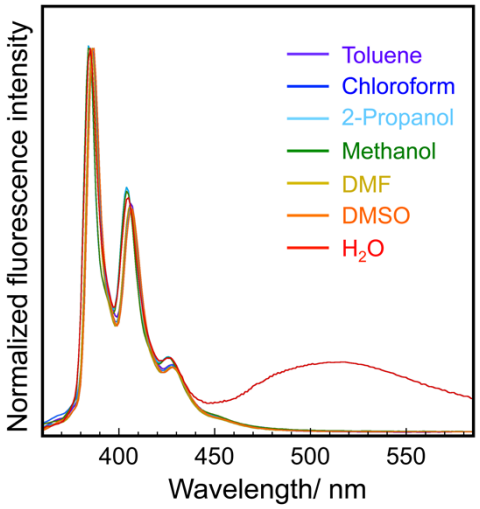

Fig. S11 Fluorescence spectra of 1-ethynyl pyrene (6 μM) exited at 440 nm in various solvents. The spectra were normalized against the main peak at 385 nm.

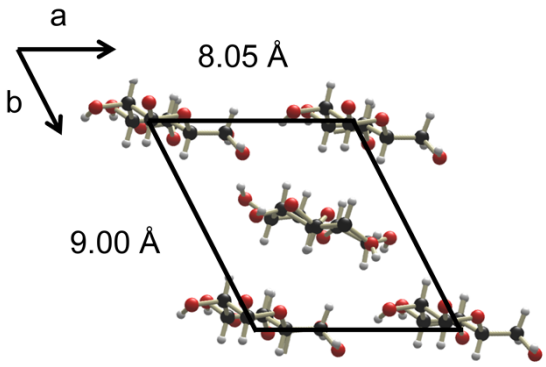

Fig. S12 Crystal lattice of the cellulose II allomorph.

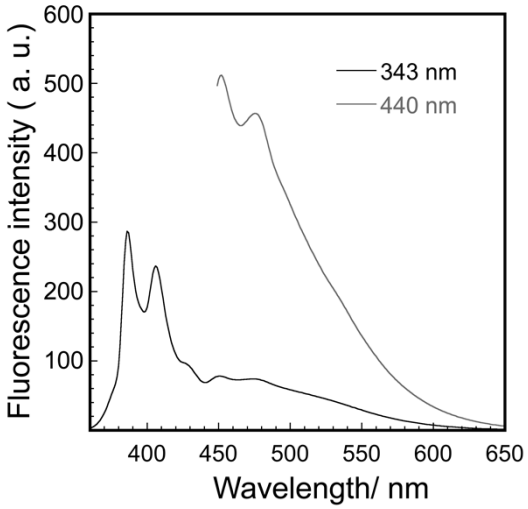

Fig. S13 Fluorescence spectra of pyrene-conjugated 2D crystalline cellulose oligomers dispersed in DMF (0.0034% (w/v)) by excitation at 343 nm (black) or 440 nm (gray).

Table S1 Weight ratio of each element for azide-containing cellulose oligomers.

C	H	N
0.4092	0.0600	0.0231

Table S2 Weight ratio of each element for pyrene-conjugated cellulose oligomers.

C	H	N
0.4507	0.0578	0.0202

Reference

1. M. Hiraishi, K. Igarashi, S. Kimura, M. Wada, M. Kitaoka and M. Samejima, *Carbohydr. Res.*, 2009, **344**, 2468-2473.