Supplemental Information

SI1. AFM Imaging and SPR Imaging Before and After Exposure to Enzyme Mixture in the Sample Chamber and Buffer in the Reference Chamber of the Fluid Cell

To verify that cellulose is removed from the substrate due to enzymatic degradation, we performed atomic force microscopy (AFM) imaging of cellulose microfibril-coated regions before and after exposure to the cellulase enzyme mixture and buffer in the SPR imaging experiment. In Figure SI1A and B, AFM images are shown corresponding to a cellulose microfibril-coated region before and after exposing the substrate to the enzyme mixture for 21 h (sample chamber of fluid cell). In Figure SI1C, corresponding AFM line scans are shown that demonstrate that the height of the cellulose microfibril-coated regions is dramatically reduced after exposure to the enzyme mixture. In Figure SI1D and E, AFM images are shown of a cellulose microfibril-coated region on the same sample that was exposed to buffer but not enzyme mixture (reference chamber of fluid cell). In Figure SI1F, corresponding AFM line scans are shown that demonstrate that the height of the cellulose microfibril-coated regions is not significantly different following exposure to the buffer.

In Figure SI2A and B, we show SPR images before and after exposure to the cellulose enzyme mixture for a SPR slide with similar initial cellulose microfibril coverage as for the AFM images shown in Figure SI1. The angle of incidence $\theta_i = 49.2^o$ was chosen to correspond approximately to the minimum in reflected light intensity for the uncoated regions, with the cellulose microfibril-coated regions appearing as brighter regions. It can be seen in Figure SI2 that exposure to the enzyme mixture for 3 h results in a significant reduction of the SPR intensity for the ROI on the microfibril-coated region in the sample chamber indicated, for example, by the white rectangles, corresponding to a decrease in the cellulose coverage. In contrast, exposure to buffer results in no measurable decrease in the SPR intensity for the ROI on the microfibril-coated region in the reference chamber, as can be seen by comparing Figure SI2 C and D. Collectively, the AFM imaging and SPR imaging results are consistent with the removal

of the cellulose microfibrils by exposure to the enzyme mixture but not by exposure to the buffer.

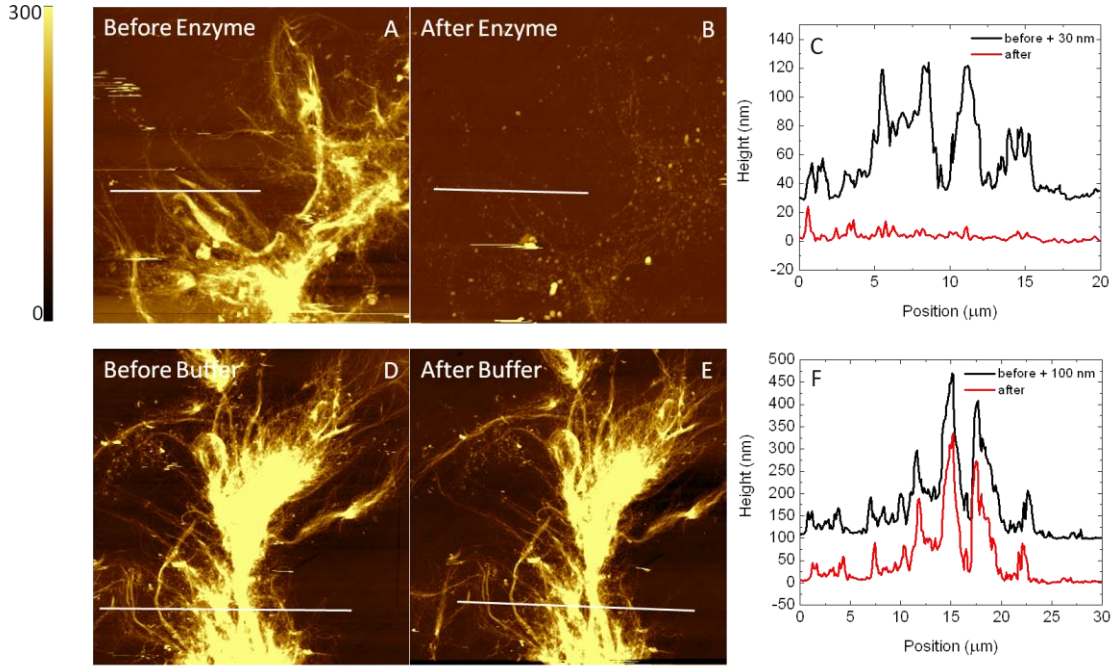

Figure SI1: AFM topography images and line scans for cellulose microfibril-coated regions. The images in parts A and B correspond to the same area on the sample before and after exposure to the cellulase enzyme mixture for 21 h. The line scans in part C correspond to the white lines in parts A and B. The images in parts D and E correspond to the same area on the sample before and after exposure to the buffer for 21 h. The line scans in part F correspond to the white lines in parts D and E. Each of the AFM images corresponds to a sample area of 40 μm × 40 μm.

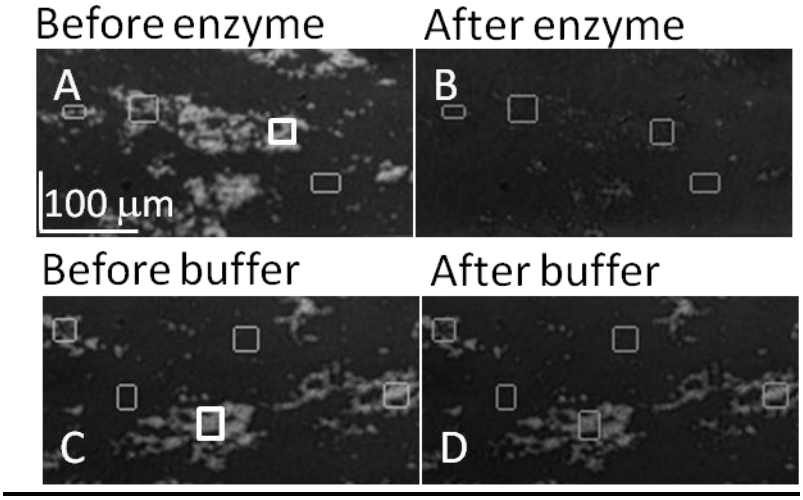

Figure SI2: SPR images for cellulose microfibril-coated regions. The images in parts A and B correspond to the same area on the sample before and after exposure to the cellulase enzyme mixture for 3 h. The sample area shown in parts A and B is from a SPR slide with similar initial cellulose microfibril coverage as for the AFM images shown in Figure SI1. The images in parts C and D correspond to the same area on the sample before and after exposure to the buffer for 3 h. The sample area shown in parts C and D is from a SPR slide with similar initial cellulose microfibril coverage as for the AFM images shown in Figure SI1.

SI2. Comparison of Bulk Enzyme Assay and SPR Imaging Measurement of Enzymatic Degradation

To verify that the cellulose microfibrils are degraded by exposure to the cellulase enzyme mixture, we performed a colorimetric carbohydrate assay[S1] on SPR slides with a high degree of surface coverage of cellulose microfibrils that were exposed to the enzyme mixture. This assay is used to measure the production of glucose that results from enzymatic degradation of cellulose by monitoring the change in color of the cellulose suspension through the use of *p*-hydroxybenzoic acid hydrazide (PAHBAH) in a basic environment. We performed the assay twice using two different cellulose microfibril-coated SPR slides with an enzyme concentration of 20 μg/mL in 50 mM citrate buffer at

pH 5.4 at 50 ^{o}C. To measure the glucose concentration after exposure to the cellulase enzyme mixture for 17 h for each of the cellulose microfibril-coated SPR slides, we removed 3 aliquots of 125 μL of the suspension, added 125 μL of the colour reagent (0.5 M PAHBAH, 0.3 M sodium hydroxide, 0.05 M sodium sulfite, 0.02 M tri-sodium citrate, 0.01 M calcium chloride; all components obtained from Sigma Aldrich) to each of the aliquots, pulse centrifuged the solution (Sorvall Legend Micro 21R microcentrifuge), heated the mixture to 100 ^{o}C for 8 min, cooled the mixture in a water bath to room temperature, pipetted 50 μL of the mixture into a microplate, diluted with 150 μL of Milli-Q water, and measured the absorbance at a wavelength of 415 nm using a Molecular Devices Vmax kinetic microplate reader. Exposure of the cellulose microfibril-coated SPR slides to the cellulase enzyme mixture for 17 h yielded measured absorption values between 0.23-0.30, corresponding to a glucose level of approximately 0.4 mM, which was significantly larger than the threshold detection level of the assay (absorption value of 0.05) (see Figure SI3). This measurement confirms that exposure of the sample to the cellulase enzyme mixture resulted in the degradation of the cellulose microfibrils into glucose.

We performed a corresponding SPR imaging experiment in parallel using an SPR slide with the same high degree of surface coverage of cellulose fibrils as for the colorimetric carbohydrate assay. In Figure SI4A and B are shown SPR images collected at a fixed angle of incidence $\theta_i = 49.0^{o}$ for the SPR slide in the SPR imaging flow cell before and after 17 h of exposure to the cellulase enzyme mixture. In Figure SI4C is shown a plot of the time dependence of average reflectivity R at a fixed angle of incidence of $\theta_i = 49.0^{o}$ for the 8 × 8 pixel ROI of the SPR image shown as the rectangles in Figure SI4A and B. The reflectivity R decreases significantly with time, with a characteristic time constant $t_{1/2} \approx 1980$ s for the present data set, which is consistent with the result shown in Figure 3B of the present manuscript. Collectively, the results shown in Figures SI3 and SI4 indicate that cellulose microfibrils are being degraded into glucose due to the action of the enzyme mixture.

[S1] M. Lever, *Biochem. Med.* **1973**, *7*, 274 – 281.

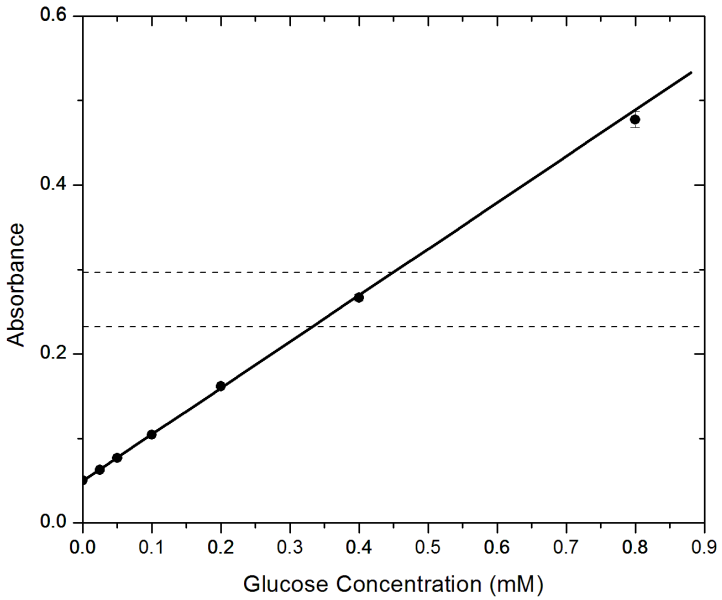

Figure SI3: Plot of light absorption at 415 nm as a function of glucose concentration. The data points were measured in triplicate using different dilutions of a master glucose solution, and the straight line corresponds to the best fit to the data points. The dashed horizontal lines correspond to the range of absorption values measured after exposing two different cellulose microfibril-coated SPR slides to the cellulase enzyme mixture for 17 h.

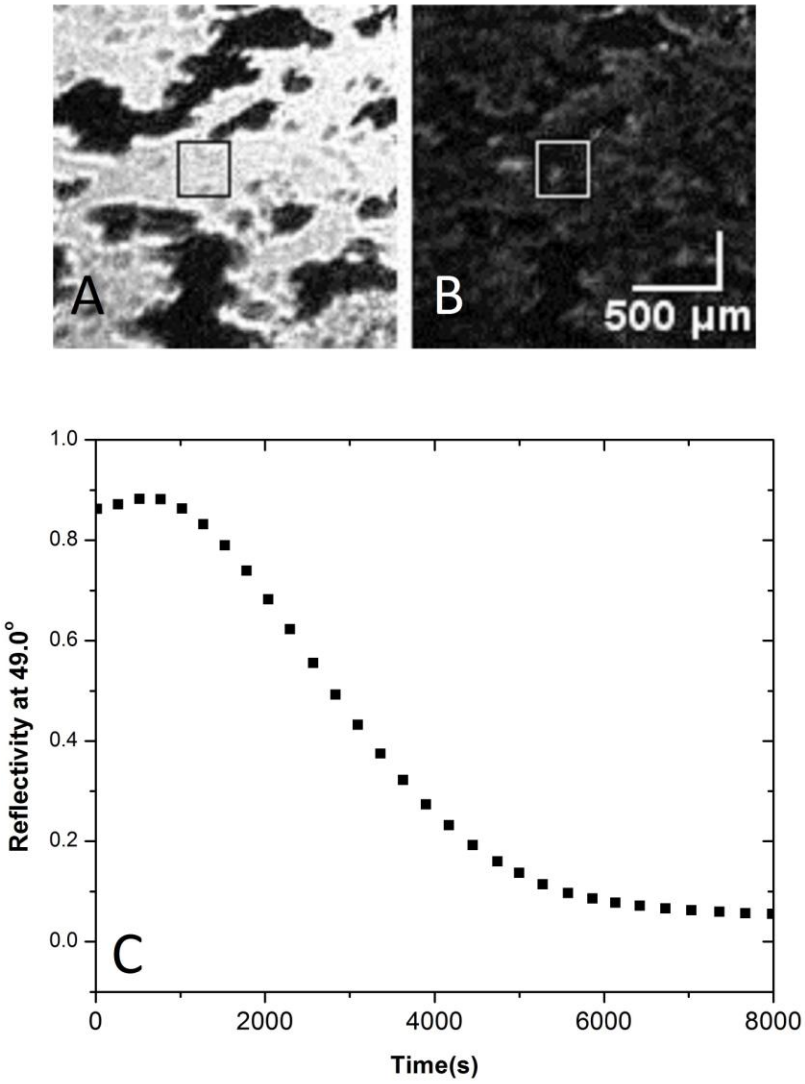

Figure SI4: SPR images before (A) and after (B) exposure to the cellulase enzyme mixture. (C) Plot of the time dependence of average reflectivity *R* at a fixed angle of incidence of $\theta_i = 49.0^o$ for the 8 × 8 pixel ROI shown as the rectangles in parts A and B.