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Supporting Information

Probing Crystal Structure and Mesoscale Assembly of Cellulose Microfibrils in Plant Cell Walls, Tunicate Tests, and Bacterial Films Using Vibrational Sum Frequency Generation (SFG) Spectroscopy

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SFG Analysis of cellulose nanowhiskers hydrolyzed from the intact biological tissues

Pure cellulose samples produced by drying solutions containing cellulose nanowhiskers were analyzed with AFM and SFG. Because of the intrinsic size distribution of cellulose crystals in biological samples, cellulose nanocrystals produced by acid hydrolysis of *Halocynthia*, *Cladophora*, *Gluconacetobacter* are thick and long; while cellulose nanocrystals from wood pulps are thin and short. Typical crystal sizes reported in the literature are 15~30 nm for *Halocynthia*,[1] 10~20 nm for *Cladophora*,[2] 10~20 nm for *Gluconacetobacter*,[3] and 3~4 nm for secondary woody cells[4] . *Halocynthia* and secondary wood cells contain cellulose Iβ only, while *Cladophora* and *Gluconacetobacter* have mostly cellulose Iα (see Table 1 in the main paper). AFM analyses showed that thick and long cellulose nanowhiskers were packed without substantial lateral packing (Figure S1a), while thin and short nanowhiskers were packed with a high degree of lateral packing at least within the small area imaged by AFM (Figure S1b). Different regions of the sample showed different degree of packing and orientation. The tight lateral packing of thin and short nanocrystals must be induced by the action of water surface tension during the dry process. However, the surface tension force did not seem to be large enough to induce packing of thick and long cellulose nanocrystals. The thick and long cellulose nanocrystals produced from *Halocyntia* (Figure S1c), *Cladophora* (Figure S1d), and *Glucoancetobacter* (Figure S1e) gave the SFG spectra with the characteristics of random packing (Figure 2b), while the thin and short cellulose nanocrystals produced from Avicel (Figure S1f) gave the spectra of the laterally well-packed system (Figure 2a). These data indicate that the SFG spectra of samples containing 'pure' cellulose nanocrystals cannot be fully understood with the crystallographic unit cell structures of cellulose; the CH_2 peak shape and CH_2/OH intensity ratio can vary depending on the packing of crystallites which also varies depending on the crystal size and deposition conditions.

Estimation of the coherence length in cellulose

The coherence length is related to the phase mismatch $\Delta k = \left|\vec{k}_{SF} - \left(\vec{k}_{VIS} + \vec{k}_{IR}\right)\right|$; calculation of the exact value of the phase mismatch for the SFG process from cellulose microfibrils dispersed in amorphous matrix is not easy. Cellulose is birefringent, the ordinary and extraordinary refractive indices of cellulose are slightly different. Even in the uniaxially aligned sample, the orientations of all cellulose crystals are not homogeneous; they can vary over a wide range from the average orientation. the refractive indice of cellulose at the wavelength of the input visible (532 nm), infrared (2700 or 3800 cm^{-1}), and SFG (442-465 nm) are estimated to be approximately 1.472, 1.41, and 1.477, respectively.[5] At the incidence angles θ_{VIS} = 60° and θ_{IR} = 56° to surface normal, the phase matching condition ($\Delta k_x = 0$) predicts that the SFG signal is emitted at $\theta_{SFG} \approx 58°$. If we use this geometry to estimate the coherence lengths in the z-direction, then $l_c \equiv \pi/\Delta k_z$ was calculated to be around 900 nm. It should be noted that this is a first order approximation since the scattering and birefringence were not taken into account.

References

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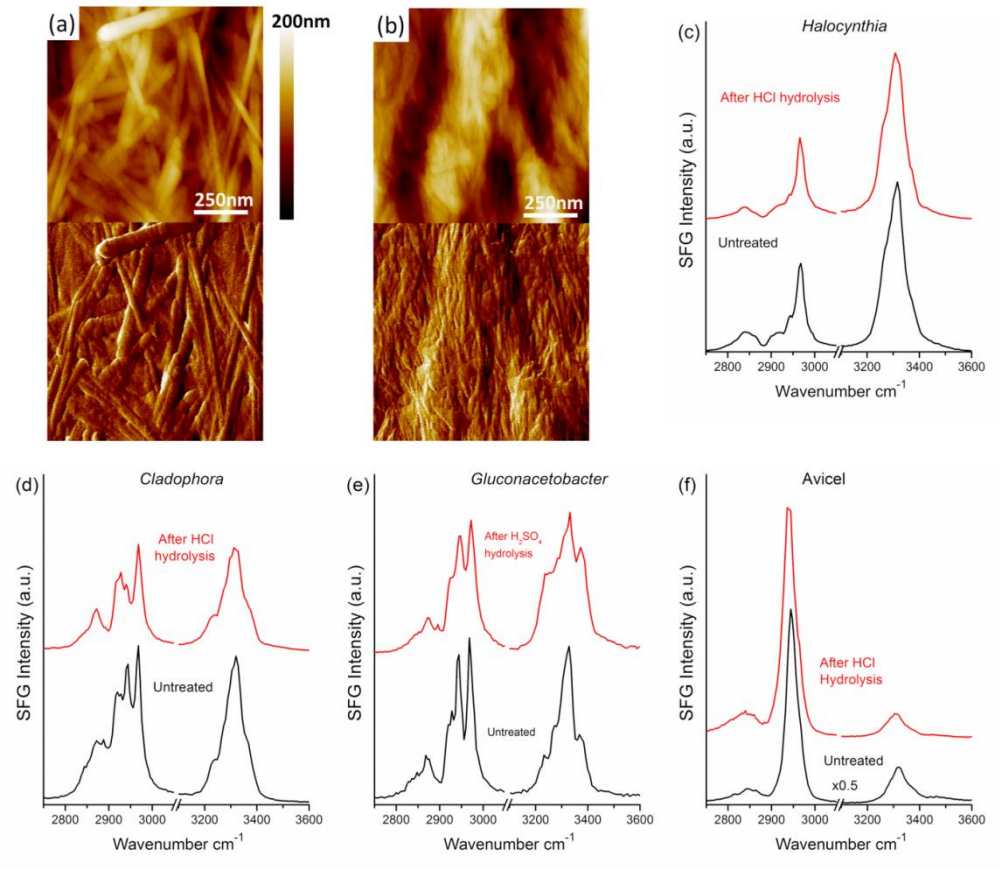

Figure S1. AFM images (topography and deformation) of cellulose nanocrystals purified from (a) *Halocynthia* and (b) Avicel. SFG spectra of cellulose nanocrystals purified from (c) *Halocynthia*, (d) *Cladophora*, (e) *Gluconacetobacter*, and (f) Avicel. The SFG intensities were adjusted for comparison of peak shape.

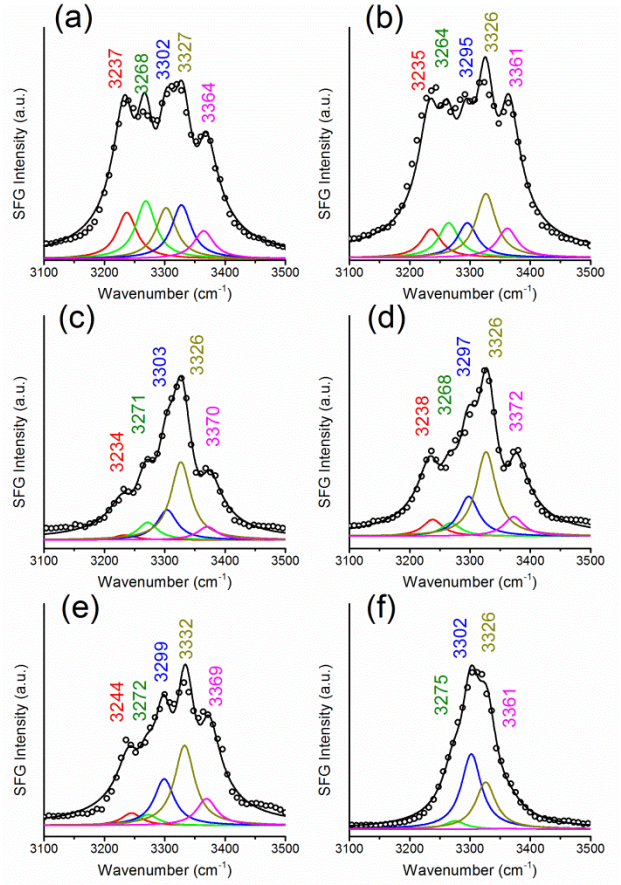

Figure S2. Deconvolution of the OH region of the SFG spectra using Equation (1) of (a) *Glaucocystis*, (b) *Oocystis* (c) *Cladophora*, (d) *Valonia*, (e) *Gluconacetobacter*, and (f) *Halocynthia* shown in Figure 3b. Solid lines are the fitted curves.

Table S1. Fitting parameters (with standard error of the mean) of the SFG spectra in Figure 3b, using Equations (2).

	Fitting parameter for the peak position ω_q (cm^{-1})				
Species	3234-3244	3264-3275	3295-3303	3326-3332	3361-3372
Glaucocystis	3237.2 (0.7)	3268.8 (0.7)	3302.4 (0.7)	3327.7 (0.7)	3364.7 (1.0)
Oocystis	3235.8 (1.1)	3264.6 (1.0)	3295.6 (1.1)	3326.3 (0.8)	3361.9 (1.1)
Gluconacetobacter	3244.9 (1.7)	3272.4 (1.7)	3299.2 (0.9)	3332.9 (0.8)	3369.4 (1.2)
Cladophora	3234.2 (1.9)	3271.9 (0.9)	3303.8 (0.6)	3326.7 (0.4)	3370.7 (1.1)
Valonia	3238.2 (1.1)	3268.2 (1.2)	3297.9 (0.7)	3326.6 (0.5)	3372.5 (1.1)
Halocynthia	NA	3275.3 (1.4)	3302.2 (0.5)	3326.8 (0.6)	3361.8 (5.2)

	Fitting parameter for the peak amplitude α_q				
Species	3234-3244	3264-3275	3295-3303	3326-3332	3361-3372
Glaucocystis	0.20 (0.01)	0.22 (0.01)	0.21 (0.01)	0.21 (0.01)	0.15 (0.01)
Oocystis	0.18 (0.01)	0.19 (0.01)	0.19 (0.01)	0.26 (0.01)	0.18 (0.01)
Gluconacetobacter	0.13 (0.02)	0.12 (0.02)	0.24 (0.01)	0.32 (0.01)	0.19 (0.01)
Cladophora	0.09 (0.01)	0.17 (0.01)	0.22 (0.01)	0.36 (0.01)	0.15 (0.01)
Valonia	0.15 (0.01)	0.13 (0.01)	0.23 (0.01)	0.33 (0.01)	0.16 (0.01)
Halocynthia	NA	0.15 (0.01)	0.45 (0.01)	0.36 (0.01)	0.04 (0.01)