Electronic Supplementary Information (ESI) for **"Multi-scale visualization and characterization of lignocellulosic plant cell wall deconstruction during thermochemical pretreatment"** *DOI: 10.1039/c0ee00574f*

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Supplementary Material Figure/Table/Movie Legend

Figure S1. (I) SEM micrographs of untreated (i), L-AFEX treated (ii) and H-AFEX treated (iii) corn stover stem cross-section at various magnifications (a-d). (II) Comparison of cell wall perimeter (L, μm), area enclosed within lumen space (A, μm^2) and isoperimetric quotient ($4\pi A/L^2$) for untreated, L-AFEX and H-AFEX treated corn stover cell walls. Values with different alphabetical superscripts in an individual column were statistically different with $p < 0.05$ based on the Student t-test. (III) LCSM maximum intensity projection images of untreated (a) and H-AFEX treated (b) corn stover vascular bundles stained with Safranin. Sample sections were excited at 543 nm and emission was detected through an LP 560 nm emission filter (fluorescence intensity shown in green). Image (a) and (b) are projection images compiled from 20 and 15 optical sections representing a thickness of 122 and 90 μm, respectively. A Plan-Neofluar 10X/0.3 objective was used to obtain the images.

Figure S2. Untreated (U1-U10; panel I) and L-AFEX (A1-A10; panel II) treated corn stover AFM amplitude (left) and phase (right) image dataset (image size is 2.5 x 2.5 μm^2). Box-plot of surface roughness factors (nm) along with data points scatter (N=10 per set) for untreated and AFEX treated amplitude image dataset from panels I-II (panel III).

Figure S3. (I) TEM images from corn stover treated with various AFEX severities; (A-B) LA-AFEX, (C-D) L-AFEX, (E-F) HAT-AFEX, (G-H) Lt-AFEX and (I-J) LW-AFEX. Scale bars shown at the bottom of each image. (II) Immuno-gold labeling of arabinoxylan epitopes extracted to outer cell walls (A, B) of L-AFEX pretreated corn stover and AFEX corn stover water soluble extractives (C).

Movie S4. TEM tomogram movie for LA-AFEX treated corn stover cell wall. Scale bar is 500 nm. (still image snapshot shown in pdf, movie provided as separate ESI)

Movie S5. TEM tomogram movie for L-AFEX treated corn stover cell wall. Scale bar is 500 nm. (still image snapshot shown in pdf, movie provided as separate ESI)

Movie S6. TEM tomogram movie for HAT-AFEX treated corn stover cell wall. Scale bar is 500 nm. (still image snapshot shown in pdf, movie provided as separate ESI)

Movie S7. TEM tomogram movie for LW-AFEX treated corn stover cell wall. Scale bar is 500 nm. (still image snapshot shown in pdf, movie provided as separate ESI)

Movie S8. TEM tomogram movie for untreated corn stover cell wall. Scale bar is 500 nm. (still image snapshot shown in pdf, movie provided as separate ESI)

Table S9. Surface area (SA) to volume (Vol) ratios for the isosurface mesh enclosing porous regions within TEM-3D-tomograms of corn stover cell walls treated at various AFEX severities. M: Middle lamella, P: Primary wall, S1/S2/S3: Secondary walls and delam: Delamination zones.

Figure S10. (I) ESCA-based surface atomic composition of untreated, L-AFEX and water extracted L-AFEX corn stover. % Phenolic acid equivalent content of cell wall water soluble extractives was estimated via Prussian blue analysis. All experiments were conducted in quadruplicates and standard deviation was <10%. (II) NMR analysis of delocalized lignin (or 'biomass surface deposited lignin') for L-AFEX corn stover. Aromatic spectral region of lignin delocalized after AFEX pretreatment of corn stover revealed that 90% of the *p*-coumarate esters had been cleaved; examination of the aliphatic region of the spectrum (not shown) revealed that the major structural linkages (β-O-4) of lignin remained largely intact.

Figure S11. Baseline corrected Raman spectra of partly delignified and sodium borohydride bleached untreated (black; A) and L-AFEX treated (orange; B) corn stover depicting extent of cellulose III formation (350 cm^{-1} intensity increase relative to 380 cm^{-1} band intensity). Also seen in the inset is a magnified view of A and B along with spectra for HAT-AFEX treated corn stover in the absence of water (green; C) and crystalline cotton linter derived cellulose III (blue; D).

Figure S1.

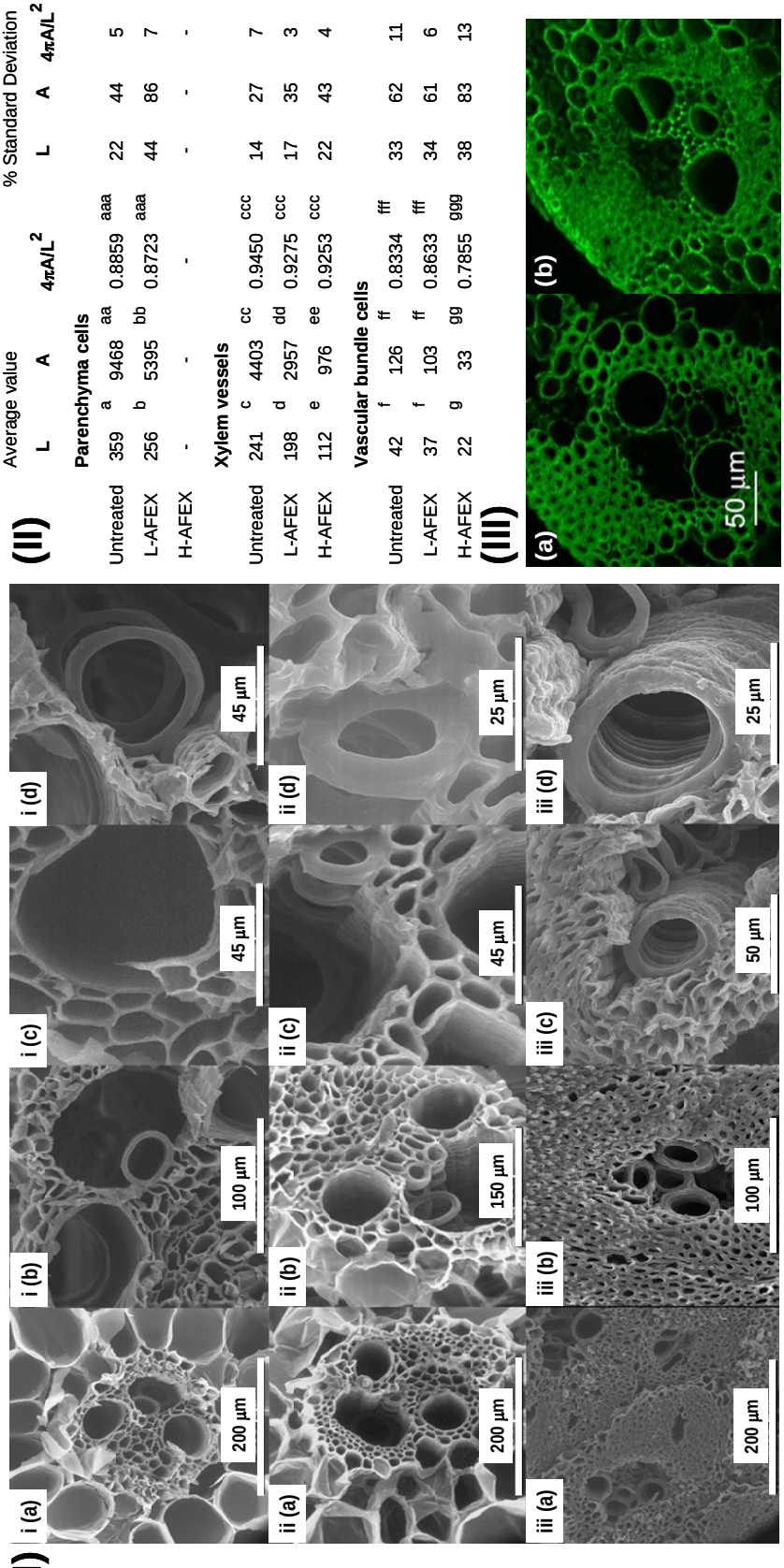

(I)
i (a)
i (b)
i (c)
i (d)
ii (a)
ii (b)
ii (c)
ii (d)
iii (a)
iii (b)
iii (c)
iii (d)
200 µm
100 µm
45 µm
150 µm
25 µm
50 µm
(II)
Average value
% Standard Deviation
L
A
4πA/L2
Parenchyma cells
Untreated 359 a 9468 aa 0.8859 aaa 22 44 5
L-AFEX 256 b 5395 bb 0.8723 aaa 44 86 7
H-AFEX - - - - - -
Xylem vessels
Untreated 241 c 4403 cc 0.9450 ccc 14 27 7
L-AFEX 198 d 2957 dd 0.9275 ccc 17 35 3
H-AFEX 112 e 976 ee 0.9253 ccc 22 43 4
Vascular bundle cells
Untreated 42 f 126 ff 0.8334 fff 33 62 11
L-AFEX 37 f 103 ff 0.8633 fff 34 61 6
H-AFEX 22 g 33 gg 0.7855 ggg 38 83 13
(III)
(a)
(b)
50 µm

Figure S2.

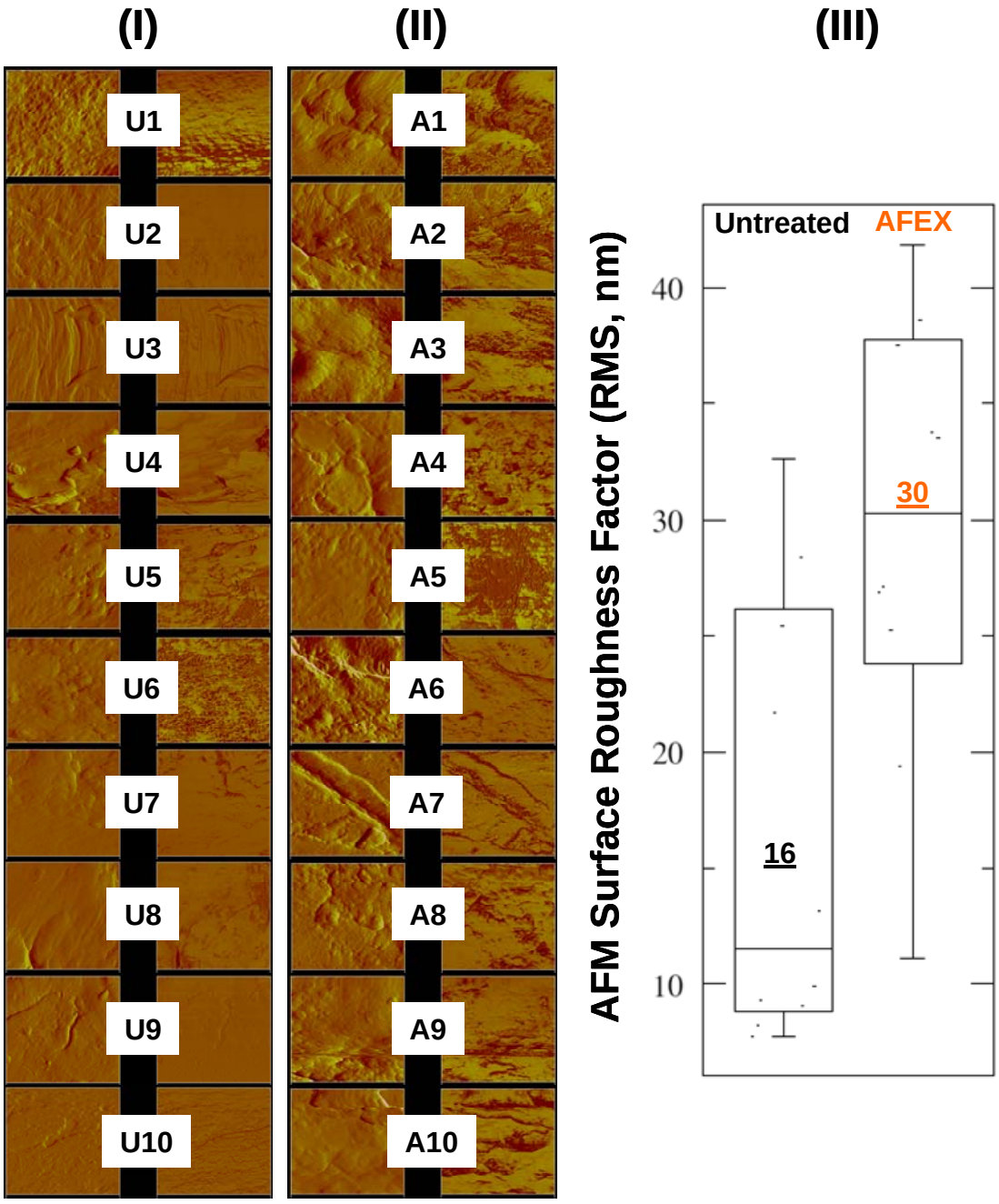
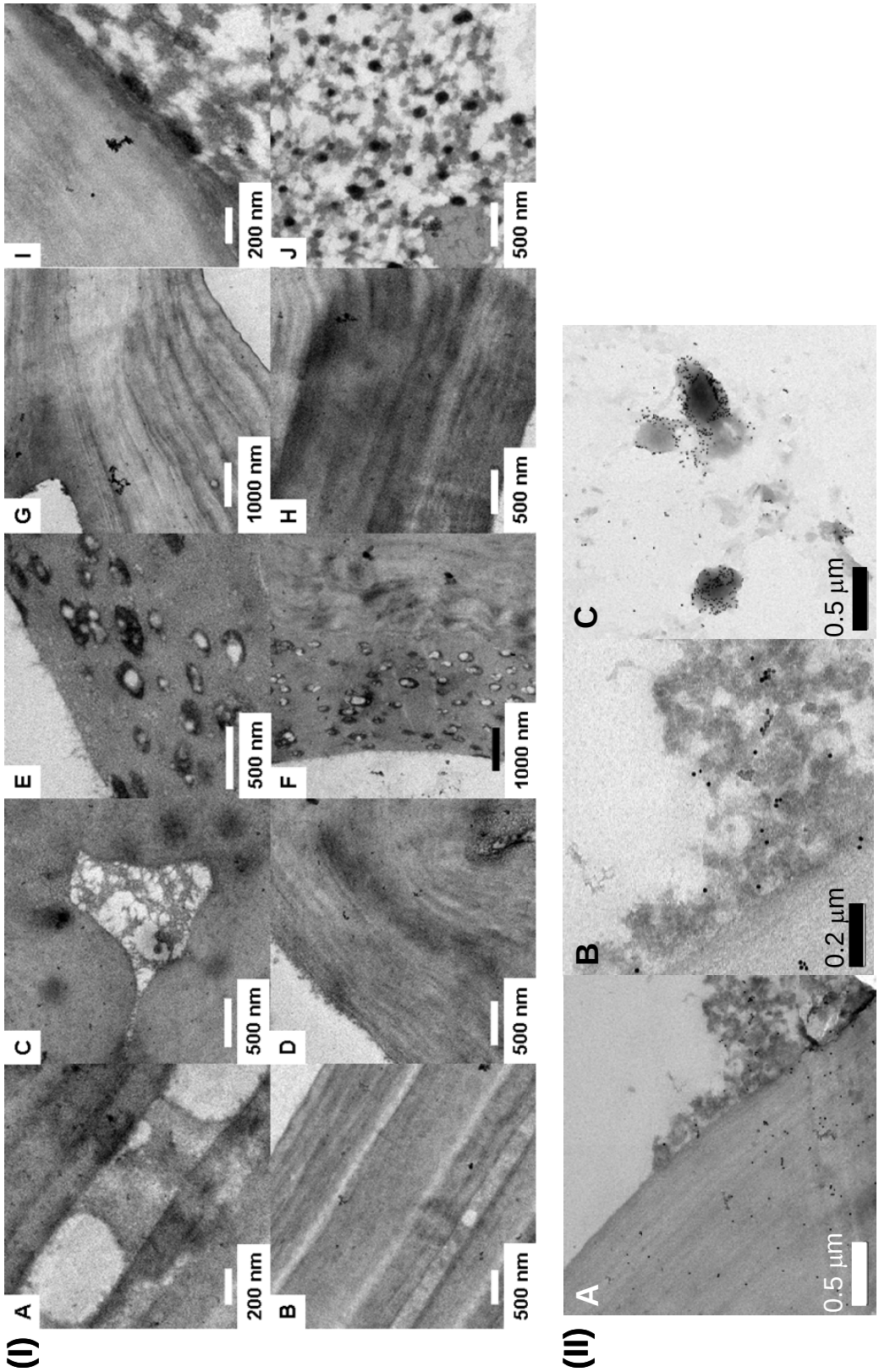
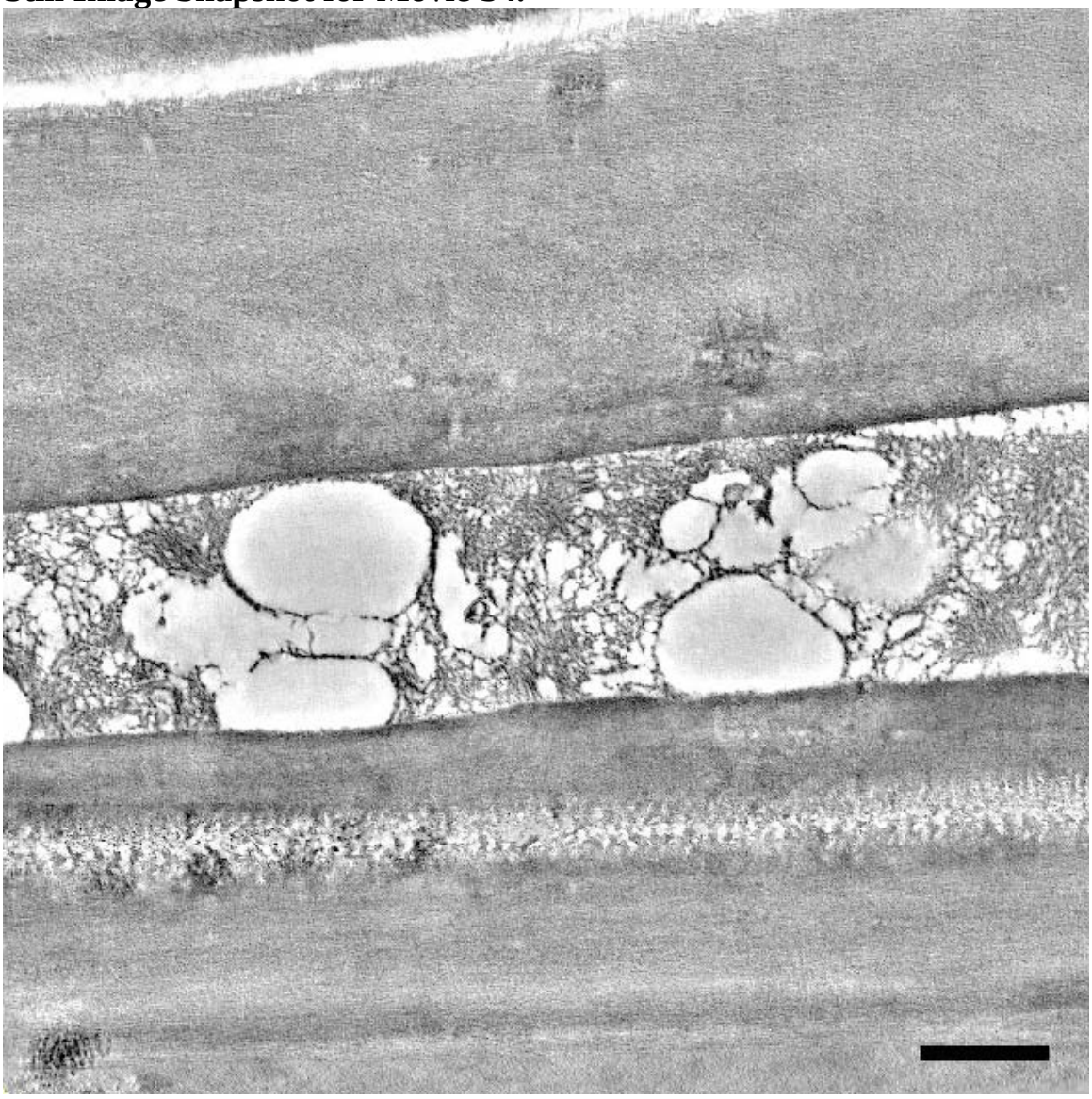


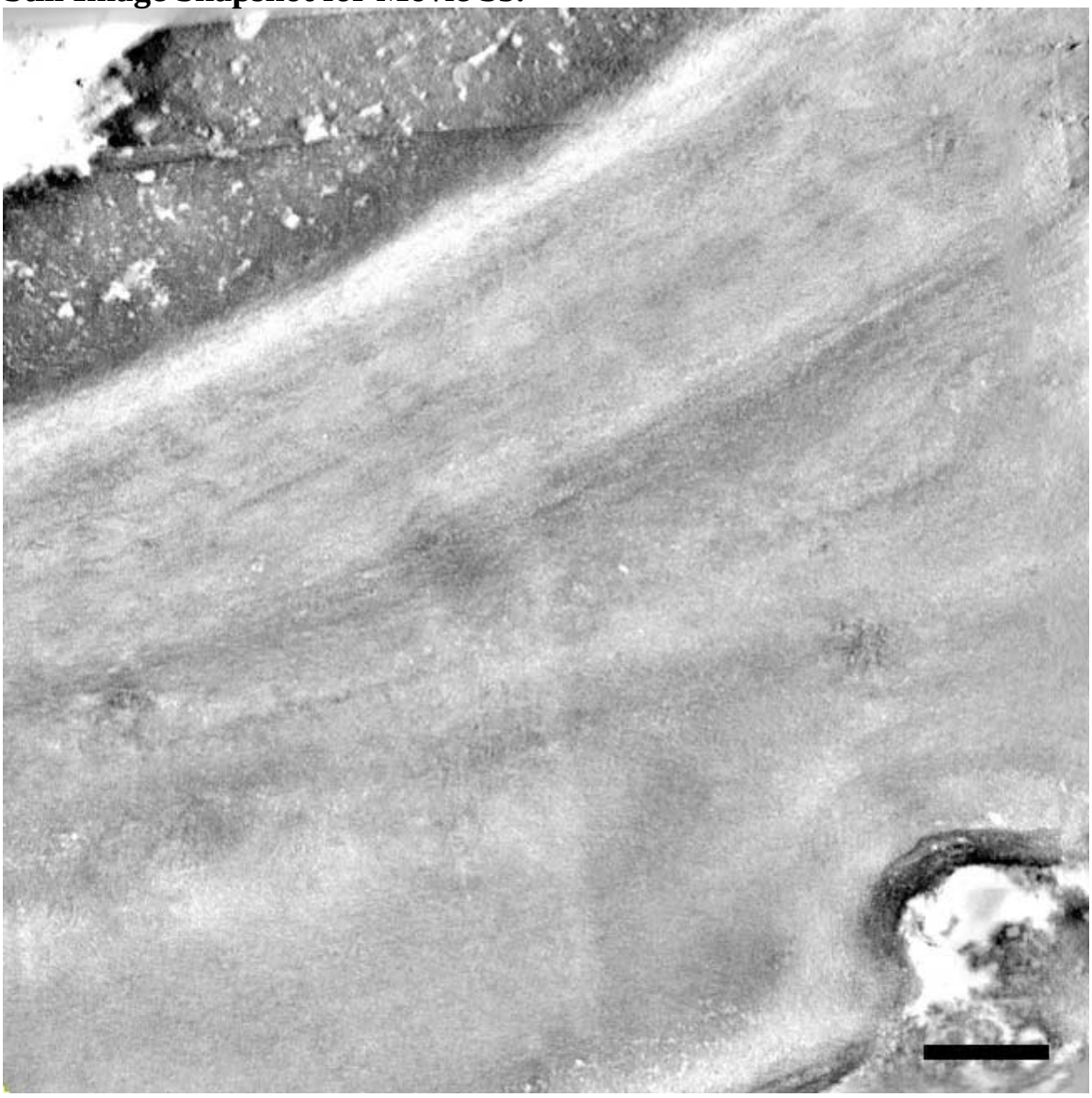
Figure S3.


(I)
A
200 nm
B
500 nm
C
500 nm
D
500 nm
E
500 nm
F
1000 nm
G
1000 nm
H
500 nm
I
200 nm
J
500 nm
(II)
A
0.5 µm
B
0.2 µm
C
0.5 µm

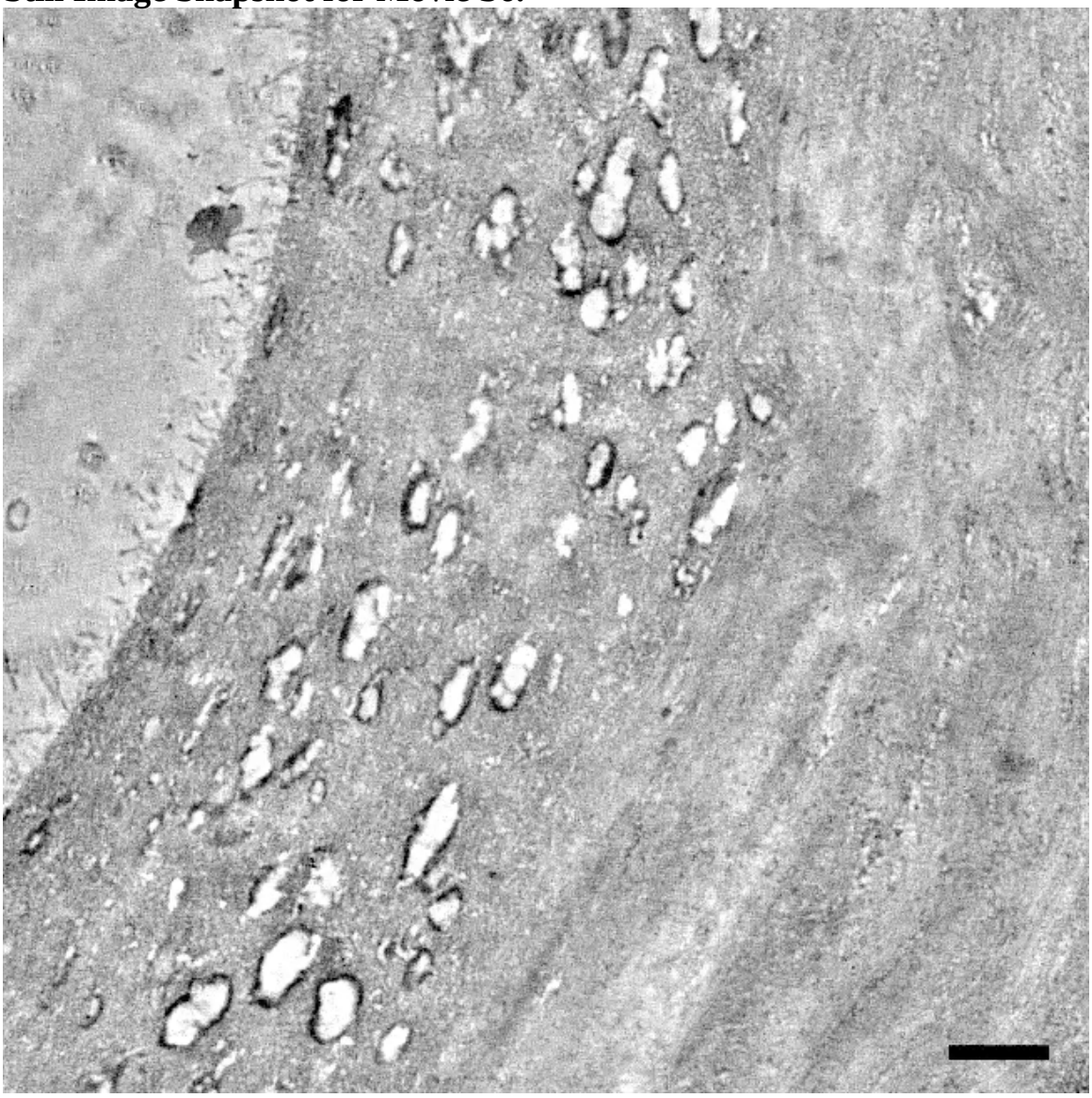
Still Image Snapshot for Movie S4.



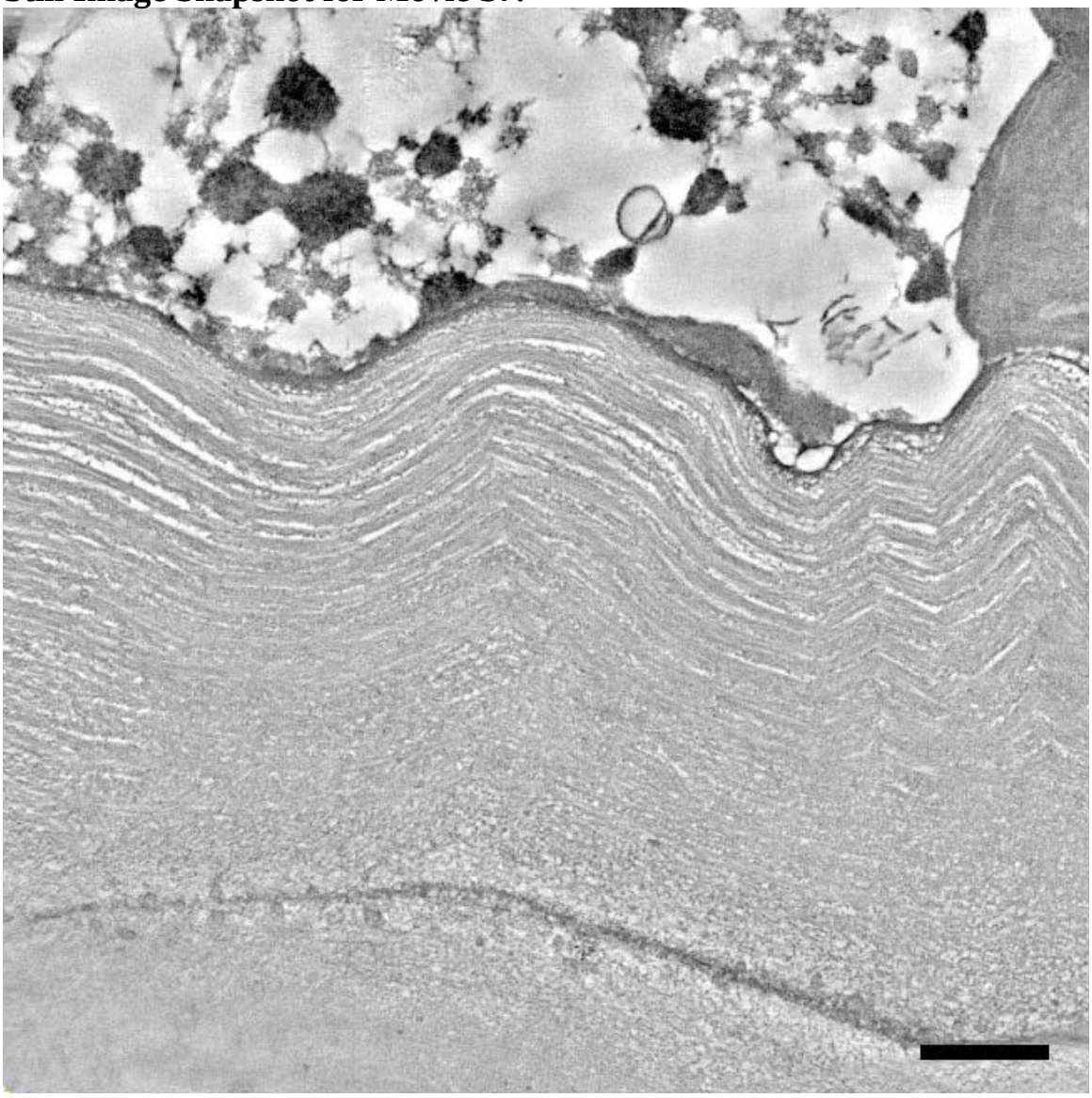
Still Image Snapshot for Movie S5.



Still Image Snapshot for Movie S6.



Still Image Snapshot for Movie S7.



Still Image Snapshot for Movie S8.

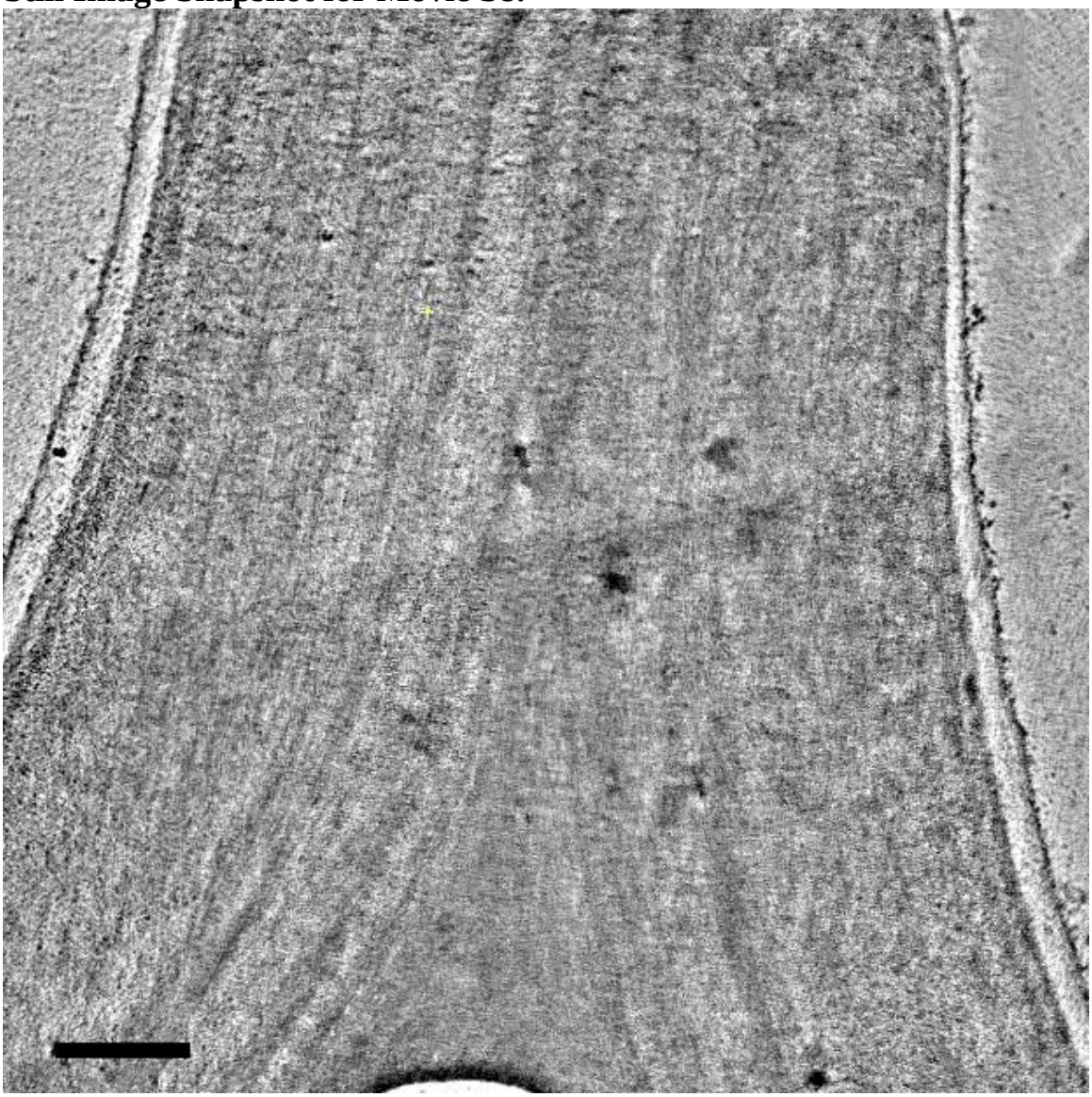


Table S9.

Location	Thickness L (nm)	SA/Vol P (1/nm)	Fractional SA/Vol $P_F = P*L/L_{total}$
(1) LA-AFEX corn stover			
M+P	223	2.81E-02	2.37E-03
S1	500	2.32E-03	4.40E-04
S1 delam	125	3.76E-02	1.78E-03
S2	865	1.83E-04	6.01E-05
S2 delam	125	5.28E-04	2.50E-05
S3	800	3.24E-05	9.83E-06
(2) L-AFEX corn stover			
M+P	260	4.50E-02	3.76E-03
S1	760	-	-
S1 delam	330	5.10E-02	5.41E-03
S2	420	-	-
S2 delam	670	3.87E-02	8.33E-03
S3	670	2.34E-02	5.04E-03
(3) HAT-AFEX corn stover			
M+P	220	4.79E-02	2.79E-03
S1	420	-	-
S1 delam	250	-	-
S2	1020	-	-
S2 delam	170	5.82E-02	2.62E-03
S3	1700	3.16E-02	1.42E-02
(4) LW-AFEX corn stover			
M+P	330	4.75E-02	7.54E-03
S1 region	780	5.75E-02	2.16E-02
S2 region	970	4.35E-02	2.03E-02

Figure S10.

(I)

	ESCA based surface atomic composition					% Phenolic content of extractives
	% C	% *C1*	% *C2*	% *C3*	% O	% N	
Untreated	71.3	56.3	*34.1*	*9.6*	26.6	1.7	0.02
L-AFEX	74.1	*64.5*	28.3	*7.1*	22.7	2.8	0.16
Extracted L-AFEX	73.4	*62.6*	29.5	*7.9*	24.1	1.7	-

(II)

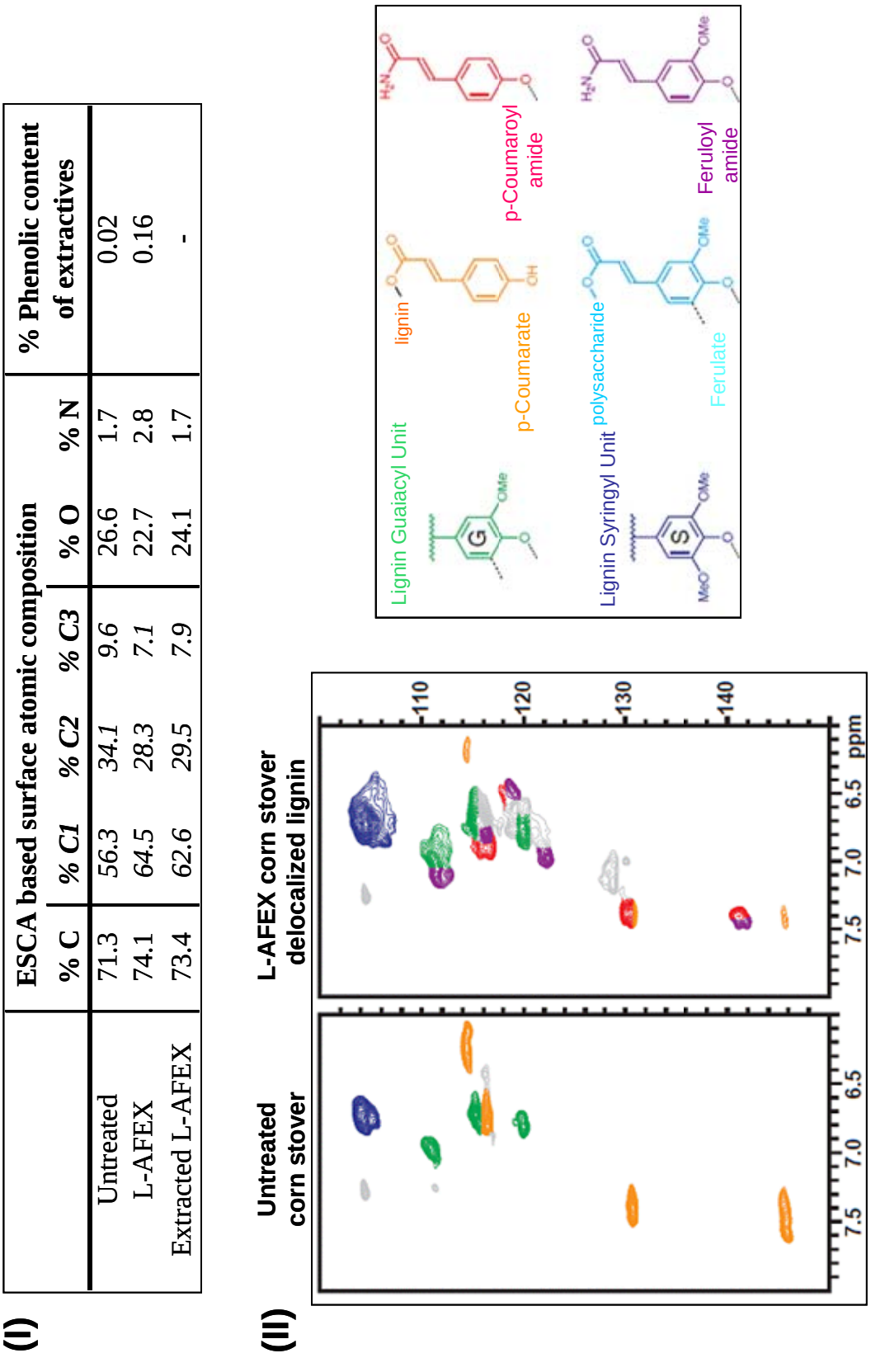
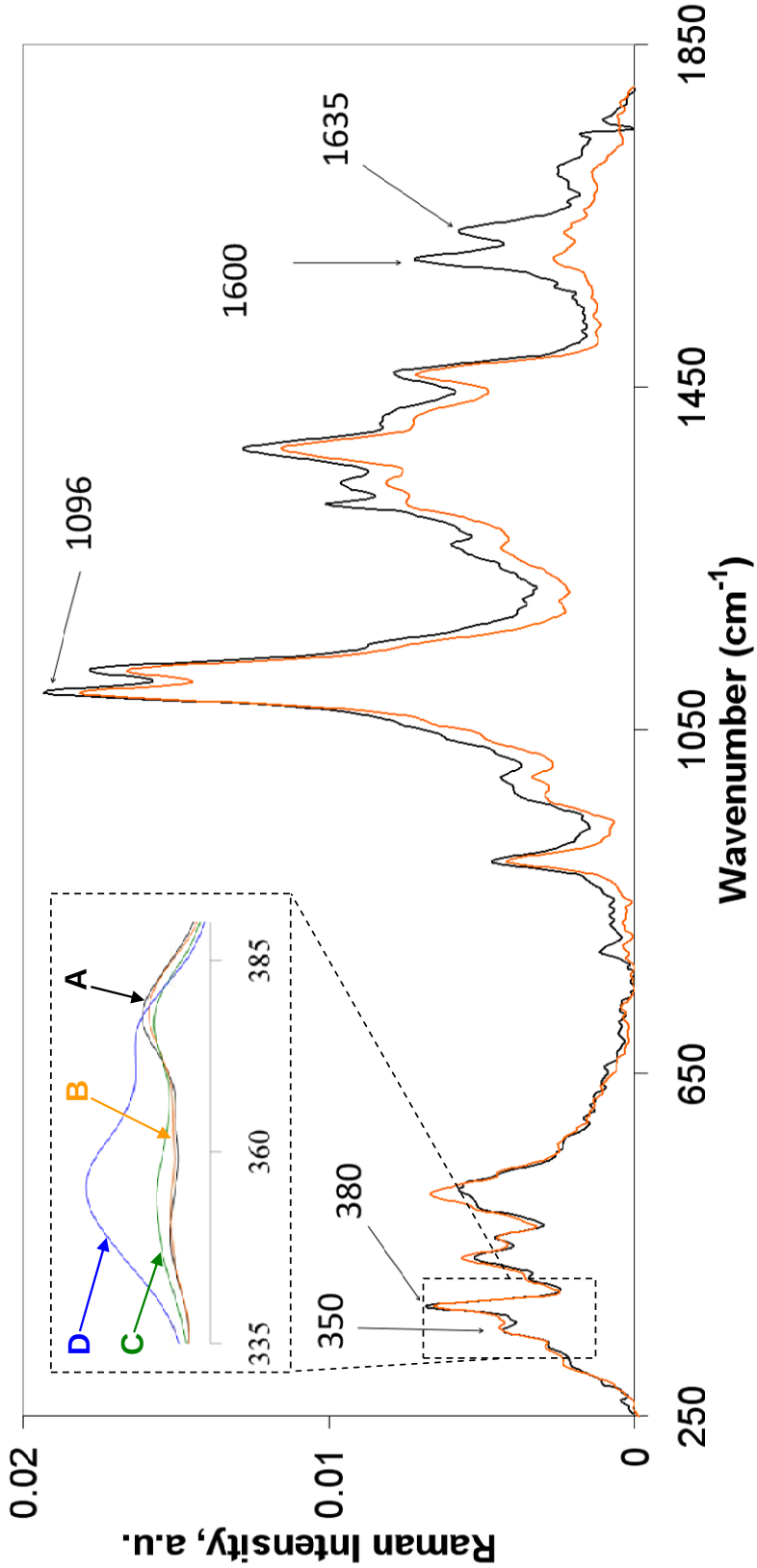


Figure S11.


Raman Intensity, a.u.
0.02
0.01
0
250
650
1050
1450
1850
Wavenumber (cm-1)
1096
1600
1635
350
380
335
360
385
A
B
C
D