Supplemental Material

A TEMPO-catalyzed oxidation-reduction method to probe surface and anhydrous crystalline-core domains of cellulose microfibril bundles

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Table S1 Example of the correction for ^{13}C-spillover in fragments of total cellobiose from TEMPO-catalyzed and $NaBD_4$-reduced cellulose from wild-type poplar. Abundances were determined by integration of the *m/z* values from electrospray ionization MS. The abundance of the undeuterated *m/z* 365 fragments was multiplied by 0.126 to estimate the spillover into *m/z* 366, and the abundance of *m/z* 366 spillover was then multiplied by 0.126 to give double spillover into *m/z* 367. The estimated spillover value of *m/z* 366 was subtracted from the uncorrected abundance of *m/z* 366 to give corrected *m/z* 366, and the spillover in the corrected *m/z* 366 determined as above. The single spillover of m/z 366 and the double spillover of m/z 365 were subtracted from the uncorrected abundance of m/z 367 to give corrected m/z 367, and this calculation was iterated for the remaining *m/z* values. The *m/z* 365 and its estimated spillover was summed to give total anhydrous abundance. The sums of corrected *m/z* 366 and *m/z* 367 gave the surface chain abundance, and the sums of corrected m/z 368 and *m/z* 369 gave the amorphous abundance

Wild-type 1

m/z	Uncorrected Abundance	Corrected *m/z* values with single and double ^{13}C-spillover 365	366	367	368	369
365	418328	418328				
366	72882	52710	20172			
367	99102	6641	2542	89919		
368	19856		320	11330	8206	
369	7276			1428	1034	4814
					130	607
						76
		477678	**23034**	**102677**	**9370**	**5498**

Anhydrous	Surface	Amorphous	Anhydrous : Surface
0.773	**0.203**	**0.024**	**3.81**

Wild-type 2

m/z	Uncorrected Abundance	Corrected *m/z* values with single and double ^{13}C-spillover 365	366	367	368	369
365	382209	382209				
366	69977	48158	21819			
367	113203	6068	2749	104386		
368	25231		346	13153	11732	
369	12080			1657	1478	8945
					186	1127
						142
		436435	**24914**	**119195**	**13396**	**10214**

Anhydrous	Surface	Amorphous	Anhydrous : Surface
0.722	**0.239**	**0.039**	**3.02**

Mean ± variance

Anhydrous	Surface	Amorphous	Anhydrous : Surface
74.8±2.6	**22.1±1.8**	**3.1±0.7**	**3.42±0.4**

Table S2 TFA-hydrolysis of non-crystalline glucose from TEMPO-catalyzed, $NaBD_4$-reduced Sigmacel cellulose. Oxidized and reduced cellulose was hydrolyzed with 2 M TFA at 120°C for 90 min, a treatment that yields amorphous and surface-chain residues but cannot hydrolyze anhydrous crystalline cellulose. Undigested cellulose was pelleted by centrifugation, and the TFA-soluble sugar collected, the TFA evaporated, and alditol acetate derivatives from the recovered sugars separated by GLC, and EIMS performed. The major diagnostic masses to differentiate undeuterated glucose (*m/z* 187, *m/z* 217, and *m/z* 289) from the 6-mono- and 6,6-dideutero-glucose quantified. After correction for ^{13}C-spillover, the sums of corrected *m/z* 188/189, *m/z* 218/219, and *m/z* 290/291 are the proportion of deuterated Glc, and this value was subtracted from the *m/z* 187. As undeuterated glucose produces two *m/z* 187 fragments and the mono- and di-deutero-glucosyl derivatives only one, the corrected abundances were divided by two to obtain the equivalent undeuterated Glc.

m/z	Uncorrected Abundance	$[M]^+$	$[M+1amu]^+$	$[M+2amu]^+$
187	18472	18472		
188	2991	2327	664	
189	6983	293	84	6606
		21093	11	832
			758	105
217	14722	14722		**7543**
218	2385	1855	530	
219	4147	234	67	3846
		16811	8	485
			605	61
289	7466	7466		**4392**
290	1535	941	594	
291	2935	119	75	2742
		8525	9	345
			679	44
				3131

	Glc	GlcA	%GlcA
187	6396	8301	0.565
217	5907	4997	0.458
289	2358	3809	0.618
			0.547

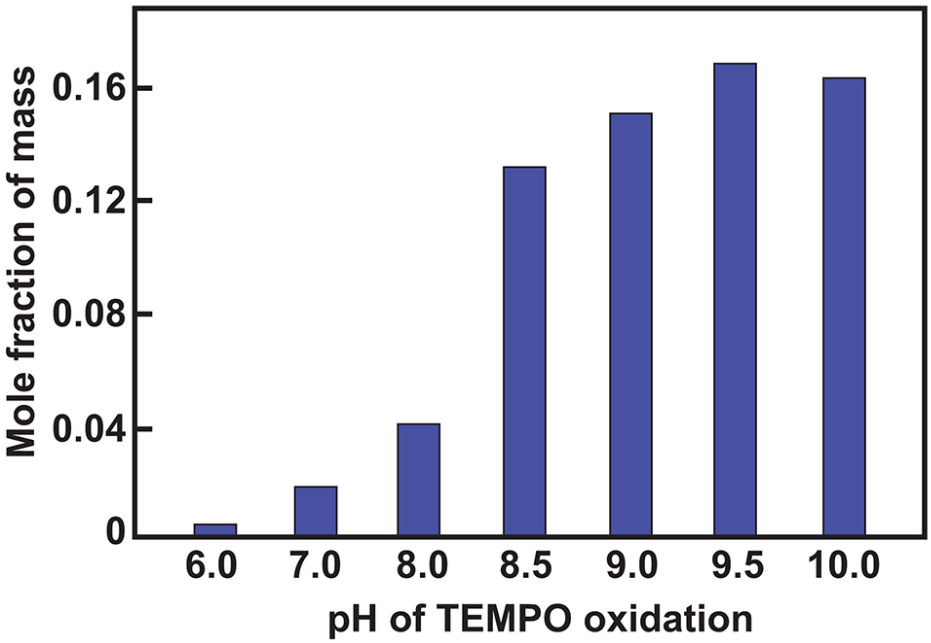

Fig. S1 TEMPO-catalyzed oxidation requires alkaline pH. Cotton cellulose was suspended in 0.1 M sodium bromide-sodium phosphate buffer at the desired pH, and pH maintained by addition of hypochlorite after addition of TEMPO. Moles of uronic acid were determined by a method that reduces interference of neutral sugars (Filisetti-Cozzi and Carpita 1991), and compared to total moles of sugar determined by a phenol-sulfuric acid assay before reactions (Dubois et al. 1956)

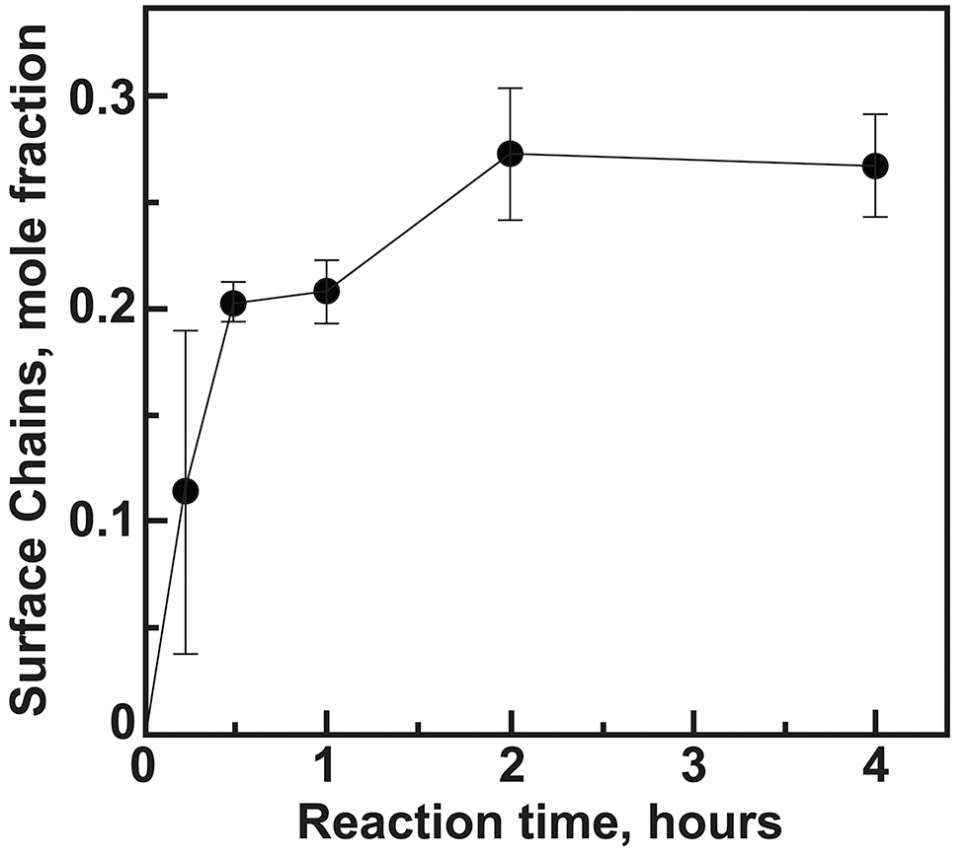

Fig. S2 Reaction time of TEMPO-catalyzed oxidation of hybrid poplar biomass. After oxidation, the reactions were stopped by addition of glacial acetic acid, dialyzed against deionized water, and freeze-dried. Uronic acids of oxidized cellulose were activated with a water-soluble carbodiimide reagent and carboxyl-reduced with $NaBD_4$ (Carpita and McCann, 1997). The oxidized-reduced materials recovered with dissolved in ice-cold TFA (–20°C) for 18 h, heated to 55°C for 2.5 h, and crashed into ethanol. Gelatinized cellulose was digested with a combination of cellulase and cellobiohydrolase, and surface chains determined after ESI-MS as the abundance of undeuterated cellobiose (*m/z* 365) compared to *m/z* 366 and *m/z* 367 after correction for ^{13}C-spillover (Fig.3). Values are mean ± variance of two samples

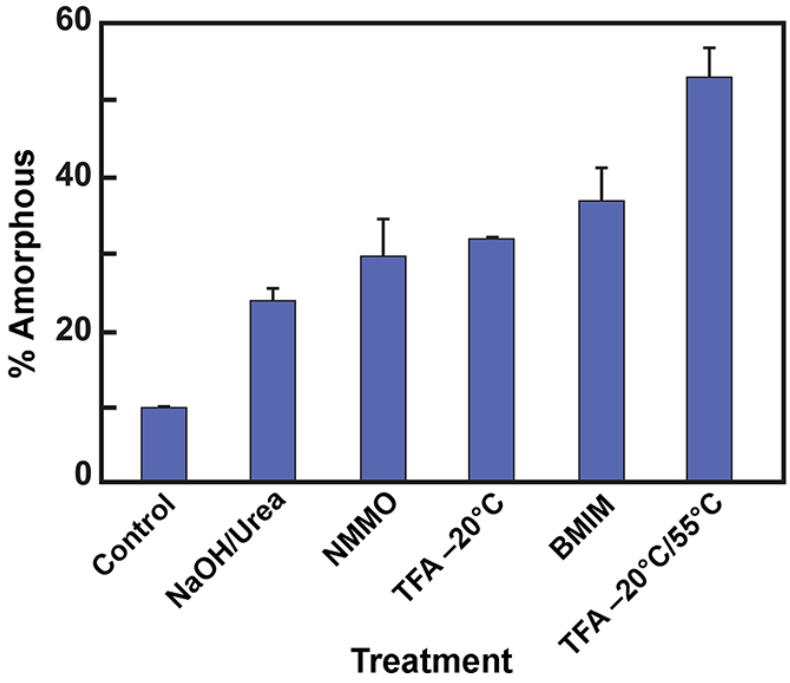

Fig. S3 Generation of amorphous cellulose. Treatment of crystalline cellulose with ionic liquids, NaOH/urea, phosphoric acid, and ice-cold TFA each convert crystalline cellulose to proportionally higher degrees of amorphous forms susceptible to hot acid hydrolysis. After treatment, the materials were dialyzed against deionized water, freeze-dried, and hydrolyzed in 2 M TFA at 120°C for 90 min. The mole fraction of soluble sugar recovered was determined by a phenol-sulfuric acid assay (Dubois et al. 1956)

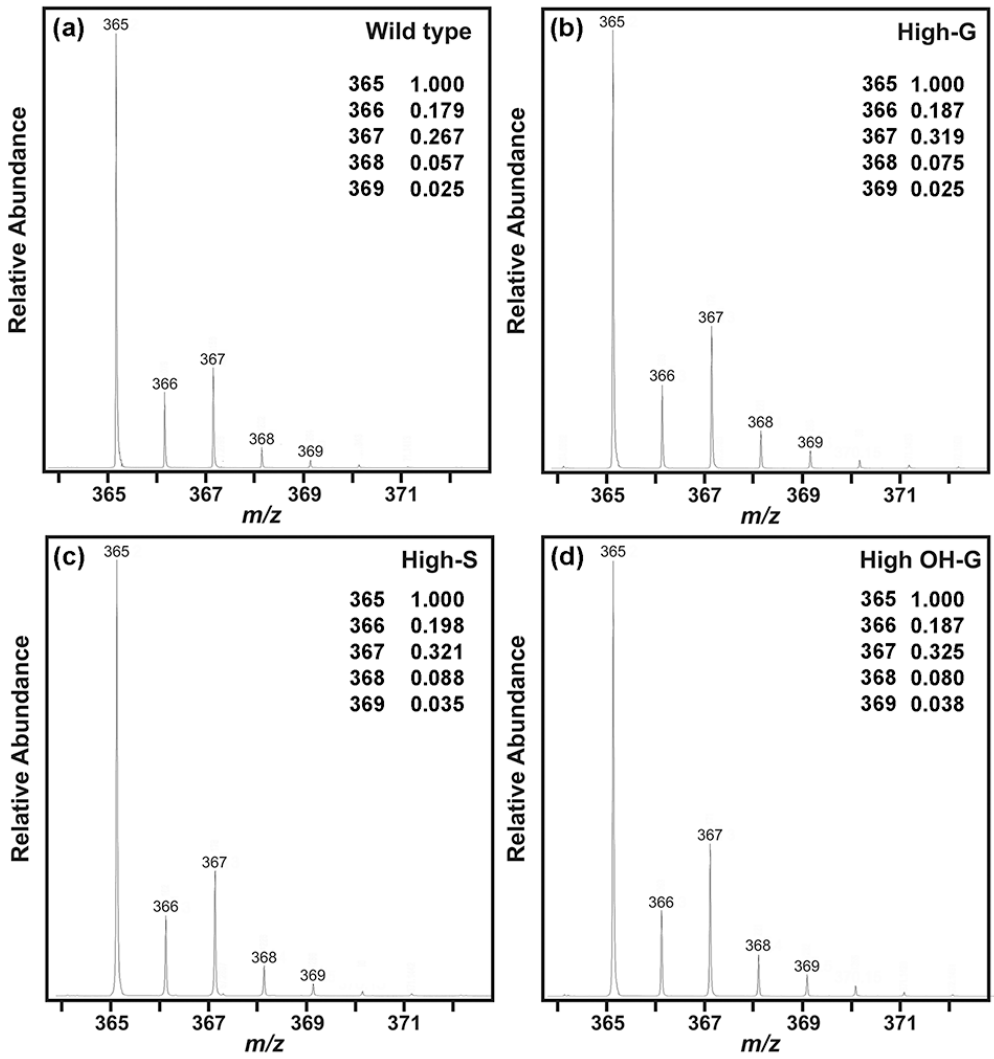

Fig. S4 Determination of the amorphous, surface and crystalline core chains of hybrid and transgenic poplar with altered lignin composition. Wood particles were TEMPO-oxidized and $NaBD_4$-reduced, and degrees of deuteration were determined in cellobiose generated by complete digestion with a combination of cellulase and cellobiohydrolase. Electrospray ionization of partly deuterated cellulose resulted in cellobiose residues with M+1 and M+2 (*m/z* 366 and *m/z* 367), indicating one glucose residue (surface chain), and M+3 and M+4 (*m/z* 368 and *m/z* 369 fragments), indicating both glucosyl residues of the disaccharide were oxidized and subsequently reduced (amorphous). Undeuterated cellobiose arises only from anhydrous domains unexposed to solvent (*m/z* 365). Inset are the normalized *m/z* abundances relative to *m/z* 365. **(a)** Wild-type hybrid poplar wood. **(b)** Transgenic poplar wood with elevated G-lignin. **(c)** Transgenic poplar wood with elevated S-lignin. **(d)** Transgenic poplar with elevated 5-OH-G-lignin

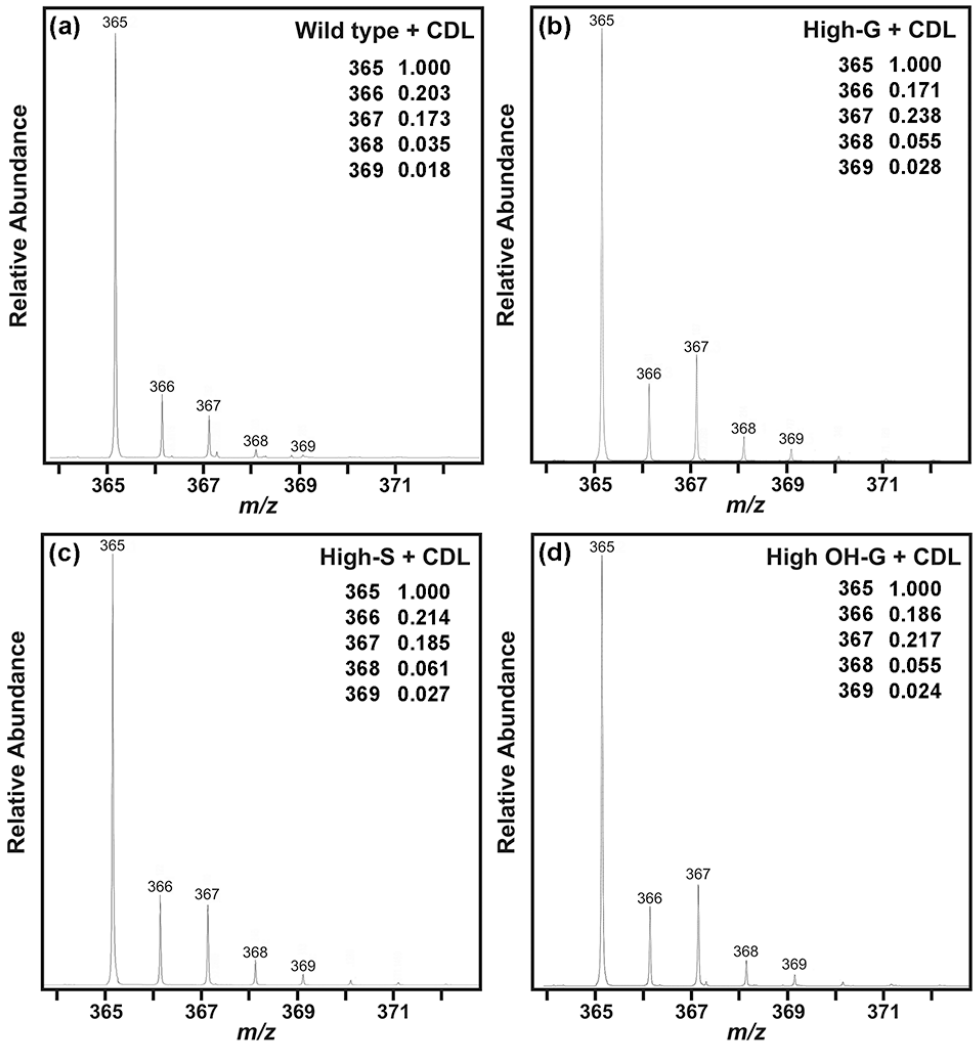

Fig. S5 Determination of the amorphous, surface and crystalline core chains of hybrid and transgenic poplar with altered lignin composition. Wood particles were catalytically delignified using a Ni catalyst as described by Luo et al. (2016). TEMPO-oxidized and $NaBD_4$-reduced, and degrees of deuteration were determined in cellobiose generated by complete digestion with a combination of cellulase and cellobiohydrolase. Electrospray ionization of partly deuterated cellulose resulted in cellobiose residues with M+1 and M+2 (*m/z* 366 and *m/z* 367), indicating one glucose residue (surface chain), and M+3 and M+4 (*m/z* 368 and *m/z* 369 fragments), indicating both glucosyl residues of the disaccharide were oxidized and subsequently reduced (amorphous). Undeuterated cellobiose arises only from anhydrous domains unexposed to solvent (*m/z* 365). Inset are the normalized *m/z* abundances relative to *m/z* 365. **(a)** Wild-type hybrid poplar wood. **(b)** Transgenic poplar wood with elevated G-lignin. **(c)** Transgenic poplar wood with elevated S-lignin. **(d)** Transgenic poplar with elevated 5-OH-G-lignin