Supplementary information for

Protective fractionation of highly uncondensed lignin with high purity and high yield: new insights into propanediol-blocked lignin condensation

Yaling Zhao[a], Deqing Zhao[a,*], Jingpeng He[a], Kaibin Ma[a], Jiatian Zhu[a], Jianrong Liu[a], Yongqi Zhang[a], Qinqin Xia[b], Ting Li[a].

[a]School of Biological Engineering, Sichuan University of Science & Engineering, Yibin, Sichuan province, 644000, China.

[b]Key laboratory of Bio-based Material Science and Technology, Ministry of Education, Northeast Forestry University, Harbin, 150040, China

1. Experimental section

1.1 Cellulolytic enzyme lignin (CEL) preparation

Enzymatic digestion: 8g of extracted moso bamboo powder was weighed and placed in a star ball mill for ball milling for 24h. 6g of ball milled raw material was added into a conical flask, and the appropriate amount of cellulase and xylanase were weighed and dissolved in acetate-sodium acetate buffer solution at pH 5.0 and transferred into the conical flask containing raw material, controlling the enzyme digested substrate with a mass fraction of 6%. The conical flask was placed in a thermostatic oscillator and processed at 50℃ for 72h with an oscillation speed of 150 r/min. After the first enzymatic digestion, the mixed system was centrifuged with buffer solution for 2-3 times and the precipitate was collected and transferred to the conical flask, and the process of the first enzymatic digestion was repeated. At the end of the second enzymatic digestion, the centrifugation residue was washed with deionized water for 3 times, and the resulting precipitate was freeze-dried to obtain crude enzymatic lignin.

Purification: 100mL of 1,4-dioxane/water solution at 96% volume fraction was added to the crude enzymatic lignin, shaken well and stirred overnight. The supernatant was collected by centrifugation, and the precipitated portion was washed at least twice by adding 96% 1,4-dioxane/water solution. The supernatant collected by combined centrifugation was evaporated under reduced pressure at 40℃. To the resulting substrate, a small amount of acetone/water

solution with a volume ratio of 9:1 was added, dissolved, and then added drop by drop into 200mL of deionized water, and placed in the refrigerator to stand overnight. The precipitated fraction was collected by centrifugation and freeze-dried to obtain purified enzymatic lignin.

1.2 Calculation formula for lignin yield, recovery rate

The lignin yield and recovery rate were calculated from the recovered lignin obtained after DES treatment as follows：

$$Purity = \frac{Klason\ lignin\ content\ in\ DESL + Acid - soluble\ lignin\ content\ in\ DESL}{Recovery\ lignin\ content} \times 100\%$$

$$Lignin\ yield = \frac{Mass\ of\ DESL}{QInitial\ mass\ of\ moso\ bamboo \times Dryness\ of\ raw\ materials} \times 100\%$$

$$Recovery\ rate = \frac{Lignin\ yield}{Lignin\ content\ in\ moso\ bamboo} \times 100\%$$

$$Solid\ \ rate = \frac{Mass\ of\ solid}{QInitial\ mass\ of\ moso\ bamboo \times Dryness\ of\ raw\ materials} \times 100\%$$

1.3 Quantitative calculation formula for connecting keys in 2D-HSQC

Semi-quantitative calculation of the α position in the side-chain region by integration of total 100 aromatic units (Au_{100}) in the aromatic region.[1]

$$Aromatic = \left(\left(\frac{S_{2,6} + S'_{2,6}}{2}\right) + S_{condensed}\right) + \frac{G_2 + G_5 + G_6}{2}$$

$$\beta - O - 4content = \frac{A_\alpha}{Aromatic} \times 100$$

$$\beta - O - 4'content = \frac{A''_\alpha}{Aromatic} \times 100$$

Table S1. Main chemical components and content of raw materials of moso bamboo.

Ingredient	Content / %
Klason Lignin	27.25
Acid-soluble Lignin	0.0521
Benzene Alcohol Extracts	9.66
Ash	0.47
Moisture content	9.25

Table S2. Molar ratio of binary DES.

HBD	HBD:HBA/mol:mol
PG	1:2
Gly	1:2
BDO	1:3
PEG	1:5
EG	1:2

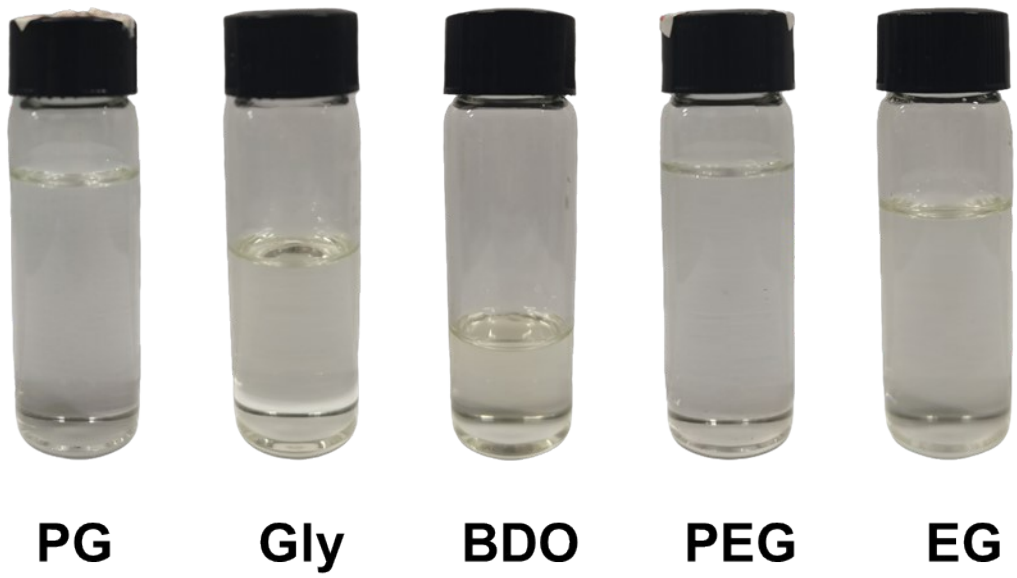

Fig. S1. Solubility of polyol-based ternary DES**.**

Fig. S2. Solid residue at different temperatures.

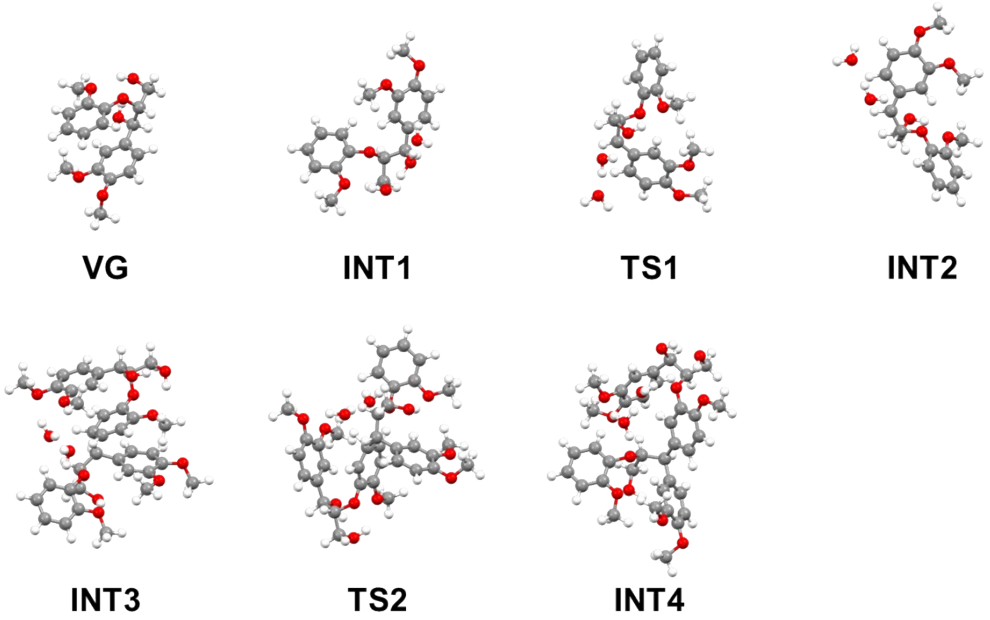

Fig. S3. The intermediate and transition state model involved in the calculation of the Gibbs free energy barrier of the condensation path.

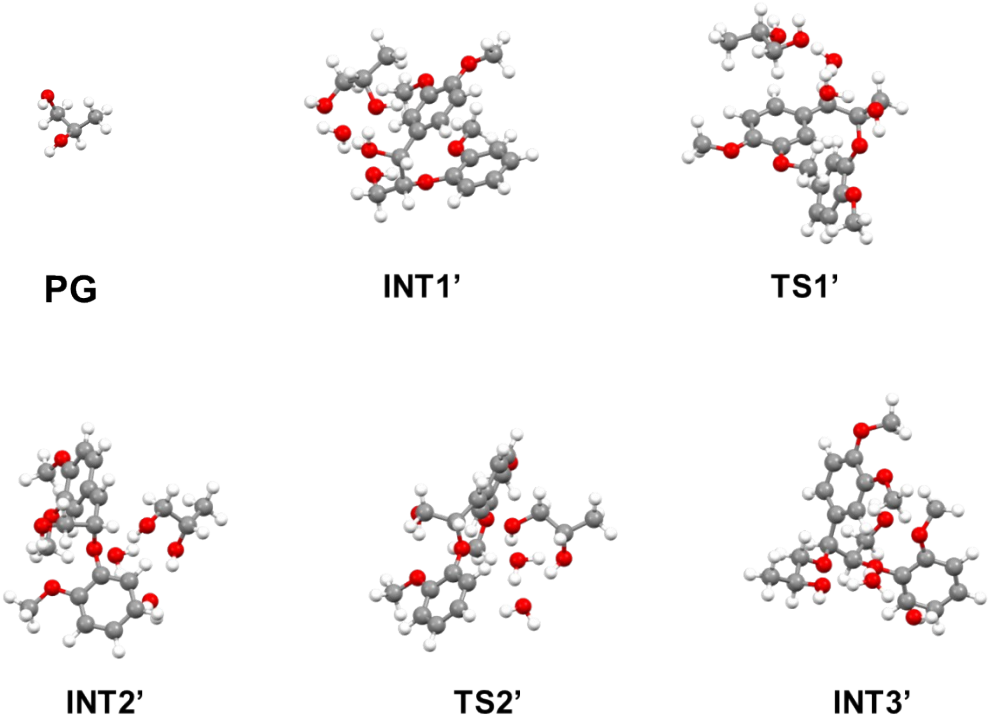

Fig. S4. The model of intermediates and transition states involved in the calculation of the Gibbs free energy barrier for lignin inhibition condensation by 1,2-propanediol.

Table S3. Assignments of main 13C- 1H cross-signals in the HSQC NMR spectra of CEL.

Lable	δC/δH(ppm)	Assignments
A_{α}	71.8/4.86	C_{α}-H_{α} in β-O-4’ units(A)
B_{α}	84.8/4.66	C_{α}-H_{α} in β-β’ resinol substructures (B)
C_{α}	86.8/5.45	C_{α}-H_{α} in phenylcoumaran substructures (C)
OMe	55.5/3.70	C-H in methoxyls
C_{β}	53.1/3.46	C_{β}-H_{β} in phenylcoumaran substructures (C)
$A_{\beta(G/H)}$	83.5/4.33	C_{β}-H_{β} in β-O-4’ substructures linked to G/H units (A)
$A_{\beta(S)}$	85.9/4.10	C_{β}-H_{β} in β-O-4’ substructures linked to S units (A)
A_{γ}	59.9/(3.35-3.80)	C_{γ}-H_{γ} in β-O-4’ substructures (A)
A'_{γ}	63.0/4.36	C_{γ}-H_{γ} in γ-acylated β-O-4’ substructures (A’)
B_{γ}	71.2/(3.82-4.18)	C_{γ}-H_{γ} in β-β’ resinol substructures (B)
C_{γ}	62.2/3.76	C_{γ}-H_{γ} in phenylcoumaran substructures (C)
$S_{2,6}$	103.6/6.72	$C_{2,6}$-$H_{2,6}$ in syringyl units (S)
$S'_{2,6}$	106.2/7.23	$C_{2,6}$-$H_{2,6}$ in oxidized (Cα=O) syringyl units (S’)
G_2	110.7/6.96	C_2-H_2 in guaiacyl units (G)
G_5	114.5/6.69	C_5-H_5 in guaiacyl units (G)
G_6	118.8/6.78	C_6-H_6 in guaiacyl units (G)
$H_{2,6}$	127.6/7.19	$C_{2,6}$-$H_{2,6}$ in H units (H)
$PCE_{2,6}$	130.0/7.46	$C_{2,6}$-$H_{2,6}$ in p-coumarate (PCE)
PCE_7	144.7/7.50	C_7-H_7 in p-coumarate (PCE)
PCE_8	113.6/6.72	C_8-H_8 in p-coumarate (PCE)
FA	110.9/7.35	C_2-H_2 in ferulate (FA)

References

1. Y. Liu, N. Deak, Z. Wang, H. Yu, L. Hameleers, E. Jurak, P. J. Deuss and K. J. N. c. Barta, 2021, 12, 5424.