Electronic Supplementary Material (ESI) for Green Chemistry.

Elucidating non-productive adsorption mechanism of cellulase with lignin fractions from hydrothermally pretreated poplar using multi-dimensional spectroscopic technologies

1. Stern-Volmer equation

Quenching of fluorescence for SL and RL on cellulase was fitted following the Stern-Volmer [1,2]:

$$F_0/F = 1 + K_{SV}[Q] \quad \text{(Equation S1)}$$

Where F_0 and F represented the fluorescence intensity of cellulase and cellulase-SL/RL; K_{sv} and $[Q]$ represented the dynamic quenching constant and the lignin's total concentration, respectively.

2. Calculating the binding parameters by FLS

To further study the interaction between SL/RL and cellulase, a double logarithmic formula was used to calculate the apparent binding constant [2].

$$\lg\left[(F_0 - F)/F\right] = lgK + nlg[Q] \quad \text{(Equation S2)}$$

where F_0 and F are the cellulase fluorescence intensities in the absence and presence of quencher (e.g., lignin), respectively; K_a is the binding constant; n is the number of binding sites; $[Q]$ is the concentration of quencher (e.g., lignin).

3. Thermodynamics parameters

The van't Hoff and Gibbs-Helmholtz equations might be used to calculate the value of ΔG as well as the value of ΔS in the case of constant ΔH[3]:

$$\ln\left(\frac{K_2}{K_1}\right) = \frac{\Delta H}{R} \cdot \left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad \text{(Equation S3)}$$

$$\Delta G = \Delta H - T \cdot \Delta S \quad \text{(Equation S4)}$$

$$\Delta G = -RT \cdot lnK \quad \text{(Equation S5)}$$

Where K_1 and K_2 (L·mol^{-1}) represented the binding constants related to temperature T_1 and T_2; T was the absolute temperature, and R was the gas constant; ΔH (kJ·mol^{-1}) and ΔS

($J \cdot mol^{-1} \cdot K^{-1}$) was the enthalpy change and entropy of the reaction system.

The interaction between lignin and cellulase is influenced by several forces, including hydrogen bonding, van der Waals forces, electrostatic interactions, and hydrophobic interactions. These forces are difficult to quantify individually and can be affected by several factors, such as temperature, pH, and ionic strength. In the process of lignin and cellulase interactions, ΔH and ΔS values can provide insights into the relative contribution of different forces to the overall interaction. For example, if the interaction between lignin and cellulase is primarily driven by hydrogen bonding, the ΔH value will be negative, indicating that energy is released during the formation of the bond. The ΔS value will be negative, indicating that the system becomes more ordered during the interaction. In contrast, if the interaction is driven by hydrophobic forces, the ΔH value will be positive, indicating that energy is required to disrupt the interactions, and the ΔS value will be positive, indicating that the system becomes more disordered during the interaction.[4] According to Ross's theory,[3] the major binding forces could be implied from the values of the ΔS and ΔH. When $\Delta S>0$, it is mainly manifested as hydrophobic and electrostatic forces; when $\Delta S<0$, it is mainly hydrogen bonds and van der Waals forces; when $\Delta H>0$ and $\Delta S>0$, it is mainly typical hydrophobic forces; when $\Delta H<0$ and $\Delta S<0$, it is mainly hydrogen bonds and van der Waals forces.

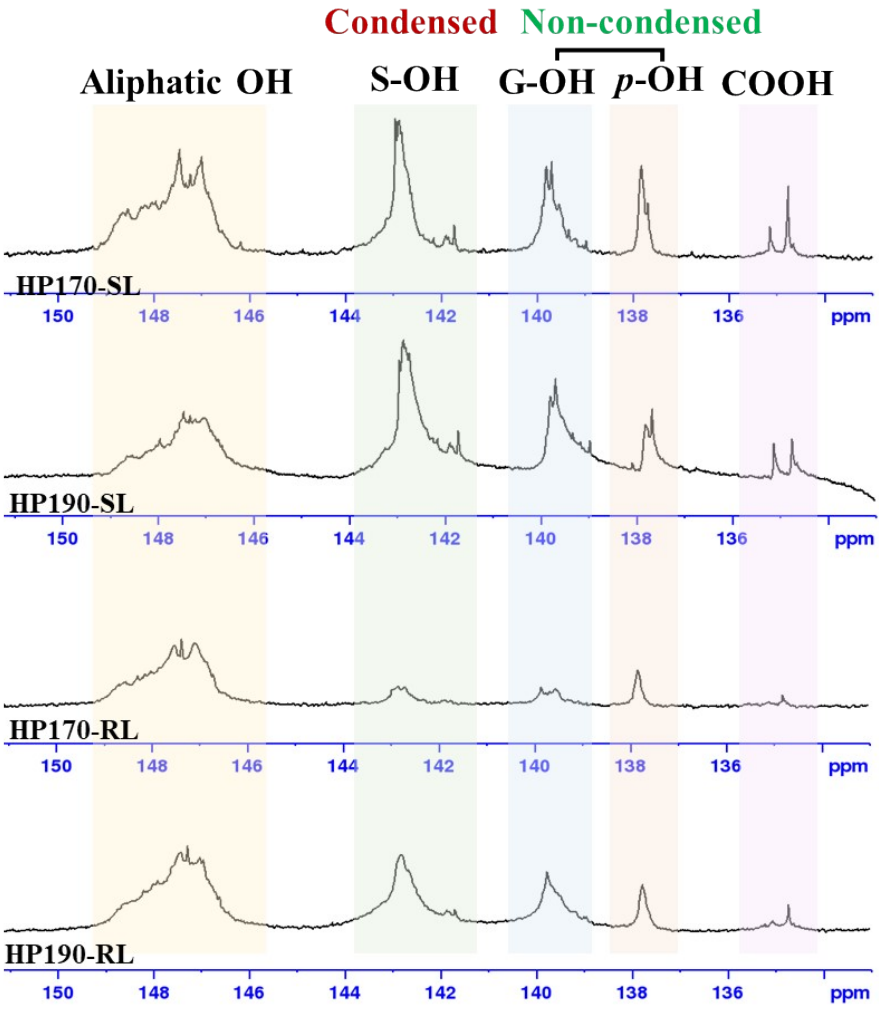

Figure S1 ^{31}P NMR spectra from the SL and RL.

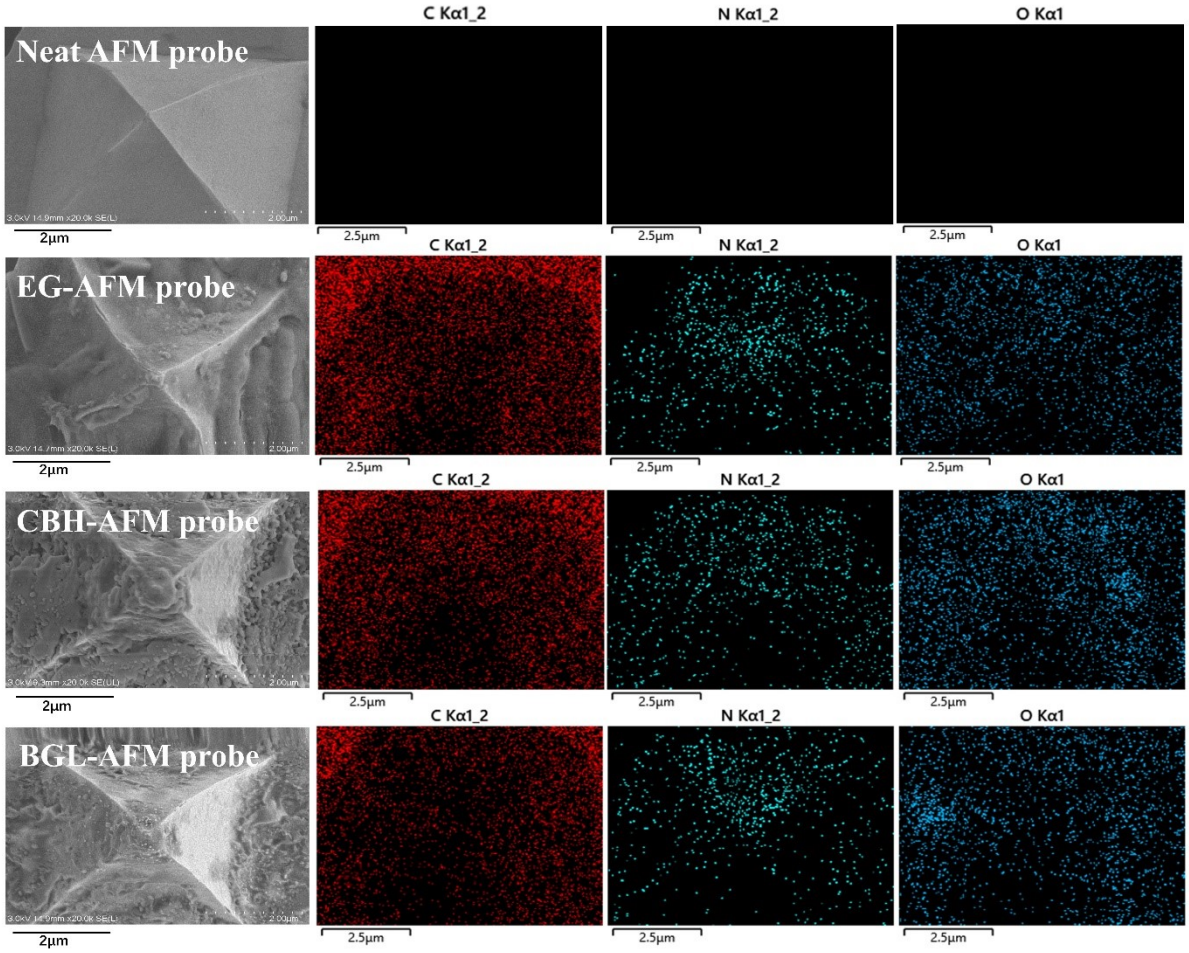

Figure S2 FE-SEM analysis morphology of neat AFM probe and AFM probe modified by different enzymes of EG, CBH and BGL.

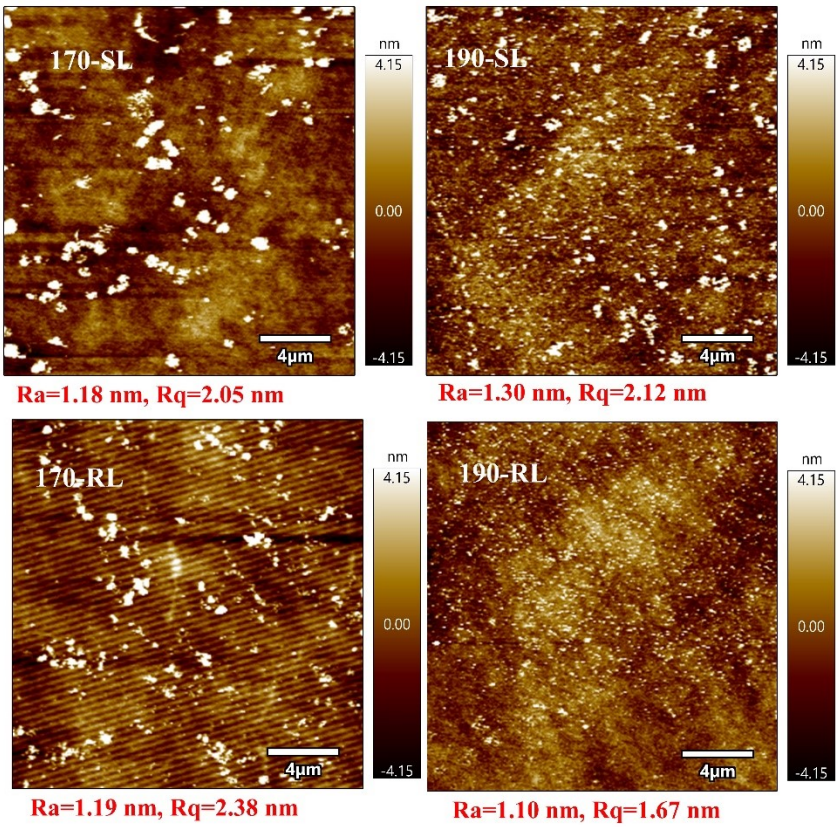

Figure S3 AFM image of lignin film from 0.5% DMSO aqueous solution.

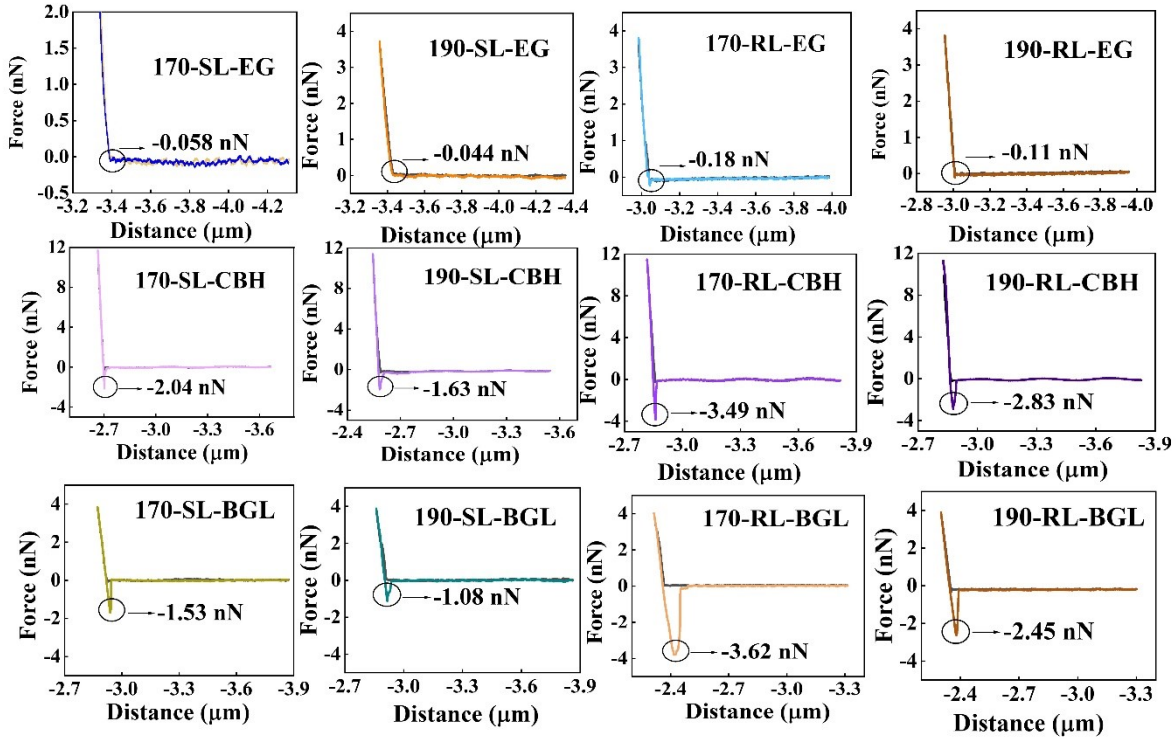

Figure S4 The force-distance curve between SL, RL film and different functionalized AFM probes (EG, CBH, BGL enzymes).

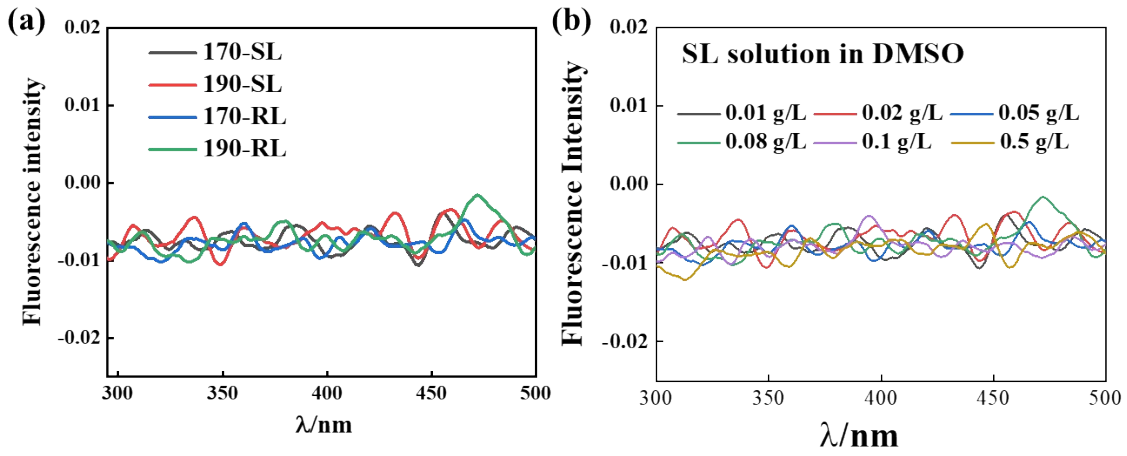

Figure S5 (a): Fluorescence emission peaks of different lignin solutions at 280 nm excitation wavelength. (b): Fluorescence emission peaks of SL DMSO solutions with different concentrations at 280 nm excitation wavelength.

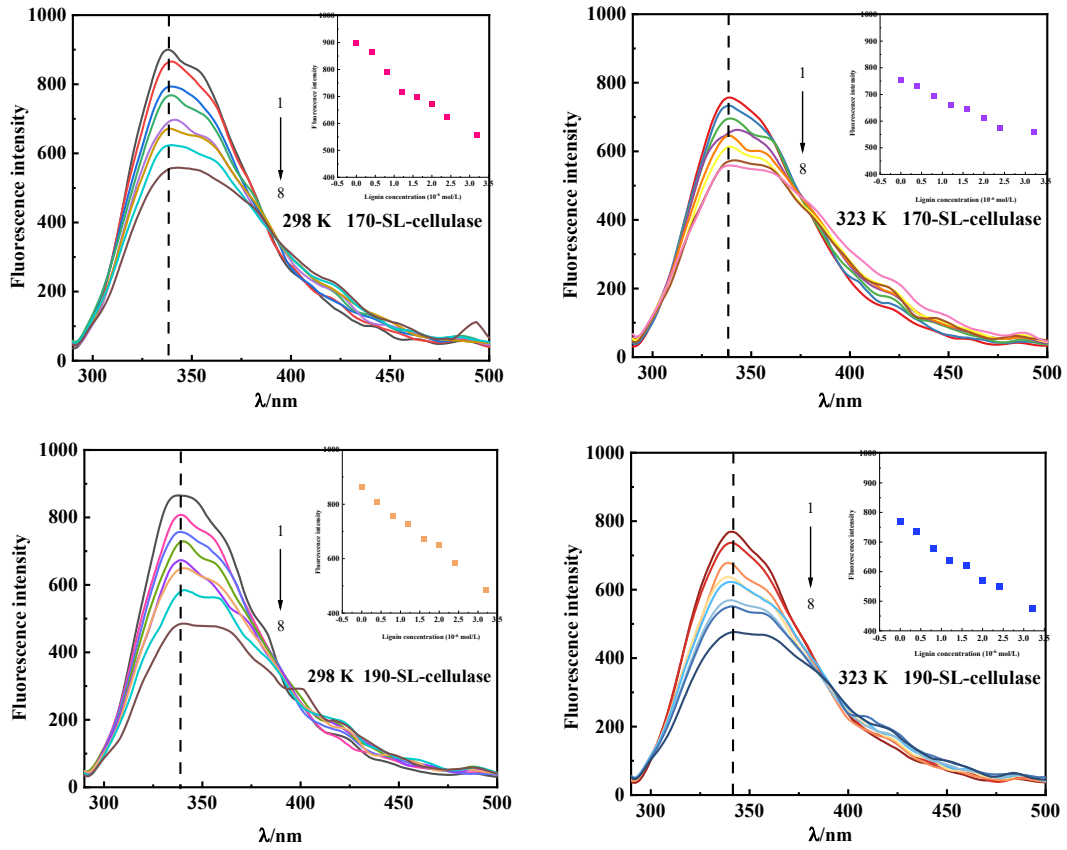

Figure S6 Fluorescence emission spectra of cellulase at 1×10^{-6} mol/L titrated with different concentrations of SL at 298 K and 323 K. λ_{ex}=280 nm, λ_{em}=300-500 nm.

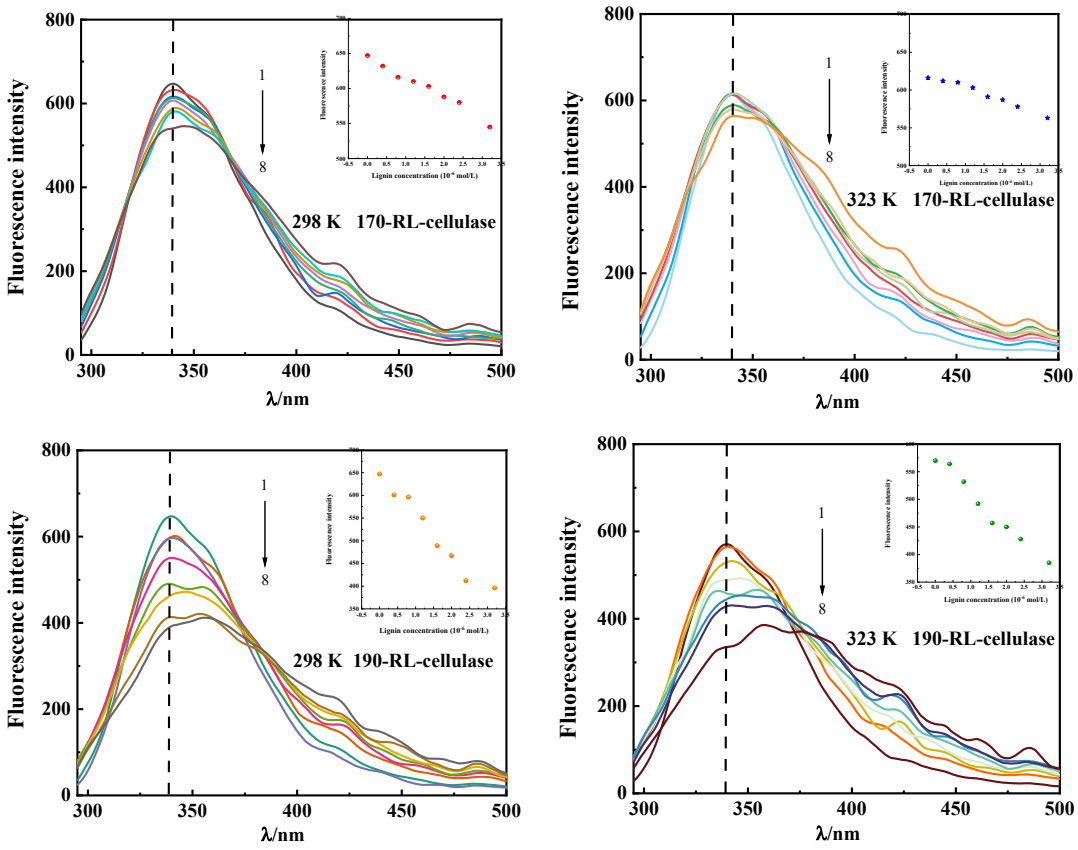

Figure S7 Fluorescence emission spectra of cellulase at 1×10^{-6} mol/L titrated with different concentrations of RL at 298 K and 323 K. λ_{ex}=280 nm, λ_{em}=300-500 nm.

References

1. J. R. Lakowicz, *Principles of fluorescence spectroscopy*, Springer, New York, 3rd ed., 2006.
2. S. Zhuang, H. Wang, K. Ding, J. Wang, L. Pan, Y. Lu, Q. Liu and C. Zhang, *Chemosphere*, 2016, **144**, 1050–1059.
3. P. D. Ross and S. Subramanian, *Biochemistry*, 1981, **20**, 3096–3102.
4. W. Norde, *Adv. Colloid Interface Sci.*, 1986, **25**, 267–340.