Electronic Supplementary Material (ESI) for Toxicology Research.

Supplementary Information

Exploring the binding interaction between Copper Ions and Candida Rugosa Lipase

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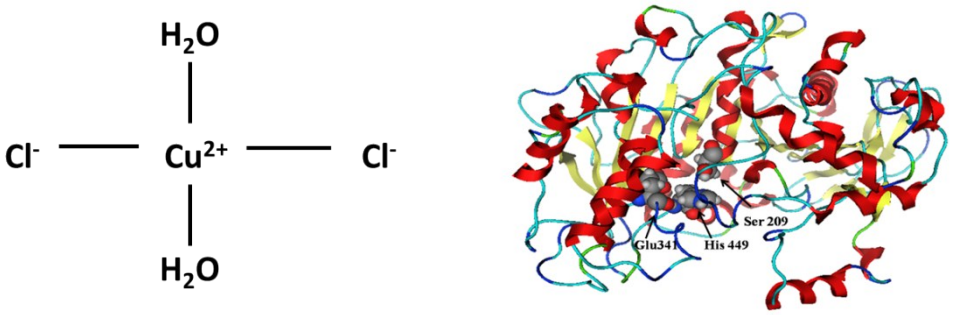

Fig.S1 Schematic illustration of molecular structure of ligands and proteins

Serine (Ser-209), histidine (His-449) and glutamic (Glu-341) were found to be the central group of the catalytic center of the CRL. In the structure image of CRL, the helix of CRL was colored as red, the coil structure of CRL was colored as blue and the strand was colored as yellow.

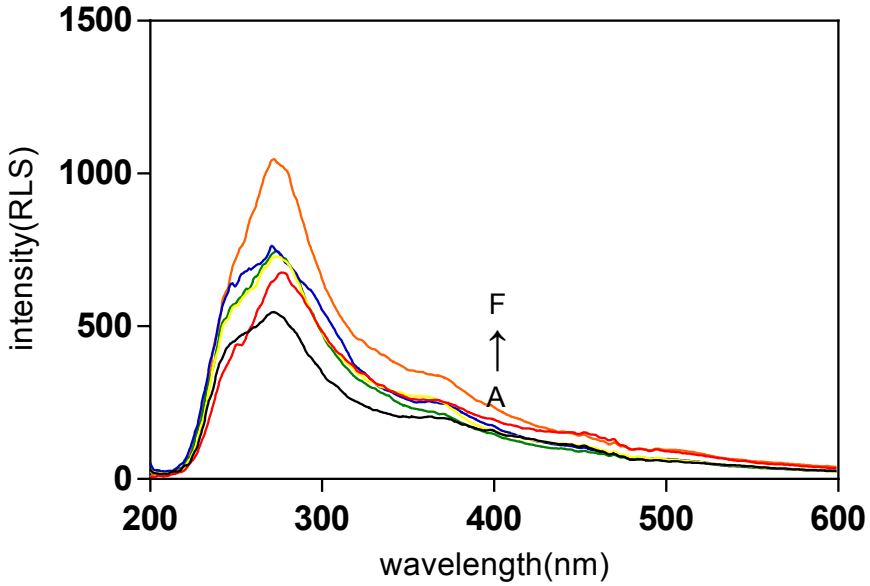

Fig.S2 RLS spectra of Cu^{2+}

Conditions:C_{Cu}^{2+}（A-F）= 0, 2×10^{-5}, 4×10^{-5}, 6×10^{-5}, 8×10^{-5}, 10×10^{-5} M; pH=5.5, T=310 K.

The resonance scattering spectrum in the presence of copper ions without the addition of CRL, to ensure that the change in resonance scattering spectra was the result of the interaction between copper and CRL.

Table S1 The inner-filter effect of Cu^{2+}-CRL system

C_{Cu}^{2+}($\times10^{-5}$ M) / Wavelength (nm)	0	2	4	6	8	10	12	14
279	0.026	0.032	0.036	0.039	0.039	0.051	0.058	0.067
279.2	0.026	0.031	0.035	0.039	0.039	0.051	0.058	0.066
279.4	0.026	0.031	0.035	0.038	0.038	0.051	0.057	0.066
279.6	0.026	0.031	0.035	0.038	0.038	0.05	0.057	0.065
279.8	0.026	0.031	0.035	0.038	0.038	0.05	0.057	0.065
280	**0.026**	**0.031**	**0.035**	**0.038**	**0.038**	**0.049**	**0.056**	**0.064**
280.2	0.026	0.031	0.034	0.037	0.038	0.049	0.056	0.063
280.4	0.026	0.03	0.034	0.037	0.037	0.049	0.055	0.063
280.6	0.025	0.03	0.034	0.037	0.037	0.048	0.055	0.062
280.8	0.025	0.03	0.034	0.037	0.036	0.048	0.054	0.061
281	0.025	0.03	0.033	0.036	0.036	0.047	0.053	0.061

The fluorescence intrinsic effect refers to a phenomenon in which fluorescence is weakened due to absorption of excitation light or emitted light by a phosphor or other light-absorbing substance when the concentration of the phosphor is large or coexist with other light-absorbing substances. But we adopted a lower concentration in this system, the absorbance value at 280 nm shown in Table1 is<0.1, at this time, the error caused by the absorption that quenching agent to excitation and / or emission wavelength is<0.5%, which means that the internal filter effect can be neglected.

Table S2 The specific parameters of copper and CRL

ligand	receptor	residue	distance
Cu 8509	CA 1813	GLY	3.32
Cu 8509	N 1811	GLY	3.74
Cl 8511	CA 1813	GLY	3.93
Cl 8511	N 1811	GLY	3.60
Cl 8511	OG 3069	SER	3.38
Cl 8511	NE 6643	HIS	3.65

Table 2 shows that copper chloride was close to the active center of CRL (Ser-209, His-449, Glu-341), which is about 3Å. So the binding interaction between copper chloride and CRL could cause structural changes of CRL and affect the enzyme activity. These results help to explain the conclusion obtained from changes in molecular structure enzyme activity.

Table S3 Thermodynamic parameters of copper ions combined with CRL (T = 298 K)

	N	Ka(10^{-3} M^{-1})	ΔH(cal/mol)	ΔS(cal/mol·K)	ΔG(cal/mol)
Cu-CRL	6.60±1.04	2.91±0.619	4055±847.6	29.4	-4706.2

The number of binding sites (N) was 6.60±1.04, indicating that each CRL molecule could bind about 7 copper ions. The binding affinity constant (Ka) was (2.91 ± 0.619) × 10^{-3} M^{-1}. The enthalpy change (ΔH) and entropy change (ΔS) were all greater than 0 indicates that the reaction is an endothermic progress, the degree of chaos in the reaction system increases, and the main driving force of the process is hydrophobic. Gibbs Free Energy (Δ G)<0, indicating that the combination of Cu^{2+} and CRL is a spontaneous process.