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Supporting information for article:

Direct relationship between dimeric form and activity in the acidic Cu–Zn superoxide dismutase from lemon

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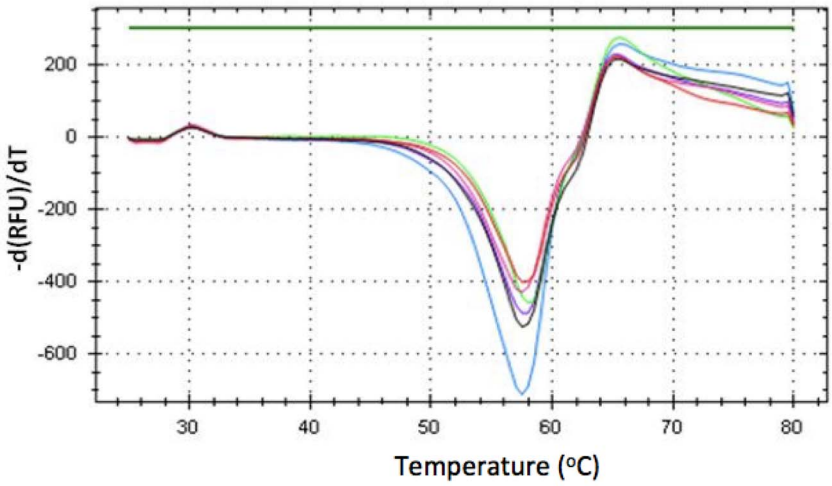

Figure S1 The melting curves of SOD_CL in LEW buffer (black), PBS at 7.4 (blue), 50 mM Tris HCl at 7.0 (red) and 8.0 (pink), 50 mM HEPES at pH 7.0 (green) and 8.0 (purple).

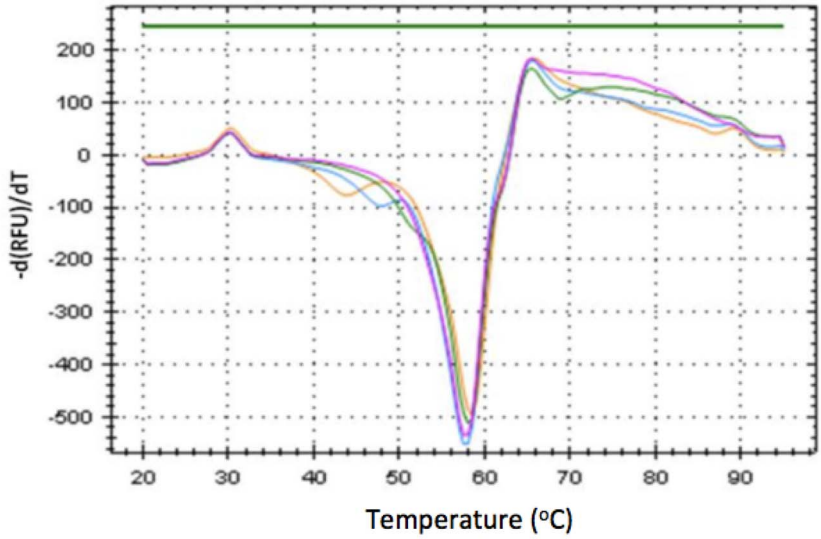

Figure S2 The melting curves of SOD_CL in Britton-Robinson buffer at pH 8.0 (pink), 9.0 (green), 10.0 (cyan), and 11.0 (orange).

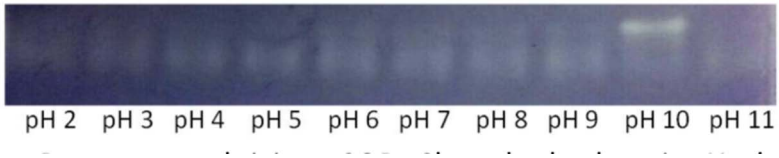

Figure S3 Activity of SOD_CL at various pH values. The intensity of the clear zone increases as the pH increases and an additional band appears at pH 10.0.

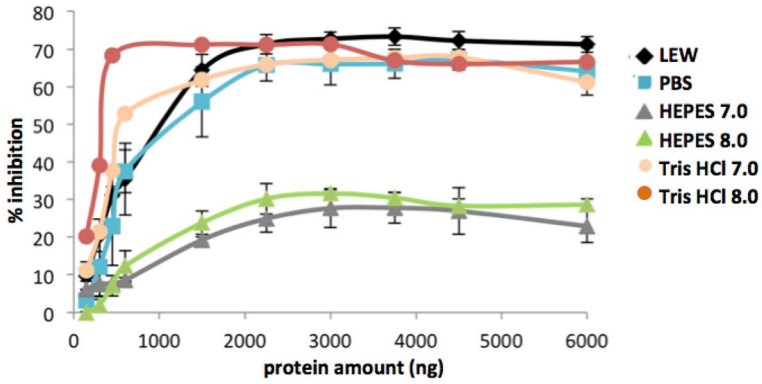

Figure S4 SOD_CL activity (presented as inhibition of oxidation reaction) in various type of buffers

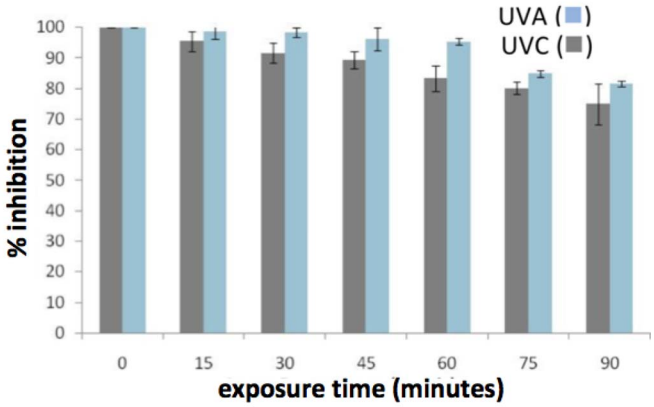

Figure S5 SOD_CL activity (presented as inhibition of oxidation reaction) after exposure to the UV irradiation.

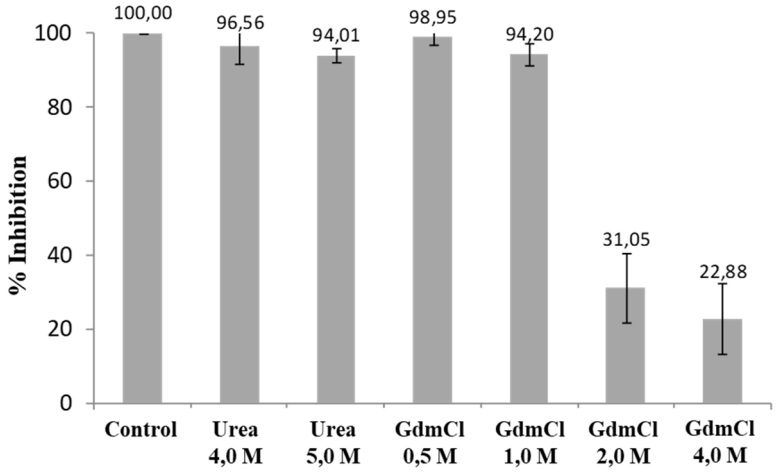

Figure S6 SOD_CL activity (presented as inhibition of oxidation reaction) in Urea/GdmCl various concentration

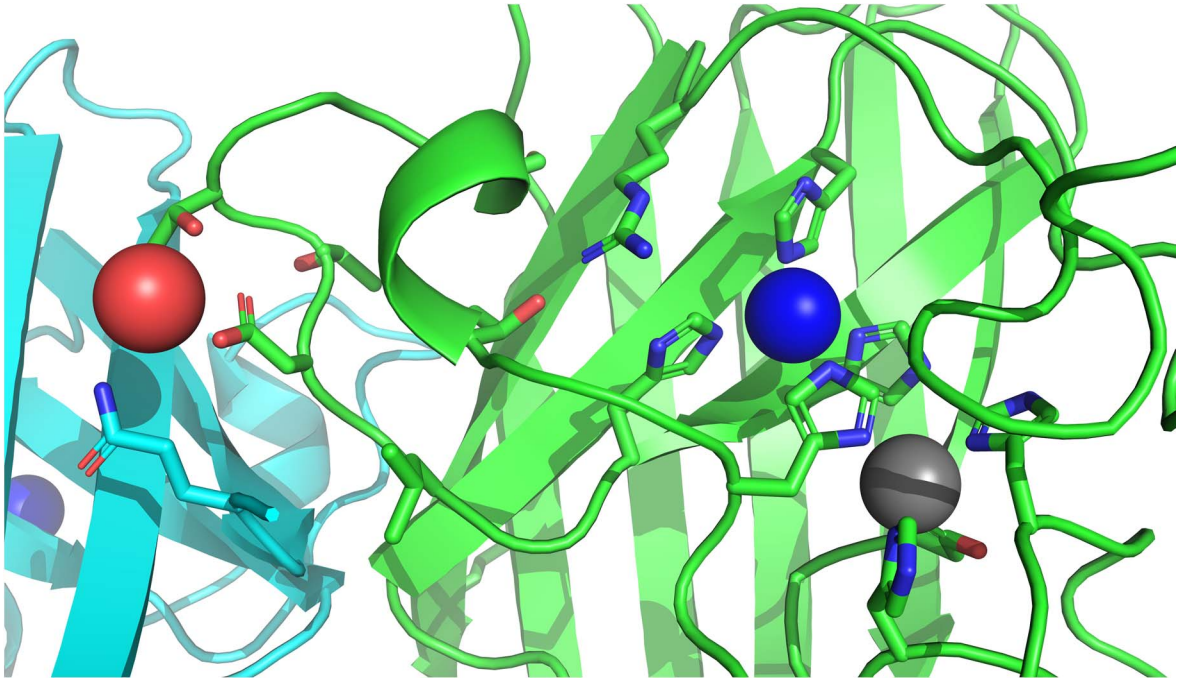

Figure S7 The chain of residues from the Zn binding site to the dimer interface. Monomer A and B are presented in green and cyan; the residues are colored accordingly. The grey, blue and red spheres are of Zn and Cu atoms, and water molecule.

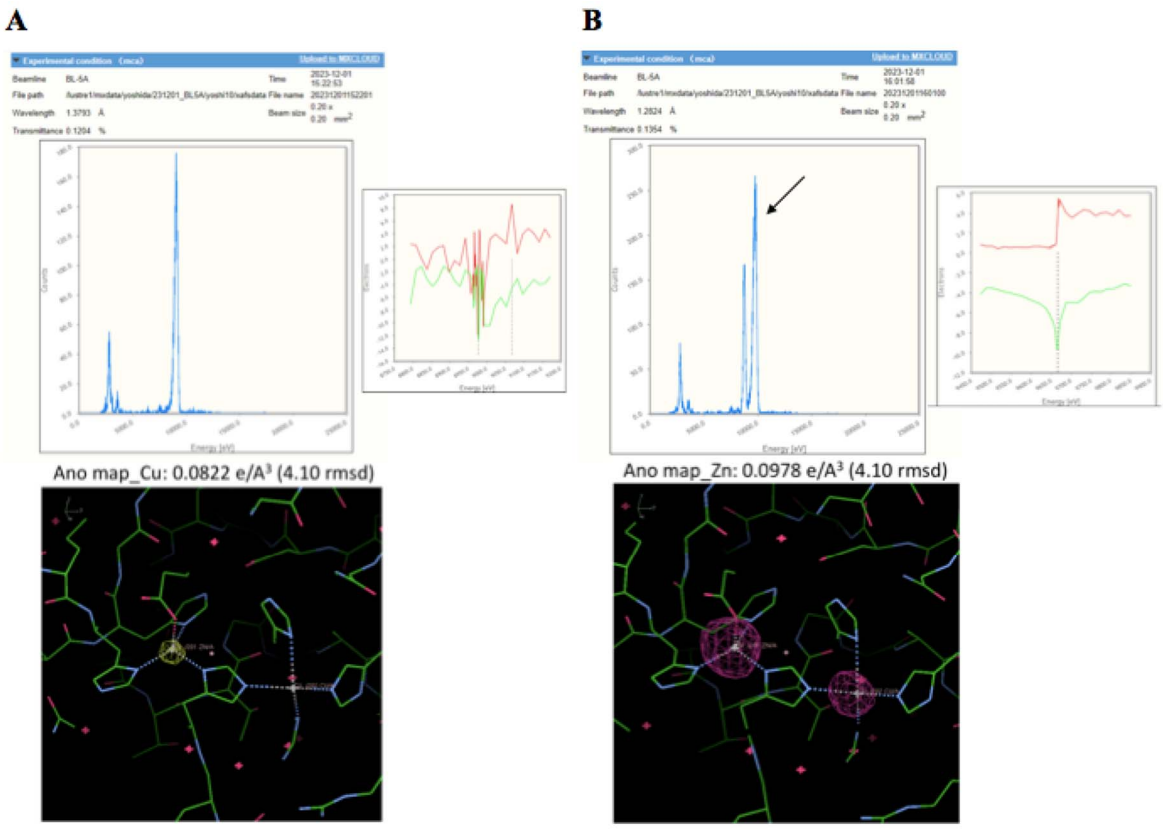

Figure S8 XAFS profile and anomalous signals for Cu (A) and Zn (B) from a crystal grown under the same conditions. The anomalous data for Cu and Zn were collected at 1.3672 Å and 1.28234 Å, respectively.

Table S1 Activity of SOD_CL upon treatment with chelating agents and/or metals

Strategy*	Treatment	Activity as compared to the untreated
1	EDTA 20 mM	0,03
	$CuCl_2$ 20 mM	1,32
	$ZnCl_2$ 20 mM	0,71
	$CuCl_2$ 20 mM + $ZnCl_2$ 20 mM	1,21
	Without treatment	1,00
2	Buffer Tris-HCl pH 8 (Metal free sample)	0,25
	Zn,Cu	1,38
	Cu,Zn	1,39
	Without treatment	1,00

Strategy 1: SOD_CL activity was measured in the presence of exogenous metals as indicated in the treatment.

Strategy 2: IMAC-mediated metalation of His-tagged SOD_Cl

Selective metalation was achieved by treat SOD_CL with EDTA to remove the metal and then reactivation by combination Cu or Zn was further assayed by independently incubating the EDTA-inactivated SOD_Cl in sequence with $CuCl_2$ or $ZnCl_2$. The treatment was done with the enzyme immobilized on Ni-NTA resin.

Each 100 µL of Ni-NTA agarose resin slurry (Roche) was transferred to five different 1.5 ml microcentrifugation tube, centrifuged for 1 minute at 2000 rpm and the supernatant removed. The Ni-NTA agarose resin slurry was equilibrated twice for 10 minutes in 1 ml of LEW buffer (50mM NaH_2PO_4, 300mM NaCl, pH 8.0,) before mixing it with the SOD_Cl.

SOD_Cl was treated by EDTA addition to a final concentration of 6 mM then add to Ni-NTA agarose resin, incubated overnight at 4°C with rotational shaking. The tube was centrifuged at 2000 rpm for 1 minute and the buffer aspirated. Metal-reactivation proteins were acquired by incubating the resin with the corresponding metal either $CuCl_2$ or $ZnCl_2$ in two step addition. To prepare metal free sample, Tris-HCl pH 8 solution was used. Elute the protein using imidazol 250 mM. Note: extra care must be paid not to transfer Ni-NTA resin along with the supernatant.