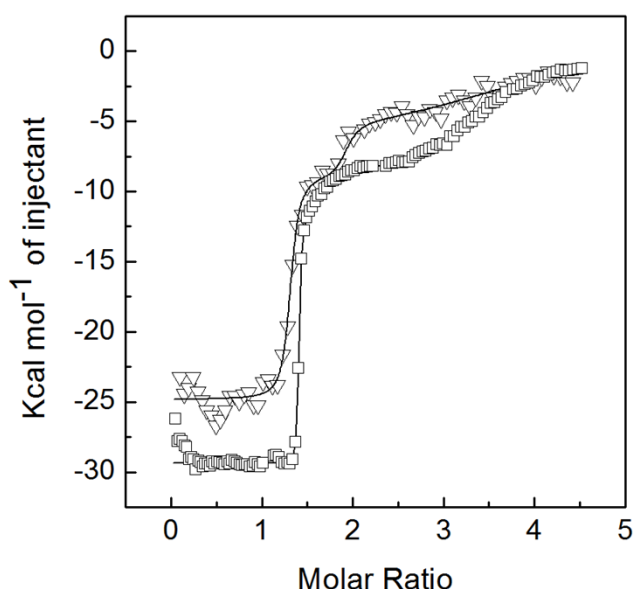


## Aberrant zinc binding to immature conformers of metal-free copper-zinc superoxide dismutase triggers amorphous aggregation

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### Supplemental Figures and Tables

**Figure S1: Comparative isothermal titration calorimetry analysis of Zn<sup>2+</sup> binding to dSOD1<sup>S-S</sup> at distinct concentrations.** Integrated data of raw heats of dilution obtained after subtracting the heat of dilution. See also Table S1. 20 μM (∇) and 80 μM (□) of dSOD1<sup>S-S</sup> at pH 7 and 37°C (see Materials and methods)

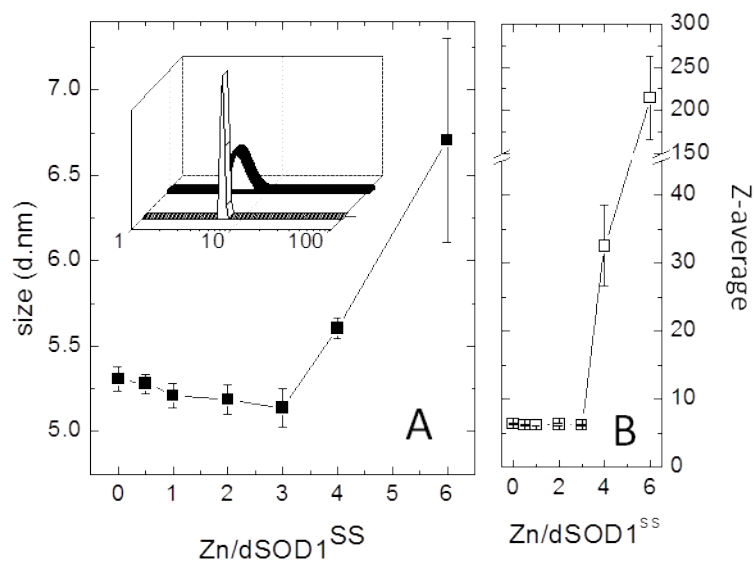


**Table S1: Estimated parameters of Zn<sup>2+</sup> binding to 20 μM of dSOD1<sup>S-S</sup> <sup>a</sup>**

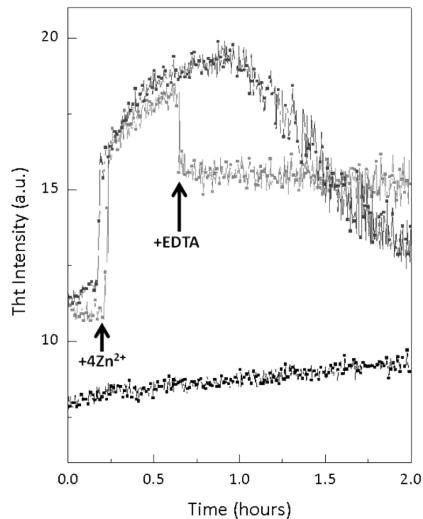
	n	K <sub>a</sub> (M <sup>-1</sup> )	ΔH (Kcal/mol <sup>-1</sup> )	ΔG (Kcal/mol <sup>-1</sup> )	ΔS (cal/mol <sup>-1</sup> /deg)
I	1.28 ± 0.02	1.2 × 10 <sup>10</sup>	-24.0 ± 0.3	-14.3	10.55
II	0.62 ± 0.10	3.9 × 10 <sup>7</sup>	-9.0 ± 1.4	-10.8	-1.77
III	1.94 ± 0.40	1.6 × 10 <sup>5</sup>	-0.1 ± 2.9	-7.4	-1.30

<sup>a</sup> Zn<sup>2+</sup> binding to dSOD1<sup>S-S</sup> (20 μM) in 50 mM TRIS pH 7.4 and 37°C. Apo SOD1 dimer contains 2 zinc-sites and 2 copper-sites, one per monomer. The roman numbering in the table refers to binding events associated with one Zn<sup>2+</sup> binding to the first zinc-site Zn1 (I), to one Zn<sup>2+</sup> binding to the second zinc-site in the apo dimer, Zn2 (II), and to binding of two Zn<sup>2+</sup> to the two copper-sites (Cu1 and Cu2) in the apo dimer.

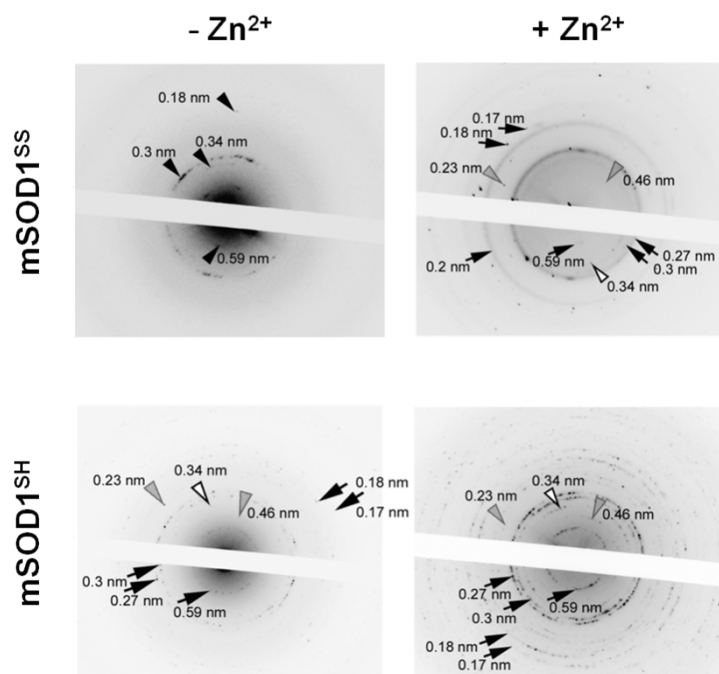
**Figure S2: Impact of increasing  $Zn^{2+}$  concentration on dSOD1<sup>SS</sup> size distribution (A) and Z-average (B) of dSOD1<sup>SS</sup>.** Measurements were performed after 1h of incubation at 37°C. See “Materials and Methods” for further details. *Error bars, S.D*



**Figure S3: ThT aggregation kinetics of dSOD1.** Black line – dSOD1 alone; dark grey - dSOD1 with addition of  $Zn^{2+}$  to a molar ratio of  $Zn^{2+}/dSOD1^{SS}=4$  at 10 minutes; light grey - dSOD1 with  $Zn^{2+}$  with addition of 10 mM of EDTA. Measurements were performed with 150  $\mu M$  of dSOD1<sup>SS</sup> at 37°C without agitation. See “Materials and Methods” for further details.



**Figure S4: Electron diffraction pattern of mSOD1<sup>SS</sup> and mSOD1<sup>SH</sup> with and without Zn<sup>2+</sup>.** Main reflections (black arrows) between 0.27 nm and 0.3 nm (second order) and at about 0.59 nm (first order); weaker reflections at 0.34 nm (white arrow); grey arrow heads label the 0.43 nm and 0.23 nm. -Zn<sup>2+</sup>, no ZnCl<sub>2</sub>; +Zn<sup>2+</sup>, ZnCl<sub>2</sub>/SOD1=4. See “Materials and Methods” for further details.



**Table S2: Qualitative analyses of diffraction pattern reflections of dSOD1<sup>SS</sup>, mSOD1<sup>SS</sup> and mSOD1<sup>SH</sup> with and without zinc.** ° 1-2 single reflections, + weak ring of reflections, ++ ring, +++ strong ring bold lines. See “Materials and Methods” for further details.

Diameter (nm <sup>-1</sup> )	d spacing (nm)	dSOD1 <sup>SS</sup>		mSOD1 <sup>SS</sup>		mSOD1 <sup>SH</sup>	
		-Zn <sup>2+</sup>	+Zn <sup>2+</sup>	-Zn <sup>2+</sup>	+ Zn <sup>2+</sup>	-Zn <sup>2+</sup>	+Zn <sup>2+</sup>
3.4	0.59	++	+++	+++	+	+++	+++
4.4	0.46	°	+	-	°	°	°
5.9	0.34	++	++	++	+	+	°
6.7	0.30	+++	+++	++	++	+++	+++
7.4	0.27	+++	+++	+++	+++	+++	+++
8.6	0.23	++	°	-	+	+	+
9.3	0.22	°	°	-	+	-	-
10	0.20	++	++	°	++	+	+
11	0.18	++	++	++	-	+	+
12.1	0.17	++	++	+	++	+	°