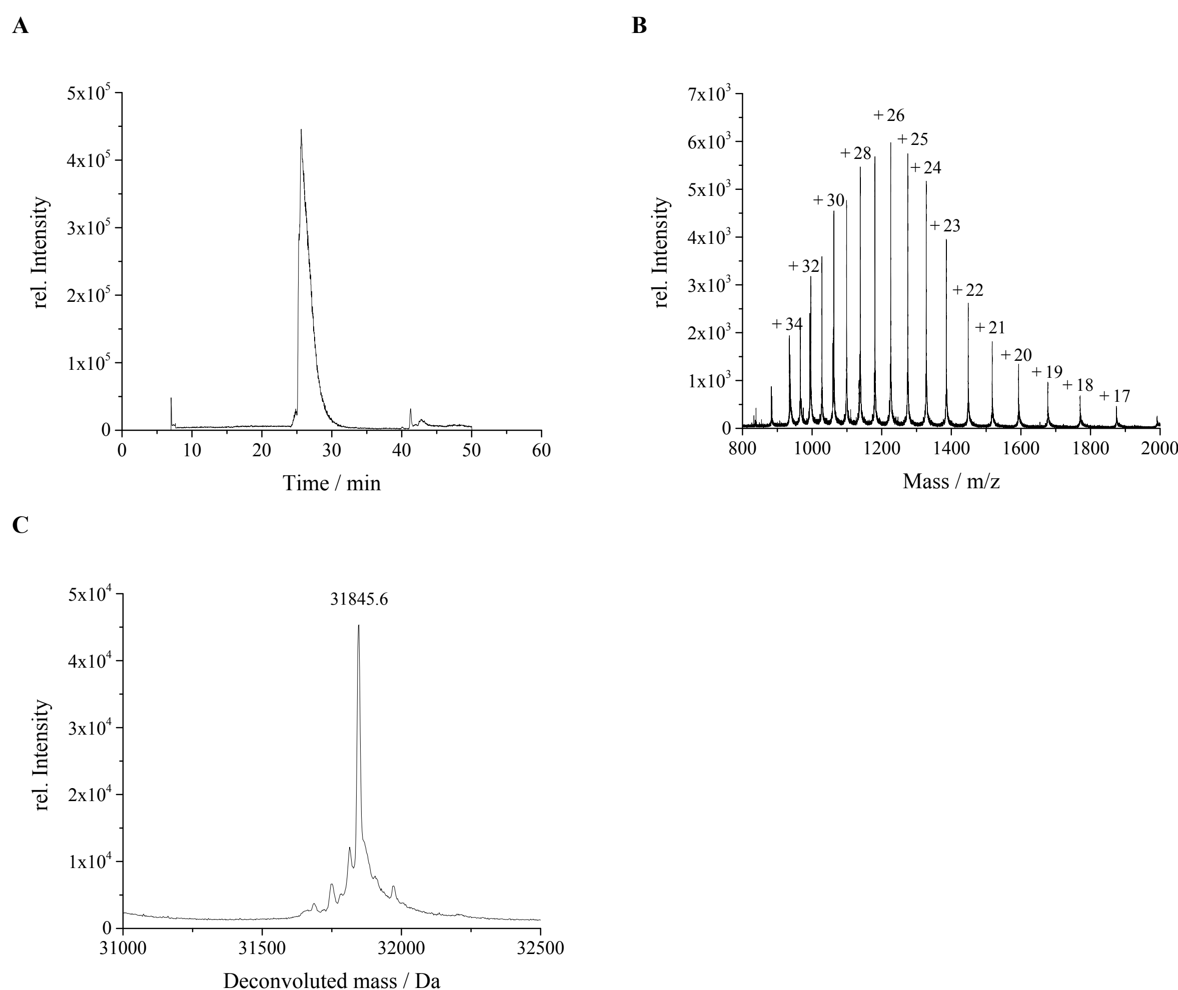
Electronic Supplementary Material (ESI) for JAAS.
This journal is © The Royal Society of Chemistry 2016

Electronic Supplementary Information

***ESI* Table 1:** Typical instrument working conditions.

HPLC	
Agilent 1200 coupled to Agilent ICP-MS 7700	
Autosampler temperature	4 °C
Injection volume	40 µL
Flow rate	0.5 mL/min
Column	MonoQ 5/50 GL
Oven temperature	30 °C
Eluent	A: 12.5 mM Tris pH 7.2
	B: A + 125 mM NH_4Ac pH 7.2
Gradient	7 % B 0 – 5 min
	7 - 50 % B 5 - 10 min
	50 % B 10 – 15 min
	50 - 86 % B 15 – 17 min
	86 % B 17 – 19 min
	86 – 100 % B 19 – 20 min
	100 % B 20 – 25 min
Agilent 1200 coupled to Bruker micrOTOF-Q	
Flow rate	0.2 mL/min
Column	Jupiter300-C4
Oven temperature	40 °C
Eluent	A: 0.1% HCOOH in H_2O
	B: 0.1% HCOOH in CH_3CN
Gradient	5 % B 0 – 5 min
	5 - 100% B 5 – 55 min
	100% B 55 – 65 min
	100 - 5% B 65 – 70 min
	5% B 70 – 80 min
Accela coupled to Element2	
Injection volume	50 µL
Flow rate	1 mL/min
Column	HiTrap (SEC) / MonoQ 5/50 GL
Oven temperature	30 °C
Eluent (SEC)	20 mM Tris + 50 mM KCl pH 7.4
Eluent (MonoQ)	A: 25 mM Tris pH 7.4
	B: A + 250 mM NH_4Ac pH 7.4
Gradient (MonoQ)	0 - 100 % B 0 – 30 min
	100 % B 30 – 35 min
ICP-MS	
Agilent 7700	
RF Power	1500 W
Sample depth	8.0 mm
Plasma gas flow	15 L/min

Spraying chamber temperature	2 °C
Isotopes monitored	63, 65, 66, 67
Collision gas	4.5 mL He / no gas



ESI **Figure 1.** ESI-MS data for the verification of the identity of native SOD1. A) Total ion chromatogram of SOD1. B) Charge distribution of SOD1. C) Mass calculation by charge deconvolution.

Equation for the determination of the Cu mass fraction in native SOD1 by single post-column IDMS

Calculation of K

$$K = \frac{R^{IUPAC}}{R_K} \quad (1)$$

$$R^{true} = K * R^{meas} \quad (2)$$

Single post-column IDMS

$$w_x = \frac{m_y * w_y}{\rho_x * V_{inj}} * \frac{M_x}{M_y} * \frac{\sum R_x}{\sum R_y} * \int_{t_1}^{t_2} \frac{R_y - R_{bx}}{R_{bx} - R_x} dt \quad (3)$$

***ESI* Table 2.** Meaning of the symbols used in the equations (1) – (3).

symbol	unit	definition
w_x, w_y	g/kg	mass fraction of Cu in sample x and spike y, resp.
m_y	g	mass flow of spike y
ρ_x	g/cm³	density of sample x
V_{inj}	mL	injection volume of sample x
M_x, M_y	g/mol	molar masses of natural Cu in sample x and isotopically labelled Cu in spike y
R_x, R_y	mol/mol	isotope amount ratio of spike and reference isotope (^{65}Cu/^{63}Cu) in sample x and spike y, resp.
R_{bx}	mol/mol	isotope amount ratio of spike and reference isotope (^{65}Cu/^{63}Cu) in blend bx (sample x + spike y)
K	1	Correction factor for mass bias
R^{IUPAC}	mol/mol	natural isotope ratio according to IUPAC
R_k	mol/mol	measured isotope ratio in reference k
R^{true}	mol/mol	isotope ratio after correction for mass discrimination
R^{meas}	mol/mol	measured isotope ratio in sample x

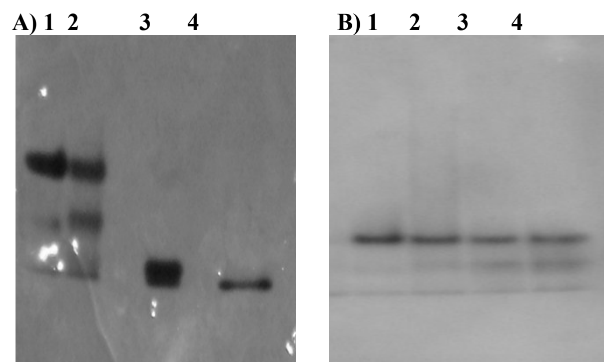
Instrumentation for characterization of produced apo-SOD1 and *SOD1

Both the apo- and remetallated SOD1 as well as the native protein, were characterized regarding metal content, protein integrity and activity.The characterization of the produced apo-SOD1 and the remetallated protein was performed with an Accela HPLC system (Thermo Fisher, Waltham, USA) and an sector-field ICP-MS (Element 2, Thermo Scientific, Schwerte, Germany) in medium resolution (typical operating parameters are also summerized in *ESI* table 1). For the quantification of Cu and Zn in the apo- and the remetallated form of the protein, external calibration using Cu and Zn standard solution and solutions prepared from ^{65}Cu and ^{68}Zn were used. Speciation of apo- and remetallated SOD1 was done using size exclusion chromatography (SEC) on a HiTrap column (Pharmacia, Uppsala, Sweden) and strong anion exchange (SAX) chromatography. Protein integrity was tested using ESI-FT-MS (solvent: 1 mM NH_4Ac buffer at pH 7.4, flow injection). An Orbitrap Discovery system (Thermo Scientific) was used for this purpose for the determination of the molecular mass of the produced apo- and spike protein material using MagTran 1.02 program for the deconvolution of the spectra. The positive fourier transformation (FT) mode with a resolution of 30.000 and an extended mass range from 600 - 3000 Da was used in ESI-FT-MS. The activity was determind according to Marklund[15] using an UV-vis spectrophotometer (Lambda 25, Perkin Elmer, Cambridge, UK). An electrophoresis system from Atto (Tokyo, Japan) was used for native PAGE. Gels were self-casted and contained 7 % polyacrylamid and were stained with Coomassie blue.

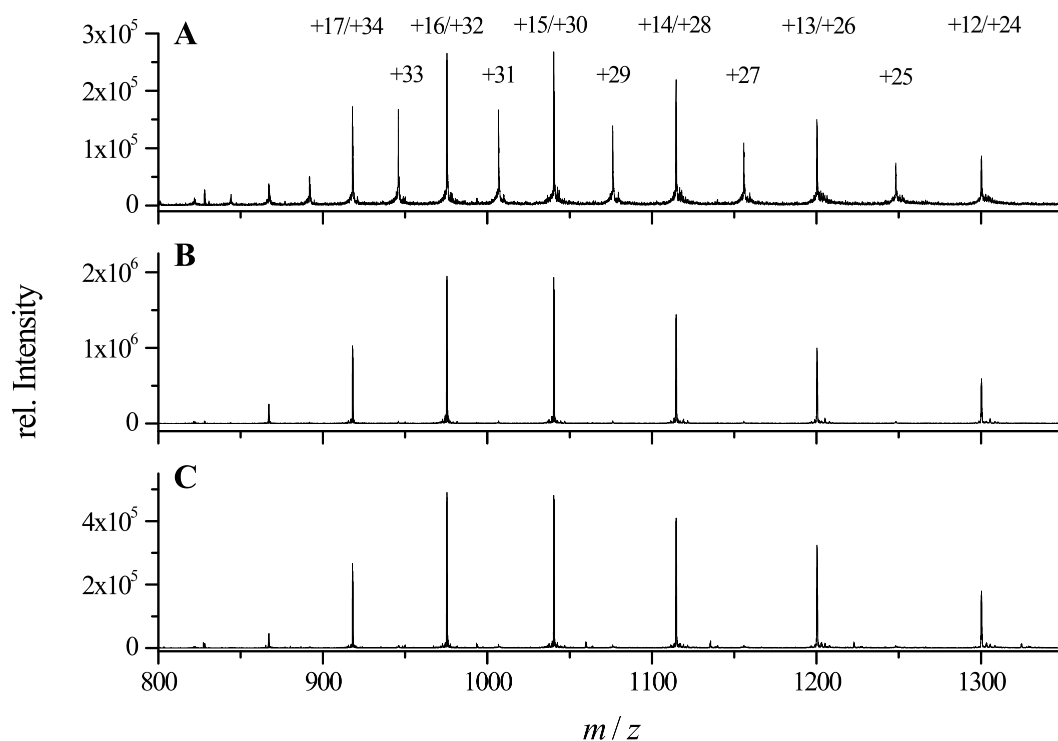
***ESI* Table 3.** Results of ICP-MS measurements expressed as protein concentration of produced apo-SOD1 and isotopically labelled SOD1 (n = 9).

	total Cu ng/mg	RSD %	^{65}Cu ng/mg	RSD %	Total Zn ng/mg	RSD %	^{68}Zn ng/mg	RSD %	Activity U/mg	RSD / %
native SOD	5.96	4.5			4.38	6.7			5690	4
apo-SOD A	1.91	2.5			1.61	1.1			260	20
*SOD A	6.49	4.5	4.46	4.4	5.62	5.6	3.55	4.1	4918	11

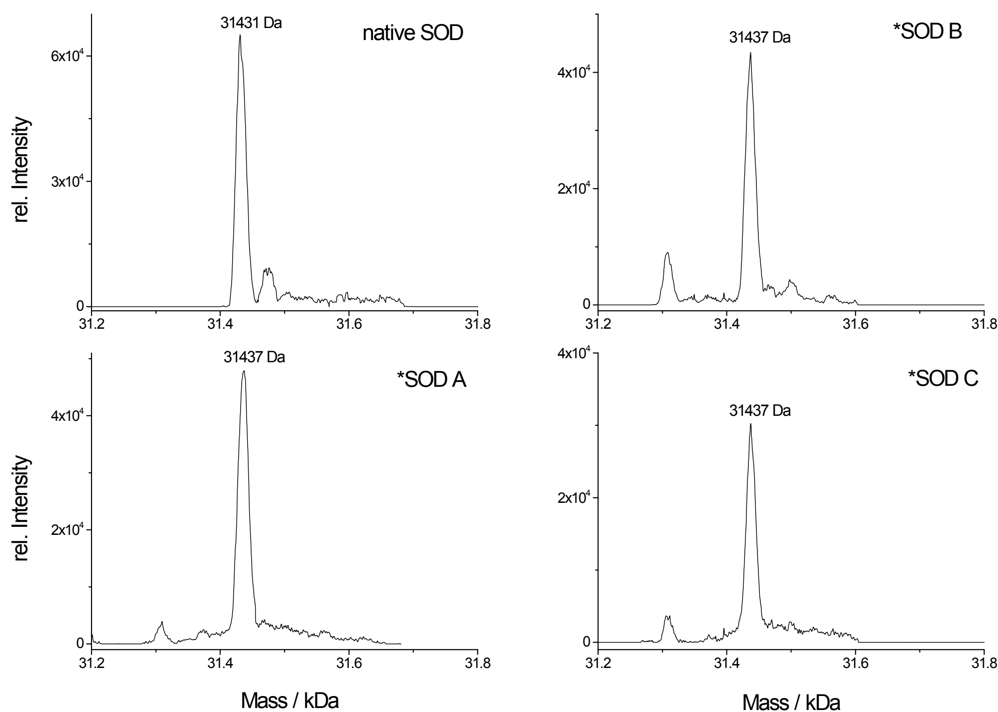
apo-SOD B	0.53	3.4			0.34	2.3			164	14
*SOD B	6.3	4.5	6.1	4.5	6.99	3.9	6.15	3.6	5752	15
apo-SOD C	0.72	4.2			0.72	7.2			311	24
*SOD C	4.3	4.7	3.76	4.4	6.23	5.1	5.18	3.8	4733	1



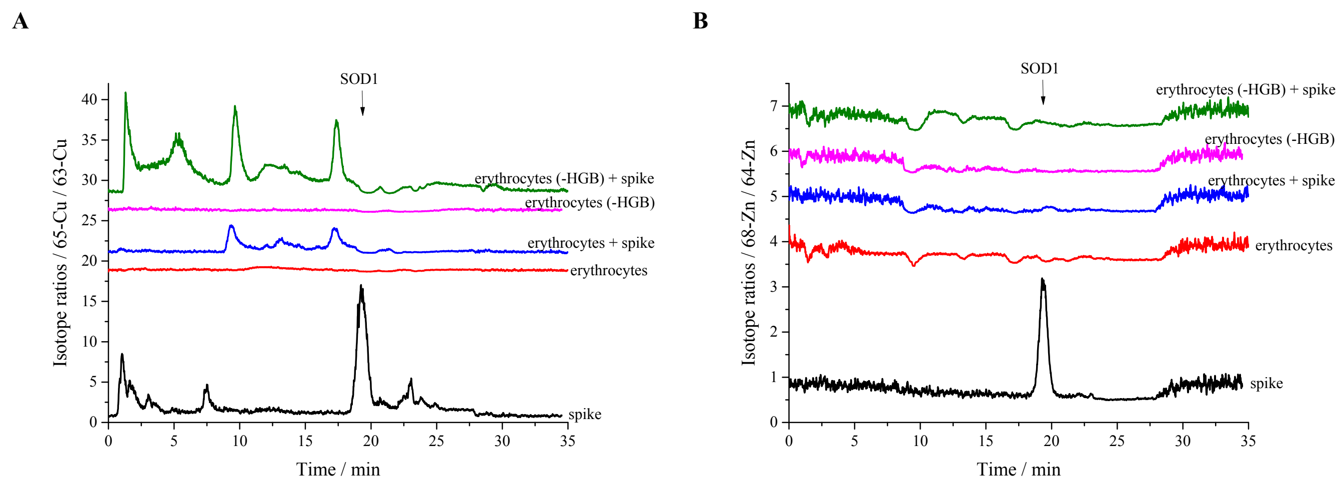
***ESI* Figure 2.** Native PAGE to determine the structural state of A) the apo-SOD1 after production using methods A, B and C, resp., compared to native SOD1. Lane 1: native SOD1, lane 2: apo-SOD1 method A, lane 3: apo-SOD1 method B, lane 4: apo-SOD1 method C and B) the remetallated SOD1 spikes compared to native SOD1. Lane 1: native SOD1, lane 2: *SOD1 A, lane 3: *SOD1 B, lane 4: *SOD1 C.



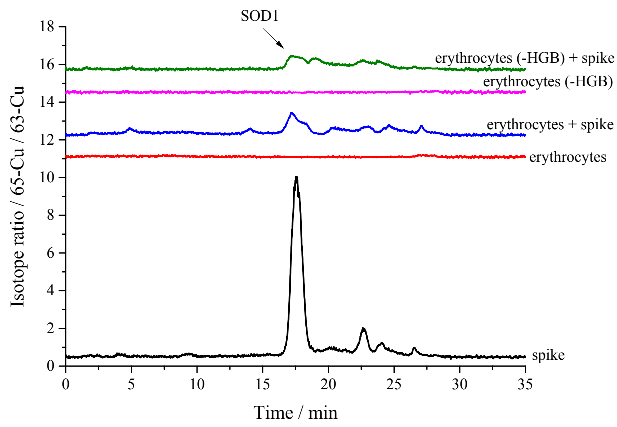
***ESI* Figure 3.** Charge distribution of apo-SOD1 prepared according to A) method A, B) method B and C) method C.

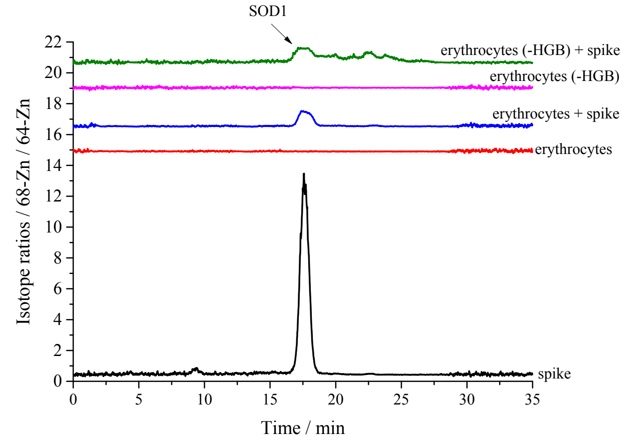


***ESI* Figure 4.** Mass calculation by charge deconvolution for SOD1 and *SOD1 prepared according to method A, B and C.

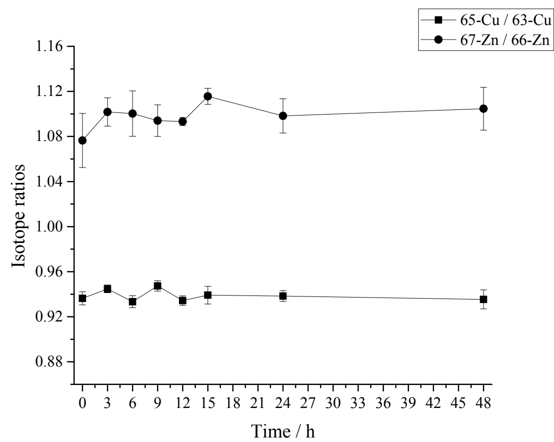


***ESI* Figure 5.** Stability of *SOD1 C on MonoQ 5/50 GL column: A) ^{65}Cu / ^{63}Cu ratios and B) ^{68}Zn / ^{64}Zn ratios for pure spike material, erythrocyte lysate, erythrocyte lysate (Hb precipitated) and both of the latter spiked with *SOD C. Y-axis is set-off for clarity purposes.





***ESI* Figure 6.** Stability of *SOD1 B on MonoQ 5/50 GL column: A) ^{65}Cu / ^{63}Cu ratios and B) ^{68}Zn / ^{64}Zn ratios for pure spike material, erythrocyte lysate, spiked erythrocyte lysate, erythrocyte lysate (Hb precipitated) and spiked erythrocyte lysate (Hb precipitated). Y-axis is set-off for clarity purposes.



***ESI* Figure 7.** $^{65}Cu/^{63}Cu$ resp $^{67}Zn/^{66}Zn$ isotope ratio of human SOD spike in erythrocyte lysate over a time period of 48 h. The error bars represent the standard deviation (n = 3).