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SUPPLEMENTARY INFORMATION

Modulating Glyoxalase I Metal Selectivity by Deletional Mutagenesis: Underlying Structural Factors Contributing to Nickel Activation Profiles

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Table S1: Percentage of amino acid sequence identity and similarity (in brackets) of various glyoxalase I pertinent to the present investigation[1].

Organisms	*E. coli*	*H. sapiens*	GloA1	GloA2	GloA3
E. coli		27(37)	75(82)	64(75)	33(43)
H. sapiens			31(41)	28(42)	50(64)
GloA1				72(81)	34(45)
GloA2					34(46)
GloA3					

[1]organism name followed by National Center for Biotechnology Information (NCBI) accession number. *E. coli* (NP_310387), *H. sapiens* (AAB49495) and *P. aeruginosa* (GloA1, GloA2 and GloA3 with NCBI access numbers AAG06912, AAG04099 and AAG08496, respectively) were determined using web-based software, SISA: Sequence Identities and Similarities (http://imed.med.ucm.es/Tools/sias.html). The sequence identity was calculated by taking gaps into account using the following equation; PID = 100(identical position)/(length of the alignment). The global sequence similarity was calculated using BLOSUM62 matrix with gap penalties of 10 for the cost of creating the gap and 0.2 for the cost of extending the gap.

Table S2. Calculated molecular mass, recovery yield and theoretical pI for wild-type GloA2, wild-type GloA3 and GloA3 protein variants.

Sample	[#]***Molecular mass (Da)***	***Recovery yield (mg)/L culture***	***Recovery yield (mg)/g cell pellet***	[#]***Theoretical pI***
GloA2	14925.8	97	18	5.54
GloA3	20083.5	232	29	5.00
delbGloA3	18292.5	52	6	4.95
delcGloA3	19462.9	111	20	5.39
deldGloA3	19587.0	85	10	4.99
delbcGloA3	17672.0	25	6	5.35
delbdGloA3	17796.0	47	8	4.94
delcdGloA3	18966.4	33	7	5.38
delbcdGloA3	17175.5	10	1.5	5.34

[#] Molecular mass and theoretical pI of the monomeric enzyme were calculated from amino acid sequences using the web-based software, ProtParam tool from ExPASy Proteomics Server (http://ca.expasy.org/tools/protparam.html). Calculations based on proteins lacking the N-terminal methionine.

Table S3. Kinetic data for wild-type GloA2, wild-type GloA3 and GloA3 protein variants prepared from *E. coli* expression systems that were grown and expressed in the absence as well as in the presence of 1 mM metals ($ZnCl_2$ or $NiCl_2$) in the growth media. Purified enzymes isolated from cells grown in the presence of metal chlorides were also incubated in the presence of 5 equivalents of those particular metals overnight at 4 °C before performing assays using 0.02-1 mM substrate (MG-GSH), pH 6.6, at room temperature.

Enzyme	***Metal chloride***	***V_{max} (μmol/min/mg)***	***K_m (mM)***	***k_{cat} (s^{-1})***	***k_{cat}/K_m ($M^{-1}.s^{-1}$)***	****Relative k_{cat}/K_m***
GloA2	Ni^{2+}	377 ± 6	0.13 ± 0.01	188	1.4×10^6	-
GloA3	-	589 ± 42	0.24 ± 0.04	395	1.7×10^6	0.6
	Zn^{2+}	831 ± 45	0.21 ± 0.01	556	2.6×10^6	1
	Ni^{2+}	1000 ± 67	0.13 ± 0.01	669	5.3×10^6	2
delbGloA3	-	2.6 ± 0.3	0.71 ± 0.09	1.6	2253	0.7
	Zn^{2+}	4.6 ± 0.5	0.88 ± 0.16	2.8	3182	1
	Ni^{2+}	2724 ± 174	1.06 ± 0.09	1661	1.6×10^6	503
delcGloA3	-	145 ± 9	2.23 ± 0.18	94	4.2×10^4	0.3
	Zn^{2+}	109 ± 9	0.56 ± 0.05	71	1.3×10^5	1
	Ni^{2+}	950 ± 43	0.71 ± 0.03	616	8.7×10^5	7
deldGloA3	-	183 ± 41	1.67 ± 0.49	120	7.2×10^4	0.2
	Zn^{2+}	258 ± 61	0.41 ± 0.19	168	4.1×10^5	1
	Ni^{2+}	374 ± 14	0.72 ± 0.01	244	3.4×10^5	0.8
delbcGloA3	-	0.030 ± 0.006	0.06 ± 0.01	0.02	333	1.1
	Zn^{2+}	0.116 ± 0.005	0.28 ± 0.01	0.07	250	1
	Ni^{2+}	2.9 ± 0.6	0.22 ± 0.03	1.7	7727	33
delbdGloA3	-	0.043 ± 0.003	0.05 ± 0.02	0.03	600	1.6
	Zn^{2+}	1.237 ± 0.023	1.93 ± 0.05	0.73	378	1
	Ni^{2+}	451 ± 45	0.98 ± 0.09	268	2.7×10^5	714
delcdGloA3	-	58 ± 2	0.64 ± 0.01	36	5.6×10^4	0.6
	Zn^{2+}	132 ± 14	0.80 ± 0.08	83	1.0×10^5	1
	Ni^{2+}	341 ± 18	0.38 ± 0.02	216	5.7×10^5	6
delbcdGloA3	-	0.014 ± 0.001	0.55 ± 0.07	0.01	18	0.4
	Zn^{2+}	0.027 ± 0.002	0.50 ± 0.05	0.02	40	1
	Ni^{2+}	17.39 ± 0.04	0.97 ± 0.01	10.0	1.0×10^4	250

*Relative k_{cat}/K_m was calculated from the k_{cat}/K_m of each specific sample relative to that of its Zn^{2+}-bound form.

Table S4. Kinetic data for wild-type GloA2, wild-type GloA3 and various GloA3 "B-loop" protein variants (delbGloA3, delbdGloA3 and delbcdGloA3) showing the different catalytic activities in the presence of Ni^{2+} and Zn^{2+} ions. The proteins were expressed and overproduced without additional metals added to the bacterial growth media. Proteins were purified and any bound metals were removed by dipicolinic acid (DPA) incubation as previously described for GloA3. Metals ($NiCl_2$ or $ZnCl_2$) were then added to solutions of these apo-enzymes. The resulting enzymes were incubated overnight at 4 °C before performing assays using 0.02-1 mM substrate (MG-GSH), pH 6.6, at room temperature. No loss of activity was observed for overnight incubations compared to 15 minute incubation times.

Enzyme	***Metal chloride***	k_{cat} (s^{-1})	K_m *(mM)*	k_{cat}/K_m $(M^{-1}.s^{-1})$	[#]***Relative*** k_{cat}/K_m
GloA2	Ni^{2+}	238	0.12 ± 0.01	2.0×10^6	-
GloA3	[†]Zn^{2+}	226	0.27 ± 0.02	0.8×10^6	1
	[$]$Zn^{2+}$	556	0.21 ± 0.01	2.6×10^6	3.2
	[£]Zn^{2+}	738	0.48 ± 0.02	1.5×10^6	1.8
delbGloA3	Zn^{2+}	4.8	1.37 ± 0.49	3472	1
	Ni^{2+}	1375	0.98 ± 0.16	1.4×10^6	404
delbdGloA3	Zn^{2+}	0.89	1.02 ± 0.21	873	1
	Ni^{2+}	167	1.06 ± 0.22	0.2×10^6	181
delbcdGloA3	Zn^{2+}	0.02	0.43 ± 0.03	53	1
	Ni^{2+}	14	1.22 ± 0.19	1.1×10^4	212

[†] The purified holoGloA3 was assayed in the presence of 5 equivalents of metal ion

[$] The purified GloA3 that was overproduced and expressed in the presence of 1 mM $ZnCl_2$ in bacterial growth medium and assayed in the presence of 5 equivalents of metal ion

[£] The purified GloA3 that was overproduced and expressed in the presence of 2 mM $ZnCl_2$ in bacterial growth medium and assayed without additional metal ion added to the assay

[#] Relative ratio of k_{cat}/K_m was calculated from the k_{cat}/K_m of each specific sample relative to that of its Zn^{2+}-reconstituted form.

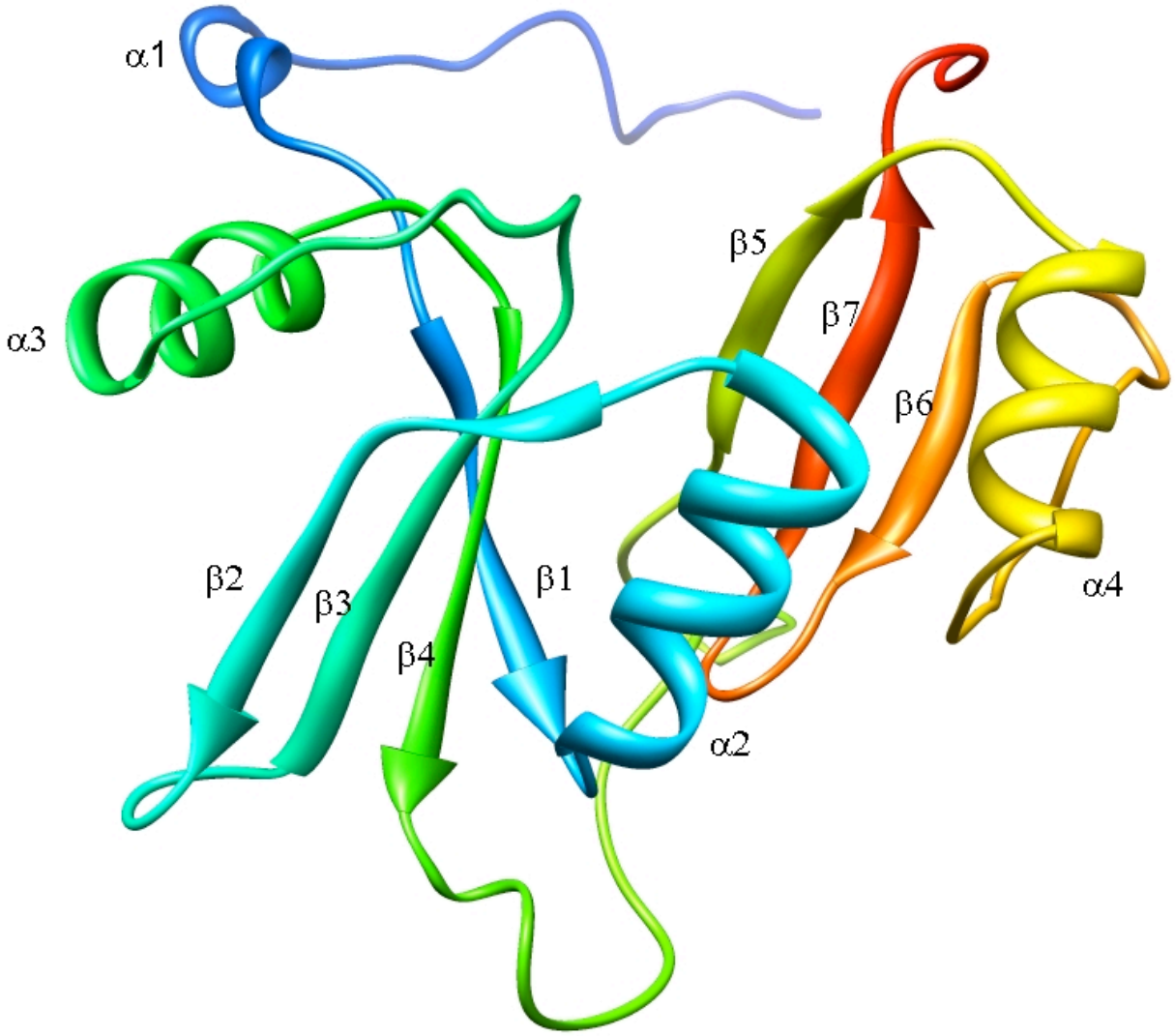

Figure S1. The predicted subunit structure of GloA3 as determined by use of the Geno3D web-based program [1] using human GlxI (PDB: 1QIN) as a template.

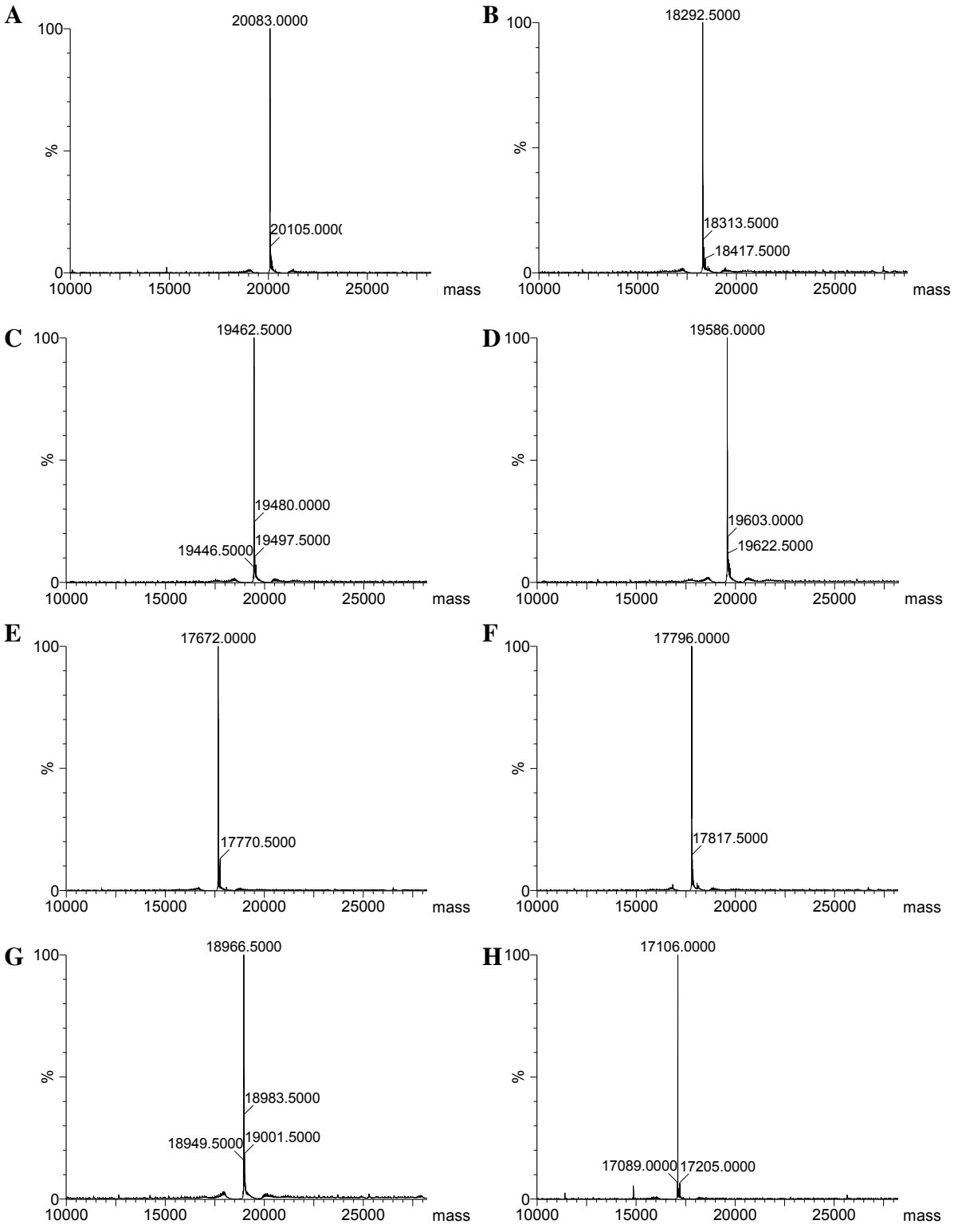
A
20083.0000
20105.0000
B
18292.5000
18313.5000
18417.5000
C
19462.5000
19480.0000
19497.5000
19446.5000
D
19586.0000
19603.0000
19622.5000
E
17672.0000
17770.5000
F
17796.0000
17817.5000
G
18966.5000
18983.5000
19001.5000
18949.5000
H
17106.0000
17089.0000
17205.0000
100
%
0
10000
15000
20000
25000
mass

Figure S2. The electrospray mass spectrum of individual subunits of *(A)* GloA3 (calculated MW is 20083.5 Da), *(B)* delbGloA3 (calculated MW is 18292.5 Da), *(C)* delcGloA3 (calculated MW is 19462.9 Da), *(D)* deldGloA3 (calculated MW is 19587.0 Da), *(E)* delbcGloA3 (calculated MW is 17672.0 Da), *(F)* delbdGloA3 (calculated MW is 17796.0 Da), *(G)* delcdGloA3 (calculated MW is 18966.4 Da) and *(H)* delbcdGloA3 with MW of a single subunit at 17106.0 Da (calculated MW is 17175.5 Da). Unintentional single mutation (Arg→ Ser) introduced into triple deletion at position 69 of the protein gene product as explained in Experimental Procedures. Electrospray mass spectrum for this protein variant is consistent with this single mutation being present (calculated MW for this variant is 17106.4 Da).

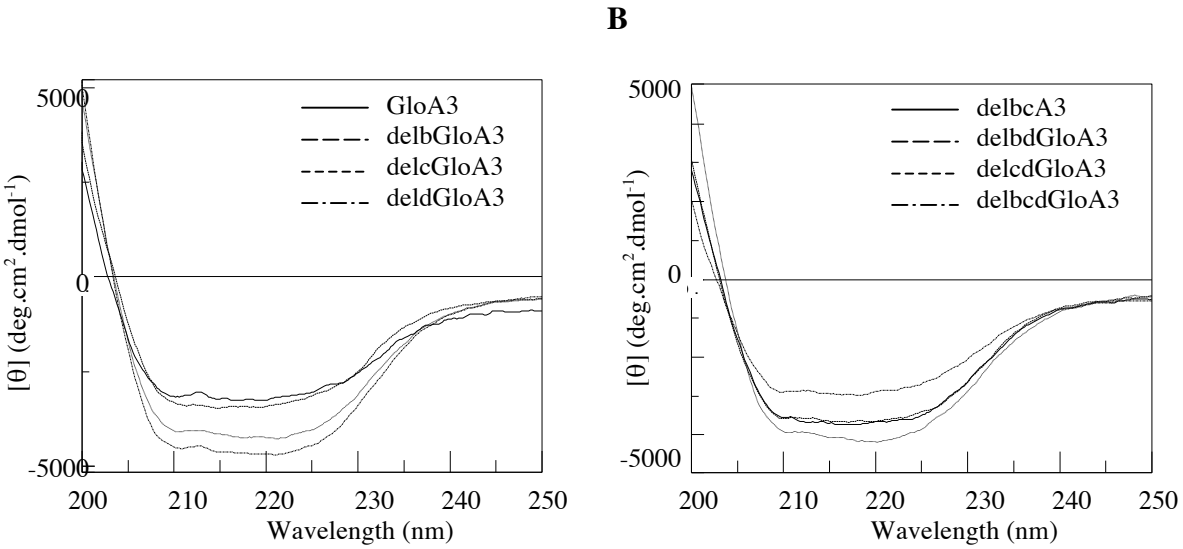

Figure S3. The CD spectra of the Ni^{2+}-bound enzymes (0.3 mg/mL) including *(A)* GloA3, delbGloA3, delcGloA3 and deldGloA3 and *(B)* delbcGloA3, delbdGloA3, delcdGloA3 and delbcdGloA3 in 50 mM Tris (pH 7.5) and 150 mM NaCl.

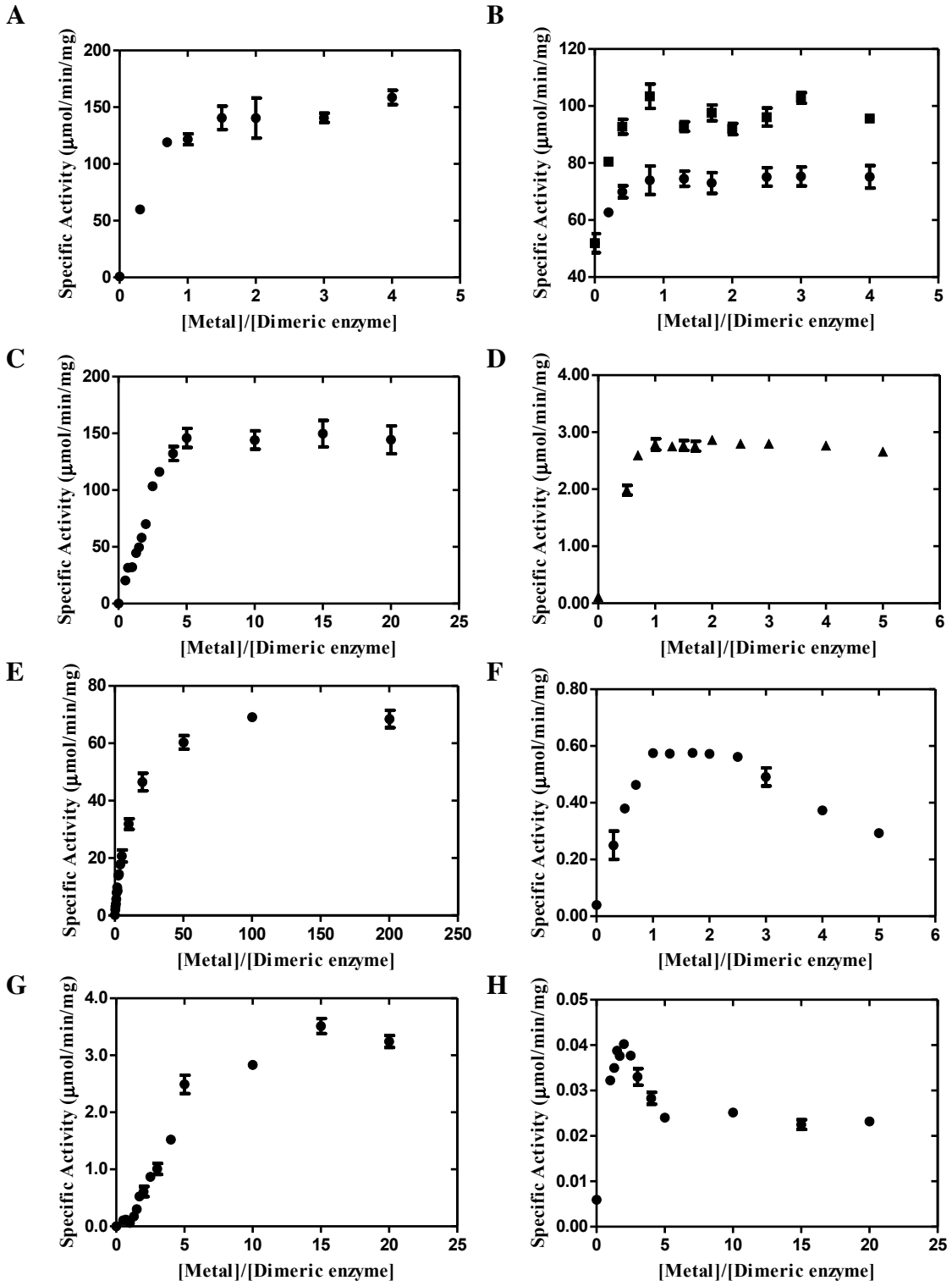

Figure S4. Metal titration profiles for *(A)* GloA2 with Ni^{2+}, *(B)* GloA3 with Ni^{2+} (■) and Zn^{2+} (●), *(C)* delbGloA3 with Ni^{2+}, *(D)* delbGloA3 with Zn^{2+}, *(E)* delbdGloA3 with Ni^{2+}, *(F)* delbdGloA3 with Zn^{2+}, *(G)* delbcdGloA3 with Ni^{2+} and *(H)* delbcdGloA3 with Zn^{2+}. The apo-enzymes of GloA3 protein variants were incubated with metals overnight at 4 °C, while GloA2

and GloA3 were incubated with metals for 15 min. The assay was performed using 0.5 mM MG-GSH, pH 6.6, at room temperature.

References for Supplementary Information

[1] Combet, C., Jambon, M., Deleage, G. and Geourjon, C. (2002) *Bioinformatics*. **18**, 213-214.